ANALYSIS OF EXTRUDED READY-TO-EAT BABY FOODS PREPARED USING COMPOSITE FLOURS FROM ORANGE-FLESHED SWEET POTATOES, SOYBEANS, AND AMARANTH SEEDS

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DECLARATION AND RECOMMENDATION

Declaration

I hereby certify that this thesis is my original work and confirm that it has never been submitted anywhere for the award of any degree.

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Recommendation

This thesis has been submitted with our approval as the official university supervisors.

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DEDICATION

This work is dedicated to my wife Jovia, my daughter Abiela, my parents Jack and the late Kate, my sisters Gloria, Geddy, and Christine, my brother Bright, my sister-in-law Merica, Jeannette, Genevieve, and my friend Faustin for their unshakable love, care, prayers, encouragement, and support from all sides.

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ABSTRACT

There is severe food insecurity for many people who live in sub-Saharan Africa. The situation is worse for children under the age of five years who are undernourished. This is caused mainly by poverty limiting access to quality foods, as well as poor breastfeeding, and complementary feeding practices. Therefore, the purpose of this study was to develop and analyze the physicochemical properties, microbial, sensory properties, and shelf-life of extruded RTE baby foods prepared using composite flours from orange-fleshed sweet potatoes (OFSP), amaranth seeds, and soybeans. Ready-to-eat (RTE) baby foods which are conveniently distributed to the poor could be a way of mitigating this challenge. Additionally, there are advocacies for the utilization of locally available food resources for sustainable food production. Extrusion technology has been used in many parts of the world to process RTE foods. However, as with most food technologies, there are always questions regarding optimum working conditions, nutritional quality and safety, consumer outlook, and shelf stability that need to be addressed. The ingredients were optimized using the extreme vertices method of mixture design using Minitab Software. The optimum value for model verification was 57% OFSP, 24% amaranth seeds, and 19% soybeans flour, to achieve targeted values of crude protein (14%), total minerals (4.7%), and Vitamin A (813.6 Retinol Activity Equivalent μg/100g). The optimum extrusion cooking conditions were established as 90^{o}C die temperature, 35% feed moisture content, and 400 rpm screw speed based on Box-Behnken Experimental Design of Response Surface Methodology under Design Expert Software. A completely randomized design (CRD) in a factorial experimental design was employed using statistical analysis software (SAS) for the production of extruded ready-to-eat baby foods. The results revealed that extrusion cooking and blend proportions significantly ($p<0.05$) affected the physicochemical properties, microbial, sensory properties, and shelf-life of extrudates. The extrusion cooking significantly ($p<0.05$) reduced moisture content (59.5%), protein content by 5.56%, anti-nutrient content (65.24 - 97.43%), and vitamin A content (21.33%). On the other hand, there were significant ($p<0.05$) increases in carbohydrate (12.31%), total minerals (10.44%), dry matter (7.06%) contents, energy value (6.38%) and energy-to-protein ratio (11.28%). Blend proportions and extrusion cooking have significant ($p<0.05$) positive effects on the protein quality of the extrudates in terms of *in vitro* protein digestibility and available lysine. The shelf-life of extrudates was found to be about 4 to 6 months. Therefore, the use of extrusion cooking techniques for OFSP, amaranth seeds, and soybeans composite flours has the potential for the production of value-added OFSP food products. These findings can be used to achieve food and nutrition security in developing countries.

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LIST OF ABBREVIATIONS AND ACRONYMS

AACC:	American Association of Cereal Chemists
AIC:	Akaike's Information Criterion
ANOVA:	Analysis of Variance
AOAC:	Association of Official Analytical Chemists
BBD:	Box-Behnken experimental Design
BIC:	Schwarz's Bayesian Criterion,
C(p):	Conceptual Predictive Criterion
CAGR:	Compound Annual Growth Rate
CESAAM:	Centre of Excellence in Sustainable Agriculture and Agribusiness Management
CRD:	Completely Randomized Design
DF:	Degree of Freedom
FAO:	Food and Agriculture Organization of the United Nations
GABA:	Gamma-Aminobutyric Acid
HSD:	Honest Significant Difference
IVPD:	*In vitro* Protein Digestibility
KALRO:	Kenya Agricultural and Livestock Research Organization
MENA:	Middle East and North Africa
MIMO:	Multiple in multiple out
OFSP:	Orange Fleshed Sweet Potato
PDA:	Potato Dextrose Agar
PEM:	Protein-Energy Malnutrition
PRESS:	Predicted Residual Error Sum of Squares
RCBD:	Randomized Complete Block Design
RSM:	Response Surface Methodology
SAS:	Statistical Analysis Software
SSA:	Sub-Saharan Africa
SSE:	Standard Error of the Estimate
TVC:	Total Viable Count
UNICEF:	United Nations International Children's Emergency Fund
VAD:	Vitamin A Deficiency
WHO:	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Food insecurity is a main significant challenge that persists in many parts of the world, mainly in developing countries. The undernourishment situation is approximately 702.7 million humans, and 830.2 million severely food insecure people in the globe (FAO *et al*., 2022). Undernourishment and severe food insecurity seem to be growing in nearly all areas of Africa, and South America, while undernourishment in most regions of Asia is stable. Many countries in sub-Saharan Africa (SSA) rely on a narrower food base, a situation that minimizes variety in food production and contributes to food insecurity (FAO *et al*., 2018; Mugalavai, 2013).

Climate change is one of the factors which affect food security. In addition, climate change aggravates the already unacceptably excessive levels of hunger and under-nutrition in developing countries. Some of the countries in SSA where malnutrition is widespread include Burundi, Ethiopia, Rwanda, Tanzania, Uganda, and Kenya. Most cases of child mortality and morbidity are linked to poverty and food insecurity. Diseases associated with poor nutrition intake such as protein-energy malnutrition (PEM), vitamin A deficiency (VAD), and anaemia (Global Nutrition Report, 2016; Hjelm, 2016) also lead to the death of children. Some of the causes of PEM are the inadequate nutritional quality of traditional complementary food, inappropriate complementary feeding practices, and the increased cost of protein-based complementary foods (Aka *et al*., 2010). The interaction of poverty, poor complementary feeding practices, and poor health has numerous adverse effects on the general welfare of children and is additionally exposed to risks such as growth retardation, poor cognitive development, illness, and death among children in developing countries (Anigo *et al*., 2007).

The quality and quantity of dietary protein and calorie intake affect physiological maintenance and growth as a result of malnutrition (Path & Koch, 2018). Vitamin A deficiency (VAD) is also a primary public health problem in low- and middle-income countries, where it is estimated that 190 million children less than five years of age are affected and leading to many adverse health effects, including death (Imdad *et al*., 2017). Blending diverse foods can be an efficient technique for combating malnutrition since it delivers a wide range of necessary elements such as carbohydrates, protein, fats, vitamins, and minerals. The orange-fleshed sweet potato (OFSP) has been used as an ingredient in infant foods to boost nutrients specifically vitamin A. However, it is low in protein, fat, and some minerals (Amagloh & Coad, 2014).

Therefore, soybean incorporation could help to increase protein, fat, and minerals. The protein quality of both orange-fleshed sweet potato and soybean (*Glycine max* (L.) Merrill) has low levels of lysine, tryptophan, and methionine content. These limiting amino acids are present in significantly amounts in amaranth seed than that found in cereals and legumes (Mendoza & Bressani, 1987). Unlike true cereals, pseudocereals contain high amounts of essential amino acids, particularly methionine, lysine, arginine, tryptophan, and sulfur-containing amino acids (Akkoc *et al*., 2019). Therefore, the incorporation of amaranth seed flour into the orange-fleshed sweet potatoes and soybeans flour blends could help to produce nutritious food with high-quality protein-based ready-to-eat baby food for under-five years old children.

The situation of food and nutrition for under-five children can be improved with the aid of considering plants that have domestication potentials, such as the amaranth grain (*Amaranthus hybridus* L), which is relatively rich in protein (12-19%) and minerals such as calcium (190.7 mg/100g), magnesium (220.4 mg/100g), potassium (326.8 mg/100g), phosphorous (323.2 mg/100g), iron (13.9 mg/100g), zinc (5.2 mg/100g), manganese (6.3 mg/100g), sodium (8.1 mg/100g), copper (0.6 mg/100g) on dry matter basis when compared to commonly consumed cereal grains such as maize, brown rice, sorghum, wheat, oat, millet, barley, and rye. Amaranth grain is a highly neglected crop, but it is very easily grown, as well as its stress resistance, and increased yield (Mburu *et al*., 2012; Mugalavai, 2013). Cereals and pseudocereals also contain good amounts of bioactive compounds including dietary fibres, phenolic acids, carotenoids, β-glucans, as well as other phytochemicals such as tocopherols, alkylresorcinols, and flavonoids associated with the prevention of diseases (Akkoc *et al*., 2019). Among pseudocereals, amaranth, quinoa, and buckwheat have the most commercial potential. Pseudocereal grains are mostly made up of carbohydrates and proteins, which account for 55–75% (Venskutonis & Kraujalis, 2013) and 12–16% (Mota *et al*. 2016), respectively.

Traditional cereals such as wheat, maize, rice, and barley, as well as minor cereals such as sorghum, millet, oats, rye, and spelt, are becoming more popular worldwide. Cereals‘ principal nutritional components are starch and non-starch carbohydrates, which account for around 87% of their total weight, while protein content ranges from 6 to 15% (Pojic & Tiwari, 2021).

Some of the extruded foods available on the market include snacks and grain products such baby food, weaning food, cereal bar, bread crumb, instant porridge, pre-cooked flours, instant concentrates, functional components (Guo & Dong, 2019; Pinkaew *et al*., 2012; Riaz, 2016), texturized plant protein (Bhattacharya *et al*., 2009; Moscicki, 2011), modified starches

(Nehmer *et al*., 2007; Smith, 1992), encapsulated flavours (Camire, 2000), dairy products such as sodium caseinate (Manoi & Rizvi, 2009), breakfast cereals (Bouvier *et al*., 2001; Riaz, 2016; Singh *et al*., 2019), drinks like beverage powder (Kazemzadeh, 2001), muscle foods like restructured fish/meat mince (Choudhury & Gautam, 2003), spice products and flavours (Wojtowicz *et al.*, 2010), confectionery (Moscicki, 2011; Muliji *et al*., 2003), animal feed (Muthukumarapan, 2011; Singh, 2016; Williams, 2000), baked goods and pasta products (Guo & Dong, 2019; Moscicki, 2011; Wojtowicz, 2011), and fats and oils such as stabilization of rice bran (Kim *et al*., 1987). Cereals are poor in protein and lysine is the limiting amino acid in cereals. As a result, combining them with pseudocereals and soybeans might increase protein content while conserving energy.

Sweet potato (*Ipomoea batatas*) is one of the most crucial foods security-promoted tuber crops in the world, particularly in SSA (Sanoussi *et al.*, 2016). Sweet potato has excellent energy content (438 kJ/100 g edible component). It can produce more suitable energy for human consumption per hectare per day than cereals, such as wheat and rice (Abu *et al*., 2000). Orange-fleshed sweet potato tubers are rich in starch, sugar, vitamin C, β-carotene, iron, and several other minerals (Laurie *et al*., 2012; Oloo *et al*., 2014). The main dry matter components for sweet potato are carbohydrates which in most cultivars make up 90% of dry matter. The protein content in sweet potato is generally low, ranging from 1.0 to 14.2% dry weight basis (dwb) (Dhungana *et al*., 2014). Sweet potato protein is good quality and contains high amounts of essential amino acids except for tryptophan and total sulphur amino acids (Wanda, 1987). Grain legumes are the most important sources of dietary proteins in most developing countries because animal proteins are expensive (Ku *et al*., 1976). Domestic cooking and processing techniques have been considered to significantly reduce the antinutrients and thus increase the nutritional value of legume grains (Sharma & Sehgal, 1992).

Maintaining and enhancing the nutritional quality of food during food processing is always an area of research that is potentially important (Singh *et al*., 2007). The increased consumer demand for gluten-free, high-value cereals that are sustainable, safe, and nutritious is not the only factor driving innovation in the food industry; there is also a need to mitigate processing‘s adverse impact on the environment by using as little energy as possible. However, there are several safety-related obstacles to the commercialization of grain byproducts, such as the existence of hazardous substances and significant antinutritional factor concentrations (Pojic & Tiwari, 2021). Furthermore, the issues of heat-labile nutrients including lysine owing to high-temperature processing is a major challenging problem in most traditional cooking methods (Singh *et al.*, 2007). Extrusion cooking is a continuous operation that forces food material through a confined region or die under controlled conditions (Sudhakar *et al.*, 2023).

Extrusion is a financially feasible technology due to its widespread use in the food industry and customer demand for extruded products. The functional food sector is looking for processed foods with new designs and nutritional benefits. Extrusion technology is without a doubt the greatest method for meeting these requirements (Sudhakar *et al.*, 2023). Extrusion is a continuous process that combines several unit operations, including mixing, cooking, kneading, shearing, shaping and forming, to produce a wide range of products (Fellows, 2022). Extrusion cooking of ready-to-eat (RTE) cereals provides many benefits in comparison to conventional processing methods. Extrusion cooking allows for quicker processing, lower processing costs, high productivity, significant nutrient retention, less space for the plant required for processing equipment, little labour, and greater flexibility leading to more types of end-products (Alam *et al.*, 2016; Guy, 2001; Sudhakar *et al.*, 2023). Extruded foods are used by consumers in a variety of forms, including breakfast cereals, infant food, and ready-to-eat snacks (Sudhakar *et al.*, 2023).

1.2 Statement of the Problem

Consumers are drawn to increase the consumption of ready-to-eat cereal products which are low in nutritional quality. Changing dietary preferences and trends for healthier or more convenient foods should be adopted. Apart from the high cost of quality protein-based complementary foods, there is also limited access to nutritious food, poverty, inadequate healthcare, breastfeeding, complementary feeding, poor hygiene, inadequate dietary diversity, and disease burden. Thus, they aggravate protein-energy malnutrition (PEM) and micronutrient deficiencies (Vitamin A deficiency and iron deficiency) in children. Malnutrition is still very common in many parts of the world. Sub-Saharan Africa (SSA), specifically in Eastern Africa has one of the highest levels of stunting worldwide. Malnutrition is mainly caused by insufficient intake of nutrients in terms of quality and quantity.

On the other hand, heat-labile nutrient issues in most traditional cooking methods are becoming increasingly prevalent across the world. Lysine is an essential amino acid necessary for human health and plays a crucial role in protein synthesis, metabolism, growth and development of a child. However, heat-labile lysine is prone to degradation and denaturation under high-temperature cooking processes, which can lead to a decrease in its bioavailability and nutritional value in food products. The extent of heat-induced lysine degradation during different food processing methods, and its implications for food quality and nutrition are not fully explored. This issue is of great concern as it directly affects the nutritional value and protein quality of various food products consumed by individuals, especially children.

Most traditional cooking methods cause the potential loss of heat-labile nutrients such as lysine and other free amino acids. Indigestible protein-sugar complexes are created during the heat treatment of food, thereby reducing the nutritional value of that food. In addition, the complexes inhibit the absorption of nutrients and their metabolism in the body. To mitigate food insecurity among the poor, there are suggestions to utilize locally available food crops for sustainable food production and technologies that would produce shelf-stable food products that can be distributed with little storage challenges.

RTE foods are good candidates to offer a solution. Extrusion technology takes a shorter period to prepare food while significantly maintaining its quality as compared to most traditional cooking methods. Extrusion cooking is also relatively cheaper, fast speed, high productivity, versatility, distinctive product shapes, great nutritional retention, energy efficacy, environmentally friendly, and it could be used to produce convenient food products for under-five children. However, as with most food technologies, there are always questions regarding optimum working conditions, nutritional quality and safety, consumer perspective about the food product and shelf-life that need to be ascertained. Therefore, there is a need to develop new and unique products for healthful and nutritious qualities using emerging technologies such as extrusion cooking at the same time optimizing extrusion cooking conditions to minimize the degradation of lysine and enhancing its bioavailability.

1.3 Objectives of the Study

1.3.1 General Objective

To contribute towards sustainable food and nutrition through analysis of ready-to-eat baby foods prepared using composite flours from orange-fleshed sweet potatoes, soybeans, and amaranth seeds using extrusion cooking technology.

1.3.2 Specific Objectives

i. To evaluate the impact of optimizing the ingredients on the protein, total mineral, and vitamin A levels in composite flour made from orange-fleshed sweet potatoes, soybeans, and amaranth seed flour.
ii. To determine the influence of optimizing extrusion cooking parameters on functional qualities of ready-to-eat foods made from orange-fleshed sweet potatoes by using RSM

iii. To determine the influence of blending and extrusion cooking on the physicochemical composition and sensory characteristics of extruded ready-to-eat baby foods made from blends of orange-fleshed sweet potatoes, soybeans, and amaranth seeds flours.
iv. To determine the influence of blending and extrusion cooking on the protein quality of extruded ready-to-eat baby foods from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour blends.
v. To determine the influence of blending, storage temperature and storage time on microbiological load and shelf-life of extruded ready-to-eat baby foods made from orange-fleshed sweet potatoes, soybeans, and amaranth seed flour blends.

1.4 Hypotheses

i. There is no significant impact of optimizing the ingredients on the protein, total mineral, and vitamin A levels in composite flour made from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour.
ii. Optimization of extrusion cooking parameters has no significant influence on the functional properties of extrudates.
iii. There is no significant influence of blending and extrusion cooking on the physicochemical composition and sensory characteristics of extruded ready-to-eat baby foods made from blends of orange-fleshed sweet potatoes, soybeans, and amaranth seeds flours.
iv. Blends and extrusion cooking have no significant influence on the protein quality of extruded ready-to-eat baby foods from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour blends.
v. Blending, storage temperature and storage time have no significant influence on the microbiological load and shelf-life of extruded ready-to-eat baby foods from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour blends.

1.5 Justification

Malnutrition is a serious global concern that disproportionately affects children in many parts of the world. While there has been progress in reducing malnutrition rates, there are still important gaps that must be filled including undernutrition, stunting, wasting, micronutrient deficiencies dues to limited access to healthcare, climate change and conflict, socioeconomic inequalities, inadequate complementary feeding and breastfeeding, and limited access to nutritious foods. On the other hand, cereals and pseudocereals contain many healthy functional components, including dietary fibres (insoluble and soluble), prebiotic carbohydrates, resistant starch, proteins, minerals, vitamins, and antioxidants (Mišan *et al*., 2021). They could be used as raw materials for the production of nutritious diets due to their numerous health benefits.

Furthermore, there is great potential to improve food and nutrition security in Africa, especially in SSA due to the presence of a great diversity of locally available raw materials which are highly nutritious and drought tolerant (Mazike *et al*., 2022). There has been limited product diversification, innovation, and value addition on the amaranth seeds and OFSP value chain; and a lack of indigenous pseudo-cereals in modern commercialized and industrialized markets to make them more attractive, convenient, and accessible and therefore remain underutilized. OFSP has been used to increase the vitamin A content of infant foods, however, it is low in protein and fat. Soybean inclusion may aid in the rise of protein, fat, and minerals. However, the protein quality of both OFSP and soybean is low in some amino acids while amaranth seeds have a high level.

In Africa, the production, processing, and consumption of African indigenous vegetables have been neglected (FAO, 2016; Mazike *et al*., 2022; Nyaruwata, 2019). Traditional processing in Africa is labour intensive with women contributing up to 80% of labour; hence, more complex processing such as extrusion and flaking is not being practised by smallholder farmers on the African continent (Palacious-Lopez *et al*., 2017). Furthermore, mechanization provides opportunities for sustainably intensifying production, value addition and food systems development, and improved local economies and livelihoods. However, African food production systems are the least mechanized across all continents (FAO, 2016). The use of roots and tubers blended with amaranth seeds could potentially increase their demand and drive their production and development.

Moreover, some of the most common traditional processing methods being used such as direct sun-drying results in loss of nutrients and therefore, identify the best methods for maintaining nutrients but at the same time diversifying value-added products using advanced food processing technologies such as rolling, canning, extrusion, malting, and flaking could help (Mazike *et al*., 2022). Extrusion cooking is an efficient, versatile, high-temperature short-time process in which food raw materials are cooked using a combination of moisture, pressure, temperature, and mechanical shear (Boakye *et al*., 2022; Fellows, 2022). In Africa, dried leafy vegetables are the most common value-added product, whereas in Asia and South America, popped amaranth and extruded products from amaranth flour are common value-added products, respectively (Onyeoziri *et al*., 2017).

This study produced new RTE baby foods, optimum ingredients, optimum extrusion cooking conditions, and the Arrhenius models for shelf-life prediction. In addition, this research provides valuable insights into the impact of heat-labile lysine degradation on food quality and nutrition. The findings will contribute to the development of guidelines and recommendations for extrusion cooking methods which minimize lysine loss, thus preserving the nutritional value and protein quality of food products. Ultimately, this research can help improve dietary practices and promote healthier food choices for individuals with lysine-deficient diets or specific nutritional requirements. The findings from this study will be useful for researchers, policy-makers, food processors, and consumers.

Insights from the literature review and the developed nutrient-dense extrudates can help understand how amaranth seeds, soybean, and OFSP flour blends can be utilized to reduce the food and nutrition insecurity burden in SSA. This will contribute towards the achievement of Sustainable Development Goals **(**SDGs) number 1, 2, and 3 which aim to end poverty; end hunger, and all forms of malnutrition, achieve food security and improved nutrition; and ensure health and well-being for all, at every stage of life, respectively. This research advances knowledge, advancements in technology, food safety, nutritional enhancements, and product quality, all of which have major implications for the food industry, public health, and consumer well-being.

1.6 Scope and Limitations

This study was concerned with the production and evaluation of extruded RTE products from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour blends. The study focused on the analysis of proximate, micronutrient, anti-nutritional factors, sensory, physical, microbial, and protein quality. Consumer acceptability test was used during sensory evaluation and untrained panellists (mothers who have under-five years children) were used for sensory evaluation of extruded ready-to-eat baby foods because under-five years children are verbal scale limited. On the other hand, due to time limitations, further analysis like bioassays, packaging, a market study of extruded ready-to-eat baby foods, and running a full-length storage trial for determining shelf-life was not carried out.

CHAPTER TWO
LITERATURE REVIEW

2.1 Malnutrition Prevalence in Under-five Children

Malnutrition is an atypical physiological condition that is due to insufficient, unbalanced, or excessive intake of macronutrients and/or micronutrients. Malnutrition consists of under-nutrition and over-nutrition in addition to micronutrient deficiencies (FAO *et al*., 2022). Although significant progress has been made in reducing child stunting, levels remain unacceptably high. Currently, the report reveals that 22.0% of under-five years children are stunted and 6.7% are wasted globally in 2020 (Global Nutrition Report, 2021). Africa stands at 30.7%, SSA at 32.3% as shown in Table 2.1, while Eastern Africa has one of the highest levels of stunting cases with 32.6% and 5.2% of wasting under-five children globally (FAO *et al*., 2022). In addition, it keeps having an impact on more than 50 million children under-five years in the world which brings these children at high risk of morbidity and mortality (FAO *et al*., 2018).

Protein-energy malnutrition (PEM) is described as a clinical situation that occurs due to inadequate protein and calorie consumption, specifically in children (Chukwu *et al*., 2017). Under-nutrition is the recognition of being underweight (inadequate age weight), stunting (inadequate age length/height), and waste (low height weight). Stunting consequences from the cumulative effects of infant under-nutrition and infection all through the essential 1000-day period covering pregnancy and the first two years of a child's life. These children are exposed to risks that consist of diminished cognitive and physical development. An estimated 93 million children under the age of five (one in seven children worldwide) are suffering from underweight negative effects and 45% of child deaths are associated with under-nutrition (WHO, 2015).

In developing countries, malnutrition accounts for half of the deaths of children under 5 years of age. Africa and Asia have high levels of all forms of malnutrition. More than half of all stunted children under the age of five were living in Asia, while more than one-third were observed in Africa in 2015. More than two-thirds of all wasted children under five lived in Asia in 2015, while more than a fifth lived in Africa. All stunted children (66%) live in countries with lower-middle incomes (WHO, 2015). The signs of increasing hunger and food insecurity are a caution that there is extensive work to be done to make certain that “no one is left behind” on the road in the direction of a world with zero hunger (FAO *et al*., 2018).

Table 2. 1 Prevalence of undernourishment, severe food insecurity, and selected forms of malnutrition (%)

Region	Undernourished People in 2019-2021	Severely Food-Insecure People in 2019-2021	Wasting (< 5 years of age children) in 2020	Stunting (< 5 years of age children) in 2020
World	9.0	10.7	6.7	22.0
Africa	19.1	22.0	6.0	30.7
Sub-Saharan Africa	22.0	24.8	5.9	32.3
Eastern Africa	29.2	27.3	5.2	32.6
Kenya	26.9	26.1	4.2	19.4
Uganda	-	23.2	3.5	27.9
Rwanda	35.8	-	1.1	32.6
Burundi	-	-	4.8	57.6
Ethiopia	24.9	19.6	7.2	35.3
Somalia	-	41.6	-	27.4
Eritrea	-	-	-	49.1
Tanzania	22.6	25.8	3.5	32.0
South Sudan	-	62.3	-	30.6
DRC	39.8	39.2	6.4	40.8

Source: FAO *et al*. (2022)

A majority of the world's population suffers from inadequate dietary protein and calorie intake, both qualitatively and quantitatively. Physiological maintenance and growth are impaired in all such instances, resulting in malnutrition (Pathak & Kochhar, 2018).

2.2 Impact of Different Food Processing Methods on Food Quality

Drying, milling, malting, fermentation, germination, frying, roasting, and baking are the most frequent cereal and pseudocereal processing methods, and they are characterized by a slow rate of innovation (Pojic & Tiwari, 2021). The secondary processing includes baking, extrusion and/or puffing, flaking, frying, steaming, and roasting. Secondary processing renders cereal grains more pleasant, digestible, and acceptable for consumption. Pasta, bread and baking items, morning cereals, and cereal-based confectionary treats are examples of secondary processed products. A number of modifications may occur during processing that impacts the nutritional and functional aspects of cereal or pseudocereal products, influencing the availability and digestibility of macro and micronutrients, as well as shelf life (Sasthri *et al.*, 2021). Depending on the nature and intensity of the factors, any processing method modifies the nutritional content of the grains. This is reflected in either a decrease in nutrients, phytochemicals, and antinutrients or an increase in nutrient digestibility or availability. It is critical to understand these changes and choose optimal processing processes to get the greatest nutritional and physiological advantages from cereal-consuming food (Oghbaei & Prakash, 2016).

Milling is a mechanical process for reducing solid materials (cereals and pseudocereals) to smaller particles or powders. Milling is the primary processing step used in the cereal sector, which can be done using either a wet or dry milling method (Sasthri *et al.*, 2021). The milling procedure eliminates anti-nutrients found in the bran of grains, such as phytic acid, lectins, and tannins, however, this method's primary disadvantage is that it also reduces crucial minerals (Gupta *et al.*, 2015). The increased surface area of foods induced by size reduction may also result in a loss of nutritional content due to the oxidation of fatty acids, carotenes, and heat-sensitive, oxygen-sensitive, and light-sensitive components (Fellows, 2023). In addition, primary processing involves milling, which is frequently paired with fractionation, pearling, and malting (Sasthri *et al.*, 2021). Dry milled foods have a low enough a_w to store for several months without significant changes in nutritional content, eating quality, or microbiological safety. Moist foods, on the other hand, decay quickly if other preservation techniques, such as cooling, freezing, or heat processing, are not used. Particle sizes have a significant impact on the textural or rheological qualities of meals. Milling and pulping modify texture by physically reducing particle size and, in wet foods, physically damaging cells. This results in the formation of new surfaces that allow enzymes and substrates to become more closely combined, which can result in the faster degradation of colour, fragrance, flavour, and texture (Fellows, 2023).

Dehydration (or drying) is the controlled use of heat to remove the bulk of the water ordinarily contained in food by evaporation or, in the case of freeze drying, by freezing (Fellows, 2023). Drying is one of the most efficient methods for lowering the water activity of foods and improving their shelf life. The most prevalent methods for lowering the moisture content of foods are sun drying and hot air drying (Kohli *et al.*, 2023). Drying via traditional methods, such as dehydration or desiccation, needs a long time to reduce moisture content and requires high temperatures, which damages food products (Shwetha & Preetha, 2017). The mentioned problems will reduce the quality of the food products. Furthermore, freeze-drying is a costly operation, and spray-drying is only appropriate for liquids. The ultrasonic technique can dry fruits, vegetables, fish, and meat due to enhanced mass transfer and quicker diffusion (Prasad *et al*., 2023). The heat used to dry food or concentrate liquids by boiling eliminates water and so protects the food by reducing water activity (a_w). However, heat causes a loss of sensory qualities as well as a drop in nutritional quality (Fellows, 2023). A study found that freeze-drying may be a promising strategy for food preservation due to its capacity to preserve broad bean physical qualities, antioxidant potential, and bioactive compounds. Sun drying, on the other hand, is a low-cost, convenient method that is time-consuming and dependent on weather conditions (Li *et al*., 2023).

There was no influence on minerals, with more than 92% of Fe, Ca, and Zn preserved in sun-dried, boiling, and sun-dried leaves. The study found that submitting cowpea leaves to sun drying or a combination of boiling and sun drying results in a little loss of carbohydrates and proteins but a large loss of bioactive chemicals (Wasswa *et al*., 2021). The Black sesame seeds shells were severely damaged and their colour intensified after the steaming and sun-drying treatments. The rate of oil extraction rose as the protein content and relative amount of monounsaturated fatty acid (MUFAs) and polyunsaturated fatty acid (PUFAs) dropped (Cheng *et al*., 2021). The disadvantages of open sun drying include a long drying period (typically at least one week), the need for broad expanses to distribute the products in thin layers, and the usage of fields that could otherwise be utilized for other agricultural operations. The old open-air drying process should be replaced by a more regulated, efficient, and dependable industrial drying technology (Xie *et al*., 2022). Another study discovered that drying methods such as tray drying (TD), sun drying (SD), oven drying (OD), and microwave drying (MWD) lowered all important nutrients such as protein, fat, fibre, and vitamin C. For all important nutrients such as protein, minerals, and antioxidants, the freeze-drying (FD) sample had the lowest per cent loss (Liao *et al*., 2022). When proper freezing, storing, and thawing protocols are followed, the nutritional content and sensory quality of foods are only a little affected. Slow freezing, temperature abuse during frozen storage, and thawing, on the other hand, can all degrade food quality (Fellows, 2023).

During traditional cooking, the food product may be subjected to a range of temperature conditions, which might result in undercooked or overcooked situations. These factors have a negative impact on food quality (Prasad *et al*., 2023). The basic goal of all industrial cooking is to modify the organoleptic quality of food to fulfil market demands for certain flavours, scents, colours, or textures, as well as to assure that the products being produced are microbiologically safe. Following chilling, freezing, or packing, preservation is obtained. Baldwin (2012) highlights many variations in organoleptic quality, some of which occur quickly and others more slowly. Because it is difficult to keep food at a low temperature for long enough for gradual changes to occur, most traditional cooking is focused on quick changes. The exact temperature control of sous vide cooking provides for both sorts of organoleptic quality alterations, particularly changes in texture. Cooking with dry heat exposes the food to either direct heat from a flame or indirect heat from hot air or oil encircling the food. These techniques heat food at higher temperatures (up to 300^{o}C), which results in a number of organoleptic modifications that are different from moist heat cooking. Important qualities include surface browning, flavour development as a result of Maillard reactions, and crust development as a result of surface protein denaturation, notably in roast beef products (Fellows, 2023).

Fermentation refers to any microorganism-controlled food processing procedure. It aims to improve functional qualities such as texture, flavour, fragrance, and nutritional status by bioconverting food components and releasing specified metabolites. Alcohols and acids are the principal byproducts of food fermentation. However, fermentation techniques are also used in industry to produce and then isolate essential commercial enzymes and metabolites. However, due to the broad variety of process and product factors, optimizing the kinetics of commercial fermentation operations is difficult (Paliwal & Dutta, 2023). The primary benefits of fermentation as a processing method include (1) the use of mild pH and temperature conditions, which preserve the nutritional properties and sensory characteristics of the food; (2) enrichment of foods with vitamins, proteins, essential amino acids, or fatty acids; or detoxification by removal of antinutritional factors (e.g., phytate, trypsin inhibitor) or toxins (e.g., aflatoxins) (Hasan *et al*., 2014); (3) the manufacture of food with different flavours, scents, or textures, many of which are impossible to accomplish using other methods; (4) minimal energy usage due to the moderate working conditions; and (5) relatively simple technologies with cheap capital and operational expenses. Today, these include fermented grain goods, meat and fish products, alcoholic drinks, dairy products, and fruit, vegetable, and legume products (Guizani *et al*., 2020; Stanbury *et al*., 2017).

The modest conditions utilized in food fermentations result in a few of the negative alterations to sensory qualities and nutritional quality evident in many other unit activities. Indeed, fermentation has the potential to improve food quality indicators such as texture, odour, flavour, appearance, nutritional value, and safety (Fellows, 2023). Although fermentations generally do not change a food's mineral composition, hydrolysis of chelating substances (e.g., phytic acid) during fermentation may enhance their bioavailability. Cereal fermentation boosts their nutritional value by boosting the amount and quality of proteins and accessible lysine. Microorganisms can also use or release carbohydrates, vitamins, vital fatty acids, and amino acids, as well as eliminate antinutrients (such as oxalate, protease and -amylase inhibitors, lectins, condensed tannins, and phytic acid) (Di Cagno *et al*., 2016), natural toxins and mycotoxins. Fermentation does not necessarily result in higher vitamin concentrations because bacteria require vitamins for development as well. The vitamin composition of food varies depending on the microorganisms and raw materials employed, although there is usually an increase in B-vitamins (Finglas *et al*., 2014).

Samtiya *et al*. (2020) examine the effect of fermentation on toxins and antinutritional components in plant food. These include decreases in trypsin inhibitors, phytates, and oligosaccharides that cause flatus. Fermentation of fibre-rich foods such as soya bean germ, wheat germ, rice bran, or fermented bread yields unique bioactive chemicals with immunological, glycemic, and antiinflammatory properties (Torino *et al*., 2013). Fermentation might be a good way to reduce bacterial contamination in food. Fermented millet products are indicated as probiotics for treating diarrhoea in young children (Manisseri & Gudipati 2012; Nduti *et al*., 2016). The quantity of B-vitamins in fermented cereal products is influenced by the extent of dough fermentation. For many years, bread flour has been routinely fortified with B vitamins and, more recently, with folic acid in several nations (Fellows, 2023).

Soaking is commonly used as an early washing and conditioning step in the processing of cereal grains and seeds. Soaking is a gradual process that involves water absorption and subsequent transportation of water particles or moisture within the seed. Water flow in the seed and moisture migration inside the endosperm is driven by molecular diffusion, which is influenced by elements such as physical structure, soaking water temperature, time, and grain-to-water ratio. Apart from water migration, the solid molecules and chemicals leached out of the wheat grains owing to solubilization in the existing aqueous environment (Paliwal *et al*., 2023). Seed germination often activates the enzyme phytase, which degrades phytate and results in lower phytic acid concentrations in samples. Germination frequently alters the nutritional content, biochemical properties, and physical characteristics of foods. This approach is most commonly used to reduce the anti-nutritional content of grains (Laxmi *et al*. 2015; Oghbaei & Prakash, 2016; Onyango *et al*. 2013). Furthermore, the most beneficial condition for mung bean germination is 48 hrs soaking, followed by 3 days of germination at 35°C. Mung bean sprouting has been shown to increase its nutritional value in terms of increased nutrient content, lower phytic acid content, and increased enzyme activity and protein content (Fayyaz *et al*., 2018).

Baking, like dehydrating and smoking, is an old activity that continues to be a major business across the world. Baking and roasting are both unit operations that employ hot air to change the eating quality of food products. In popular use, baking refers to flour-based food products or fruits, whereas roasting refers to meats, cocoa and coffee beans, nuts, and vegetables (Fellows, 2023). Roasting is carried out when cereal or pseudocereal grains are placed in a hot airflow and heat is transferred to the surface, mainly by convection. The water vapour formed is carried away from the drying surface in the airstream. The temperature for roasting usually ranges from 140 to 160°C, applied for 20–30 minutes (Sasthri *et al*., 2021). Lysine is the limiting amino acid in wheat flour, and its breakdown by the Maillard reactions during baking is consequently nutritionally significant. The loss of lysine during the manufacturing of maize breakfast cereals is 88%, which is compensated for by fortification. Tryptophan (44%), methionine (48%), and lysine (61%) are the amino acids lost in cookies. Higher baking temperatures, longer baking times, and higher concentrations of reducing sugars all result in increased losses. The Maillard reactions also produce carcinogenic acrylamide in bread products (Cheng *et al*., 2014) and roasted nuts (Lukac *et al*., 2007).

The extent of thiamin loss in cereal food is regulated by the baking temperature and the pH of the food (losses are greater at higher pH values). Thiamin loss in pan bread is around 15%, while in cakes or biscuits artificially leavened with sodium bicarbonate, losses can range from 50% to 95%. Although vitamin C is also degraded during baking, it is frequently used as an enhancer in bread dough. Other vitamin losses are negligible. High-fibre bread and products containing omega-3 fatty acids, lutein, fructans, or oligosaccharides are examples of nutraceutical bakery products. Because the baking time and temperature required to achieve the appropriate sensory properties in the food are adequate to kill vegetative bacterial, yeast, or fungal cells, baked foods are substantially devoid of microorganisms (Fellows, 2023). Baking is the technique of cooking a food combination without exposing it to an open flame. In terms of cooking, it is a method of converting any semi-liquid mass mixed with suitable ingredients into a soft, spongy texture by using leavening agents such as baking powder or baking soda, where the semi-liquid mass is dried and filled with air, giving the end product a spongy texture. Baking is a regulated heating process that is often performed in ovens, either microwave ovens or conventional baking ovens with temperature and timing controllers (Ananthanarayan *et al*., 2019).

Post-baking contamination from the bakery air, slicing machinery, or bakery employees is the most common source of food safety issues. As a result, baked items should be cooled and wrapped as quickly as possible under proper hygiene conditions. Bakery items with a short shelf life are sold within a few days of creation and hence require just minimum packaging to keep them clean and avoid crushing (Fellows, 2023). The generation of crust colour owing to the Maillard reaction and sugar caramelization, the production of taste and fragrance compounds, the formation of hazardous chemicals (e.g., acrylamide), and a loss in the nutritional content of proteins all occur during baking (Sasthri *et al*., 2021). Some mycotoxin types, such as aflatoxins, fumonisins, and zearalenone, are reduced by 20%-50% during dough fermentation and 20% during baking, whereas others, such as ochratoxin A, are completely unaffected by baking. D-values of pathogenic bacterial spores are greater than those of vegetative cells. They may be able to withstand the baking temperatures and grow to levels that pose a public health risk (Fellows, 2023).

Pathak and Kochhar (2018) revealed various food processing methods which can increase nutrient content, the bioavailability of nutrients, food safety, storage stability, palatability, and convenience of supplemental foods that may be ideal for infant feeding. Such technologies used for weaning food processing typically involve roasting, germination, milling, baking, cooking, drying, fermentation, and extrusion. Extrusion cooking technology in this case is a useful method.

2.3 Extrusion Cooking in Food Processing

Extrusion cooking is a multistep and multifunctional method that produces a wide range of food products such as pasta, cereal flakes, bread sticks, flatbreads, snacks, and textured vegetable protein (Sasthri *et al*., 2021). Extrusion cooking is a high-temperature short-time (HTST) procedure that kills microorganisms and inactivates naturally existing enzymes in raw materials. Many heat-sensitive components may be kept under HTST conditions, resulting in great organoleptic quality and nutritional value preservation (Fellows, 2023). Extrusion cooking allows for quicker processing, relatively cheaper, no process effluents, high productivity, significant nutrient retention, less space for the plant required for processing equipment, little labour, and greater flexibility leading to more types of end-products (Alam *et al*., 2016). Extrusion cooking provides several advantages, including improving protein digestibility and total dietary fibre while lowering lipid oxidation, microbial contamination, and anti-nutritional factors, all of which are important in the production of a range of extruded food products (Mathad *et al*., 2022). Furthermore, the ability of extrusion cooking to improve cereal safety by lowering mycotoxin levels and antinutritional components has been proven (Sasthri *et al*., 2021). Many starch- and sugar-based products are preserved by achieving a low water activity (a_w) of 0.1-0.4. Semimoist protein-based meals can be kept by packing in materials with a strong oxygen and moisture barrier. Other extruded items that are further processed include pasta drying, infrared toasting of crispbreads, and frying (Fellows, 2023).

Extrusion is a flexible processing technique that combines many unit operations into a single process. It is continuous processing equipment capable of conveying, mixing, shearing, and cooking (transforming) food materials as they pass through it. Extrusion is an interesting technology that can be categorized as "integrated processing technology" because it integrates multiple unit operations into a single device (Pichmony & Ganjyal, 2020).

The screw typically has a deep channel (20–30% of diameter) with no compression ratio and operates at a relatively low speed (below 50 rpm). Cooking extrusion usually uses medium-shear and high-shear extruders and the products receive significant energy by viscous heat dissipation, heat transfer through the barrel, and often with steam injection. The screws run at relatively higher speeds (>100 rpm) with a shallower channel depth (Yacu, 2011). Extrusion is a very important process and is used in the food industry. Extrusion cooking is a short-term high-temperature process that involves simultaneous, combined thermal and pressure treatment with mechanical shaving, leading to changes in effects such as cooking, sterilization, drying, melting of food, cooling, conveying, puffing, texturizing, mixing, kneading, conching (chocolate), freezing, forming (Berk, 2009).

Extrusion cooking produces a wide range of finished products with a minimal processing time from low-cost raw materials (Alam *et al*., 2016). The extrusion technology can be used to process a variety of products by modifying some ingredients and processing conditions on the same machine; various shapes, sizes, structural, textured, and sensory properties and colours of snack foods can be generated using an extruder (Dubey & Bhattacharya, 2015). Extrusion is used for the production of many food products including expanded and non-expanded products. Product requirements generally depend on consumer requirements and regulatory requirements. Consumer requirements are sensory attributes such as taste, texture, colour, shape, and microbiological safety while regulatory requirements are filling weight, and nutritional claims (Rungsardthong, 2014).

2.4 Ultrasound and Supercritical Carbon Dioxide in Extrusion

The adherence of food items inside the extruder influences the operation condition of the machinery and the quality of the extruded foods during extrusion. A similar sticking/adherence concern was noticed during large-scale cooking and the demolding of food products from the processing vessel. The US can generate vibration in the processing vessels and extrusion tubes, preventing food products from adhering/sticking to the vessels and extrusion tubes (Prasad *et al*., 2023).

Extrusion with supercritical fluids is a hybrid process that combines extrusion and supercritical fluid technologies. It makes use of supercritical carbon dioxide as a viscosity-lowering plasticizer and expansion/foaming agent. Sauceau *et al*. (2011) provided a thorough overview of the first implementation of this technology, which was designed for the agro-food industry. The expanded products manufactured with supercritical carbon dioxide have good textural qualities and are generated at lower temperatures (around 100^{o}C) than those produced with traditional steam-based extrusion (130–180^{o}C). The process aids in the full retention of all introduced minerals, as well as the retention of 55–58 % of vitamin A and 64–76 % of vitamin C. All essential amino acids, such as lysine, are held at relatively high rates (98.6%), and no losses due to a Maillard reaction or oxidation were discovered using this technique (Paraman *et al*., 2012).

2.5 Characterization of Extruded Products

The complex existence of extruded cereal products manufactured from whole cereal flour, water, and various additives makes it hard to recognize their properties. Extrusion conditions also affect the interactions of the ingredients (Kirjoranta *et al*., 2012). As a result, new methods for better characterization of extrudates have newly been created. Without using dye or complicated sample preparation, X-ray microtomography was found to be an effective and easy technique for analysing cellular structures of extrudates from cereal products (Penttilä *et al*., 2011).

2.6 Ready-To-Eat Products

Ready-to-eat foods have become one of the most common types of food on the market. Extrusion has the most applications in the ready-to-eat sector, such as breakfast cereals, salty or sweet snacks, co-extruded snacks, croutons for soups and salads, pet and fish foods, nutritious pre-cooked food blends for children, and confectionery food (Singh *et al*., 2007).

2.7 Extruded Food Products Growth

Extruded products are among the quickest segments of the food sector. For years, extrusion has become a key component in the development of new and innovative products, and it still has a lot of growth opportunities (Alam *et al*., 2016; Yadav, 2015). In the 1940s, expanded corn snacks were one of the first extrudates to be launched and produced commercially. Extruded snacks come in a range of sizes, colours, and flavours, and can be found in supermarkets, grocery shops, corner stores, gas stations, vending machines, and cinemas, or even at sporting events and theme parks (Yadav, 2015).

2.8 Types of Extruded Cereal Foods

The first is extruded puffed food, which is made primarily of corn powder, potato or bean powder, and starch. Puffed small foods, breakfast cereals, extruded vegetarian breakfast foods, extruded tissue protein, and other extruded puffed foods are some of the most common examples of extruded puffed foods. The extruded non-puffed products are another type which including such rice noodles, rice cakes, pasta, and other similar products (Guo & Dong, 2019).

2.8.1 Extruded Cereal and Snack Food Market

Puffed food is a component of extruded cereal products that would be recognized as a rising star in the snack food industry. The top two nations in the salt-flavoured snack business are still the United States and China, followed by India, Mexico, Japan, and the United Kingdom. According to a study by market research firm Mintel on salt-flavoured snacks, China and India are the two fastest-growing salt-flavoured snack markets (Liu, 2016). Extruded snacks get the highest growth potential in the snack food sector (Dubey & Bhattacharya, 2015). Puffed food will evolve toward healthier goods in the future as people's living standards and health consciousness increase. Furthermore, as a result of the effects of market upgrading, customers are increasingly pursuing high-end and high-quality food brands. This necessitates that puffed food businesses keep promoting nutritious, high-end, and healthy food (Anon, 2013).

The global demand for savoury snacks is rapidly expanding, reaching approximately $ 50 billion per year with a volume of approximately 6 million tonnes. North America accounts for more than half of the industry, with Western Europe accounting for around 20%. The global average per capita intake is 1.5 kg, but this ranges greatly from more than 9 kg in the United States to less than 0.5 kg in India and less than 0.1 kg in China. Chips as well as other snack foods produced from maize and potato contribute to more than 60% of the market, with nuts coming in second with 15% and the remaining made up of many different other snacks (Yadav, 2015).

2.8.2 Extruded Cereal Staple and Market

Breakfast cereal income in the world totalled \$32.5 billion in 2016. In the five years from 2007 to 2012, the compound annual growth rate was 6.1%. Breakfast cereal sales in the supermarket and catering sectors accounted for 91% of overall sales. Although consumers purchase breakfast cereals primarily via the retail channel, sales of breakfast cereals from restaurants have achieved \$2.9 billion and are continuing to rise. The United States continues to be the largest growing consumer market, and then China, the United Kingdom, France, and Germany. Breakfast cereal sales in the United States were \$8.1 billion in 2012, \$6.2 billion in China, \$4.2 billion in the United Kingdom, \$1.7 billion in France, \$1.5 billion in Germany, and \$10.9 billion in many other nations (Anon, 2014).

2.9 Application of Extrusion Technology

Due to its flexibility, extrusion technology has a diverse range of applications in the food industry; Table 2.2 shows a few of them when compared to other common food techniques, extrusion processing is useful due to its potential application in a broad range of diverse processes, flexibility, cost reductions, high efficiency, and great quality foods (Fellows, 2022; Riaz, 2000).

Table 2.2 Application of extrusion cooking

Application of extrusion cooking	Application of extrusion cooking
Bread crumbs	De-germination of spices
Precooked starches	flavour encapsulation
Anhydrous decrystallization of sugar to make confectioneries	Enzymatic liquefaction of starch for fermentation into ethanol
Chocolate	Quick-cooking pasta products
Pre-treated malt and starch for brewing	Oilseed treatment for subsequent oil extraction
Stabilization of rice bran	Preparation of specific doughs
Gelatin get confectioneries	Destruction of aflatoxins or gossypol in peanut meal
Caramel, licorice, chewing gum	Precooked soy flours
Corn and potato snack	Gelation of vegetable proteins
Co-extruded snacks with internal filling	Restructuring of minced meat
Flat crisp bread, biscuits, crackers, cookies	Preparation of sterile baby foods

Pre-cooked flour, instant rice puddings	Oilseed meals
Cereal-based instant dried soup mixes or drink bases	Sterile cheese processes
Transformation of casein into caseinate	Animal feeds
Pre-cooked instant weaning foods or gruels	Texturized vegetable proteins

2.10 Extrusion Equipment and Role during Extrusion Cooking

Extruders comprise two screws coated in a grooved or smooth barrel. A motor powers the screw(s), which is normally equipped with a gearbox to help with differing screw speeds throughout the operation. Furthermore, the end of the barrel is normally equipped with a die that pushes the material out from the extruder. Each extruder component is critical to the proper operation of the system (Pichmony & Ganjyal, 2020). Different parts of an extruder are shown in Figure 2.1.

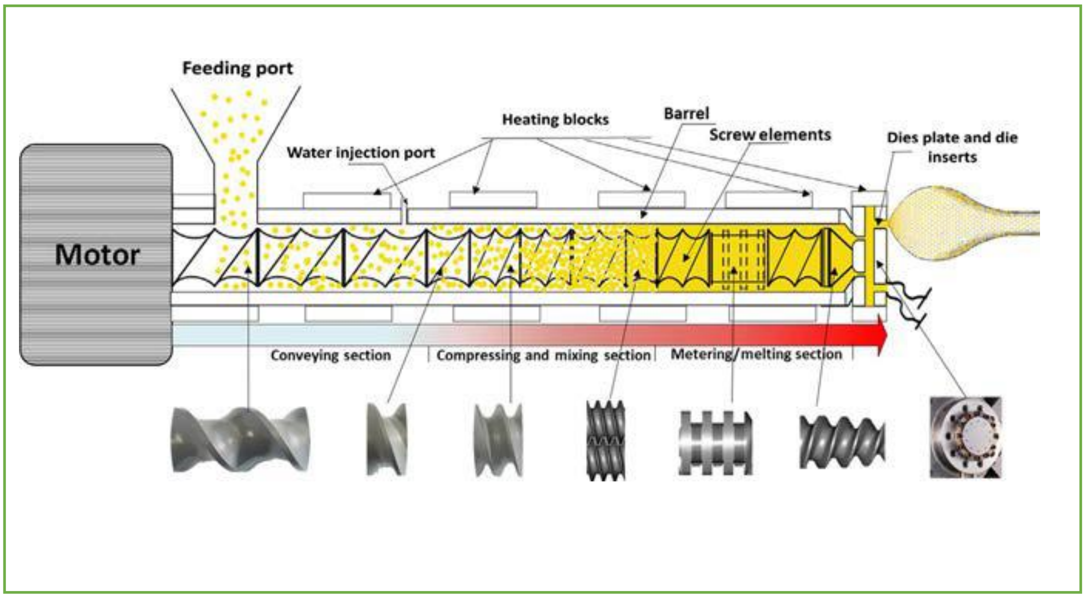


Figure 2. 1 Schematic design of a generic extruder (Ganjyal & Hanna, 2004).

2.10.1 Barrel

Barrels, like all other vital components of extruders, play a critical role. The main purpose of the barrel is to protect the screws. The inner lining of the barrel does play a role in the shear generation, especially in single-screw extruders. The inner surface of the barrel of a twin-screw extruder is normally smooth (Kowalskia & Ganjyal, 2020).

2.10.2 Screws

Screws, whether single or twin-screw, are an essential part of the extrusion process. The screws are responsible for conveying food, compressing the material, providing shear to the material, and supporting friction generation between the food particles (Kowalskia & Ganjyal, 2020).

2.10.3 Dies

Dies play various roles in the extrusion process. They effectively aid in the development of back pressure in the final zone of the extruder by restricting melt flow. They also assist in realigning the melt polymers in the die, providing additional shear, and providing the final form to the extruded food products (Kowalskia & Ganjyal, 2020).

2.11 Extrusion Process as a Multiple Input and Multiple Output (MIMO) System

There are two major energy inputs required in the extrusion process: (i) energy transferred as a result of screw rotation; (ii) heat energy passed from the heater through the barrel walls. This causes enough heat to be generated in the barrel, resulting in moisture evaporation or melting of the solid material used for extrusion cooking (Sudhakar *et al*., 2023).

Extrusion can be thought of as a MIMO (multiple input, multiple outputs) process (Eerikäinen *et al*., 1994). Extrusion process conditions can be roughly divided into three groups, as shown in Figure 2.2: (1) independent parameters (input parameters), (2) system parameters (dependent parameters), and (3) product properties (output parameters) (Gu *et al*., 2017).

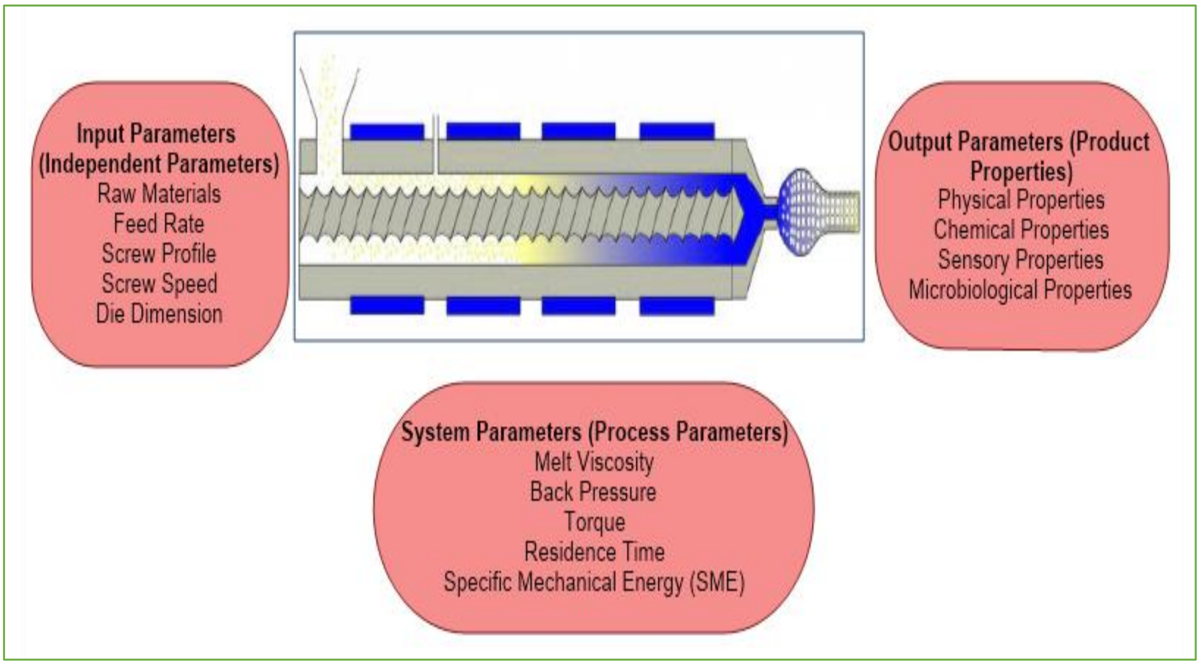


Figure 2. 2 Extrusion process as a MIMO system with modification (Gu *et al*., 2017).

The "independent parameters" are all the process parameters that can be operated by the system operator. Ingredient formulation, the moisture content in the input, screw configuration and speed, barrel temperature, die dimension, die cutter speed, and other factors are among them. On the other hand, specific mechanical energy (SME), residence time, die-back pressure, melt viscosity, and torque are among the "system parameters". The extruder conditions obtained as a result of the independent parameters are referred to as system parameters (Kowalskia & Ganjyal, 2020).

2.12 Structure and Physico-Chemical Changes during Extrusion Cooking

Extrusion Cooking causes various changes in the food components such as gelatinization of starch, denaturation of proteins, and melting of lipids (Sudhakar *et al*., 2023). The level of physicochemical changes and the quality of the final extruded products are affected by heat transfer to the food material during processing (Cotacallapa-Sucapuca *et al*., 2021). Gelatinization, solubilization, and dextrinization or complex formation of starches, denaturation, polymerization or crosslinking and texturization of proteins, the partial or complete deactivation of enzymes, browning reactions, denaturation of vitamins, and inactivation of antinutritional factors are some of the changes that occur during the extrusion process (Shah *et al*., 2021).

During extrusion cooking, a mixture of moisture, pressure, temperature, and mechanical shear induces molecular transformation and chemical reactions, plasticizing and preparing food products in a minute (Navale *et al*., 2015). The thermal and shear energy applied to raw foods induces mechanical, chemical, and nutritional transformations such as starch gelatinization and degradation, protein denaturation, vitamin degradation, anti-nutrients and phytochemicals, flavour development, increased bioavailability of minerals, aflatoxins destruction, and increased digestibility of fibre (Camire, 2003; Riaz *et al*., 2009; Singh *et al*., 2007). The fundamental causes of these changes as well as the effect of system conditions and composition of food mix. Great attention is focused on the physicochemical and chemical changes in protein, starch, and dietary fibre (Navale *et al*., 2015). Similar to other heat methods, extrusion cooking has some special positive features as the material is subjected to extreme mechanical shear. It helps break the covalent bonds in biopolymers and the intense structural disruption and mixing facilitate the modification of the functional and textural properties of food ingredients (Carvalho & Mitchelle, 2008). In addition, the process of extrusion cooking denatures undesirable enzymes, sterilizes the product, and retains natural food colours and flavours (Bhandari *et al*., 2001; Fellows, 2022).

Proteins are a group of highly complex organic biomolecules that are made up of a sequence or chain of amino acid molecules which play a crucial role as a principal component of the protoplasm of the cell structure thereby considered essential to life (Singh *et al*., 2007). The major biological functions of protein are the replication of Deoxyribonucleic acid (DNA), building blocks of cells, formation, and stabilization of foams and emulsions. Extrusion improves protein digestibility via denaturation, which exposes enzyme-access sites. The nutritional quality of protein is dependent on the quantity, digestibility, and availability of essential amino acids. Amino acid profile, digestibility, and available lysine are considered important factors for protein quality determination (Camire, 2001; Guerrieri, 2004; Walstra, 2003). The Maillard reaction takes place between free amino protein groups and carbonyl groups of reducing sugars and contributes to browning and flavour development and also decreases the availability of amino acids and protein digestibility (Benyi *et al*., 2016).

Carbohydrates in foods are important, not only as energy sources but also as ingredients that impart texture and as dietary fibre that contributes to human health. Their use as food ingredients is large in terms of both quantities consumed and the variety of applications and products (BeMiller, 2019). Carbohydrates range from simple sugars to more complex molecules, like starch and fibre. Control of sugars during extrusion is critical for the nutritional and sensory quality of the products. Several researchers have reported sugar (sucrose) losses during extrusion due to the conversion of sucrose into glucose and fructose (reducing sugars), and the loss of these reducing sugars during Maillard reactions with proteins (Camire *et al*., 1990). Sugars also depress gelatinization by increasing the temperature needed for initiation and depression of the enthalpy of gelatinization. Oligosaccharides like raffinose and stachyose can induce flatulence (Omueti & Morton, 1996).

Starchy tubers and grains provide important energy and satiation in most diets. Starch, the storage form of energy for plants, is a polysaccharide made up of glucose units. The molecule of starch is composed of amylose and amylopectin but in different proportions within the starch grains; providing each starch with its characteristic properties in cooking and gel formation. Humans and other monogastric species cannot easily digest ungelatinized starch. Extrusion cooking is unique because gelatinization takes place at much lower levels of moisture (12-22%) than is necessary for other forms of food processing (Camire, 2001). The presence of lipids, sucrose, dietary fibre, and salts, also affects gelatinization (Jin *et al*., 1994).

Dietary fibre is a form of carbohydrate or edible component of plants that cannot be digested and absorbed in the human small intestine with full or partial fermentation in the large intestine. This contains polysaccharides, oligosaccharides, lignin, and associated substances of plants. Soluble fibres include pectins, β-glucans and galactomannan gums facilitate beneficial physiological effects and can help lower blood cholesterol and regulate blood glucose levels and laxation (Camire, 2001). The insoluble fibres are composed of cellulose, hemicellulose, and lignin, which stimulate an increase in faecal bulk and reduced the transit time of faeces through the large intestine (Rod´riquez *et al*., 2006). Vasanthan *et al*. (2002) found that the total dietary fibre content of barley flour increased by extrusion cooking. An increase in total dietary fibre (TDF) in waxy barley was the result of an increase in soluble dietary fibre. The increase in insoluble and soluble dietary fibres has resulted in increased TDF content for regular barley flour.

While lipids serve as a concentrated form of energy, chronic diseases such as heart disease, cancer, and obesity are associated with excess dietary lipid intake. Lipids are used as plasticizers and lubricants in extrusion cooking. Generally, foods containing less than ten per cent lipids are extruded because greater quantities of lipids reduce slip within the extruder barrel, making extrusion difficult, particularly for expanded products. Oils and fats produce large effects on the processing of starch at levels of 1–2% and higher levels may reduce the degradation of the starch polymer to such an extent that no expansion is obtained from a recipe. Amylose-lipid complex formation can also reduce starch digestibility (Camire, 2001; Guy, 2001). High-fibre foods may abrade the interior of the extruder barrel and screws, resulting in increased mineral content. However, loss of mineral bioavailability may occur in foods containing high levels of dietary fibre and phytate (Camire, 2001).

2.13 Parameters Affecting Extrudates during Extrusion Cooking

Extrusion technology has had a significant impact on the food industry because of its multiple advantages over conventional processing processes (Shah *et al*., 2021). The extrusion process parameters play an important role in determining the quality output of the extruded food. Figure 2.3 illustrates the processing parameters and the raw materials during the process of extrusion cooking. The process controlling the product depends on various primary and secondary extrusion process parameters (Chessari & Sellahewa, 2001). Extrusion techniques can be used to prepare a variety of products from various food groups by altering minor or main components and processing conditions (Shah *et al*., 2021).

Process parameters such as barrel temperature, screw speed, feed composition, moisture content, throughput, screw configuration, and die geometry generate system variables such as mechanical and thermal energy inputs and residence time. These variables influence nutritional value, texture, flavour, colour, and microbiological quality (Ganesan & Rajauria, 2021). Product quality can vary widely depending on the type of extruder, the configuration of the screw, the feed moisture levels in the barrel session, the speed of the screw, and the feed rate (Ding *et al*., 2005). Vitamins are heat-labile elements that can degrade thermally during extrusion cooking. The temperature, moisture content, and screw speed are all factors that influence vitamin retention (Shah *et al*., 2021).

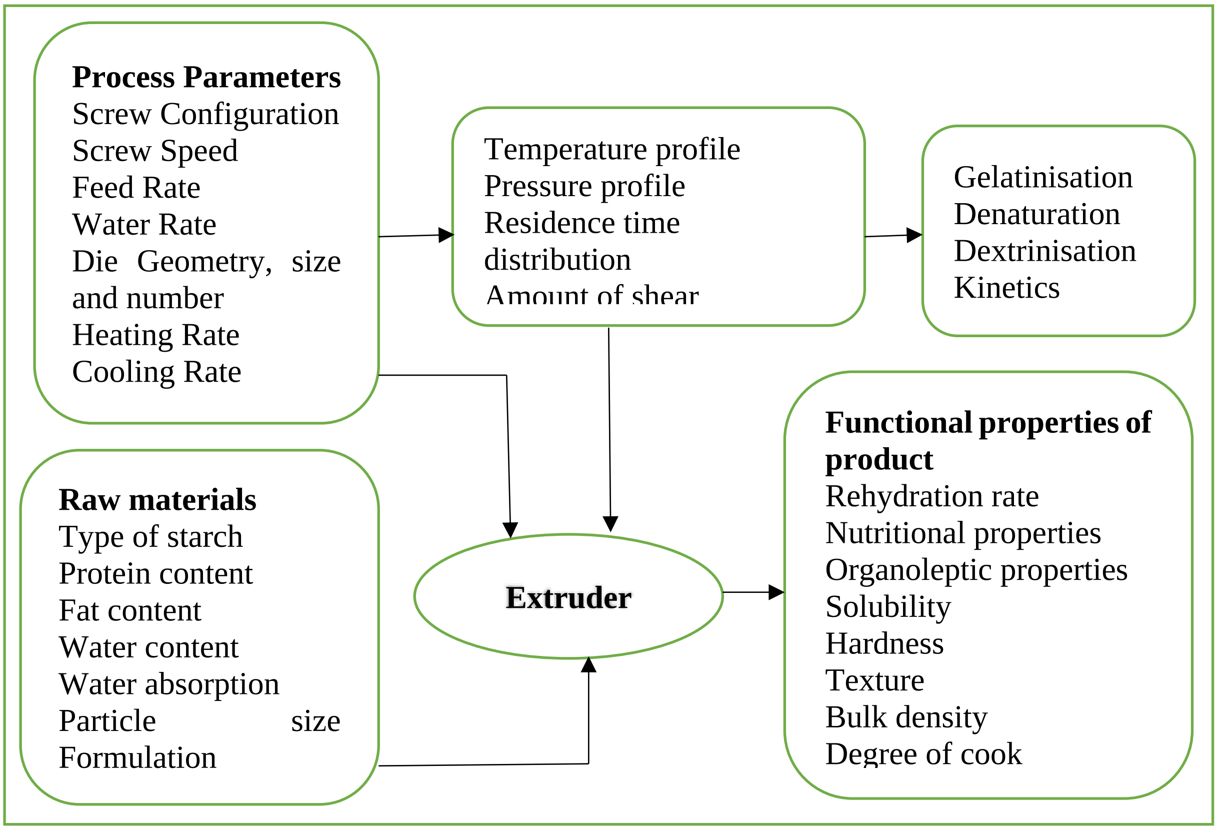


Figure 2. 3 Interaction of raw material properties, process variables, and product with modification (Shah *et al*., 2021).

Vitamin deterioration during extrusion is caused by increased temperature, screw speed, feed moisture, feed rate, and die diameter (Dalbhagat *et al*. 2019). The texturization process in the formation of high-moisture meat analogues is influenced by the protein content of the feed material, screw profile, screw speed, moisture content, and cooking temperature (Shah *et al*., 2021). Specific mechanical energy (SME), a scale-independent measure of mechanical energy, is regarded as an important system parameter used to control extrusion processing operations, with a significant impact on the preservation of valuable amounts of macro and micronutrients (water-soluble vitamins) in extrudates. It may also have an effect on the preservation of bioactive components such as polyphenols, dietary fibre, and alpha-galactosides (Cotacallapa-Sucapuca *et al*., 2021).

2.13.1 Screw Speed

It is not possible to explain the effect of each variable separately. Masibo *et al*. (2020) observed that the screw speed, temperature, and moisture level of the food combination during extrusion all had a substantial impact on the final properties of the extrudates. It is well known that screw speed governs the mechanical energy input. Increasing screw speed elevates the friction between the product and the screw and thus more mechanical energy is produced. On the other hand, increased screw speed results in inadequate cooking due to shorter residence time (Singh, 2016). They reported that the degree of gelatinization increased with decreasing screw speed due to the increase in residence time. The combination of mechanical and thermal processes involved in the extrusion process destroyed anti-nutrients such as phytate, polyphenols, oxalates, and trypsin inhibitors, the levels of which were reduced by 54.5, 73.3, 36.8, and 72.3%; respectively; at high die temperature (115, 140, and 165^{o}C) and high screw speed (400 rpm) in wheat bran, rice bran, barley bran, and oat bran (Nikmaram *et al*., 2017). While the concentration of mealworm powder remained constant, an increase in processing parameters, such as barrel temperature and screw speed, resulted in a larger pore size and expansion ratio (Shah *et al*., 2021).

2.13.2 Barrel Temperature

Extruder barrel temperature is reported to have a profound effect on the product properties depending on the degree of cooking. Extrusion cooking helps to inactivate heat-labile anti-nutrients present in plant proteins. Nutritional properties are reduced when barrel temperatures are high and moisture levels are low (Mathad *et al*., 2022). Increasing the barrel temperature resulted in a harder and more fibrous product that resembled a meat analogue. Higher temperatures enhanced fibre quality while having no influence on elasticity. Because of the high screw speed and temperature, as well as the low moisture content, larger concentrations of spirulina may be used, resulting in a more nutritional meat analogue. Higher barrel temperatures and feed moisture of less than 58% (wt/wt) resulted in a rapid increase in the expansion ratio (ER) and a reduction in final product bulk density (Masibo *el al*., 2020). The barrel temperature has a beneficial effect on the expansion ratio, crispness, and water solubility index (WSI) while having a negative effect on bulk density, hardness, and water absorption index (WAI) (Sahu *et al*., 2022).

Nutritional quality has been shown to increase with moderate conditions (short duration, high moisture, low temperature), whereas a negative effect on the nutritional quality of the extrudate occurs at a high temperature (at least 200^{o}C), low moisture (less than 15%), or inadequate components in the mix (Singh *et al*., 2007). High-temperature short-time extrusion can minimize losses in vitamins and amino acids as well as denature antinutritional factors, such as destroying toxins or killing microorganisms. It can also increase the ' protein content and digestibility ' and change the shape texture, colour, and flavour of the product (Harper, 1978). Increasing barrel temperature from 140 to 160^{o}C resulted in a 14 to 15% increase in WSI, whereas a reduction in WSI was discovered when barrel temperature increased to 180^{o}C, likely due to the complex molecular weight established and the reduction in the solubility index (Altan *et al*., 2009). Extrusion parameters such as the temperature profile along the barrel or moisture content can significantly improve protein digestibility and thus their utilization (Masibo *et al*., 2020). Despite using a high-temperature profile along the extruder barrel, Guldiken *et al*. (2020) observed a decline in the digestibility of chickpea and barley extrudates due to high moisture content. According to research, protein molecular changes such as denaturation and polypeptide bond unwinding occur during extrusion.

2.13.3 Moisture Content

Moisture can be added directly to feed, injected into the barrel, or added as steam to the preconditioner or barrel. Water acts as a plasticizer, lubricating the feed material and reducing the friction and heat occurring during extrusion. Increasing moisture will decrease viscosity, torque, and product temperature and increase bulk density (Rungsardthong, 2014). Increasing the moisture content during extrusion may result in a change in the amylopectin molecular structure of the material, thus decreasing melt elasticity, reducing expansion, and increasing the bulk density of the extrudate (Ding *et al*., 2005; Thymi *et al*., 2005). The increase of feed moisture from 16 to 17% significantly increased the water absorption index (WAI) of the rice extrudate (Thongkum *et al*., 2010). Greater expansion and crispiness of snacks were attained at a die temperature of 160°C, a blend moisture content of 11.2% db, and a screw rotation speed of 200 rpm, comparable to the maximum SME achievable (Carmo *et al*., 2019). Moisture content has been shown to decrease protein solubility. Mozafarpour *et al*. (2019) discovered a modest decrease in the solubility of soy protein concentrate when the moisture level was increased from 18% to 25% (wt/wt) during extrusion. On the other hand, soy protein concentrate extruded with moisture content less than 18% (wt/wt) showed smaller-sized protein aggregates, resulting in greater protein solubility, as proven previously by Osen *et al*. (2015). The feed moisture content had a beneficial impact on bulk density, hardness, and WAI while having a negative impact on ER, crispness, and WSI of the product (Sahu *et al*., 2022).

2.13.4 Feed Formulation

The qualities of raw materials have a major impact on material transfer in the extruder, melt viscosity, and final product characteristics. Extrusion technology enables the rapid and effective conversion of a wide range of raw materials into a wide range of food products by varying feed formulation and operating conditions on the same extrusion equipment (Shah *et al*., 2021). Feed composition had the greatest influence on specific mechanical energy (SME) and product parameters such as water absorption index (WAI), water solubility index (WSI), bulk density (BD), expansion ratio (ER), breaking strength (BS), colour values (L*, a*, and b*), and overall acceptability (OA) (Altaf *et al*., 2020).

2.13.5 Screw Configuration

Screw configuration refers to the arrangement and combination of various screw components, and varied screw configurations affect system and product properties (Zhang *et al*., 2020). The screw configuration, also known as the screw profile, is an important feature of the extrusion process because it affects the amount of shear applied to the material being extruded. The degree of material transformation and, eventually, the quality of finished products is directly related to the level of shear. Screw configuration is one of the independent parameters in the MIMO representation of the extrusion system (Pichmony & Ganjyala, 2020). It is impossible to estimate the impact of screw shapes on product quality because screw behaviour is unknown. Because these factors hamper a systematic examination of the screw geometry effect on product quality, it is uncertain how much changing the screw configuration (e.g., during the process transfer from a lab-scale extruder to a larger production extruder) may affect product quality (Bauer *et al*., 2021).

The screw design makes it easier to set up multiple screw configurations to generate low or high shear. In general, amorphization should be aided by a high-shear screw setup with vigorous mixing. As a result, the degree of amorphization had a direct effect on crystallinity and dissolution rate (Butreddy *et al*., 2021). Ten alternative screw configurations with varying component-die distance and element spacing were created. The effects of screw configuration on chemical characteristics and ginsenosides content of extruded ginseng were considerably noticed using different screw configurations as experimental parameters (Zhang *et al*., 2020). The customized screw configuration with four mixing zones was tailored for high mixing capacity and longer residence time to achieve complete co-crystal formation (Srinivasan *et al*., 2021).

2.13.6 Mass Flow Rate

The mass flow rate (MFR) is the amount of material extruded per unit time. The MFR is influenced by the drag flow created by screw rotation and the pressure created at the die end owing to constriction (Singha *et al*., 2018). Feed moisture (FM) composition and extruder SS have a considerable impact on the MFR of millet-based extrudates. MFR decreased for pearl millet and Bambara groundnut-based extrudates, whereas FM and screw speed (SS) increased (Filli *et al*., 2013). Rao *et al*. (2018) found a lower MFR for extruded snacks made from foxtail millet, finger millet, pearl millet, sorghum, and rice. MFR is significantly affected by the viscosity of the material inside the barrel. Reduced FM and SS enhance melt viscosity due to reduced shear, which provides increased resistance to flow and, as a result, lowers the MFR of the extrudates (Dalbhagat & Mishra, 2019).

2.13.7 Specific Mechanical Energy

Specific mechanical energy (SME) is the quantity of energy needed to extrude per unit mass of feed material in the form of work (KJ/kg) and is proportional to the melt viscosity within the barrel (Kharat *et al*., 2019). The amount of SME input directly influences macromolecular changes and interactions between the various components during extrusion cooking (Chen *et al*., 2010; Singha *et al*., 2018). The macromolecular changes may be starch gelatinization or protein denaturation (Dalbhagat *et al*., 2019; Singha *et al*., 2018). With a rise in FM, there was a reduction in SME. However, SME tends to rise for the gelatinization of starch at low FM levels because the gelatinization process needs more mechanical energy at low moisture content (Dalbhagat *et al*., 2019).

2.13.8 Die Pressure and Torque

Die pressure is influenced mainly by the material's melt viscosity, which is a measure of resistance to the movement of feed material through the die. It is also strongly influenced by the geometrical structure of the die opening. Material accumulation at the die end raises die pressure (Dalbhagat *et al*., 2019). The torque of the extruder is the amount of work required to drive the feed material through the barrel and die as well as rotate the screw within the barrel during full and no load conditions (Dalbhagat *et al*., 2019; Singha *et al*., 2018).

2.14 Effect of Extrusion on Nutrient Content of Food

The effects of the extrusion cooking process on the nutritional quality of food are still ambiguous. The nutritional value may be affected favourably or unfavourably by extrusion cooking. Beneficial effects include destroying anti-nutrients composition, gelatinization of starch, increased soluble dietary fibre, and reducing enzymatic lipid oxidation. Protein digestibility and bioavailability of sulphur amino acids may be improved, and trypsin inhibitors and lectins may be inactivated (Fellows, 2022; Nikmaram *et al*., 2017; Singh *et al*., 2007). Extrusion may cause chemical and physicochemical changes in macronutrients like carbohydrates, proteins, and lipids, thus affecting their digestibility and bioavailability. Extrusion changed the starch components of barley and oats in the solubility of dietary fibre and enhanced the functional properties of cereal products (Shivendra *et al*., 2010; Zhang *et al*., 2011). The same may happen to micronutrients, e.g., vitamins, phytochemical antioxidants such as phenolics, carotenoids, and other food bioactive (Brennan, 2011; Zeng *et al*., 2016; Zhang *et al*., 2017).

Losses of vitamins in extruded foods differ by food type, moisture content, processing temperature, and holding time. In general, losses are low in cold extrusion (the ambient temperature of food products continues to remain) whereas when the food is usually heated above 75°C, the process is defined as hot extrusion (also referred to as extrusion cooking or thermal extrusion). The high-temperature short-term (HTST) cooking conditions, and the rapid cooling of food products resulting from the die, cause comparatively small losses of most vitamins and essential amino acids (Harper, 1979). Vitamins are even more severely affected during extrusion. Fat-soluble vitamins, such as vitamin A, including the provitamin A (e.g., β-carotene) and vitamin E, and water-soluble vitamins such as vitamin C, B_1, and folic acid have been most sensitive to extrusion cooking. Vitamins of the B group, such as B_2, B_6, B_{12}, niacin, Ca-pantothenate, and biotin remain relatively stable during extrusion (Riaz *et al*., 2009). Oxidation occurring to the vitamins, phenolics, and carotenoids during extrusion cooking may be prevented or reduced by adding natural antioxidants such as ferulic acid and benzoin to the feed of cereal foods (Viscidi *et al*., 2004).

In soy flour, the changes to proteins depend on the formulation and processing conditions. High temperatures and the presence of sugars cause Maillard browning (Fellows, 2022). The Maillard reaction with any reducing sugar, including fructose, glucose, and galactose. results in a decrease in protein quality due to the loss of amino acid residues and decreased protein digestibility. In addition, Maillard products generated during extrusion cooking may also prevent free amino acids and other nutrients such as zinc from being absorbed and metabolised (Dills, 1993). Lower temperatures and low concentrations of sugars lead to increased protein digestibility, due to the rearrangement of the protein structure. The destruction of anti-nutritional components in soy products improves the nutritive value of texturized vegetable proteins (Fellows, 2022).

2.15 Effect of Extrusion on Anti-nutritional Factors

Anti-nutritional factors are substances that restrict the use of nutrients and/or the consumption of plants or plant products used as human food and play a vital role in determining plant use for humans. In plant foods, anti-nutritional factors are responsible for the deleterious effects of nutrient and micronutrient absorption (Gemede & Ratta, 2014).

Oxalate forms close bonds between oxalic acid and other minerals including calcium, magnesium, sodium, and potassium, thereby forming oxalate salts that block nutrients from being available in the body (Nachbar *et al*., 2000). Massey *et al*. (2001) reported a relatively high oxalate content of 11 soybean cultivars which ranged from 0.67 to 3.5 g/100 g of dry weight. Amaranth grains contain a moderate amount of oxalate (178 – 220 mg/100 g) and can thus be taken at a moderate level regularly. Too much soluble oxalate in the body inhibits the absorption of soluble calcium ions because oxalates bind the calcium ions to form insoluble complexes of calcium oxalates (Kariuki *et al*., 2013).

Phytate acts as a largely negative ion in a large pH range and its existence in food also harms the bioavailability of divalent and trivalent minerals in the body (Mueller, 2001). In addition, saponins which are toxic and provide a bitter taste and astringency in food were also found to reduce the bioavailability of nutrients and reduce enzyme activity and it affects the digestibility of proteins by preventing various digestive enzymes, like trypsin and chymotrypsin. It has negative effects like growth impairment and decreases their intake of food owing to bitterness and throat irritation (Liener, 2003).

Furthermore, the process of extrusion inactivates anti-nutritional factors (trypsin inhibitors, haemagglutinins, tannins, and phytates) (Bhandari *et al*., 2001; Fellows, 2022). In a sesame oilseed meal, a single-screw extrusion cooking was used to reduce tannin. The process variables identified for the experiment were: extruder screw speed 63.18–96.80 rev min^{-1}; barrel temperature 63.18–96.80°C and moisture content of raw oilseed meal 31.59–48.41%. Mukhopadhyay and Bandyopadhyay (2003) found the extrusion cooking technology to be very effective in reducing antinutrients namely tannin sesame meal. In another research conducted by Singh *et al*. (2009) extrusion cooking parameters such as screw speed of 300-r.p.m, feed rate of 27-kg h^{-1}, die size of 5/32 inches and die temperature ranging from 93–97°C showed the total degradation of trypsin inhibitor activity in extruded blends of broken rice and wheat bran with up to 20% wheat bran. The main variables for the degradation of trypsin inhibitors are, in conclusion, high extrusion temperatures, longer residence time, and lower feed moisture content (Nikmaram *et al*., 2015).

2.16 Effect of Extrusion on Sensory Characteristics

One of the main features of extrusion technology is the production of characteristic textures. The extent of changes in starch achieved by operating conditions and feed produces a wide range of product textures (Fellows, 2022). Extrusion cooking is an effective and economic continuous process in which the physical, nutritional, and sensory properties of the raw material can be changed to the desired degree (Schiavone *et al*., 2001). The responding factors texture, bulk density, and expansion index are dependent on the raw materials used and the configuration of the extrusion cooker. Starch and similar components like maltodextrins are responsible for higher expansion indices and lower bulk density while components like fat, sugar, and salt have the contrary effect. Regarding the formation of water vapour during the discharge of the molten material through the die, the water content in the flour and water addition has the greatest influence on the expansion (Zweytick, 2008).

In extrusion cooking, the high-temperature short-term (HTST) conditions have only minor effects on the natural colour and flavour of foods. However, in many foods, synthetic pigments added to the feed material, such as water or oil-soluble powders, emulsions, or lakes, determine the colour of the product. Fading of colour due to the expansion of food products, excessive heat or protein reactions, and reducing sugars or metal ions in some extruded foods may be a problem (Fellows, 2022).

2.17 Effect of Extrusion on Contaminating Microorganisms

Destruction of microorganisms during extrusion cooking is advantageous in foods. Little research has been done on bacterial spores being destroyed during extrusion cooking (Nikmaram *et al*., 2015). Cold extrusion is a processing method that is usually used in a single-screw extruder where food products are kept at room temperature and then used to produce products like pasta and various meat products (Riaz, 2000). During commercial processing, the food is not heated which can result in the existence of some microorganisms in the food products. Viable micro-organism remains unaffected by the processing technique of cold extrusion, but the process is defined as hot extrusion when the food is normally heated above 75ºC. This high-temperature short-time (HTST) process is relatively effective in reducing the level of microbial contamination. Extrusion cooking studies on microbial inactivation often target a single product matrix and characterize a limited set of processing parameters (Anderson *et al*., 2017).

Extrusion at higher barrel temperatures in zone 2 showed significant spore destruction, for example, at 120 and 140°C (Likimani & Sofos, 1990). Two similar experiments investigated the destruction of *Bacillus stearothermophilu*s liquid or freeze spore suspensions during the extrusion processing of a mixture of starch-protein-sucrose biscuits with a twin-screw extruder (Bouveresse *et al*., 1982). Their results showed a significant reduction in spore numbers (105-108°C), at high temperatures (150-180°C) during extrusion. Extrusion cooking destroys aflatoxins and reduces the microbial count (Navale *et al*., 2015; Singh *et al*., 2007).

2.18 Raw Materials in Extrusion

Cereal flours are the most common raw materials used in the production of extruded products, but other ingredients derived from other food sources may also be used if they meet the requirements of the equipment. Pseudocereals, fruits and vegetables, legumes, pulses, oilseeds, roots and tubers, nuts and seeds, and meats are also used as raw materials (Shah *et al*., 2021). Starch and protein-based materials are the key raw materials used in the extrusion process. The structure of the extruded products can be developed from starch or protein-polymer transformations during the process. The physicochemical changes in biopolymers that can occur during extrusion cooking include binding, cleavage, loss of native conformation, fragmentation, re-association, and thermal degradation. Physical losses may change the composition of the raw ingredients such as water evaporation (Riaz, 2000).

2.18.1 Nutritional Quality of Cereals

Plants are the major source of the human diet with over 50,000 edible plants available worldwide. Rice, maize, and wheat alone account for 60% of the world’s food energy intake. Cereals are the most dominant staple foods (51%) of the world's average diet, followed by animal-based foods (meat, fish, milk, and egg) (13.5%), fruits and vegetables, pulses and nuts (8.2%), and roots and tubers (5.3%). In the average African diet, the main staple foods are (energy) cereals (46%), roots and tubers (20%), and animal products (7%) (FAO, 2021). Grains and pulses have historically been considered “food for poor people”. But, as their nutritional values continue to be exposed, whole foods and functional foods are becoming very popular. Cereals and other grains contain not only important macronutrients such as protein, lipids, and carbohydrates but also many essential vitamins, minerals, and phytochemicals. Dietary fibres and phytochemical antioxidants, especially insoluble fibres and bound phenolics, and their roles in human health, particularly intestinal health, have been emerging areas of research (Hua & Rong, 2016; Monk *et al*., 2016).

2.18.2 Role of Cereals in Extrusion

Cereals are comprised mostly of starch. This starch provides the structure and texture of extruded foods. The cereals also provide a moderate level of protein, varying levels of fat and fibre, and low levels of sugar, vitamins, and minerals. The most common cereals used for snack products are corn, wheat, rice, and oats (Frame, 1994). Cereal grains are commonly used as important raw materials for the production of extruded snack food owing to their good expansion characteristics the high starch content. Cereals are rich in carbohydrates and fibres but relatively low in protein content, so cereals have to be enhanced with the protein component in the extruded products (Devi *et al*., 2013).

2.18.3 Bioactive Compounds in Cereals

Cereals are considered good sources of many bioactive components such as dietary fibres (e.g., β-glucan, lignan, inulin, arabinoxylan, and resistant starch), phenolic compounds (e.g., phenolic acids, alkylresorcinols, and flavonoids), carotenoids (e.g., lutein and zanthein), anthocyanins, vitamins (e.g., B and E), and minerals (e.g., iron, zinc, magnesium, and phosphorus) (Ragaee *et al*., 2013).

2.18.4 Amaranth Grain (Amaranthus hybridus **L***)*

Amaranth is an ancient grain that originated in the South American Andes. It is known to have a high protein content, roughly 13.5%, making it an excellent source of protein fortification as well as bioactive substances (Ramos-Diaz *et al*., 2015). Grain amaranth has lately emerged as a viable food crop due to its tolerance to weather stress and high biomass and grain yield potential. Grain amaranth is being used as a healthful diet to supplement regular cereals. Grains can be found in breakfast items such as bread, multigrain crackers, pastas, pancakes, and popped products (Henrion *et al*., 2021). While amaranth has a little colour effect, it has a unique, earthy flavour that can permeate a food product even at low inclusion levels (about 3%). Extruded amaranth additionally has bitter and unpleasant aftertaste flavours (Ramos-Diaz *et al*., 2015). Whole amaranth flour has a carbohydrate content of 56%-66%, a fibre content of 10%-12%, a protein content of 13%-16.5%, a fat content of 2%-6.5%, and an ash content of 3%. (Robin *et al*., 2015; Srichuwong *et al*., 2017). This flour also has a low amylose level, accounting for 1.2% of total starch content (Srichuwong *et al*., 2017). Amaranth grain has a significantly higher lysine content (6.48 g/100g) which is absent in cereal grains and makes it particularly attractive for use as a blending food to increase the biological value of processed foods (Chavez-Jáuregui *et al*., 2006). Amaranth flour has a high-water absorption rate, which is beneficial in extrusion, but it also has a high-fat content, which reduces expansion (Pichmony *et al*., 2020).

2.18.5 Potential Health Effects of Bioactive Compounds in Amaranth Grain

It is high in bioactive components including beta-carotene, L-ascorbic acid, polyphenols, lutein, and anthocyanins. It is being used as an antipyretic in herbal medicine in India and Nepal to alleviate labour pain. Amaranth has also been used to treat bladder pain, toothache, blood disorders, piles, and dysentery as a diuretic, astringent, hepatoprotective, and haemorrhage agent. Amaranth has anti-inflammatory, anthelmintic, and antioxidant effects when tested *in vivo* (Iftikhar & Khan, 2019).

The storage proteins of amaranth seeds have an outstanding amino acid balance but are also considered to even have antioxidant, antihypertensive, antiproliferative, antithrombotic, cholesterol-lowering, and immune regulatory functions (Suarez & Añón, 2018). The amaranth crop was preferred for fortification since it takes a huge concentration of lysine, which is lacking in other cereals. Amaranth is also rich in minerals, especially magnesium, calcium, dietary fibre, and unsaturated oils. Tocotrienols are a rare type of vitamin E contained in amaranth oil which inhibits the enzyme that regulates cholesterol biosynthesis. Squalene is found in amaranth oil in higher concentrations than in other vegetable oils. Squalene transports oxygen to different cells in the body, boosting the immune system by retaining LDL (low-density lipoprotein) in the blood cholesterol and preventing cancer-like diseases (Jimoh *et al*., 2018).

Amaranth is a gluten-free ingredient since its protein has fewer prolamins. Its peptides are both antihypertensive and anti-inflammatory. Popped amaranth can indeed be consumed with fruits and milk for a nutritious meal. It may be used to make breakfast cereals and soup. It can even be baked whole to produce salty or spicy “polenta” for breakfast. Amaranth leaf extract contains anticancer properties in liver, breast, and colon cancer cells. The vegetable component provides antitumor properties as well (Bhat *et al*., 2015).

Grain amaranth also has greater levels of vitamins A, E, and folic acid as compared to other starchy staples. The nutraceutical qualities of grain amaranth have indeed been discovered (Tibagonzeka *et al*., 2014). Amaranth grain consumption, for example, was shown to have nutritional and health benefits ranging from general well-being development to the mitigation and recovery of specific diseases and symptoms, and also the speedy response of chronically malnourished children and a rise in the body mass index of people who might have been formerly impaired with HIV / AIDS (Tagwira *et al*., 2006).

2.18.6 Amaranth Grain Products

Amaranth grain has also been utilized to generate acceptable soups and fried and baked food products. Grain amaranth seems to have the potential to improve the nutritional status of malnourished people (Muyonga *et al*., 2008). The roasted and sprouted amaranth grains are consumed, whereas the flour is used as a thickener in gravies, soups, and stews. Because of its nutritious qualities, amaranth flour could also be used to bake cookies, muffins, pancakes, pasta, flatbreads, and other pastries (Singh & Singh, 2011). The use of amaranth flour in the food system is heavily reliant on its functional properties. Hydration, fat absorption, emulsification, foaming, and viscosity are all essential functional characteristics of flours that influence their use in a variety of products formulations (meat, confectionery, and bakery products) (Shevkani *et al*., 2014). Furthermore, amaranth products and seeds contain a well-balanced amount of bioactive substances that have antioxidant properties. Non-saponifiable grain lipids comprise tocopherols, squalene, sterols, and many others (Ogrodowska *et al*., 2014).

2.18.7 Legumes/Pulses in Extrusion Cooking

Pulses, often known as grain legumes, are dry edible seeds that come with a pod. Pulses provide critical micronutrients, complex carbohydrates, dietary fibre, and high protein content (Ravichandran & Upadhyay, 2022). Extrusion of legume flours has the potential to produce extruded ready-to-eat foods that partially or completely replace cereals. The inclusion of legume flours improves the nutritional value of cereal-based end-products by increasing the quantity of important amino acids, fibres, proteins, and micronutrients while decreasing the content of trypsin inhibitors, lectins, phytic acid, and tannins (Pasqualone *et al*., 2020).

Soybean (*Glycine max,* [L] Merrill), a versatile pulse is the main food in many parts of the world. Soybeans (Glycine max L. Merr.) are exceptional amongst legumes because they are low in saturated fat and high in protein, complex carbohydrates, fibre, and oil. Besides their excellent nutritional profile, they are also good sources of functional components such as isoflavones, soyasaponins, and tocopherols (Messina, 1999). It is the richest, most cost-effective, and best supply of vegetable protein available to mankind. Soybeans are high in protein and have an excellent source of essential amino acids necessary for the growth, maintenance, and reproduction of the body; they also contain high levels of polyunsaturated fat and a lack of cholesterol and lactose. Soybeans are also an excellent source of minerals and vitamins (Iwe, 2003). Due to the presence of these bioactive compounds, soybeans are widely used in the food industry for developing functional foods that have several health benefits.

Soybeans in the form of soybean carbohydrates, soybean oil, soy proteins, and soy germ are widely used in a range of food products for their functional benefits (Messina, 1999).

2.18.8 Nutrient Potential of Legumes

Legumes are processed to enhance digestibility, palatability, sensorial properties, nutritional qualities, and nutrient bioavailability (Tharanathan & Mahadevamma, 2003). Pulses are commonly referred to as "poor man's meat" since they are regularly consumed by people in developing nations due to their high protein content. They are a good source of complex carbohydrates, protein, dietary fibre, and vitamins and minerals. Carbohydrate is the most abundant substance present in pulses, with an average range of 60-65%. Starch has the most carbohydrates. The content of oligosaccharides and dietary fibre varies according to the degree of husk removal. Pulses have a larger percentage of amylose and complex carbohydrates, which makes them high in resistant starch content and low in glycemic index (GI) food. The presence of oligosaccharides in pulses has been related to anti-colon cancer potential by boosting bifidobacteria proliferation in the gut (Ravichandran & Upadhyay, 2022).

Dietary fiber included in beans may help to reduce the prevalence of diseases such as diabetes, cancer, and diverticulitis. Pulses contain both edible and non-digestible carbohydrates: flavonoids and isoflavones. Stachyose is the most abundant oligosaccharide in lentils, accounting for more than 50 mg/g, while raffinose accounts for 39.9 mg/g in chickpeas (De Angelis *et al*., 2021). Cereal grains and legume seeds are known to contain a plethora of phytochemicals that can act as antioxidant and anti-inflammatory agents when consumed. These bioactive compounds are considered reasons for the many health benefits of cereal foods (Wang *et al*., 2015).

2.18.9 Sweet Potato **(*Ipomea batatas*)**

Sweet potato (*Ipomea batatas*) is an incredibly important crop cultivated in more than 100 countries in many regions of the world. The sweet potato is one of Africa's most important root and tuber crops. Over 7 million tons of sweet potatoes, approximately 5% of total production, are produced in Africa, many of which are grown in the East and South African regions (Olapade & Ogunade, 2014). Sweet potato is a very important food crop in the tropical and subtropical regions and thus has the nutritional benefit of increasing production and consumption for both rural and urban communities of these regions (Fesco & Boudion, 2002; Woolfe, 1992). Sweet potato in Rwanda is a major nutritional staple crop. The rural poor and urban people who depend on it for their livelihood in Rwanda consume sweet potatoes

(Ingabire & Vasanthakaalam, 2011). Sweet potato contains 63–70% moisture content, 18.86–29.86% carbohydrates, 0.71–9.84% protein, 0.14–2.8% crude fibre, 0.43–2.0% ash, and 1.1–2.0% fat (Ukom *et al*., 2009; Ingabire & Vasanthakaalam, 2011).

Orange-fleshed Sweet Potato (OFSP) varieties are among the foods with the greatest potential for reducing VAD owing to their very high beta-carotene content and general acceptability as a food (Burri, 2011). The beta-carotene content of Orange-Fleshed Sweet Potato may vary between 1,240 and 92,940 μg/100 g beta-carotene (Burri, 2011; Fonseca *et al*., 2008). It is reported that a daily intake of 6–33 or 68–381 g / day of OFSP by children and lactating mothers respectively is sufficient to provide 100% of the requirements for vitamin A (Burri, 2011). OFSP has been used in baby foods as an ingredient to improve the vitamin A content. OFSP is nevertheless low in protein and fat and must be blended with high protein and lipid-containing ingredients such as soy, fish powder, and soybean oil to produce suitable nutritional products (Amagloh & Coad, 2014).

2.19 New Product Development

The development of food products requires far more than producing the ideal recipe. Industries are engaged in new product development to attract new consumers, grow into new global markets, increase revenues, increase brand awareness or increase market shares. Product development also takes a bit of consumer acceptance, correct timing, and luck (Aramouni, 2017). The process of food product development is summarized in Figure 2.4.

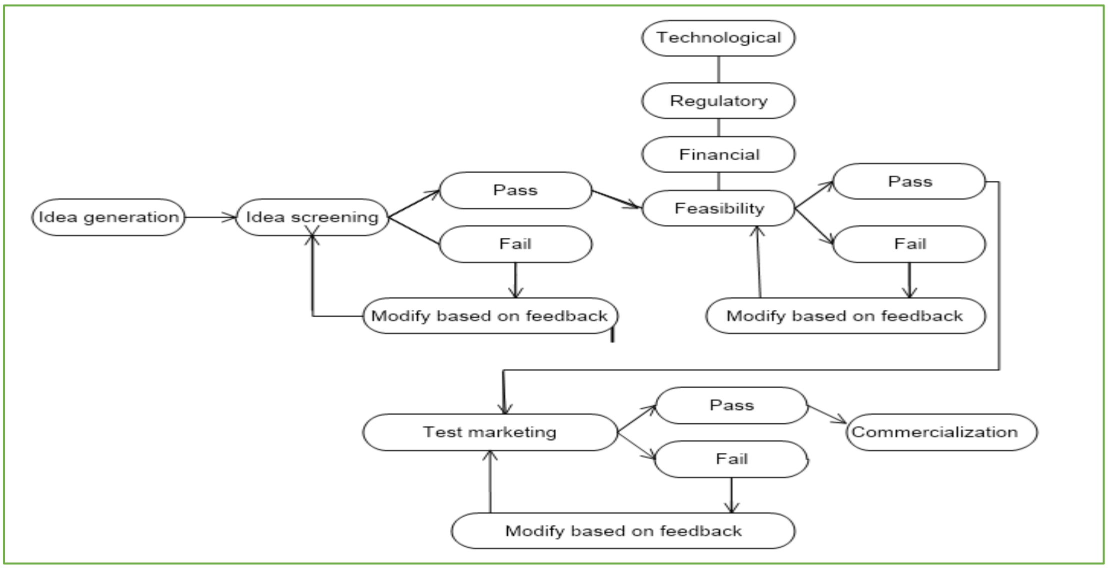


Figure 2. 4. The process of product development with modification (Aramouni, 2017).

2.20 Shelf-life Testing of Food

Shelf life is described as the time during which the food product will (1) keep safe; (2) be sure to maintain the intended sensory, chemical, physical and microbiological characteristics; (3) adhere to any declaration of nutritional data on the label; and (4) be acceptable to the consumer (Earle & Earle, 2008). It is also very crucial for food products and must meet the requirements of health, quality, organoleptic, and appearance to ensure consumer acceptance (Phimolsiripol & Suppakul, 2016).

2.20.1 Types of Shelf-Life Tests and Design

The shelf-life test may be split into three categories (Kilcast & Subramaniam, 2000) including (1) **Static tests:** Product that is kept under a given set of environmental conditions. This test takes a long time to observe changes and it is expensive. It contains no information about the effects of stress and comes closest to the conditions of distribution. (2) **Accelerated tests:** Product stored in a range of environmental conditions (usually temperature or relative humidity). The conditions of this experimental test are chosen to cover the estimated range observed and can be accomplished in a relatively short period. It also gives kinetic data, and the test conditions must not alter the normally expected path that affects shelf life. The findings have to be carefully interpreted.

Accelerated shelf-life testing (ASLT) may refer to any process capable of determining product stability based on information collected in a significantly shorter time than the product's actual shelf life. ASLT refers to any deterioration mechanism that a valid model may be described quantitatively. This model can follow changes in shelf life that express the value of a deterioration marker or the extent of product failure under a given history of storage and handling. The mechanisms of deterioration could be chemical, physical, biochemical, or microbial (Mizrahi, 2011) and (3) **Use/Abuse tests:** The product is cycled via environmental variables. The test is used for product and packaging evaluation as a unit. Such experiments utilize cycles of variables equivalent to or above what is expected under real conditions. It has been used to assess transport impacts.

2.20.2 Sampling Schedule

The sampling plan depends on the typical shelf life: (1) Short-shelf-life products (up to 1 week for example ready meals), Samples may be taken daily for testing. (2) Medium-shelf-life products (up to 3 weeks for example some ambient cakes and pastry), Samples may be collected during days 0, 7, 14, 19, 21, and 25. (3) Long-shelf-life products (up to 1-year example of some breakfast cereals), Samples may be collected at intervals of 0, 1, 2, 3, 6, 12, and (maybe) 18 months (Kilcast & Subramaniam, 2000).

2.20.3 Kinetic Reactions

The simplest method for testing shelf-life is the kinetic reaction approach. The kinetic data are used to determine how the degradation cycle works as a function of time to estimate the shelf-life. It is the principle of quantifying the quality of products based on a change in reaction (Corradini & Peleg, 2007). The basic process of the kinetic model approach comprises the following steps: (a) Selection of the desired kinetically active factors for an acceleration of the deterioration process. (b) Running a kinetic analysis of the process of deterioration at such rates of the accelerating factors that the deterioration rate is quick enough. (c) Extrapolating the data to normal storage conditions by measuring kinetic model parameters (Figure 2.5) and (d) Using the extrapolated information or even the kinetic model to estimate shelf life at actual storage conditions (Mizrahi, 2011).

The forms of the quality function of the food for an apparent zero-, first-, second-, or n^{th}-order reaction are shown in Table 2.4 and Figure 2.5, showing the different patterns of the reaction orders. The reaction rate for zero-order reactions is independent of the concentration of a reactant. Reactions in first order depend on a single reactant, and the exponent value is one. The reaction rate may be proportional to one concentration squared or to the average of two concentrations for second-order reactions (Labuza & Riboh, 1982).

Some reactions responsible for shelf-life loss based on a typical physicochemical, chemical, or microbial index typically involve zero order (e.g., overall frozen food quality, Maillard browning) and first order (e.g., loss of vitamins, oxidative colour loss, microbial growth), as shown in Table 2.4 and Figure 2.5. The easiest and most often used approach for determining the order of the reaction is the integration process (van Boekel, 2008).

Table 2. 2 Quality function of reaction order

Apparent reaction order	Quality function
0	$A_t - A_0$ or A_t/A_0
1	ln $(A_t - A_0)$ or ln (A_t/A_0)
2	$1/A_t - 1/A_0$
n (n ≠1)	$(1/n - 1) \times (A_t^{1-n} - A_0^{1-n})$

The process begins with (1) assumption of the order of reaction; (2) integration; (3) linearization by linear regression; (4) linearization of experimental data; and (5) if data match a straight line, then the assumption is correct if it does not restart. The simple selection is a higher coefficient of determination (R^2), and the slope is the reaction rate constant.

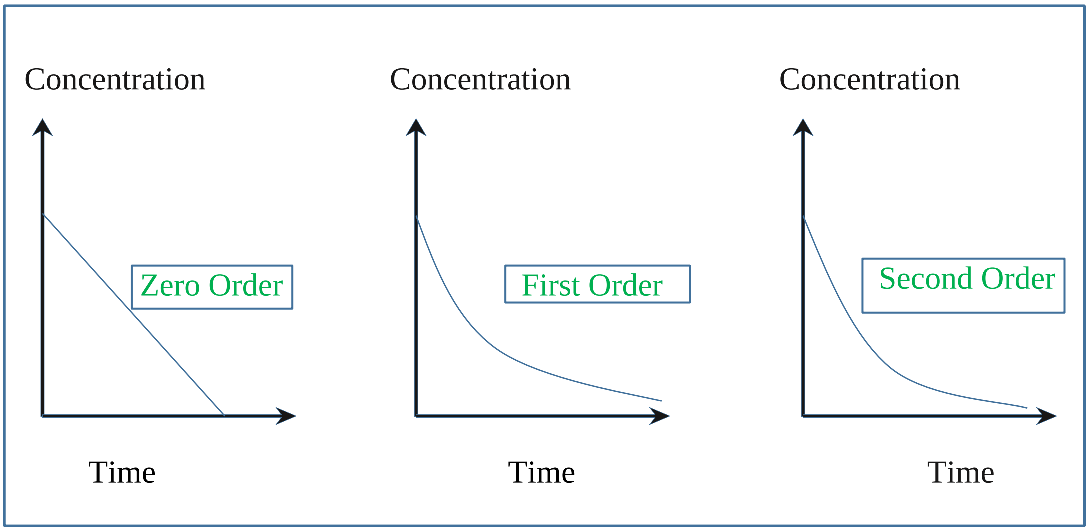


Figure 2. 5 Pattern of concentration changes in different reaction orders with modifications (Mizrahi, 2011).

2.20.4 Accelerated Shelf-Life Simulation

Food industries need a relatively short time to collect the information necessary to determine the shelf-life of their food products. Accelerated shelf-life prediction may refer to any method capable of evaluating product stability, based on information gathered in a considerably shorter period than the actual shelf-life testing of the specific product (Steele, 2004). To reduce the time needed to estimate a shelf life, the principles of accelerated shelf-life simulation include: (1) the idea is that storing food at higher temperatures will show any adverse effects on its storage behaviour, and hence shelf life can become evident in a shorter period. (2) Under standard storage conditions, extrapolation will estimate the shelf-life using data obtained from accelerated determination.

2.20.5 Steps of Accelerated Shelf-Life Simulation

When conducting the accelerated shelf-life test, the compositional factors must be maintained constant (Hough, 2010). The microbial safety and quality parameters need to be established and described as the priority for product shelf-life. Main deteriorating reactions are then identified which will cause a loss of quality and unacceptability by consumers. In the case of correct packaging, cost-effective packaging is used except for the impact of packaging materials. The various packaging materials are to be used for comparison. Therefore, other environmental factors, such as temperature, relative humidity, light are chosen as the ideal kinetically active factors for the acceleration of the deterioration phase.

The next step is to assess the length of the product at each test temperature and the frequency of testing between tests at temperature. The total number of samples to be stored at each temperature value is calculated. The final step is to run a kinetic analysis of the process of deterioration at such rates of the accelerating variables. Nevertheless, if the rate of deterioration is too rapid or too slow, the sampling frequency can be increased or reduced as required. The shelf-life prediction will be determined by establishing the shelf-life plots to determine shelf-life under normal storage conditions utilizing the extrapolating data or the kinetic model to estimate shelf-life under actual storage conditions (Phimolsiripol & Suppakul, 2016).

2.20.6 Arrhenius Model

The Arrhenius model is a classic model, which relates the rate of a chemical reaction to temperature changes. The model is commonly applied in several temperature-affected processing and storage tests (Corradini & Peleg, 2007; Phimolsiripol *et al*., 2011). It is noted, however, that the determination of shelf life is accurate only for the exact combination of product composition, packaging, and processing condition (Phimolsiripol & Suppakul, 2016). The steps of the Arrhenius model for assessing shelf life are summarized in five steps namely (1) find the reaction order according to the principle of kinetic reaction. (2) Follow the Arrhenius relationship. (3) Plot the Arrhenius relationship. (4) Fit the curve using linear regression. (5) Slope of the plot between ln k versus 1/T is Ea/R.

2.21 Food Packaging

Packaging plays a vital role in many food preservation operations. Packaging has many functions, including containment, preservation, communication/education, handling/transportation, and marketing. Packaging helps maintain during storage the quality and properties of foods attained via processing. The packaging protects the food material from microbiological contaminants and other environmental factors. The package also helps prevents light-induced changes in stored food products and minimizes loss of moisture (Clark *et al*., 2014).

2.21.1 Steps for Determining Packaging

Aramouni (2017) demonstrated the eight steps for selecting packaging namely (1) Defining food properties, (2) Defining package technical and functional requirements, (3) Defining package marketing and design requirements, (4) Identifying legal and regulatory requirements, (5) Selecting potential package design and materials, (6) Establishing the feasibility of packaging with equipment and material, (7) Estimating time and cost constraints and (8) Shelf-life testing and market testing.

2.22 Experimental Designs

The experiment design is key in producing food product development ventures. Well-planned studies save time and can be conducted more easily. The statistical design of experiments gives assurance that the data you gather is accurate. Experimental design is mainly used in product formulation, process development, sensory analysis, and market analysis (Aramouni, 2017). Guidelines for designing and experimenting include (1) Recognition of and statement of the problem, (2) Selection of the response variable(s), (3) Choice of factors, levels, and ranges, (4) Choice of experimental design, (5) Performing the experiment, (6) Statistical analysis of the data and (7) Conclusions and recommendations (Rigdon *et al*., 2022).

2.22.1 Completely Randomized Design (CRD)

The most basic experimental design is a completely randomized design, in which the treatments are assigned completely randomly in the experimental plots. These treatments would be assigned in n homogenous plots (Bhuyan, 2021; Madhyastha *et al*., 2020). The CRD is used in laboratory, dairy science research and in green-house experiments (Bhuyan, 2021). A completely randomized design is one in which the researcher assigns the experimental units to the treatments completely at random, with the only constraint being the number of observations to be taken on each treatment. For experiments with no blocking factors, completely randomized designs are utilized (Dean *et al*., 2017). A completely randomized design (CRD) is an experimental design by which the analyst evaluates the samples randomly to various procedures (Aramouni, 2017).

The advantages of CRD are as follows: (i) It is simple to use a completely randomized design in practice; (ii) The d.f. of error of this design becomes large because the total d.f. is divided into only two parts, one for treatment and one for error; (iii) If homogeneous plots are available, the design can be used for any number of treatments; and (iv) The data analysis of this design is simple and easy. Even if one or more observations are missing, the analysis is straightforward. However, the disadvantages of CRD are as follows: I If the plots used in the experiment are not homogenous, the design is unsuitable; and (ii) If the number of treatments is considerable, a large number of homogeneous plots are required to conduct the experiment.

As a result, the plots may lose their homogeneity, and (iii) the design is unsuitable for agricultural experiments in the field (Bhuyan, 2021).

2.22.2 Randomized Complete Block Design (RCBD)

A Block Design involves randomly assigning two or more treatments to experimental plots divided into two or more blocks (Madhyastha *et al*., 2020). A block design is one in which the researcher divides the experimental units into blocks, allocates treatments to blocks, and distributes the experimental units within each block to the treatments completely at random (Dean *et al*., 2017). A randomized complete block design (RCBD) is limited randomization of treatments where the units are sorted into blocks, and the treatments are allocated randomly to units within each block. The block design shall be deemed complete if at least one unit in each block is allocated for each treatment. The block design will otherwise be incomplete (Aramouni, 2017). The RCBD is the most extensively utilized architectural research plan in teh medical, agricultural, biological, and engineering sciences (Basavarajaiah & Murthy, 2020).

2.22.3 Response Surface Methodology (RSM) and Optimization

Response Surface Methodology is a set of mathematical and statistical approaches that can be used to model and analyze problems in which a response of interest is influenced by multiple variables and the goal is to maximize this response (Montgomery, 2017). Response Surface Methodology provides many advantages for optimization over one factor at a time approach and also does not take the interaction of factors into account (Esfahanian *et al*., 2013). Response Surface Methodology (RSM) offers statistically based predictive models which can be used to identify optimum process configurations (Alsanusi & Bentaher, 2015). RSM typically is useful in situations where several factors influence one or more performance characteristics or responses. It can also be utilized to optimize one or more responses to meet a given set of specifications. More specifically, RSM offers an adequate empirical analysis of the surfaces of experimental results for non-linear responses (Behera *et al*., 2012). The standard response surface designs, such as the central composite design (CCD), the Box-Behnken design (BBD), and their derivatives (such as the face-centred cube), are extensively utilized since they are relatively broad and flexible designs (Montgomery, 2017).

2.22.4 Extreme Vertices of Mixture Design

Mixture design is a statistical and analytical strategy for developing a robust prediction model for the given response that optimizes the composition (Poornesh & Bhat, 2021). Extreme vertex designs are combination designs that provide us with a sub-portion of the simplex. This condition is typically generated when the components have both lower and upper-bound constraints. The goal of an extreme vertices design is to choose design points that cover the design space efficiently (Danielraj *et al*., 2020; Tufa *et al*., 2021).

2.22.5 Factorial Design

Factorial design (FD) is a research method that uses two or more variables or factors to conduct research in such a way that all potential treatment combinations of selected subjects, each subject is used for practical purposes (Basavarajaiah & Murthy, 2020; Rigdon *et al*., 2022). A factorial experimental design means that in each entire trial or replication of the experiment, all potential combinations of the factor levels are evaluated (Rigdon *et al*., 2022). The levels of the factors can be arbitrarily labelled "low" and "high" (Montgomery, 2017). The effect of a factor is defined as the change in response caused by a change in the level of the factor. This is commonly referred to as a main effect because it refers to the principal components of interest in the experiment (Rigdon *et al*., 2022). Factorial experiments can be used in chemical and industrial studies that include a high number of variables (Madhyastha *et al*., 2020).

2.23 Impact of Maillard Reactions on Food Quality

The Maillard reactions between protein and sugar decrease the nutritional value of protein and reduce heat-sensitive vitamins, and variations in protein and amino acid profiles are also triggered by extrusion cooking. Extrusion may change carbohydrates, dietary fibre, protein, amino acid profile, vitamins, and mineral contents of the extrudate with both positive and negative effects on the food (Singh *et al*., 2007).

2.23.1 Maillard Reactions in Food

Common browning of foods during heating or storage is normally due to a chemical reaction between reducing sugar (most frequently D-glucose) and a primary amino group (most frequently on a protein molecule). The reaction is called the Maillard reaction (named after the chemist who first studied and described it). The overall process is called nonenzymic (or nonenzymatic) browning (sometimes Maillard browning) to differentiate it from the more rapid, enzyme-catalysed browning commonly observed in freshly cut fruits and vegetables (such as apples and potatoes) (BeMiller, 2019). Browning occurs via several reactions and can take several different routes through the reaction pathway, but the reaction sequence can be divided into three basic stages: (1) Condensation of a reducing sugar with an amine followed by a molecular rearrangement, (2) Molecular dehydrations and cyclizations, and (3)

Condensations of the products from stage 2 with themselves and with amino compounds (BeMiller, 2019).

2.23.2 Factors Affecting the Extents of Maillard Browning Reactions

Reaction variables that can be controlled to increase or decrease the Maillard browning reaction are indicated in five steps namely (1) Temperature (decreasing the temperature reduces the reaction rate), (2) pH (decreasing the pH reduces the reaction rate), (3) Adjustment of water content (maximum reaction rate occurs at water activity values of 0.6-0.7 about 30% moisture), (4) The specific sugar (related to the percentage in the acyclic form) (more reactive sugars, with or without specific amino acids, may be employed to create browning reactions. Nonreducing carbohydrates such as sorbitol may be used to avoid browning. Bisulphite ions somewhat lower the concentration of reducing sugar) and (5) The presence of transition metal ions, such as Fe (II) and Cu (I) ions, undergo one-electron oxidation under energetically favourable conditions (a free-radical reaction may be involved near the end of the pigment-forming process). The presence of metal ions is the most difficult to control, but fortunately is the least important factor (BeMiller, 2019).

2.24 Weaning Foods Prepared from Different Processing Methods

Extruded weaning foods supplemented with minerals and vitamins are manufactured from a blend of cereals and legumes to provide adequate protein and energy for developing children. The extrusion technology is used to make both commercial weaning foods and emergency infant foods provided by relief organizations (Fellows, 2022). The highly nutritious complementary porridge was processed from soybean, orange-fleshed sweet potato, amaranth grains, and pumpkin seed flour blends. The study recommended the utilization of complementary porridges as strategies for improving the energy, protein, vitamin A, iron, and zinc in complementary foods used in Tanzania (Marcel *et al*., 2021).

Sweet potato flour, soybean flour, wheat flour, whole milk powder, and sugar were mixed in various proportions to make weaning foods. Commercial weaning food had the highest grade, followed by a sample comprising 10% sweet potato flour and 25% soybean flour (Haque *et al*., 2013). Another study showed that OFSP-based composite flours enriched with amaranth and skimmed milk powders have the potential to significantly improve the nutritional condition of children aged 6-59 months in developing countries (Tumuhimbise *et al*., 2019).

The highly soluble, fully gelatinized flakes or pellets can also be ground into a powder and rehydrated with hot water to make porridge. The products are microbiologically safe because of the high-temperature extrusion. When put in moisture-proof and airtight packaging, the low water activity (a_w) assures a shelf life of more than 12 months. Alternative weaning foods comprise RTE “rusk“ products that resemble aerated cookies and are designed to disintegrate in saliva when consumed (Fellows, 2022).

The study discovered that locally available food commodities such as sorghum, soybean, and orange-fleshed sweet potato can be used to make a protein-rich supplementary diet capable of addressing childhood malnutrition. A protein-rich weaning diet equivalent to commercial weaning food (cerelac) may be strategically made from a combination of fermented sorghum (59%), soybean (31%), and OFSP (10%) (Adejuwon *et al*., 2020). Complementary Food generated through Solid-State Fermentation (SSF) of Fonio, Soybean, and Orange-Fleshed Sweet Potato Blends developed nutrient-rich complementary food of an acceptable quality that may be made from blends utilizing SSF for optimum baby growth and development (Okoronkwo *et al*., 2023).

2.25 Conclusion: Research Gaps

There is a lot of proof from the literature that protein-energy malnutrition (PEM) and micronutrient deficiencies like vitamin A, iron, and zinc deficiencies are high in African children, mainly in sub-Saharan Africa. In addition, food insecurity continues rising, and still challenges for tackling. Child malnutrition is a complicated issue caused by a variety of causes, and closing the gaps in combatting it necessitates a multifaceted strategy. While there has been improvement in recent years, there are still numerous critical gaps that must be filled such as lack of access to sufficient and nutritious food, poverty, limited food availability, ensuring access to nutritious food for all children, access to quality healthcare, lack of awareness and knowledge about proper nutrition practices among parents and caregivers, educating communities about the importance of a balanced diet, breastfeeding, complementary feeding, and proper hygiene practices, poor water, sanitation, and hygiene, sustainable agriculture, diversification of food production, and support for small-scale farmers, enhancing agricultural practices, promoting nutrient-rich crops, and reducing post-harvest losses are crucial to ensure food security and address malnutrition.

On the other hand, traditional weaning food processing can have various limits, such as a lack of nutritional diversity, restricted nutrient bioavailability, insufficient energy density, texture constraints, limited flavour exposure, and a lack of hygiene and safety. While food fermentation has many advantages, it also has some drawbacks, including unpredictability, extended processing time, food safety risks, limited control over final product attributes, allergenicity concerns, scale-up challenges, and nutrient loss or alteration (degradation or inactivation of heat-sensitive vitamins and enzymes, reducing nutritional value). Sun drying is a conventional food preservation method which involves subjecting food to sunlight for a prolonged length of time in order to eliminate moisture. While sun drying can effectively preserve and extend the shelf life of certain foods, it also has some effects on their nutritional values, such as vitamin loss (vitamin C and vitamin B complex), mineral retention, beta-carotene degradation, the potential for nutrient damage, oxidation and nutrient breakdown, enzyme destruction. Sun drying has several limitations including limited scale and control, nutrient loss, prolonged drying time, inconsistent drying, dependence on weather, contamination risks, and uneven drying.

Using traditional processing methods in the context of food quality can also result in a number of gaps and challenges, including inconsistent quality control, limited traceability, longer processing time (which can impact the freshness, nutritional value, and sensory qualities of the food), inefficient preservation techniques (salting, smoking, or canning), limited nutritional retention and nutrient loss in food, scaling difficulties, and a lack of real-time monitoring and data analysis capabilities. Traditional food processing methods can occasionally result in losses of heat-labile nutrients. Certain vitamins (B vitamins, Vitamin C), enzymes (amylase), phytochemicals (polyphenols and carotenoids), and free amino acids (lysine, cysteine, methionine, tryptophane), are examples of heat-labile nutrients. Food extrusion provides several benefits in the food processing industry, including versatility, efficient and continuous processing, improved nutritional value, antinutrient destruction, textural modifications, shelf stability and extended shelf-life, uniformity and consistency, improved food safety, waste reduction, and environmental friendliness.

There is little research conducted on the analysis of available lysine in extruded ready-to-eat foods. In most heat-treated foods with proteins and reducing sugars, Maillard reactions are formed at some point in which cross-linkages are formed between epsilon amino groups (ε-NH_2) and reducing sugars. Thus, the protein-sugar complex is formed. The formed complex has an unreactive (unavailable) lysine attached to reducing sugar and prevents the availability, absorption, and metabolism of various nutrients within the human body. As a result, the nutritional value of foods is decreased. In addition, it is impossible to find the content of reactive (available) and unreactive (unavailable) lysine in protein-sugar complexes when a technical standard analysis method of amino acids in foods is used, however, only the total lysine content is detected. Therefore, the rapid dye-binding method was used to determine available lysine in extruded ready-to-eat baby foods.

CHAPTER THREE

OPTIMIZATION OF PROTEIN, TOTAL MINERALS, AND VITAMIN A CONTENT OF ORANGE-FLESHED SWEET POTATOES, SOYBEANS, AND AMARANTH SEEDS FLOUR BLENDS

Abstract

Food and nutrition security remains a major challenge facing the world and especially the developing world. This situation could be mitigated by the utilization and blending of locally available food crops. In this study, the nutritional contents of orange-fleshed sweet potatoes (OFSP), amaranth seeds, and soybeans composite flours were optimized by Extreme Vertices Mixture Design using Minitab Software. The software generated 11 experimental runs from the flour blends. Each blend was analysed for proximate, minerals and vitamin A contents. The analysis of blends lead to some useful conclusions, the most important of which yielded high protein content (15.83%) and fat (6.16%) for blend C1 (50:25:25 for OFSP, amaranth seeds, and soybean flour respectively), fibre (5.18%) for blend C11 (60:15:25 for OFSP, amaranth seeds, and soybean flour respectively), total minerals (4.83%) for blend C2 (54.5:24:21.5 for OFSP, amaranth seed, and soybean flour respectively), energy value (359.75 kcal/100g) for C3 (50:30:20 for OFSP, amaranth seeds, and soybean flour respectively), while blend C6 (75:15:10 for OFSP, amaranth seeds, and soybean flour respectively) was higher in carbohydrate (66.60%), energy-to-protein ratio (37.98 Kcal/g of Protein) and vitamin A content (890.03 RAE µg/100g) than others. Generally, blend C1 was the highest in iron content (2.64 mg/100g), Zinc (0.56 mg/100g), magnesium (81.25 mg/100g) and calcium (58.10 mg/100g). The blend C6 was higher in sodium content (41.63 mg/100g) and potassium (65.18 mg/100g) than others, while blend C11 was high in manganese content (0.59 mg/100g) and the highest copper content (0.95 mg/100g) was observed in blend C8 (54.5: 26.5:19 for OFSP, amaranth seeds, and soybean flour respectively). The most significant observation of this study is that the optimum blend was 57%, 24%, and 19% OFSP, amaranth seeds, and soybean flour, respectively, for the production of protein (14%), total minerals (4.7%), and vitamin A content (813.6 RAE µg/100g). These findings could be applicable in cases of processing nutritious foods for people in need in an economical way and promoting the utilization of orange-fleshed sweet potatoes.

3.1 Introduction

World hunger remains a significant challenge, and the COVID-19 pandemic has exacerbated the situation. The prevalence of undernourishment has increased by 1.5% within a year (FAO *et al*., 2021). A healthy diet is crucial for defending against diseases (Global Nutrition Report, 2020). Currently, 2.37 billion people worldwide are severely food insecure, with Asia accounting for half of that number (1.2 billion), followed by Africa with one-third (799 million), and Latin America and the Caribbean with 11% (267 million) (FAO *et al*., 2021). The pandemic has made it even more challenging to access and afford healthy, sustainably produced food (Global Nutrition Report, 2020).

Many children under the age of five suffered from stunting and wasting globally. Child malnutrition remains a significant challenge, particularly in Africa and Asia (FAO *et al*., 2021). Although global progress has been made in reducing vitamin A deficiency (VAD) among children under five years old, no improvement has been observed in South Asia and sub-Saharan Africa, where 44% and 48% of children in that age group, respectively, are still affected (UNICEF, 2018). Additionally, in 2019, 3 billion people globally were unable to afford healthy diets, with Africa and Latin America, and the Caribbean experiencing the impact from 2017 to 2019 (FAO *et al*., 2021).

Therefore, there is a need to develop nutritious and affordable food options using locally available crops. Sweet potato (*Ipomoea batatas* (L.) Lam.) is a root crop rich in fibre, beta-carotene, and vitamin C, especially in orange-fleshed cultivars (Sullivan, 2016). Orange-fleshed sweet potato (OFSP) has great potential for reducing vitamin A deficiency due to its high beta-carotene content and acceptability as a food product (Burri, 2011). Sweet potatoes, both the roots and leaves, are abundant in various essential nutrients for the human body. Sweet potato has also gained attention for its nutritional quality and has been processed into different staple products such as flour, flakes, granules, paste, purées, chips, canned products, beverages, bread, noodles, pancakes, and snacks (Mu & Singh, 2019). By-products such as sweet potato juice, residues, peel, and cirrus are generated during the manufacturing processes, which contain functional components like protein, dietary fibre, pectin, anthocyanin, and chlorogenic acid that play significant roles in biological functions (Mu *et al*., 2017).

According to FAOSTAT (2021), global sweet potato production reached 91.82 million metric tons (MT) in 2019, with Asia producing the highest amount (59.10 MT), followed by Africa (27.87 MT) and America (3.87 MT). Among the top ten sweet potato producers, China leads worldwide with 51.79 MT, accounting for 56.41% of global production, followed by Malawi (6.44%), Nigeria (4.51%), Tanzania (4.27%), Uganda (2.12%), Indonesia (1.97%), Ethiopia (1.91%), Angola (1.83%), the US (1.58%), and Vietnam (1.53%) (Tridge, 2021). The nutritional properties of a particular food are extremely necessary for quality requirements for consumers.

In this study, OFSP was blended with soybean (*Glycine max* (L.) Merrill) and amaranth seed (*Amaranthus hybridus* L) flour for the production of a composite flour which is highly nutritious. Amaranth has both a higher protein content (12–18%) than other cereals, as well as a marginally greater lysine content and acceptable levels of tryptophan and methionine, which are found in cereal and legume grains in small quantities (Mendoza & Bressani, 1987). Amaranth is a good source of lipids, dietary fibre, and minerals (magnesium, phosphorus, copper, manganese, and so on). Amaranth also contains a lot of polyphenols (flavonoids) that have a lot of antioxidant activity. Caffeic acid, p-hydroxybenzoic acid, and ferulic acid are the main phenolics present in amaranth seeds (Singh & Singh, 2011). The amaranth seed was preferred for fortification because it takes a huge amount of lysine, a nutrient that is lacking in other cereal grains (Jimoh *et al*., 2018) and its high biological value creates the potential to be used as a blending product to boost the nutritional value of processed foods (Chavez-Jáuregui *et al*., 2006). Beta-carotene, L-ascorbic acid, polyphenol, lutein, and anthocyanins are among the many biological activities contained in them (Iftikhar & Khan, 2019). The storage proteins of amaranth seeds have been shown to have an outstanding amino acid balance, as well as antioxidant, antihypertensive, antiproliferative, antithrombotic, cholesterol-reducing, and immune regulatory activities (Suarez & Añón, 2018). In contrast to traditional starchy staples, grain amaranth contains a higher concentration of vitamins A, E, and folic acid (Tibagonzeka *et al*., 2014).

Soybeans are the most largely produced crop worldwide. According to FAOSTAT (2021), the global production of soybean was 333.67 MT in 2019. Brazil is the leading country with 34.25%, followed by the US (29.01%), Argentine (16.56 %), and China with 4.71% (Tridge, 2021). Soybeans are rich in protein and a good source of essential amino acids for body development, protection, and reproduction; they do have a lot of polyunsaturated fat and are low in cholesterol and lactose. Soybeans are a good source of nutrients and vitamins as well (Iwe, 2003). The main objective of this study was to optimize crude protein, ash (total minerals), and vitamin A contents in the composite flour to contribute to food and nutrition security.

3.2 Materials and Methods

3.2.1 Raw Materials

Orange-fleshed sweet potato, variety, Kenspot 5, and soybean of variety DPSB 19 were bought from KALRO (Kenya Agricultural and Livestock Research Organization), Njoro, Nakuru, Kenya, and amaranth seeds of variety Katumani Amaranth (KAM) 001 was procured from KALRO (Kenya Agricultural and Livestock Research Organization), Katumani, Machakos, Kenya.

3.2.2 Production of Orange-fleshed Sweet Potatoes Flour

Orange-fleshed sweet potato tubers were washed with potable water to remove dirt and adhering soil and particle then peeled using a stainless-steel kitchen knife. The tubers were chopped to 2 mm size using a universal kitchen machine (C-1RB, No. 3601150, URDORF.ZURICH, Suisse). After blanching in hot water at 80^{o}C for 3 min for enzyme inactivation, drying was carried out to achieve a moisture content of 10% at 55^{o}C for 24 h. The dried potato was milled into flour of 100 µm of particle size using a Perten milling machine (S-14105 Huddinge, Perten Instruments AB, Finland) and then packaged and stored (Honi *et al*., 2017).

3.2.3 Production of Amaranth Seeds Flour

The grains were cleaned, carefully sorted, washed with portable water dried in an oven (Memmert GmbH+Co.KG, D-91126 Schwabach FRG, Germany) to a moisture content of about 10.20±0.10, then milled using a Perten milling machine (S-14105 Huddinge, Perten Instruments AB, Finland), and then the flour of 100 µm of particle size was stored in paper bags inside of a polyethylene packaging at 15^{o}C of temperature before analysis (Shevkani *et al.*, 2014).

3.2.4 Production of Soybeans Flour

To begin the flour processing, the soybeans were sorted to remove any debris or damaged seeds. The soybeans were cleaned and soaked overnight at room temperature (25^{o}C) in tap water (soybean: water ratio 1:3, w/v) then manually drained, rinsed, and partially de-hulling of hydrated beans. Drying was done in the oven at 55^{o}C for 18 h to achieve a moisture content of 10%, then milled into 100 µm of particle size. Soybean flour was packed in a sealed polyethylene container and kept at 15^{o}C before analysis and food formulation (Shokunbi *et al*., 2011).

3.2.5 Experimental Design

Minitab software version 19.2020.1 (Minitab, 2020) was used to formulate mixture components. On a proportional basis, the extreme vertices method of mixture design was used with constraints that included orange-fleshed sweet potatoes flour at 50-75%, amaranth seeds flour at 15-30%, and soybeans flour at 10-25%, with the total mixture being 100%. This method has been useful in situations where all the components can vary from 0 to 1.0 or 0-100%. Furthermore, extreme vertices designs are useful in analysing the response of a mixture system with upper and lower constraints (Cleland & McCluskey, 2013; Mclean & Anderson, 1966). The model generated 11 runs (Table 3.1) which are composite flour from orange-fleshed sweet potatoes, amaranth seeds, and soybeans flour at different proportions. The proximate composition, minerals, and vitamin A content of each composite flour were determined in triplicate determination. In addition, the optimization of ingredients was carried out using a response optimizer based on the protein, total minerals, and vitamin A content of the composite flour.

3.2.6 Determination of Dry Matter Content in Composite Flour

The dry matter content was determined by air oven (AACC International, 2010), Method 44-15.02 with modification. Briefly, 2 g ground portions were prepared by rapidly mixing with a spoon or spatula and weighing in dishes for moisture. Dishes were sealed and measured at the same time. Pre-weighed weights were deducted, and the sample weight was registered. After the oven had recovered its temperature, the samples were heated at 103^{o}C for precisely 60 min. The dishes were removed from the oven, insulated (using rubber finger insulators), and cooled in desiccators.

Table 3. 1 Extreme vertices method of mixture design

Run Order	Standard Order	Design Points	Ingredients Ratios				Bounds of Mixture Components	
Composite Flour (CF)	StdOrder	PtType	OFSP	Amaranth Seeds	Soybean	Total	Lower	Upper
C1	2	1	50	25	25	100	50	75
C2	8	-1	54.5	24	21.5		15	30
C3	1	1	50	30	20		10	25
C4	10	-1	67	19	14			
C5	5	1	60	30	10			
C6	4	1	75	15	10			
C7	9	-1	59.5	19	21.5			
C8	7	-1	54.5	26.5	19			
C9	11	-1	59.5	26.5	14			
C10	6	0	59	23	18			
C11	3	1	60	15	25			

C1-C11: Composite flour number 1 to composite flour number 11 with their corresponding ingredients ratios from OFSP, amaranth seeds and soybean flour

Weights of dried samples were measured once they had reached room temperature about 25°C. Weight loss was calculated as dry matter.

Moisture content (%) $=\frac{Dry\ weight\ of\ sample}{Total\ weight\ of\ sample} \times 100$

$\text{Dry matter } (\%) = 100 - \%\text{ Moisture content}$

3.2.7 Determination of Crude Fat in Composite Flour

The crude fat content was determined by the Soxhlet extraction (AACC International, 2010), Method 30-25.01. Sample (2 g) that had been previously dried in a vacuum oven at 100°C, under pressure not exceeding 100 mm Hg (for about 5 h ± 30 min) were weighed. The sample was quantitatively transferred to the extractor and was extracted with petroleum ether for 4 h at a condensation rate of 5–6 drops/sec to 6 h at a 2–3 drops/sec rate. Then ether was removed from the collection flask or beaker at low-temperature volatilization before oven drying. Fat that was remaining in the previously dried and the tared fat beaker was dried or flask in the oven at 100°C for 30 min until constant weight. It was desiccated, cooled, and weighed.

$$\text{Crude fat } (\%) = \frac{(\text{Weight of extract} - \text{Blank}) \times 100}{\text{Weight of sample}}$$

3.2.8 Determination of Crude Protein in Composite Flour

Crude protein was determined by the Kjeldahl method and boric acid with modification (AACC International, 2010), Method 46-12.01. A finely ground sample (1 g) was placed in a digestion flask. A polyethylene packet of catalyst (9.9 g potassium sulphate, 0.41 g mercuric oxide, 0.08 g copper sulphate, and approximately 0.10 g pumice stone) and 25 ml concentrated H_2SO_4 were added to the flask. Digestion was carried out until the solution was clear for 30 min; then the solution was removed and cooled. Boric acid-methyl red-methylene blue indicator solution (50 ml) was placed in a flask under the condenser tube with the tip of the condenser tube immersed under the surface of the solution. It was added to the flask and cooled with 300 ml of tap water. Concentrated NaOH (50 ml) was added, and then the flask was connected to the condenser with a tight-fitting rubber stopper and swirled. It was boiled until all of the ammonia had distilled and then the receiving bottle was set down to drain the condenser tube. The distillate was titrated to neutrality with standard 0.1N HCl, using a burette graduated in 0.1 ml. the volume of acid used was read, directly from the burette. The blank determination was run using all ingredients except the sample. The burette reading was corrected for nitrogen in reagents as shown below using blank reading.

$$\% \text{ Protein} = \frac{(\text{Volume of standard acid } \times \text{N of HCl}) \times 1.4007 \times \text{f}}{\text{Sample Weight (g)}}$$

where N = Normality of HCl; f = 6.25

3.2.9 Determination of Crude Ash in Composite Flour

Ash content was determined by muffle furnace at 550 °C for 12 h (AACC International, 2010), Method 08-01.01 where well-mixed sample (3 g) was placed into a crucible that had already been burned, cooled in desiccators, and weighed shortly after reaching room temperature. The sample was placed in a muffle furnace at 550°C and incinerated until light grey ash was obtained or constant weight was achieved. The sample was cooled in the desiccator and measured shortly after it reached room temperature.

$$\text{Ash (g/100 g)} = \frac{(\text{Weight of residue}) \times 100}{\text{Sample weight}}$$

3.2.10 Determination of Crude Fibre in Composite Flour

The crude fibre was determined gravimetrically after chemical digestion according to AACC International (2010), Method 32-10.01. The ground sample (2 g) was extracted with petroleum ether. The sample was transferred to a 600 ml beaker while avoiding fibre contamination from the paper. Prepared ceramic fibre (1.5 – 2.0 g), 200 ml boiling 1.25% H_2SO_4, and containing 1 drop diluted anti-foam were added. The beaker was positioned with a pre-adjusted hot plate on the digestive apparatus and boiled precisely for 30 min, with periodic rotation to keep the solids from side-tracking. The beaker was removed. Using the filter screen, suction was turned on and the screen was inserted into the beaker, the face of the screen was kept just under the surface of the liquid until all liquid was removed. Without breaking the suction or raising the filter, 50 – 75 ml boiling water was added. After the wash was removed, repeated three times with 50 ml of washing. The filter was removed from the beaker and drained all water from the line by raising above the trap level. The mat and residue were returned to the beaker by breaking the suction and blowing back. Boiling 1.25% NaOH (200 ml) was added and boiled for exactly 30 min. The beaker and filter were removed as above. It was washed with 25 ml boiling 1.25% H_2SO_4 and three 50 ml portions of boiling water without breaking suction. Free excess water was drained by raising the filter. The filter was lowered into a beaker and washed with 25 ml of alcohol. The line was drained, the suction was broken, and the mat was removed by blowing back through the filter screen into the ashing dish. The residue was treated. The mat and residue were dried at 130 ± 2°C for 2 h and then cooled in a desiccator and measured. It was ignited at 600 ± 15°C for 30 min and cooled and reweighed in a desiccator.

$$\% \text{ Crude Fibre} = \frac{(\text{W}_1 - \text{W}_2) \times 100}{\text{W}_3}$$

where W_1: Loss in weight on the ignition, W_2: Loss in weight on ceramic fibre blank

W_3: Weight of sample

3.2.11 Determination of Carbohydrate Content in Composite Flour

The total carbohydrate content was determined by the difference method where the summation of moisture content, crude ash, crude fibre, crude protein, and crude fat was deducted from the total of 100% (Gbadebo & Ahmed, 2021).

3.2.12 Determination of Energy Value and Energy-To-Protein Ratio in Composite Flour

The calorific value was computed by summing up the values obtained by multiplying the values with Atwater constants for carbohydrates, crude fat, crude fibre, and crude protein with the factors 4, 9, 2, and 4, respectively (Menezes *et al*., 2016). Energy-to-protein ratio was calculated by dividing the energy value of the sample by its corresponding crude protein content.

3.2.13 Determination of Vitamin A Content in Composite Flour

The beta-carotene was determined by UV/Visible spectrophotometer according to Rodriguez-Amaya and Kimura (2004) method with modification. Ground samples were saponified with potassium hydroxide and extracted with acetone. Carotene was eluted with 4% acetone (in hexane) and read by a spectrophotometer (UV-1700, Shimadzu, Japan). Sample (2 g) was weighed, homogenized and colour was extracted using a mortar and pestle with small portions of acetone until the residual was colourless. All extracts were combined into a 50 ml volumetric flask. The extract (25 ml) was taken into the round-bottomed flask and it was then evaporated to dryness in a rotary evaporator at about 60^{o}C. Petroleum ether (1 ml) was added to the evaporated sample to dissolve the beta-carotene. The elute was received into a 25 ml volumetric flask and the absorbance was read at 450 nm. Vitamin A in retinol activity equivalent was converted following the method of Trumbo *et al*. (2003). The β-carotene was calculated from the beta-carotene standard curve.

$$Conc = \frac{0.4}{0.12} \times \frac{Absorbance \times Final\ volume}{Weight\ of\ sample} \times Dilution\ factor$$

$$Vitamin\ A\ (\text{RAE}\ \mu g/100g) = \frac{\beta - Carotene}{12}$$

3.2.14 Determination of Mineral Content in Composite Flour

Determination of minerals (Ca, Fe, Mg, Mn, Zn, and Cu) were carried out by Atomic Absorption Spectrophotometry (Model AA-6300, Serial No A30524300916 SA, Shimadzu Corporation, Japan) (AACC International, 2010), Method 40-70.01. The sample (2 g) was accurately weighed into an ashing crucible. The blank (empty crucible) was placed alongside the flour samples. The crucibles were placed in a muffle furnace set at 500°C and allowed to ash for a minimum of 6 h. The crucibles were removed from the furnace and cooled to room temperature. To each crucible, 10 ml of HCl was added and then covered with a watch glass. The solution was boiled and evaporated nearly to dryness on a hot plate. The residue was not allowed to cake up on the crucible. The residue was redissolved in 20 ml 2N HCl and boiled gently. The crucibles were removed from the hot plate and cooled to room temperature. The watch glasses were rinsed into crucibles with water. Solutions were quantitatively transferred into separate 100-ml volumetric flasks using a funnel. It was diluted to 100 ml and mixed then absorption of the solution was directly measured. For calcium, there is a sufficient supply. In this case, 5 ml of Lanthanum (La) stock solution was added to achieve a final dilution of 1%. For example, 20 ml solution in a 25-ml flask, and so on.). The instrument was set up and at least 4 standard solutions within the analytical range before and after each group of 6–12 samples were read. After each sample, the burner was rinsed with water to restore the zero-absorption level. The calibration curve was prepared from an average of each standard before and after the sample group. The concentration of samples was read from the plot of absorption against µg/ml. The following calculation was used:

$$Element\ (ppm) = \frac{\mu g/ml}{Sample\ weight\ (g)} \times 100$$

3.2.15 Determination of Sodium and Potassium Content in Composite Flour

Determination of minerals (K and Na) was carried out by Atomic Absorption Spectrophotometry (Model AA-6300, Serial No A30524300916 SA, Shimadzu Corporation, Japan) (AACC International, 2010), Method 40-71.01. The sample (2 g) was accurately weighed into an ashing crucible. The control blank crucible was included. The sample was a carefully charred mass on a hot plate, or over a Bunsen burner. The sample was not allowed to ignite. The dish with the charred sample was placed in a cold muffle furnace and slowly raise temperature to 525°C and ashed overnight. The crucibles were removed from the furnace and cooled to room temperature. Hydrochloric acid (10 ml) was added to each crucible and covered with a watch glass. The solution was gently heated on a hot plate to dissolve. The watch glasses were rinsed into crucibles with water. Solutions were quantitatively transferred into separate 100-ml volumetric flasks. The crucibles were rinsed several times with water and rinsings were added to the flask, and cooled. It was diluted to volume and mixed and undissolved ash was allowed to settle. The dilutions were made to obtain solutions within the range of the instrument. For each sample and blank, a sufficient Concentration of sample (Cs) stock solution was added to achieve a final dilution of 0.1% (i.e., 5 ml Cs to 25-ml flask, 10 ml Cs to 50-ml flask). The instrument was set up. After each sample, the burner was rinsed with water to restore the zero-absorption level. The calibration curve was prepared from an average of each standard before and after the sample group. The concentration of samples was read from the plot of absorption against μg/ml. The following calculation was used:

$$mg/100g = \frac{(C_s - C_b)}{S \times 10} \times \mathrm{V} \times \mathrm{D}$$

where Cs = Concentration of sample (μg/ml), C_b = Concentration of blank (μg/ml), V = Original volume (ml), D = Dilution volume (ml)/aliquot for dilution (ml) if original solution is diluted, S = Sample weight (g).

3.2.16 Data Analysis

The data for proximate composition, minerals, and vitamin A content were statistically analysed using SAS version 9.4 TS Level 1M6 (SAS Institute Inc., 2016), and Minitab software (version ® 19.2020.1) was also used for the analysis of data from mixture design. The software generated experimental runs with randomization. The mean values were analysed statistically by analysis of variance (ANOVA). The coefficient of determination (R^2) value of the model was conducted to explain and predict the variability in the response data to fit the model. The regression models equations and graphical representations were observed. Each determination was performed in triplicate and the results were expressed as means ± standard deviation. Statistical differences between means ($p < 0.05$) were tested by Tukey's honest significant difference (HSD).

3.3 Results and Discussion

3.3.1 Proximate Composition of Raw Materials

The results indicated significant ($p<0.05$) differences in all tested parameters. The highest protein content was 31.53% from soybean flour followed by amaranth seeds flour at

19.80% then lastly OFSP had very low protein content (2.95%) but higher carbohydrate content (75.73%) and observed the highest energy-to-protein ratio (118.37 Kcal/g protein) (Table 3.2). Soybean flour was also high in total minerals (5.98%), crude fat (13.13%) and crude fibre (6.29%), energy value (389.19 kcal/100 g) but very low in carbohydrate content (33.09%) and energy-to-protein ratio (12.35 Kcal/g of Protein) compared to others.

The dry matter content found in amaranth seeds flour (89.80%) was in the agreement with 89.50% reported by Miranda-Ramos *et al*. (2019) for *A. hypochondriacus*. The high protein content of amaranth seeds in this study was 19.80% which falls within the trends (13.8 – 21.5% for *A. cruethus*, 13.1 - 21% for *A. caudatus*, 15 - 16.6% for *A. hypochondriacus*, and 16-16.5% for *A. hypondriacus* x *A. hybridus*) reported by Mlakar *et al*. (2009). The carbohydrate content was found to be 54.94% and the energy value to be 363.94 kcal/100g which is close to the value 60% and 391 kcal/100g respectively obtained by Narwade and Pinto (2018). The crude fat obtained in this experiment (6.23%) confirms well to the trends (5.6 - 10.9%) reported by Mlakar *et al*. (2009) and is also very close to the value 5.94% reported by Miranda-Ramos (2019) *A. hypochondriacus*. The fibre content was 4.47% which is within the range of 3.1 - 5.0% reported by Mlakar *et al*. (2009). Another study conducted by Antoniewska *et al*. (2018) reported 51.70% of carbohydrate content which is close to the value found in this study. The ash content of amaranth seeds flour was 4.36% which is similar to the finding range (2.5 - 4.4% for *A caudatus*) reported by Mlakar *et al*. (2009). The Energy-to-Protein Ratio was 18.39 kcal/g of protein which is slightly below the value of 24.44 kcal/g of protein from the study carried out by Narwade and Pinto (2018).

Apart from amaranth seeds, the fibre content of OFSP was 3.66% and current similar results (3.78%) were obtained by Omoba *et al*. (2021). The fat, ash, and carbohydrate content got in this study is slightly close to the current findings (3.37%, 3.37%, and 78.65% respectively) reported by Adetola *et al*. (2020). The energy value in OFSP was 349.53 which is below the value (359.71 kcal/100g) reported by Chikpah *et al*. (2020). This may be caused to different varieties of OFSP. The protein content was 2.93% and this finding is in line with the value (2.90%) reported by Pereira *et al*. (2019). The Energy-to-Protein Ratio was 118.37 kcal/g of protein. This value is high and it could be attributed to the very low protein content of OFSP observed in this study. On the other hand, soybean flour had the highest energy value (389.19 kcal/100g) than amaranth seeds and OFSP flour. The ash (5.98%) and fibre content (6.29%) of soybean flour obtained is very close to the findings (5.76% and 7.26%, respectively) reported by Adetola *et al*. (2020).

Table 3. 2 Nutritional composition of OFSP, soybean and Amaranth Seeds flour

Sample	Dry Matter (%)	Crude Protein (%)	Crude Ash (%)	Crude Fat (%)	Crude Fibre (%)	Carbohydrate (%)	Energy Value (Kcal/100 g)	EPR (Kcal/g of Protein)
OFSP	90.00 ± 0.00^{b}	2.95 ± 0.04^{a}	4.60 ± 0.19^{a}	3.05 ± 0.28^{a}	3.66 ± 0.13^{a}	75.73 ± 0.53^{c}	349.53 ± 0.21^{a}	118.37 ± 1.69^{c}
AS	89.80 ± 0.10^{a}	19.80 ± 0.26^{b}	4.36 ± 0.12^{a}	6.23 ± 0.21^{b}	4.47 ± 0.29^{a}	54.94 ± 0.51^{b}	363.94 ± 0.42^{b}	18.39 ± 0.26^{b}
soybean	90.00 ± 0.00^{b}	31.53 ± 0.46^{c}	5.98 ± 0.25^{b}	13.13 ± 0.24^{c}	6.29 ± 0.60^{b}	33.09 ± 0.50^{a}	389.19 ± 1.14^{c}	12.35 ± 0.18^{a}

Values are means ± Standard deviation replicated three times. The significant difference in means is indicated by various superscripts along columns at p 0.05. AS: Amaranth seeds, EPR: Energy-to-Protein Ratio.

The crude protein and carbohydrate findings in this study, confirm well with the value (34.5% and 35.2 % respectively) reported by Serna-Saldivar *et al*. (2019). The fat content obtained was slightly above the value (10.95%) by Ndife *et al*. (2014) but below the high value (25.53%) obtained Adetola *et al*. (2020). This high-fat content may be due to different varieties of soybean. The fibre content was similar to the value (6.74%) reported by Ndife *et al*. (2014). The Energy-to-Protein ratio was 12.35 kcal/g of protein which is slightly above the value of 10.24 kcal/g got in the previous study conducted by Adetola *et al*. (2020).

Grain and cereals are one of the major staple foods in different areas of the world. They are considered to be good sources of different many bioactive components, carbohydrates, and fibre but are very low in protein content (Devi, 2013, Ragaee *et al*., 2013). In addition, grain amaranth is exceptional because of its high protein content than most cereals, and also bioactive compounds (Iftikhar & Khan, 2019). The amaranth health advantages have also been established in homoeopathic medicine as well as ayurvedic medicines. Amaranth seeds and leaves are both utilized as herbal treatments and have nutraceutical qualities (Narwade & Pinto, 2018). The moisture contents of all flour produced in this experiment are below 14%. The moisture content of flour above 14% favours mould growth and infestation by insects during storage (Manley, 2000).

3.3.2 Nutritional Composition of Composite Flour from OFSP, Amaranth Seeds, and Soybean Flour

The results of this analysis are summarized in Table 3.3. All tested parameters in composite flour differ significantly ($p < 0.05$). By carefully analysing the proximate results, the highest protein content and fat content were observed in blend 50:25:25 for OFSP, amaranth seeds, and soybean flour respectively while the lowest value was observed at a ratio of 75:15:10 for OFSP, amaranth seeds, and soybean flour respectively but the highest carbohydrate content was obtained in this blend. This is because increasing amaranth seeds and soybean in the mixture increased the protein content and fat content. However, an increase of OFSP flour in the mixture resulted in a reduction of the protein content and an increase in the carbohydrate content and energy-to-protein ratio. The ash content was observed high in blend 54.5:24:21.5 and the lowest was seen in blend 75:15:10 while the highest fibre content was obtained in 60:15:25 for OFSP, amaranth seeds, and soybean flour respectively and the lowest was seen in blend 75:15:10. Generally, blend 75:15:10 was the lowest in crude protein, fat, ash, fibre content, and energy value but the highest in carbohydrate content and energy-to-protein ratio and vitamin A content among others.

Table 3. 3 Nutritional compositions of composite flour from OFSP, soybean, and Amaranth Seeds flour

CF	Dry Matter (%)	Protein (%)	Ash (%)	Crude Fat (%)	Fibre (%)	CHO (%)	EV (Kcal/100 g)	EPR (Kcal/g of Protein)	Vitamin A (RAE µg/100g)
C1	88.67±0.58^{a}	15.83±0.31^{e}	4.75±0.14^{bc}	6.16±0.19^{e}	4.94±0.81^{ab}	56.99±0.47^{a}	356.56±0.89^{ef}	22.53±0.49^{a}	780.17±0.38^{a}
C2	88.90±0.17^{abc}	14.50±0.50^{de}	4.83±0.06^{d}	5.07±0.12^{abc}	5.00±0.10^{ab}	59.50±0.43^{bc}	351.61±0.70^{ab}	24.27±0.89^{abc}	800.00±0.17^{c}
C3	89.66±0.30^{abc}	15.77±0.25^{e}	4.64±0.06^{abc}	5.77±0.25^{cde}	4.70±0.18^{ab}	58.78±0.48^{ab}	359.49±0.34^{g}	22.80±0.38^{ab}	785.03±0.15^{b}
C4	89.55±0.43^{abc}	11.50±0.50^{b}	4.61±0.03^{abc}	4.86±0.31^{ab}	4.71±0.17^{ab}	63.87±0.46^{e}	354.62±0.23^{d}	30.88±1.34^{e}	860.02±0.32^{i}
C5	89.82±0.31^{bc}	13.00±0.01^{c}	4.52±0.08^{a}	4.82±0.29^{ab}	4.30±0.26^{ab}	63.19±0.83^{e}	356.68±0.64^{ef}	27.45±0.06^{cde}	837.03±0.15^{h}
C6	88.87±0.06^{abc}	9.26±0.65^{a}	4.50±0.10^{a}	4.32±0.28^{a}	4.18±0.16^{a}	66.60±0.95^{f}	350.68±0.73^{a}	37.98±2.58^{f}	890.03±0.15^{j}
C7	88.90±0.17^{abc}	13.62±0.40^{cd}	4.79±0.11^{c}	5.17±0.29^{bcd}	4.94±0.05^{ab}	60.38±0.32^{bc}	352.37±0.33^{bc}	25.90±0.79^{abcd}	825.03±0.15^{g}
C8	89.67±0.58^{abc}	14.68±0.35^{de}	4.65±0.05^{abc}	5.38±0.42^{bcde}	4.79±0.01^{ab}	60.16±1.04^{bc}	357.36±0.64^{f}	24.35±0.63^{abc}	801.03±0.15^{d}
C9	89.26±0.22^{abc}	12.93±0.40^{c}	4.56±0.04^{ab}	4.99±0.01^{abc}	4.33±0.29^{ab}	62.44±0.45^{de}	355.07±0.50^{de}	27.48±0.83^{cde}	825.03±0.15^{g}
C10	88.72±0.65^{ab}	13.54±0.50^{cd}	4.70±0.10^{abc}	5.50±0.50^{bcde}	4.87±0.32^{ab}	60.12±0.72^{bc}	353.86±0.41^{cd}	26.16±0.97^{bcd}	820.00±0.50^{f}
C11	89.87±0.15^{c}	12.82±0.83^{bc}	4.80±0.02^{c}	5.97±0.07^{e}	5.18±0.15^{b}	61.11±0.94^{cd}	359.75±0.57^{g}	28.14±1.77^{de}	810.07±0.31^{e}

Values are means ± Standard deviation replicated three times. Statistical tests were conducted to see whether the means followed by different superscripts along columns were significantly different at $p < 0.05$. Where CHO is carbohydrate, and EV is energy value.

OFSP has been used to reduce Vitamin A deficiency due to its excellent beta-carotene content but is very low in protein and fat (Amagloh & Coad, 2014). Therefore, enhancing OFSP and amaranth seeds with soybean which is very high in protein resulted in high nutritional quality composite flour. The results indicated that the highest protein content and fat content observed in the composite could be due to high soybean and amaranth seeds flour inclusion in the mixture. Therefore, increasing OFSP flour in the blend reduced the protein and fat contents. However, it increases the carbohydrate (66.60%), energy-to-protein-ratio (37.98 kcal/100g of protein), and vitamin A content (890.03 RAE µg/100g). Soybean flour inclusion in the blends contributed to a high amount of total minerals, fat, fibre, and energy values observed in the composite flour. The tested parameters were significantly ($p<0.05$) different. Using these results, it was possible to investigate the impact of various blend proportions on protein, ash, vitamin A.

The highest moisture content in the composite flour was 11.33% which is below the maximum (15.5%) moisture content designated for wheat flour. High moisture levels in flour can cause caking, a condition epitomized by particle agglomeration into aggregates (Tortoe *et al*., 2017). In addition, microbial growth can take place and chemical deterioration of flour. This reduces the quality of the produced flour and loses its intended uses. On the other hand, the low moisture content is one of the major factors which contribute to achieving long shelf-life or storage stability of a particular food.

The protein content ranged from 9.26 – 15.83%, ash content ranged from 4.5 - 4.83%, crude fat content ranged from 4.32 - 6.16%, fibre content ranged from 4.18 - 5.18%, carbohydrate ranged from 56.99 - 66.60%, energy value ranged from 350.68 - 359.75 kcal/100g. Apart from the ash content, these results have led to high levels of agreement of trends in the most current study conducted by Feyera *et al*. (2021) for composite flour from finger millet, soybean, sweet potato, and ground nut where protein (4.37 - 17.16%), fat (0.02 - 15.58%), fibre (2.85 - 13.43%), carbohydrate (61.99 - 71.57%), and energy value (297.67 - 428.80 kcal/100 g) were reported. Energy is necessary to maintain the body's different processes, such as breathing, circulation, physical work, and protein synthesis (Institute of Medicine, 2006). The highest fibre content of the composite flour was 5.18%. High fibre consumption lowers the risk of digestive disorders and colon cancer. Another advantage is its capacity to regulate blood glucose levels (Raihan & Saini, 2017).

The highest protein content (15.83%) was observed in blend 50:25:25 for OFSP, amaranth seeds, and soybean flour respectively. Proteins are extremely complex biomolecules. They are the most varied group of physiologically significant compounds and are frequently recognized as significant compounds required for living. They are important in cellular metabolism, defence, communication, transport, storage, and recognition; all of these functions are necessary for the construction, function, and control of the cells of the body. Proteins collaborate with other biomolecules to carry out biological activities (Singh & Tripathi, 2020).

The highest ash content of the composite was 4.83% which is very close to the value (4.88%) reported by Adetola *et al*. (2020) from composite flour from OFSP, soybean, and carrot. This difference could be attributed to a high level (34.76%) of soybean flour inclusion in that mixture. High ash content indicates the high presence of minerals in the flour. The energy-to-protein ratio ranged from 22.80 - 37.98 kcal/g of protein which can be used as a daily ration nutrient. The vitamin A content obtained in this study confirms well to trends (215.0 - 811.6 RAE) reported by Mitra (2012), for different orange-fleshed sweet potato genotypes. These findings could also be applicable in cases of insufficient protein, mineral, energy intake, and vitamin A situations to provide the human body with these nutrients.

3.3.3 Optimization of Protein Content, Total Minerals, and Vitamin A Content of Composite Flour

These results were obtained using the methods described in this work and optimized using Minitab Software. Optimization has been used in various areas including food science and technology. It has also been used extensively and successfully in food processing engineering like process optimization and also in ingredient optimization. The optimum blend proportions of the composite flour and the predicted response values are illustrated in Figure 3.1. The values showed that the optimum value for model verification was 57, 24, and 19% for Orange-fleshed sweet potatoes, amaranth seeds, and soybeans flour respectively for all three response variables.

The results demonstrated the high adequacy of the regression model to predict the responses where the coefficient of determination (R^2) of the model was 0.9973, indicating that the model explained 99.73 of the variability in the response data fitted the model. The target was to optimize the responses to the target value of 14%, 4.7%, and 813.6 RAE µg/100g, crude protein, total minerals, and Vitamin A content, respectively. This value could be used as a daily recommended intake of 13 g of protein for children aged 1- 4 years and 800 RAE µg/100g as a daily recommended intake for pregnant women (Stathers *et al*., 2018). The coefficient of determination for each component was 0.9995, 0.9927, and 0.9997 for crude protein, ash (total minerals), and Vitamin A, respectively.

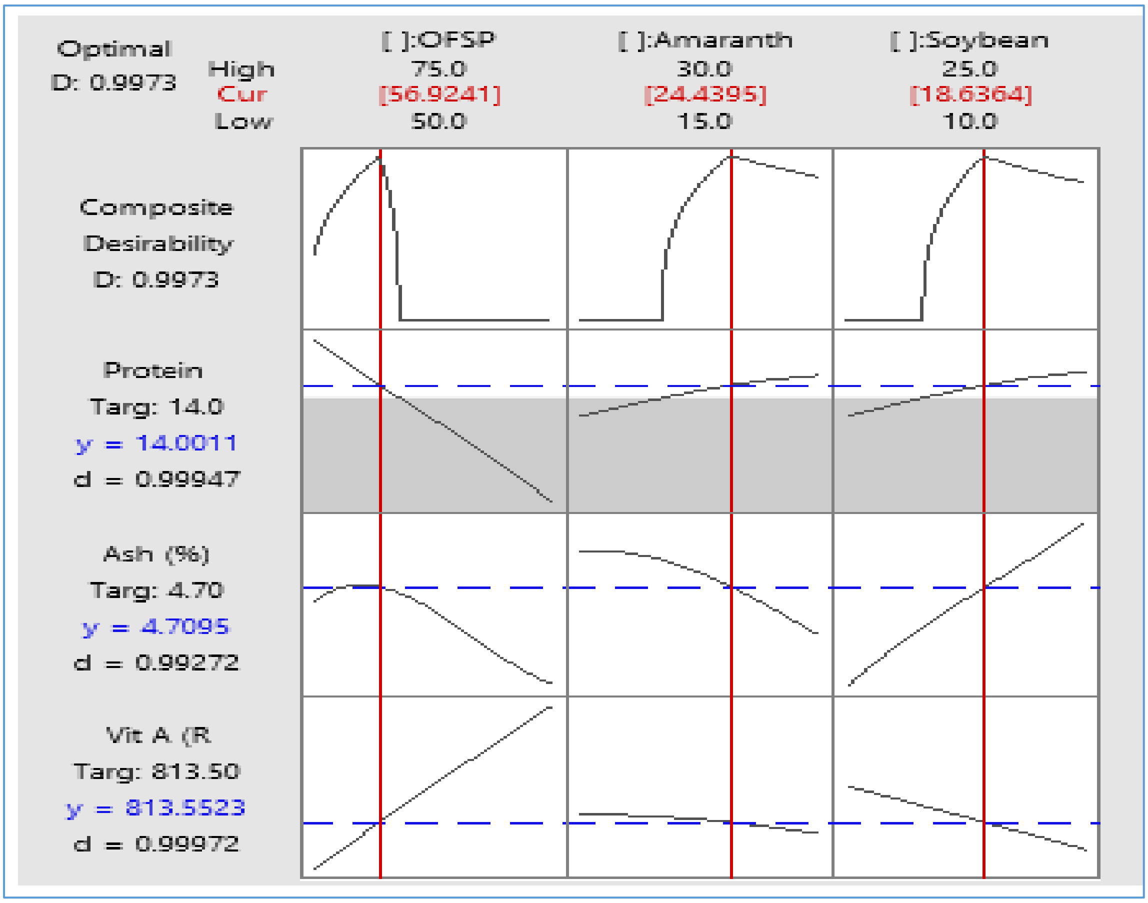
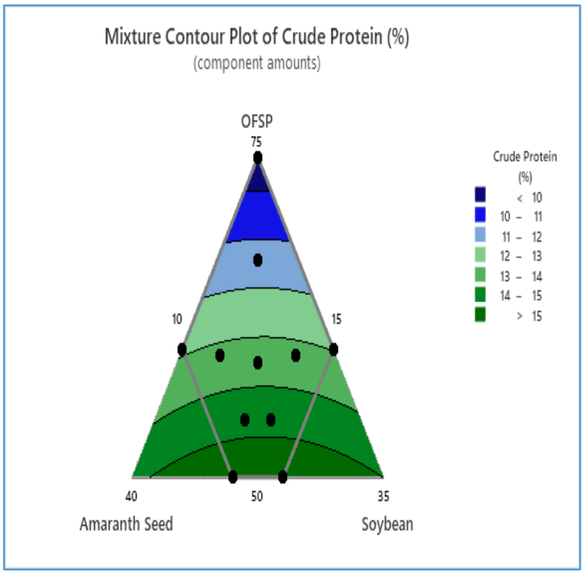
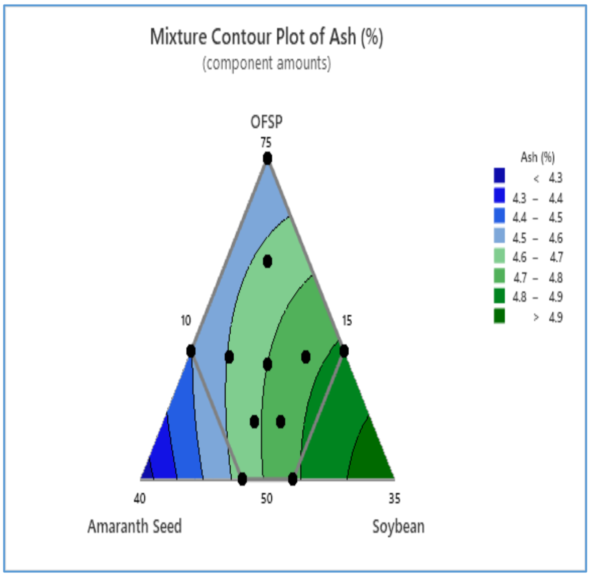


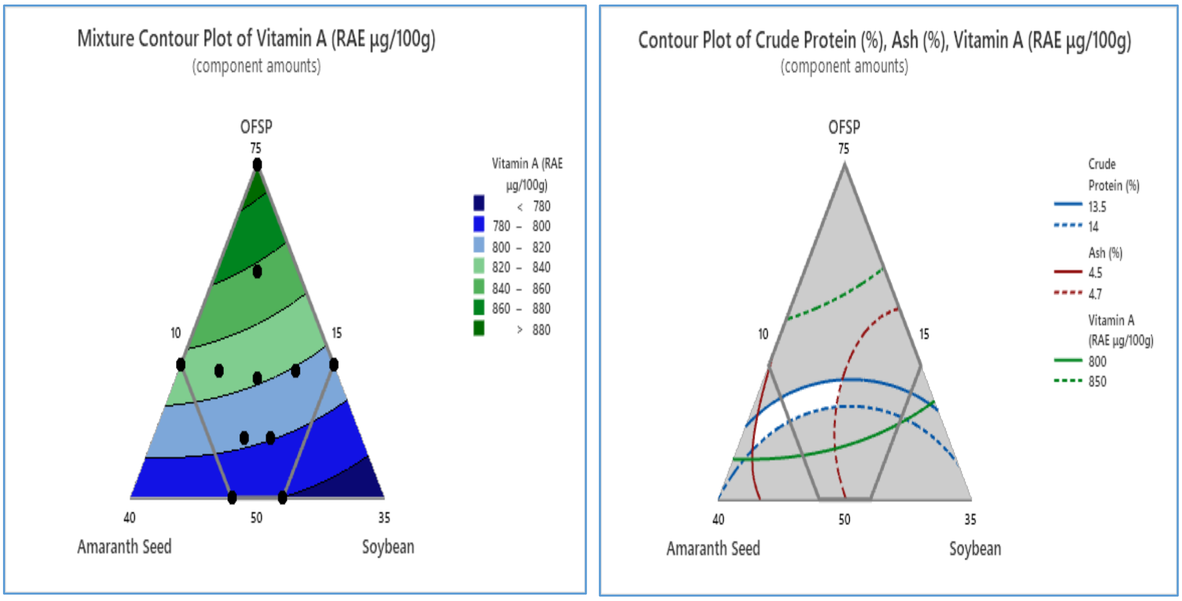
Figure 3. 1 Variable optimization results. Key: D: Overall coefficient of determination of experiment (composite desirability), y= Optimum response value for each response, and d= Coefficient of determination for each response.



a



b



c d

Figure 3. 2 Proportion of each component concerning protein content (a), Proportion of each component concerning ash content (b), Proportion of each component concerning vitamin A content (c) and Overlaid counter-plot for ingredients mixture composition for the optimum blend (d).

Based on available data in Figure 3.2 a, it can be concluded that the highest protein content lies between amaranth seeds and soybean flour inclusion in the mixture while its reduction observed in the OFSP flour region. Figure 3.2b shows the proportion of OFSP, amaranth seeds, and soybean flour concerning ash content where increasing soybean in the mixture resulted in high ash content. Figure 3.2 c shows the contribution of OFSP, amaranth seeds, and soybean flour concerning vitamin A content where increasing OFSP flour in the mixture resulted in high vitamin A content followed by amaranth seeds flour. Finally, Overlaid counter plot (d) shows the optimum feasible (white) region for all ingredients.

3.3.4 Mixture Analysis of Composite Flour

After conducting regression for mixture data, it was observed that all response parameters tested were highly and significantly predicted data for the production of composite flour from Orange-fleshed sweet potatoes, amaranth seeds, and soybeans flour blends except for dry matter and energy value. The highest coefficient of determination was observed in protein with R^2=99.21 while the least was in crude fat with R^2=83.42%. The desirability (R^2) and regression model of each tested parameter are shown in Table 3.4

Table 3. 4 Regression model equations for response parameters

Response	Model	R^2
Crude protein	$Y=2.32X_1+12.7X_2+12.4X_3+20X_1X_2+16.7X_1X_3+60.4X_2X_3$	99.21%
Crude ash	$Y=3.66X_1-1.41X_2+5.19X_3+10.35X_1X_2+2.35X_1X_3+6.74X_2X_3$	90.41%
Crude fibre	$Y=2.10X_1-3.0X_2-3.2X_3+13.0X_1X_2+22.7X_1X_3+15.9X_2X_3$	92.24%
Crude fat	$Y=4.65X_1+25.6X_2+24.9X_3-31.4X_1X_2-15.2X_1X_3-50.8X_2X_3$	83.42%
Carbohydrate	$Y=78.16X_1+146.2X_2+14.9X_3-151.5X_1X_2+53.7X_1X_3-160.2X_2X_3$	98.03%
EPR	$Y=71.15X_1+109.4X_2+75.2X_3-223.5X_1X_2-150.4X_1X_3-198.1X_2X_3$	99.17%
Vitamin A	$Y=979.7X_1+284X_2+551X_3+564X_1X_2-164X_1X_3+494X_2X_3$	99.07%

EPR= Energy-to-Protein ratio, Y=Crude protein, Crude ash, Crude fibre, Crude fat, Carbohydrate, Energy-to-Protein ratio, and Vitamin A content. X_1, X_2, and X_3 are OFSP, Amaranth seeds, and soybean, respectively.

3.3.5 Micro and Macro Minerals of Composite Flour

The mineral results shown in Figure 3.3 are from composite flour from OFSP, amaranth seeds, and soybean flour. The results showed significant differences ($p<0.05$) in the macro and micro minerals composition of composite flour. Minerals play a crucial role in the human body because of their biochemical function, assisting cells in absorbing and storing energy from substances in foods (Harris, 2014). Minerals are inorganic compounds that are found in all body tissues and fluids and are required for the regulation of certain physicochemical processes that are vital for life. Minerals are chemical elements that the body uses in several different ways (Soetan *et al*., 2010). Minerals are classed as macro if they occur in large quantities in the system and as micro or trace if their concentration is less than the critical mass threshold. Microminerals include Fe, Zn, Cu, Mn, Co, Ni, Mo, and I while macro minerals are Na, Ca, K, Mg, and Cl. Mineral deficiencies can be serious, life-threatening conditions that affect the body in the same way that vitamin or essential amino acid deficit does (Harris, 2014).

Calcium serves as a component of bones and teeth, as well as a regulator of nerve and muscle function (Soetan *et al*., 2010). Activates a range of processes, including fatty acid oxidation, mitochondrial ATP carrier (with magnesium), and glucose-stimulated insulin release (Huskisson *et al*., 2007).

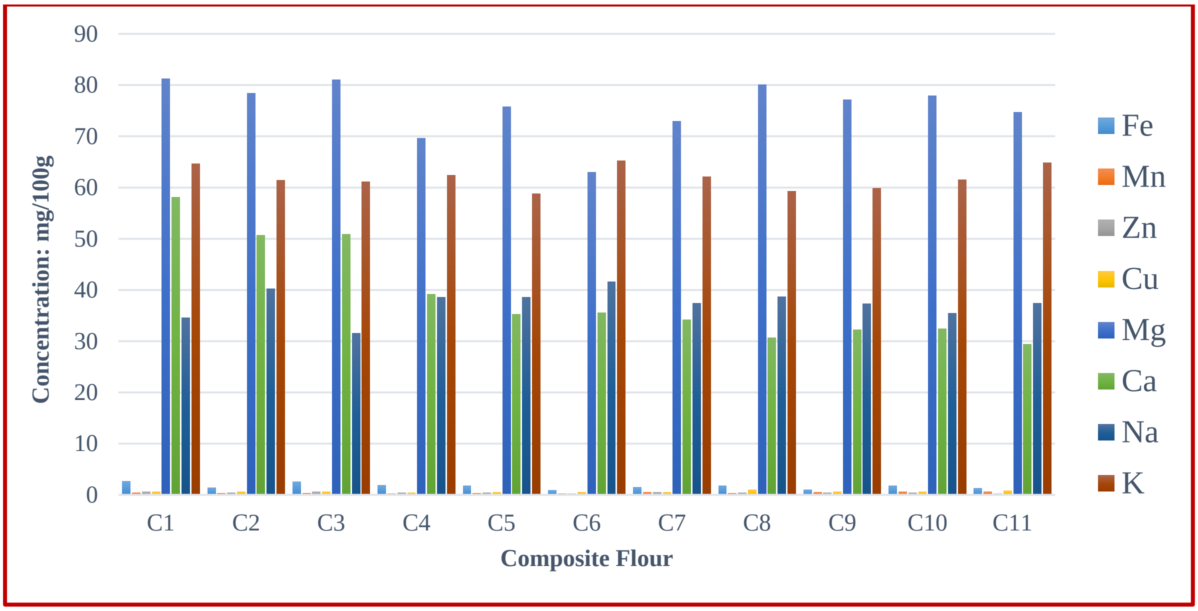


Figure 3. 3 Mean of micro and macro minerals of composite flour

where C1 (50:25:25), C2 (54.5:24:21.5), C3 (50:30:20), C4 (67:19:14), C5 (60:30:10), C6 (75:15:10), C7 (59.5:19:21.5) C8 (54.5: 26.5:19), C9 (59.5:26.5:14), C10 (59:23:18), C11 (60:15:25) for OFSP: Amaranth Seeds: Soybean, respectively.

The calcium content ranged from 29.41- 58.10 mg/100g, these values are above the results (0.28 – 1.06 mg/100g) reported by Ikegwu *et al*. (2021) for millet and soybean flour blends but below the value (110.51 - 114.05 mg/100g) reported by Adetola *et al*. (2020) for complementary foods from OFSP, soybean and carrot flour blends. The results were slightly below the value (70 mg/100g) reported by Oguntoyinbo *et al*. (2021) for wheat-banana peel flour but in agreement with the value (30 mg/100g) reported by Stathers *et al*. (2013) for sweet potato. The calcium content was high in C1 and the least was observed in C11.

Iron acts as haemoglobin in the transfer of oxygen (Soetan *et al*., 2010). Iron assists in the transport of electrons in the respiratory chain and so plays a vital role in ATP production. It is required for the production and function of red blood cells (Huskisson *et al*., 2007). The iron content of the composite flour ranged from 0.86 – 2.64 mg/100g. These findings are above the values (0.30 – 1.10 mg/100g) reported by Adeoye *et al*. (2020) for composite samples from rice flour, cassava flour, and soybean flour but below the trends (8.45 - 9.95 mg/100g) reported by Adetola *et al*. (2020) for complementary foods from OFSP, soybean and carrot flour composite. The highest value (2.64 mg/100g) of iron was observed in this study and confirms well to the value (2.55 mg/100g) of iron reported by Govender *et al*. (2019) for cooked OFSP and is close to the findings (1.94 mg/100g) found by Cayres *et al*. (2021) for the biscuit.

Oguntoyinbo *et al*. (2021) reported similar trends (1.36 - 4.76 mg/100g) for wheat-banana peel flour. The iron content was high in C1 and the least was observed in C6.

Manganese is a cofactor in different enzymes and is a constituent of mitochondrial superoxide dismutase and is essential in glycoprotein and proteoglycan production (Soetan *et al*., 2010). The manganese content of composite flour ranged from 0.17 - 0.59 mg/100g, these findings are in the agreement with the results (0.23 mg/100g) reported by Oguntoyinbo *et al*. (2021) for wheat-banana peel flour. The manganese content was high in C11.

Zinc acts as a cofactor and it is found in numerous enzymes (Soetan *et al*., 2010). The Zinc content ranged from 0.23-0.56 mg/100g, this value obtained confirms well with the value (0.45 mg/100g) reported by Govender *et al*. (2019) for cooked OFSP. In addition, the obtained results are in agreement with the value (0.40 mg/100g) reported by Oguntoyinbo *et al*. (2021) for wheat-banana peel flour. The zinc content was high in C1 and the least was observed in C11.

Copper is a component of many different enzymes (Soetan *et al*., 2010). The copper content ranged from 0.41 - 0.95 mg/100g. Similar results (0.31 mg/100g) were reported by Cayres *et al*. (2021) for pre-gelatinized composite flours. The copper content was high in C8 and the least was observed in C4. Magnesium is an active component of various enzyme systems that contain thymine pyrophosphate as a cofactor. Mg is also required for the activation of the phosphate-transferring enzymes (Soetan *et al*., 2010). The magnesium content ranged from 63.02 - 81.25 mg/100g, these findings are above the trends (184.67 - 221.33 ppm) reported by Adeoye *et al*. (2020) for composite samples from Rice Flour, Cassava Flour, and soybean flour and above the value (0.09 mg/100g) reported by Govender *et al*. (2019) for cooked OFSP. This could be due to the different raw materials and proportions used in this experiment but similar findings (60.08 mg/100g) were reported by Cayres *et al*. (2021) for pre-gelatinized composite flours. The magnesium content was high in C1 and the least was observed in C6.

Sodium is the most abundant cation in extracellular fluids. It helps to regulate plasma volume and acid-base balance, is actively engaged in the restoration of body fluid osmotic pressure, maintains normal irritability of muscles and cell permeability, stimulates nerve and muscle function, and is implicated in Na+/K+-ATPase, membrane potential general upkeep, nerve impulse transmission, and the absorptive processes of monosaccharides, amino acids, pyrimidines, and bile salts (Soetan *et al*., 2010). The sodium content ranged from 31.53 - 41.63 mg/100g, the results are high to the value (0.03 mg/100g) reported by Govender *et al*. (2019) for cooked OFSP. This may be caused by soybean and amaranth flour incorporation into OFSP flour but confirms well to the trends (30.33 – 33.10 mg/100g) reported by Sanoussi *et al*. (2016) for different OFSP. The sodium content was high in C6 and the least was observed in C3.

Potassium is the most abundant cation in intracellular fluid and is involved in acid-base balance, osmotic pressure regulation, nerve impulse conduction, muscle contraction, particularly cardiac muscle contraction, cell membrane function, and Na+/K+-ATPase. Potassium is also necessary for glycogenesis (Soetan *et al*., 2010). The potassium content was high in C6 and the least was observed in C5. The potassium content ranged from 58.76 - 65.18 mg/100g, these results are above the value (1.70 mg/100g) reported by Govender *et al*. (2019) for cooked OFSP but below the range (98.32 mg/100g) reported Cayres *et al*. (2021) for the biscuit. These could be attributed to the different raw materials used in this study. Furthermore, high temperature during ashing may reduce the minerals due to their volatility and interactions between minerals and crucibles.

3.4 Conclusion

This work reveals the optimum blending ratio of 57, 24, and 19% for Orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour respectively and nutrient content of 14%, 4.7%, and 813.6 RAE µg/100g for crude protein, total minerals and vitamin A content respectively. Furthermore, overall, this work offered a successful approach to follow for ingredients optimization. This can be easily used for the production of different food products which could meet the desired quality of end products. In addition, underutilized crops could be promoted by enhancing potential crops for the production of various nutritious foods. That could increase its applicability and value addition, convenient, functional uses, and achieve its marketability if standardized and commercialized.

CHAPTER FOUR

OPTIMIZATION OF EXTRUSION COOKING PARAMETERS ON FUNCTIONAL PROPERTIES OF READY-TO-EAT EXTRUDATES FROM ORANGE-FLESHED SWEET POTATOES

Abstract

The ability to understand the functional aspects/properties of food can help to improve the use of orange-fleshed sweet potatoes flour (OFSP) in various food products. This study was carried out to investigate the effect of extrusion cooking parameters (die temperature, screw speed, and feed moisture content) on physical (functional) properties of ready-to-eat (RTE) extrudates from orange-fleshed sweet potatoes flour using response surface methodology (RSM). Box-Behnken experiment under Design Expert software was used and 17 randomized experimental runs with 5 centre points were generated. To ensure the quality of ready-to-eat extrudates, lateral expansion, bulk density, water hydration capacity, water absorption index, oil absorption capacity, water solubility index, and swelling capacity were analysed. Co-rotating twin screw extruder was set at a constant barrel temperature of 65±5°C for three heating zones. Extrusion cooking variables were at three levels for die temperature (70, 80, and 90°C), screw speed (350, 375, and 400 rpm), and feed moisture content (30, 35, and 40%). Multiple Regression and Analysis of Variance (ANOVA) at a 5% significance level were carried out. The results showed that independent variables had a significant effect ($p<0.05$) on the functional properties of RTE foods. The lateral expansion of ready-to-eat extrudates ranged from 85 - 125%, bulk density (0.13 - 0.50 g/cm^3), water hydration capacity (4.2 - 6.17 g/g), oil absorption capacity (0.85 - 1.18 g/g), water absorption index (2.6 - 4.9 g/g), water solubility index (3.6-12%) and swelling capacity (2.4 - 6.2 ml/g). The numerical optimization generated the optimum extrusion process conditions of 90°C, 400 rpm, and 35% for die temperature, screw speed, and feed moisture content, respectively. The predicted values were 125%, 0.13 g/cm3, 6.22 g/g, 1.18 g/g, 2.99 g/g, 11.51%, and 5.05 ml/g for lateral expansion, bulk density, water hydration capacity, oil absorption capacity, water absorption index, water solubility index and swelling capacity, respectively with the overall desirability (coefficient of determination) of 0.930. The optimum conditions and the generated models can be used by food processors and academic communities to predict the quality of ready-to-eat extrudates from OFSP.

4.1 Introduction

Most traditional food processing methods are tedious and time-consuming. Extrusion is a flexible food processing technology that covers a range of operations such as mixing, shaping, kneading, forming, and cooking (Shelar & Gaikwad, 2019) into one system. In the extruder, raw materials are exposed to relatively high amounts of shear and high temperatures, resulting in mixing, compaction, phase transition, and molecular breakdown. When the extruded food products leave the extruder, high pressure is also generated at the end of the screw and the die (Pichmony & Ganjyal, 2020). Extrusion process variables also play a big role in the properties of extruded foods either chemical, physical, sensory, microbial, shelf-life of extrudates.

The functional properties of ingredients pertain to how they react during processing and cooking (Godswill *et al*., 2019). Functional characteristics are the essential physicochemical and/or organoleptic qualities of food components that provide consumers with health advantages (Kaushik *et al*., 2021). The texture, structure of nutrients, nutritional value, bioavailability of nutrients, and other characteristics of ready-to-eat food are conferred by its many ingredients. According to Chandra and Samsher (2013), variables such as water absorption capacity, hydration (water binding), oil absorption capacity, swelling capacity, solubility, emulsifying activity, emulsion stability, foam capacity, foam stability, bulk density, gelatinization, dextrinization, denaturation, coagulation, aeration, elasticity, viscosity, gelling, shortening are quality factors which must be considered while choosing an ingredient for functional food. Furthermore, components namely carbohydrates, proteins, amino acids, fats and oils, and fibre have an impact on the functional characteristics of foods and flours and must be examined to determine their type and nature, in addition to clarifying their structure and configuration (Kaushik *et al*., 2021). High temperature and short cooking times of new-generation extruders provide a variety of product characteristics, including improved starch and protein digestibility, anti-nutrient minimization, and functionality development (Pichmony & Ganjyal, 2020).

On the other hand, grains, roots, and tubers are regarded to be the most essential crops for human consumption (Castillo, 2011). Root and tuber crops, like yam, cassava, potato, and sweet potato, are the most significant food crops for direct human consumption in Africa (Sanginga, 2015). Roots and tubers provide 20% of the calories consumed in Africa. Crops like cassava, yam, and potatoes are crucial not just for food security, but also for revenue production for farmers and small enterprises, particularly for women (FAO, 2021). Roots and tubers may be cultivated in a wide range of environments and agricultural techniques. Producing diverse products from tropical roots and tubers will provide more alternatives for customers. Diverse growth potential in the processing of tropical roots and tubers may provide food processors with a broad market area within which to establish long-term development strategies (Sharma & Kaushal, 2016). People in Africa rely heavily on root and tuber foods such as yam, cassava, potato, and sweet potato (Sanginga, 2015). This is especially true in tropical locations, where the roots and tubers contribute considerably to sustainable development as well as revenue creation and food security. The characterization of tropical roots and tubers has attracted a lot of attention in recent years. For roots and tubers, it is also necessary to create and distribute the necessary processing methods and the characteristics of their products (Sharma & Kaushal, 2016).

There are plenty of sweet potatoes available, and they are also affordable to buy as raw materials. Furthermore, a vibrant development of sweet potato comprehensive processing and construction of related industrial chains have a strong economic significance in getting the benefit of this resource, broadening product applications, enhancing diet structure, and also raising the income of farmers (Mu *et al*., 2016). There are no regional or worldwide markets for sweet potato, thus it is less impacted by food price changes than most of the major cereals. During periods of high food costs, it continues to supply inexpensive meals (Sanginga, 2015).

The top two nations in the salt-flavoured snack business are still the United States and China, followed by India, Mexico, Japan, and the United Kingdom. According to a study by market research firm Mintel on salt-flavoured snacks, China and India are the two fastest-growing salt-flavoured snack markets (Liu, 2016). Extruded snacks get the highest growth potential in the snack food sector. The consumer demand for 'good for health' snack foods lead to the elusive search for something unique that attracts the consumer, and extrusion technology can meet these requirements to produce healthy snacks from health-benefiting ingredients (Dubey & Bhattacharya, 2015). Apart from that, in Africa especially the Middle East and North Africa (MENA), extruded snack foods are currently experiencing strong growth in the MENA market. According to the latest current data in the report entitled "The Middle East and North Africa Extruded Snack Food Market: Industry Trends, Share, Size, Growth, Opportunity and Forecast 2021-2026", the MENA extruded snack food market accounts for a value of US$ 2.26 billion in 2020 (IMARC Group, 2021).

There are a lot of elements that affect the final food products, including the equipment and the ingredients factors (Pichmony & Ganjyal, 2020). OFSP, a beta-carotene-rich orange sweet potato, was found to be a cost-effective method for supplying high levels of vitamin A bioavailability to vulnerable groups, such as young children and pregnant and lactating mothers (Van-Jaarsveld *et al*., 2005). Many diverse items have been made from cereal grains using extrusion technology (Pichmony & Ganjyal, 2020). Processing of many agricultural products such as grains, potatoes, juice, fruits, vegetables, meat, dairy), has resulted in a wide range of by-products that are increasingly accessible on the market (Kowalskia & Ganjyal, 2020).

On the other hand, Response Surface Methodology (RSM) is an important statistical method for experimental design, model construction, analysis of factors, and optimal search for conditions (Megha *et al*., 2018). Response surface methodology has the benefit of concurrently varying independent variables to have a useful model of overall variations in response (Awolusi *et al*., 2019). In this research, extruded ready-to-eat foods were processed from OFSP flour to have value-added products with functional characteristics convenient to consumers. This will also increase its marketability when standardized and commercialized. The main objective of this research was to optimize the extrusion cooking parameters (screw speed, die temperature, and feed moisture content) for the production of extruded ready-to-eat foods from orange-fleshed sweet potatoes flour.

4.2 Materials and Methods

4.2.1 Materials

Raw materials used in this study were bought and prepared as described in sections 3.2.1, 3.2.2, 3.2.3, and 3.2.4 of Chapter Three.

4.2.2 Extrusion Cooking Process

A co-rotating twin screw extruder (PSHJ-20, Jiangsu Xinda Science and Technology Co. Ltd, China) was used for processing extruded ready-to-eat foods from OFSP flour. The extruder had many variables to control such as the main motor, entry barrel temperature zone (pv1), Centre barrel temperature section (pv2) and end barrel temperature (pv3), and die temperature zone (pv4). The extruder was set at different conditions where screw speed (350 – 400), die temperature (70 – 90^{o}C), feed moisture content (30 – 40%), and other variables like entry barrel temperature, centre barrel temperature, and end barrel temperature were kept constant. The ratio of length and diameter of an extruder (L/D) was 28, the main motor power of 4 Kw, the total heating power of 4.5 Kw, and 500 r/min was the maximum screw speed. Extrudates were collected at the end of the barrel section (die), cut into 5 cm lengths and dried at 55^{o}C, cooled, and sealed in polyethylene plastic food freezer bags (26.8 cm x 27.3 cm) and stored at room temperature (24 ± 4^{o}C) prior for functional properties analysis.

4.2.3 Experimental Design Using Response Surface Methodology

Design Expert software version 13.0.1.0 (Stat Ease Inc, Minneapolis, MN, USA) was used for experiment design and analysis of variables. A three-factor at three levels [low, centre, high) (Table 4.1)] Box-Behnken experimental design (BBD) of response surface methodology was used to generate 17 runs with 5 centre points (Table 4.2) and the experiment was replicated three times. Centre points are employed in planned experiments to assess experimental error, allow for lack-of-fit and curvature testing, and to assure orthogonal blocking and consistent accuracy. The addition of centre point runs to experimental setup runs provides a measure of process stability and intrinsic variability, as well as a check for curvature. During the optimization process, the independent variables were screw speed (350 - 400), die temperature (70 - 90°C), and feed moisture content (30 - 40%) while the dependent or response variables were physical properties (lateral expansion, bulk density, water hydration capacity, water absorption capacity, fat absorption capacity, and water absorption index). The following second-order polynomial models were generated after regression analysis of the response.

$$\mathbf{Y_{ijk}} = \boldsymbol{\beta_o} + \sum_{\mathbf{i-1}}^{\mathbf{n}} \boldsymbol{\beta}_\mathbf{i} \mathrm{X}_\mathbf{i} + \sum_{\mathbf{i-1}}^{\boldsymbol{n}} \boldsymbol{\beta}_\mathbf{ii} \mathrm{X}_\mathrm{i}^2 + \sum_{\mathbf{i-1}}^{\boldsymbol{n}} \sum_{\mathbf{j-1}}^{\boldsymbol{n}} \boldsymbol{\beta}_\mathbf{ij} \mathrm{X}_\mathbf{i} \mathrm{X}_\mathbf{j} + \boldsymbol{\varepsilon}_\mathbf{ijk},$$

where Y_{ijk} is the dependent variable, X_i (i=1, 2, 3) are the independent variables (X_1 is the die temperature, X_2 is the screw speed and X_3 is the feed moisture content). The coefficient of the polynomial will be represented by β_0 (constant), β_i (linear effects), β_{ii} (quadratic effects), β_{ij}(interaction effects), and ε_{ijk} (effect associated with random error).

On the other hand, as for the dough moisture content, Golob *et al*. (2002) provided the following hydration equation to calculate it.

$$\mathrm{W_O} = \mathrm{S_W} \times \left(\frac{\mathrm{M}-\mathrm{M}_O}{100-\mathrm{M}}\right),$$

where W_O is the weight of water added (g), S_W is the weight of flour sample (g), M_O is the original moisture content of the flour (% wet basis), and m is the dough moisture content (% wet basis). Since the used extruder does not possess a pump stroke number and water flow rate, feed moisture content was manually calculated by considering the final dough moisture content.

Table 4. 1 Independent variables with their levels

Experimental Variables	Experimental Variable Levels		
	-1 (Low)	0 (Centre)	+1 (High)
Die Temperature (°C) [X_1]	70	80	90
Screw Speed (rpm) [X_2]	350	375	400
Feed Moisture Content (%) [X_3]	30	35	40

Table 4. 2 Box-Behnken Experimental Design of Response Surface Methodology

Run Order	Standard Order	Extrusion cooking Variables		
Sample Extrudates	**StdOrder**	**Die Temperature (°C)**	**Screw Speed (rpm)**	**Feed Moisture Content (%)**
1	1	70	350	35
2	13	80	375	35
3	17	80	375	35
4	6	90	375	30
5	2	90	350	35
6	12	80	400	40
7	11	80	350	40
8	5	70	375	30
9	15	80	375	35
10	7	70	375	40
11	4	90	400	35
12	9	80	350	30
13	14	80	375	35
14	16	80	375	35
15	8	90	375	40
16	3	70	400	35
17	10	80	400	30

4.2.4 Determination of Lateral Expansion (LE) in Extrudates from OFSP

Different lengths of sample food products were extruded using the extruder. The method of Yadav *et al*. (2021) was used with modifications. Extrudates were measured using a digital electronic Vernier calliper (Mitutoyo Digital Calliper, Japan) with a 0.01-mm accuracy. Fifteen measurements were taken for each experiment, and the average diameter was calculated. To compute the expansion ratio, the diameter of the extrudate was divided by the die diameter and the results were multiplied by 100 and the value was expressed as a percentage.

$$\text{Lateral Expansion (\%)} = \frac{D_E - D_D}{D_D} \times 100,$$

where D_E is the average extrudate diameter and D_D is the die diameter

4.2.5 Determination of Bulk Density (BD) in Extrudates from OFSP

The bulk density of extrudate was calculated using the method described by Molla and Zegeye (2020). Fifteen pieces of the extrudate were selected randomly and the average value of weight in gram (g), diameter in cm (D), and length in cm (L) was recorded. The bulk density of extrudate was calculated using the following equation:

$$\text{Bulk Density } (g/cm^3) = \frac{4M}{\pi D^2 L},$$

where M is the weight/mass of extrudate (g), D is the diameter of extrudate (cm), L is the length of extrudate (cm)

4.2.6 Determination of Water Hydration Capacity (WHC) in Extrudates from OFSP

The WHC of the extruded ready-to-eat food was estimated according to AACC (2010), Method 56-20. The sample (2 g) was weighed into a 100-ml centrifuge tube tared with a stopper and 40 ml of distilled water was added, then stoppered, and shaken vigorously to thoroughly suspend the sample. The suspension was allowed to stand for 10 min. During this time, mixing was inverted three times at end of 5-min and 10-min periods. The stopper was removed and centrifuged for 15 min at 1000 ×*g*. The centrifuge (DSC-200T, Digisystem Laboratory Instruments Inc., Taiwan R.O.C) was allowed to stop without braking. The supernatant was carefully decanted and the tube was inverted to drain. Finally, a re-stopper and weighing of the tube and contents were carried out. The following equation was used to calculate WHC.

$$\text{WHC } (g/g) = \frac{W_{TS} - W_T}{W_S},$$

where W_{TS} is the weight of the tube plus sediment, W_T is the weight of the tube and W_S is the weight of the sample on a dry basis

4.2.7 Determination of Water Absorption Index (WAI) and Water Solubility Index (WSI) in Extrudates from OFSP

WAI and WSI of the extruded products were determined according to Anderson *et al*. (1969) with modifications. A laboratory hammer mill was used to grind the extrudates to a fine powder with a particle size of around 200 µm. Weighed sample (1.25 g) was placed in a 40 ml centrifuge tube and suspended in 15 ml of distilled water before being centrifuged. The material was shaken at room temperature for 30 min, and then centrifuged at 3000 ×g for 5 min before being analysed. As soon as the supernatant was decanted into the pre-dried evaporation dish, it was carefully weighed. After the decantation of the supernatant, the sample mass (weight of the gel or absorbed water) was determined. The water absorption index (WAI) was determined as grams of absorbed water per gram of dry sample mass (1.25 g).

$$\mathrm{WAI\ (g/g)} = \frac{\mathrm{W_w - W_d}}{\mathrm{W_s}},$$

where W_w is the weight of wet sediment (g), W_d is the weight of dry residue (g) and W_s is the weight of the sample (g)

Weighing the clear supernatant of the WAI-analysed sample. The supernatant collected during the WAI analysis was evaporated for 5 h at 100°C. The residual mass of the dry solid was determined. The WSI was determined as a ratio of dry residue to the initial weight and expressed as a percentage.

$$\mathrm{WSI\ (\%)} = \frac{\mathrm{W_D}}{\mathrm{W_S}} \times 100,$$

where W_D is the weight of dry solids in the supernatant, and W_S is the weight of sample extrudates.

4.2.8 Determination of Swelling Capacity (SC) in Extrudates from OFSP

The swelling capacity (SC) was determined according to the method described by Ge *et al*. (2017) with modifications. Sample (1 g) was precisely measured before being progressively dissolved with 10 ml of distilled water. The suspension was kept at ambient temperature (24˚C ± 4˚C) for 24 h. The volume of the absorbent-treated sample was measured. Analysis was performed in triplicate and results were recorded as means. The results were presented as ml of water per gram of dry sample.

$$\mathrm{SC\ (ml/g)} = \frac{\mathrm{V_A} - \mathrm{V}_D}{\mathrm{W_D}}$$

where V_A is the volume of the absorbent treated sample (ml), V_D is the volume of the dry sample (ml) and W_D is the weight of the dry sample (g).

4.2.9 Determination of Oil Absorption Capacity (OAC) in Extrudates from OFSP

The oil absorption capacity (OAC) was determined following the method described by Song *et al*. (2018) with modification. A sample (1 g) was dissolved in 10 ml of refined soybean oil at ambient temperature overnight. After centrifugation at 3500 ×g for 15 min, the supernatant oil was removed from the tube, and sediments were weighed.

$$\mathrm{OAC}\ (\mathrm{g/g}) = \frac{W_2 - W_1}{W_0},$$

where W_0 = Initial weight of the sample (g), W_1= Weight of the sample with centrifuge tube (g) W_3= Weight of sediments with centrifuge tube (g)

4.2.10 Data Analysis

The results from this experiment were analysed for optimization of extrusion process parameters and functional properties of ready-to-eat extrudates. Data were analysed by multiple regression. In addition, the analysis of Variance (ANOVA) for responses, model significance and adequacy were carried out. The independent variables were screw speed, die temperature, and feed moisture content. The effect of independent variables, their interaction, and quadratic terms on response variables was assessed. Second-order polynomial regression models were generated for each dependent variable using Design-Expert Software version 13.0.5.0 (Stat Ease Inc, Minneapolis, MN, USA) while the normality test of response variables was carried out using Minitab Software version 20.3 (Minitab, LLC).

4.3 Results and Discussion

4.3.1 Model Fitness of Response Variables

The results from functional properties are shown in Table 4.3. On examining the data, it was found that there was sufficient evidence that all quadratic models sufficiently or significantly ($p<0.0001$) explained each tested variable. The regression coefficients for linear, interactions and quadratic effect of functional properties of ready-to-eat foods from OFSP are shown in Table 4.4. The Analysis of Variance (ANOVA) is shown in Table 4.4 and the regression model of lateral expansion, bulk density, water hydration capacity, oil absorption capacity, water absorption index, water solubility index, and swelling capacity was significantly ($p<0.0001$) explaining the response variables.

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Table 4. 3 Extrusion cooking conditions and functional properties of extruded ready-to-eat food from OFSP

Run Order	**Extrusion Cooking Variables**			**Response Variables**						
Sample Extrudates	**Die Temperature (°C)**	**Screw Speed (rpm)**	**Feed Moisture Content (%)**	**LE (%)**	**BD (g/cm^3)**	**WHC (g/g)**	**WAI (g/g)**	**WSI (%)**	**SC (ml/g)**	**OAC (g/g)**
1	70	350	35	87	0.27	4.6	4.3	3.7	3.2	1.05
2	80	375	35	97	0.25	4.95	2.6	10	3.43	0.91
3	80	375	35	98	0.24	4.6	2.8	9	3.22	0.9
4	90	375	30	125	0.18	5.8	2.9	12	3.8	1.09
5	90	350	35	108	0.35	5.5	4.8	10.3	3.4	1.12
6	80	400	40	91	0.42	4.4	3.1	10	5.3	0.91
7	80	350	40	90	0.5	4.78	4.8	9	5.6	1.01
8	70	375	30	86	0.39	4.5	3.48	3.6	3.1	1.1
9	80	375	35	100	0.29	4.97	2.8	8.7	3.2	0.92
10	70	375	40	85	0.46	4.2	4.9	3.9	4.2	0.91
11	90	400	35	124	0.13	6.17	3	11.5	5.03	1.18
12	80	350	30	92	0.25	4.3	2.6	9.8	3.07	0.97
13	80	375	35	101	0.25	4.96	2.7	9.2	3.7	0.85
14	80	375	35	104	0.24	4.95	3.2	9.7	3.5	0.9
15	90	375	40	105	0.48	5.4	4.47	9.1	6.2	1.16
16	70	400	35	90	0.41	4.6	4.29	4.4	2.4	1.02

17	80	400	30	112	0.26	5.6	2.65	10.7	4.6	1.1

Where, LE=Lateral expansion, BD=Bulk density, WHC=Water hydration capacity, WAI=Water absorption index, WSI=Water solubility index, SC=Swelling capacity, OAC=Oil absorption capacity

Table 4. 4 Regression coefficients and Analysis of Variance (ANOVA) for regression models of functional properties of ready-to-eat food from OFSP

Source	LE	BD	WHC	WAI	WSI	SC	OAC
Intercept	+100	+0.254	+4.89	+2.82	+9.32	+3.41	+0.90
Model p-value	< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****
Model Sum of Squares	2364.53	0.1895	4.91	12.14	122.11	17.47	0.1740
Model Mean Square	262.73	0.0211	0.55	1.35	13.57	1.94	0.0193
Model F-value	55.73	75.77	34.59	40.74	75.35	66.60	44.44
Linear							
X_1-Die Temperature	+14.25****	– 0.05****	+ 0.6212****	– 0.23**	+ 3.41****	+ 0.70****	+ 0.06***
X_2-Screw Speed	+5***	– 0.02*	+ 0.199**	– 0.433***	+ 0.48*	+ 0.26**	+ 0.01^{ns}
X_3-Feed MC	– 5.50***	+0.10****	– 0.18**	+ 0.71****	– 0.513*	+ 0.84****	–0.034***
Interaction							
$X_1 X_2$	+ 3.25*	– 0.10****	+ 0.17*	– 0.45**	+ 0.13^{ns}	+ 0.61***	+ 0.1320****
$X_1 X_3$	– 4.75**	+ 0.06***	– 0.03^{ns}	+ 0.04^{ns}	– 0.80**	+ 0.33**	+ 0.065**
$X_2 X_3$	- 4.75**	– 0.023*	– 0.42***	– 0.44**	+ 0.03^{ns}	– 0.458**	+ 0.06**
Quadratic							

X^2_1	$+ 3.13^{*}$	$+ 0.03^{*}$	$+ 0.27^{**}$	$+ 0.964^{****}$	$– 2.29^{****}$	$– 0.110^{ns}$	$+ 0.023^{ns}$
X^2_2	$– 0.88^{ns}$	$+ 0.01^{ns}$	$+ 0.0632^{ns}$	$+ 0.314^{**}$	$+ 0.44^{ns}$	$+ 0.21^{*}$	$+ 0.07^{****}$
X^2_3	$– 2.88^{*}$	$+ 0.10^{****}$	– 0.18*	$+ 0.154^{ns}$	$+ 0.12^{ns}$	$+ 1.03^{****}$	$– 0.06^{**}$
Lack of Fit	0.9352^{ns}	0.9085^{ns}	0.9536^{ns}	0.9232^{ns}	0.9024^{ns}	0.8641^{ns}	0.7901^{ns}
CV (%)	2.18	5.28	2.53	5.21	4.99	4.34	2.07

Where, LE=Lateral expansion, BD=Bulk density, WHC=Water hydration capacity, WAI=Water absorption index, WSI=Water solubility index, SC=Swelling capacity, OAC=Oil absorption capacity, CV= Coefficient of variation, *, **, ***, **** = Significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, $p \leq 0.0001$, respectively.

The non-significant ($p>0.05$) terms were neglected during the discussion and the significant ($p<0.05$) terms were used to explain their influences on response variables. The R^2 is a measurement of fit and is defined as the ratio of variation of the dependent variable to the total variance. Adjusted R^2 is a measurement of the amount of variation around the mean explained by the model after adjusting for the number of variables. The predicted R^2 is a measurement of how much variation in fresh data is explained by the model (Ibrahim *et al*., 2010).

The predicted R^2 in all tested response parameters was in reasonable agreement with the adjusted R^2 which means the difference was less than 0.2. The adjusted R^2 ranged from 0.9497 to 0.9768 while the predicted R^2 ranged from 0.9349 to 0.9672. The adequate precision of the model ranged from 16.38 to 28.45 which indicates an adequate signal (Mark & Patrick, 2017). Adequate precision is ideal to have a signal-to-noise ratio of at least 4, as well as a model that can be utilized to explore design space. On the other hand, no significant lack of fit was observed in all tested parameters which implies the fitness of the model. The coefficient of variation (CV) was low in all response parameters and ranged from 2.07 to 5.28 which implies that the model might be applied to the range of variables examined accurately (Nath & Chattopadhyay, 2007).

Good model fit should have adjusted $R^2 \geq 0.80$, significant $p<0.05$, low coefficient of variation (CV) value $\leq 10\%$, lack of fit values >0.1, adequate precision value > 4, low Predicted Residual Error Sum of Squares (PRESS), Schwarz's Bayesian Criterion (BIC), Akaike's Information Criterion (AIC) and Akaike information criterion corrected (AICc) (Table 4.5, Table 4.6, and Table 4.7). The models fit very well with the coefficient of determination of 0.9862, 0.9898, 0.9780, 0.9828, 0.9813, 0.9885, 0.9845 for lateral expansion, bulk density, water hydration capacity, oil absorption capacity, water absorption index, water solubility index and swelling capacity, respectively which imply high adequacy of the model (Table 4.8).

Table 4. 5 Analysis of Variance (ANOVA) for quadratic models, and comparison statistics of LE, BD and OAC

Response variables	**Source**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
	Model	2364.53	9	262.73	55.73	<0.0001****
	Residual	33	7	4.71		
	Lack of fit	3.00	3	1.00	0.1333	0.9352^{ns}
Lateral expansion (%)	Pure error	30.00	4	7.50		
	Total	2397.53	16			
	PRESS	94.88				
	BIC	87.85				
	AICc	116.19				
	Model	0.1895	9	0.0211	75.77	<0.0001****
	Residual	0.0019	7	0.0003		
	Lack of fit	0.0002	3	0.0001	0.1744	0.9085^{ns}
	Pure error	0.0017	4	0.0004		
Bulk density (g/cm^3)	Total	0.1914	16			
	PRESS	0.0063				
	BIC	-77.71				
	AICc	-49.38				
	Model	0.1740	9	0.0193	44.44	<0.0001****
Oil absorption capacity (g/g)	Residual	0.0030	7	0.0004		
	Lack of fit	0.0001	3	0.0000	0.0571	0.9797^{ns}

	Pure error	0.0029	4	0.0007		
	Total	0.1770	16			
	PRESS	0.0066				
	BIC	-70.09				
	AICc	-41.76				

Where, PRESS=Predicted Residual Error Sum of Squares, BIC=Schwarz's Bayesian Criterion, AIC=Akaike's Information Criterion, AICc=Akaike information criterion corrected, LE=Lateral expansion, BD=Bulk density, OAC=Oil absorption capacity, **** = Significant at $p \leq 0.0001$, ns= not significant, and df= Degree of freedom

Table 4. 6 Analysis of Variance (ANOVA) for quadratic models, and comparison statistics of WHC and WAI

Response variables	**Source**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Water hydration capacity (g/g)	Model	4.91	9	0.546	34.59	<0.0001****
	Residual	0.1105	7	0.0158		
	Lack of fit	0.0080	3	0.0027	0.1037	0.9536^{ns}
	Pure error	0.1025	4	0.0256		
	Total	5.02	16			
	PRESS	0.2878				
	BIC	-9.04				
	AICc	19.30				
Water absorption index (g/g)	Model	12.14	9	1.35	40.74	<0.0001****
	Residual	0.2317	7	0.0331		

Response variables	Source	Sum of Squares	df	Mean Square	F-value	p-value
	Lack of fit	0.0237	3	0.0079	0.1519	0.9232^{ns}
	Pure error	0.2080	4	0.0520		
	Total	12.37	16			
	PRESS	0.7042				
	BIC	3.55				
	AICc	31.89				

Where, PRESS=Predicted Residual Error Sum of Squares, BIC=Schwarz's Bayesian Criterion, AIC=Akaike's Information Criterion, AICc=Akaike information criterion corrected, WHC=Water hydration capacity, WAI=Water absorption index, **** = Significant at $p \leq 0.0001$, ns= not significant, and df= Degree of freedom

Table 4. 7 Analysis of Variance (ANOVA) for quadratic models, and comparison statistics of WSI and SC

Response variables	**Source**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Water solubility index (%)	Model	122.11	9	13.57	75.35	$<0.0001^{****}$
	Residual	1.26	7	0.1801		
	Lack of fit	0.1525	3	0.0508	0.1835	0.9024^{ns}
	Pure error	1.11	4	0.2770		
	Total	123.37	16			
	PRESS	4.17				
	BIC	32.35				
	AICc	60.68				$<0.0001^{****}$
Swelling capacity (ml/g)	Model	17.47	9	1.94	66.60	

	Residual	0.2040	7	0.0291		
	Lack of fit	0.0312	3	0.0104	0.2409	0.8641^{ns}
	Pure error	0.1728	4	0.0432		
	Total	17.67	16			
	PRESS	0.7696				
	BIC	1.39				
	AICc	31.89				

Where, PRESS=Predicted Residual Error Sum of Squares, BIC=Schwarz's Bayesian Criterion, AIC=Akaike's Information Criterion, AICc=Akaike information criterion corrected, WSI=Water solubility index, SC=Swelling capacity, **** = Significant at $p \leq 0.0001$, ns= not significant, and df= Degree of freedom

Table 4. 8 Regression equation models of functional properties of ready-to-eat food from OFSP

Regression equations for responses prediction	**R^2**	**Adjusted R^2**	**Predicted R^2**	**Adequate Precision**
LE $= 100 + 14.25\ X_1 + 5\ X_2 - 5.50\ X_3 + 3.13\ X^2{}_1 - 0.88\ X^2{}_2 - 2.88\ X^2{}_3 + 3.25\ X_1X_2 - 4.75\ X_1X_3 - 4.75\ X_2X_3$	0.9862	0.9685	0.9604	23.72
BD $= 0.254 - 0.05\ X_1 - 0.019\ X_2 + 0.098\ X_3 + 0.028\ X^2{}_1 + 0.01\ X^2{}_2 + 0.10\ X^2{}_3 - 0.09\ X_1X_2 + 0.06\ X_1X_3 - 0.023\ X_2X_3$	0.9898	0.9768	0.9672	28.45
OAC $= 0.90 + 0.06\ X_1 + 0.01\ X_2 - 0.034\ X_3 + 0.132\ X^2{}_1 + 0.065\ X^2{}_2 + 0.05\ X^2{}_3 + 0.023\ X_1X_2 + 0.07\ X_1X_3 - 0.06\ X_2X_3$	0.9828	0.9607	0.9629	17.83

WHC = $4.89 + 0.6212 X_1 + 0.1987 X_2 – 0.18 X_3 + 0.27 X^2{}_1 + 0.0632 X^2{}_2 – 0.18 X^2{}_3 + 0.17 X_1X_2 – 0.03 X_1X_3 – 0.42 X_2X_3$	0.9780	0.9497	0.9427	20.79
WAI = $2.82 – 0.23 X_1 – 0.433 X_2 + 0.71 X_3 + 0.964 X^2{}_1 + 0.314 X^2{}_2 + 0.154 X^2{}_3 – 0.45 X_1X_2 + 0.04 X_1X_3 – 0.44 X_2X_3$	0.9813	0.9572	0.9431	16.38
WSI = $9.32 + 3.41 X_1 + 0.48 X_2 – 0.513 X_3 – 2.29 X^2{}_1 + 0.44 X^2{}_2 + 0.12 X^2{}_3 + 0.13 X_1X_2 – 0.80 X_1X_3 + 0.03 X_2X_3$	0.9885	0.9736	0.9565	28.38
SC = $3.41 + 0.70 X_1 + 0.26 X_2 + 0.8413 X_3 – 0.110 X^2{}_1 + 0.21 X^2{}_2 + 1.03 X^2{}_3 + 0.61 X_1X_2 + 0.33 X_1X_3 – 0.458 X_2X_3$	0.9845	0.9645	0.9349	23.66

Where, X_1=Die Temperature, X_2=Screw Speed, X_3=Feed MC, LE= Lateral expansion, BD= Bulk density, WHC: Water hydration capacity, WAI= Water absorption index, WSI= Water solubility index, SC= Swelling capacity, OAC= Oil absorption capacity, R^2: Coefficient of determination

4.3.2 Effect of Extrusion Cooking Variables on the Lateral Expansion of Extruded Ready-To-Eat Food

The lateral expansion model F-value of 55.73 implies the regression model is significant. The model was strongly significant ($p<0.0001$) to explain the variable (Table 4.4). The results demonstrated the high adequacy of the regression model to predict the response where the coefficient of determination (R^2) of the model was 0.9862, indicating that the model explained 98.62% of the variability in the response data fitted the model.

The Predicted R^2 of 0.9604 is in reasonable agreement with the Adjusted R^2 of 0.9685; i.e. the difference is less than 0.2. The adequate precision of the model was 23.72 which indicates an adequate signal. Adequate precision is ideal to have a signal-to-noise ratio of at least 4, as well as a model that can be utilized to explore design space (Table 4.8). The lack of Fit F-value of 0.13, on the other hand, indicates that the Lack of Fit is not significant to the pure error. There is a 93.52% probability that a significant Lack of Fit F-value is attributable to noise (Mark & Patrick, 2017). A lack of fit is acceptable if it is s not statistically significant because the model needs to fit (Table 4.4). The following polynomial (equation 10) with significant and non-significant terms shows the effect of independent variables on the lateral expansion of extrudates:

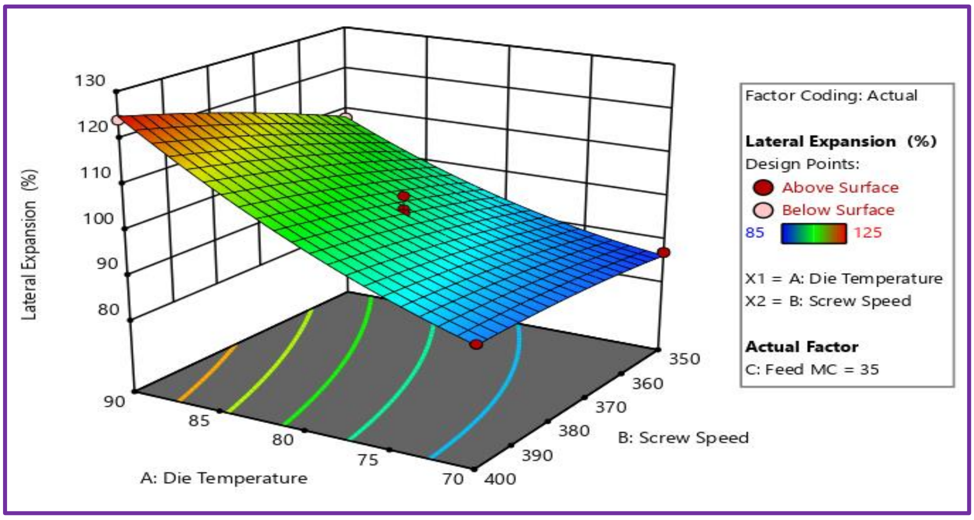
LE= $100 + 14.25 X_1 + 5 X_2 – 5.50 X_3 + 3.13 X^2{}_1 – 0.88 X^2{}_2 – 2.88 X^2{}_3 + 3.25 X_1X_2 – 4.75 X_1X_3 – 4.75 X_2X_3$ ………………......…...Equation 1

Where, X_1=Die Temperature, X_2=Screw Speed, X_3=Feed MC

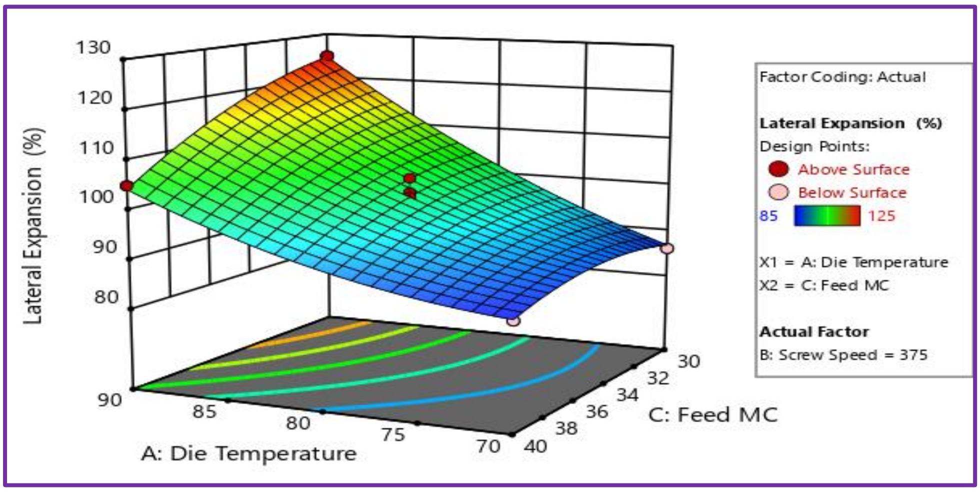
Expansion is the ratio of the diameter of the extrudate to the diameter of the die hole (Yadav *et al*., 2021). A high expansion which is a desirable quality of extruded ready-to-eat foods depends on feed moisture content, extrusion cooking temperature, feed rate, screw speed, and diameter of the die. Expansion may be a result of dough viscosity and elasticity, which are influenced by the starch, protein, and fibre ratios (Nagaraju *et al*., 2021). Extruded product expansion is proportional to the size, number, and distribution of air cells encircled by cooked material. The pressure difference between the die and the atmosphere determines the expansion of food material. When the product exits the extruder, the high pressure connected to the discharge of the die is lowered, resulting in flash loss of moisture and product expansion (Leonel *et al*., 2013).

The lateral expansion of extrudates ranged from 85 - 125%. These results are above to the value (6.19 - 6.97%) reported by Awolu and Akintade (2021) for ready-to-eat extruded snacks but in agreement with the trends (25 - 124%) reported by Cappa *et al*. (2020) for bean powders, as well as (94.50 - 112%) reported by Wani and Kumar (2016) for extrudate based on fenugreek oat and pea, and confirm well with the value (122 - 144%) reported by Yadav *et al*. (2021) for expanded extrudates. The highest value (125%) was observed at a maximum die temperature of 90^{o}C, screw speed of 375 rpm, and low feed moisture content. A similar observation was reported by Naseer *et al*. (2021) where maximum expansion was achieved at the highest barrel temperature (120^{o}C) and screw speed (450 rpm) when the moisture content of the feed material was in the intermediate range (20.50%) for functional snack food from almond press cake and pearl millet flour. Furthermore, Cappa *et al*. (2020) reported high expansion at low moisture content with an increase in die temperature and feed rate for bean powders. The quadratic equation of lateral expansion indicates that an increase in linear terms of die temperature and screw speed, interaction terms of die temperature and screw speed as well as the quadratic terms of die temperature significantly ($p<0.05$) increased the lateral expansion (Figure 4.1).

These findings are consistent with the present work of Awolu and Akintade (2021), who found that temperature and screw speed had a favourable effect on the lateral expansion of extrudates. High screw speed may also create heat, indicating that heat is a basic element that influences lateral expansion. Similarly, Wani and Kumar (2016) reported that the increased screw speed and barrel temperature led to a significant increase in lateral expansion. In addition, a similar observation was made in the current work by Yadav *et al*. (2021) that the temperature rise may promote more complete cooking of the proteins, increasing plasticity. With an increase in temperature, this enhanced plasticity may cause the extrudate to expand. In general, barrel temperature and screw speed have a favourable influence on the expansion ratio (ER) of rice-based extrudates but feed moisture content has a negative effect (Dalbhagat *et al*., 2019). In this study, the decrease in lateral expansion may result from increased linear terms of feed moisture content, interaction terms of die temperature and feed moisture content, screw speed and feed moisture content as well as quadratic terms of feed moisture content. A similar observation was made in the current work by Yadav *et al*. (2021) that high-feed moisture may minimize mechanical friction and lower the degree of boiling of starch, therefore influencing expansion. Furthermore, Wani and Kumar (2016) reported that the increase in feed moisture content led to a significant decrease in lateral expansion.



(a)



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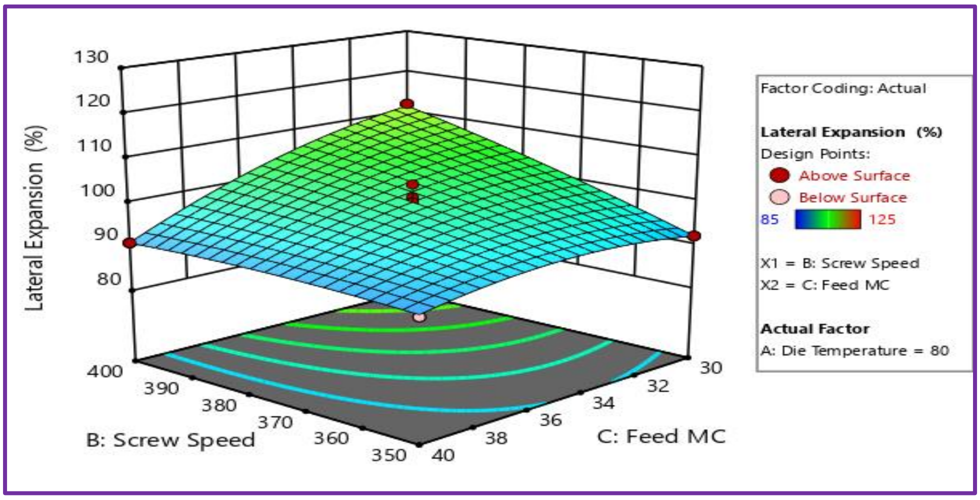


(c)

Figure 4. 1 The 3-D graphs representing the effect of die temperature and screw speed (a), die temperature and feed moisture content (b), and screw speed and feed moisture content (c) on lateral expansion

According to Rathod and Hathan (2015), increasing the feed moisture level changes the macromolecular structure of the extruded melt, lowering melt elasticity and promoting the production of more compact extrudates.

4.3.3 Effect of Extrusion Cooking Variables on Bulk Density of Extruded Ready-To-Eat Food

The bulk density of extrudates ranged from 0.13 to 0.50 g/cm^3. The findings confirm well to the results (0.23 - 0.38 g/cm3) reported by Fakolujo and Adelugba (2021) for extruded snacks from blends of *Acha* grain, Jackbean, and Pawpaw flours as well as the values (0.25 - 0.28 g/cm^3) reported by Yadav *et al*. (2021) for expanded extrudates. Furthermore, the results are above the value (0.107 - 0.152 g/cm^3) reported by Naseer *et al*. (2021) for functional snack food from almond press cake and pearl millet flour but below the value (0.41 - 0.59 g/cm^3) reported by Sobowale *et al*. (2021) for extruded snacks from whole pearl millet-based flour. The variations in bulk density of foods might be attributed to variations in starch concentration. The higher the starch concentration, the greater the likelihood of a rise in bulk density. Furthermore, bulk density is affected by elements such as geometry, measuring technique, particle size, surface characteristics, and solid density of the materials, and it may be enhanced when the particles are smaller, correctly tapped/vibrated, compatible, and packaged effectively (Iwe *et al*., 2016).

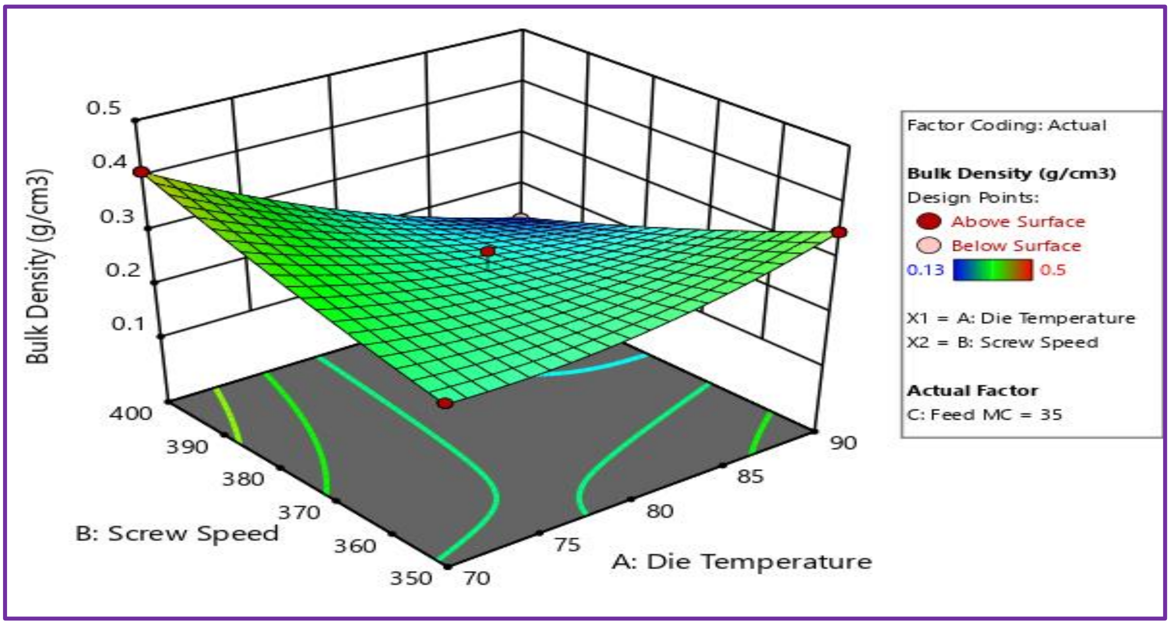
Bulk density, also known as volumetric density or apparent density, is defined as the mass of numerous particles of a material divided by the entire volume they fill. It is a functional characteristic of flours, powders, tiny particles, granules, and other split solids of foods (or food components) (Godswill *et al*., 2019). Bulk density was also thought to be desirable for easier dispersibility and for reducing paste thickness. As a result, flours with high bulk density may be employed as a thickening in various food product compositions since they assisted to lower paste thickness, which is an essential element in convalescent and child feeding. Low bulk density, on the other hand, was found to be essential in complementary food formulations (Kaushik *et al*., 2021).

On the other hand, the bulk density model F-value of 75.77 implies the model is significant. The model significantly ($p<0.0001$) predicts the variable (Table 4.4). The results demonstrated the high adequacy of the regression model to predict the response where the coefficient of determination (R^2) of the model was 0.9898, indicating that the model explained 98.98% of the variability in the response data fitted the model. The Predicted R^2 of 0.9672 is in reasonable agreement with the Adjusted R^2 of 0.9768; i.e. the difference is less than 0.2. The adequate precision of the model was 28.45 which indicates an adequate signal. For the model to be useful, adequate precision must have a signal-to-noise ratio larger than 4 (Table 4.8). On the other hand, the Lack of Fit F-value of 0.17 implies the Lack of Fit is not significant compared to the pure error. The lack of Fit F-values of this magnitude is caused by noise with a 90.85% probability (Mark & Patrick, 2017). Non-significant lack of fit is good because the model needs to fit (Table 4.4). The following polynomial (equation 2) with significant and non-significant terms shows the effect of independent variables on the bulk density of extrudates:

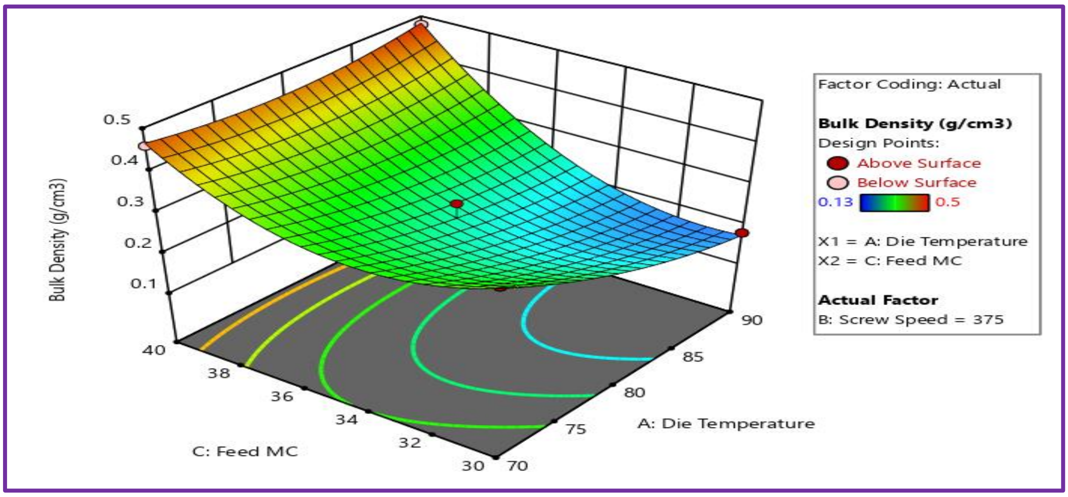
BD $= 0.254 - 0.05\ X_1 - 0.019\ X_2 + 0.098\ X_3 + 0.028\ X^2_1 + 0.01\ X^2_2 + 0.10\ X^2_3 - 0.09\ X_1X_2 + 0.06\ X_1X_3 - 0.023\ X_2X_3$ ....Equation 2

Where, X_1=Die Temperature, X_2=Screw Speed, X_3=Feed MC

The quadratic equation of bulk density indicates that all linear terms, interaction terms of all independent variables, and quadratic terms of die temperature and feed moisture content significantly ($p<0.05$) affected the bulk density of extrudates. In this study, low bulk density may be observed when increasing the linear terms of die temperature and screw speed, interaction terms of screw speed and die temperature, and die temperature and screw speed (Figure 4.2). The current study by Naseer *et al*. (2021) reported that linear terms of screw speed, temperature, and interaction terms of screw speed and barrel temperature reduced significantly ($p<0.05$) the bulk density of snack food from almond press cake and pearl millet flour. Higher barrel temperatures cause superheating of the water molecules, which explains a reduction in bulk density as barrel temperature rises (Ding *et al*., 2006).



(a)



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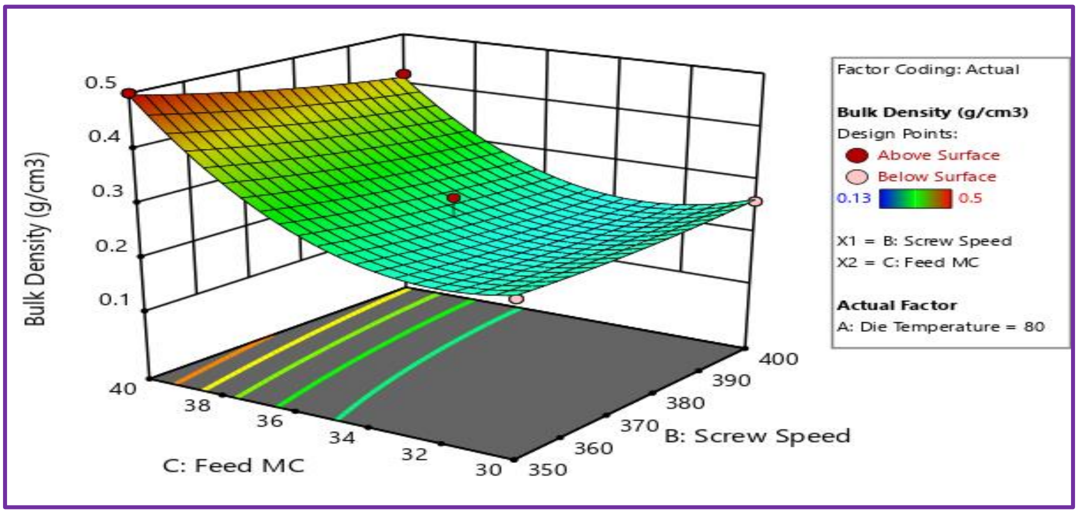


(c)

Figure 4. 2 The 3-D graphs representing the effect of die temperature and screw speed (a), die temperature and feed moisture content (b), and screw speed and feed moisture content (c) on bulk density

Increased screw speed results in an increased shearing force, which decreases the binding strength and chain length of starch and protein molecules, resulting in lower extrudate density due to easier expansion. Higher screw speed causes more structural degradation during extrusion heating, which explains the negative connection between screw speed and bulk density (Filli *et al*., 2012). The density of an extruded food product is impacted by various factors such as moisture content, temperature, and screw speed, which are inversely proportional to the expansion ratio. The decreased bulk density of extruded food products is caused by an increase in screw rotational speed and barrel temperature (Shelar & Gaikwad, 2019).

On the other hand, the linear terms of feed moisture content, interaction terms of die temperature and feed moisture content, and quadratic terms of die temperature and moisture content significantly ($p<0.05$) affected bulk density positively. According to Shruthi *et al*. (2017), higher feed moisture content throughout extrusion may lower the elasticity of the dough by plasticization of the melt, thus increasing the density of the extrudate. The substantial reliance of bulk density on feed moisture could indicate its impact on the elasticity of the starch-based product.

4.3.4 Effect of Extrusion Cooking Variables on Water Hydration Capacity of Extruded Ready-To-Eat Food

The water hydration capacity model F-value of 34.59 implies the model is significant. The model significantly ($p<0.0001$) predicts the variable (Table 4.4). The results demonstrated the high adequacy of the regression model to predict the response where the coefficient of determination (R^2) of the model was 0.9780, indicating that the model explained 97.80% of the variability in the response data fitted the model. The Predicted R^2 of 0.9427 is in reasonable agreement with the Adjusted R^2 of 0.9497; i.e. the difference is less than 0.2. The adequate precision of the model was 20.79 which indicates an adequate signal. For the model to be useful, adequate precision must have a signal-to-noise ratio larger than 4 (Table 4.8). On the other hand, the Lack of Fit F-value of 0.10 implies the Lack of Fit is not significant compared to the pure error. The lack of Fit F-values of this magnitude is caused by noise with a 95.36% probability (Mark & Patrick, 2017). Non-significant lack of fit is good because the model needs to fit (Table 4.4). Figure 4.3 shows the 3-D surface plots describing sufficient trends of WHC of extrudates.

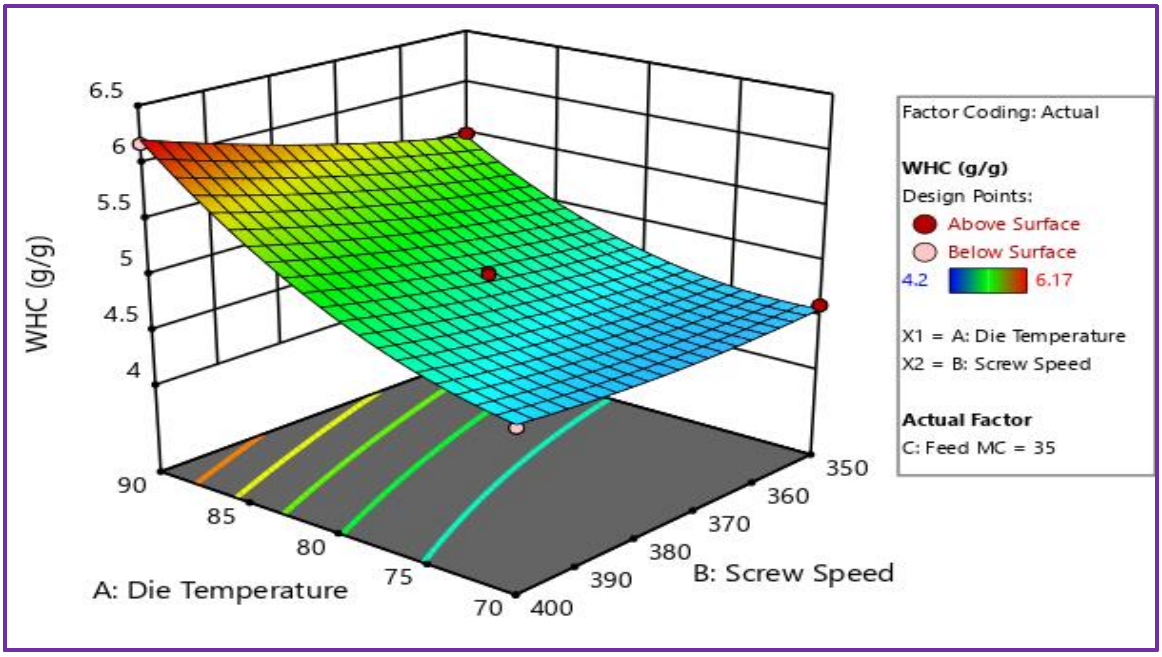
Water absorption capacity (WAC), also known as hydration capacity, is the quantity of water absorbed by wheat or food to reach the desired consistency to produce a quality product (Kaushik *et al*., 2021). It is the minimum quantity of water that should be put into the dough before it gets too sticky to work with. Water absorption that is either low or too high might have a detrimental impact on the quality of food items (Godswill *et al*., 2019). The WHC ranged from 4.2 - 6.17 g/g. The findings confirm well with the range (4.63 - 6.42 g/g) reported by Molla and Zegeye (2020) for corn-peanut flakes. Due to the significant association of protein hydration with polar components, as well as the hydrophilic interactions via hydrogen bonding, the WHC was related to capillary, pore size, and the charges on the protein molecules (Rashid *et al*., 2018). The stable organized structure of the polysaccharides might be disturbed by extrusion treatment, exposing formerly buried hydrophilic groups (hydroxyl groups) to react with water via hydrogen bonds (Chang *et al*., 2011). The following polynomial (equation 3) with significant and non-significant terms shows the effect of independent variables on the WHC of extrudates:

WHC= $4.89 + 0.6212\ X_1 + 0.1987\ X_2 - 0.18\ X_3 + 0.27\ X^2{}_1 + 0.0632\ X^2{}_2 - 0.18\ X^2{}_3 + 0.17\ X_1X_2 - 0.03\ X_1X_3 - 0.42\ X_2X_3$ ……………………………….…………………..Equation 3

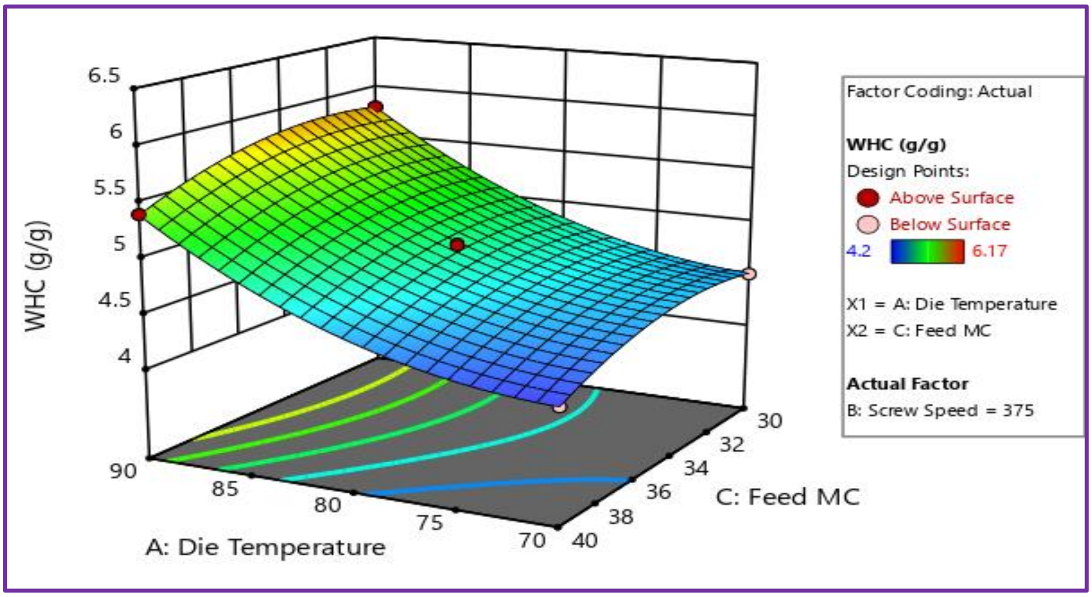
Where, X1=Die Temperature, X2=Screw Speed, X3=Feed MC

The positive coefficients of linear terms of die temperature and screw speed, interaction terms of die temperature and screw speed as well as quadratic terms of die temperature imply that WHC of extrudates may be increased with increased linear terms of die temperature and screw speed, interaction terms of die temperature and screw speed as well as quadratic terms of die temperature. Similar observations were reported by Bordoloi and Ganguly (2014) that WHC increased with extrusion temperature, whereas WHC dropped with increased moisture content at any particular extrusion temperature. A larger degree of starch gelatinization may result in higher WHC.

WHC is also heavily influenced by the degree of porosity or expansion of the extrudate since more porosity and thinner cell walls in extrudates result in increased water absorption. The linear terms of feed moisture content, interaction terms of screw speed and feed moisture content, and quadratic terms of feed moisture content significantly ($p<0.05$) affected the WHC of extrudates negatively.



(a)



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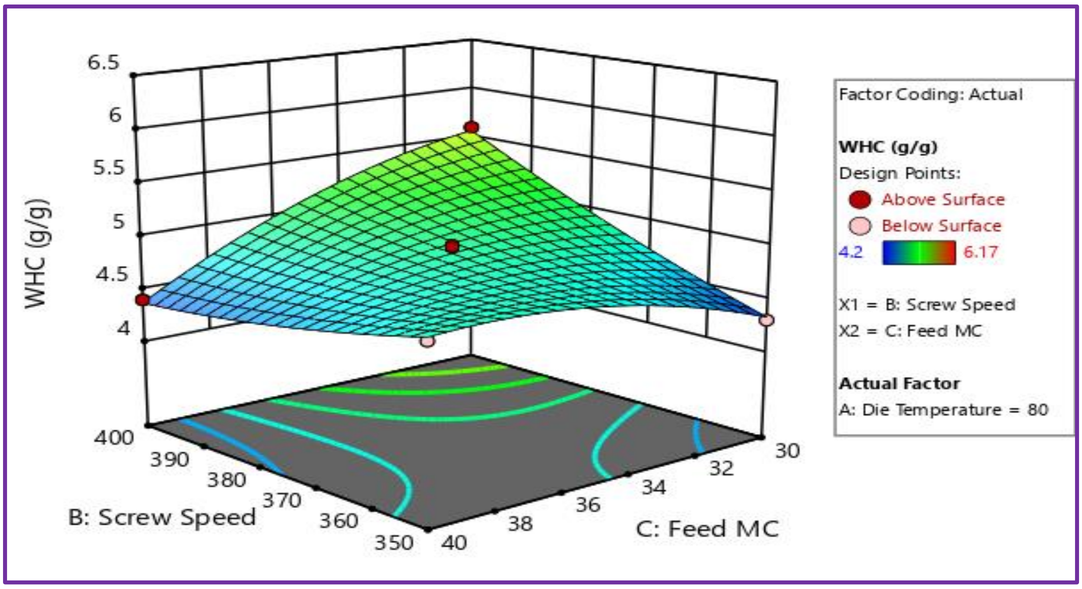


(c)

Figure 4. 3 The 3-D graphs representing the effect of die temperature and screw speed (a), die temperature and feed moisture content (b), and screw speed and feed moisture content (c) on WHC

A low value of WHC may be caused by increasing the interaction terms of screw speed and feed moisture content as well as quadratic terms of feed moisture content. WHC values dropped as screw speeds increased at constant temperatures of 140°C/160°C and % MC, and also 160°C and 17% moisture (Molla & Zegeye, 2020). The increased polymer interaction would decrease the WHC of both the starch and the proteins involved. This would also, to the extent that the interactions were covalent, decrease the amount of protein that could be resolubilized (Allen *et al*., 2007). When water is combined with flour, the water molecules hydrate the hydrophilic region of the protein, causing starches and other components to be degraded, and the process is repeated until molecules of starch and protein, particularly polar amino acid residues, form hydrogen bonds with water (Kaushik *et al*., 2021).

4.3.5 Effect of Extrusion Cooking Variables on Water Absorption Index (WAI) of Extruded Ready-To-Eat Food

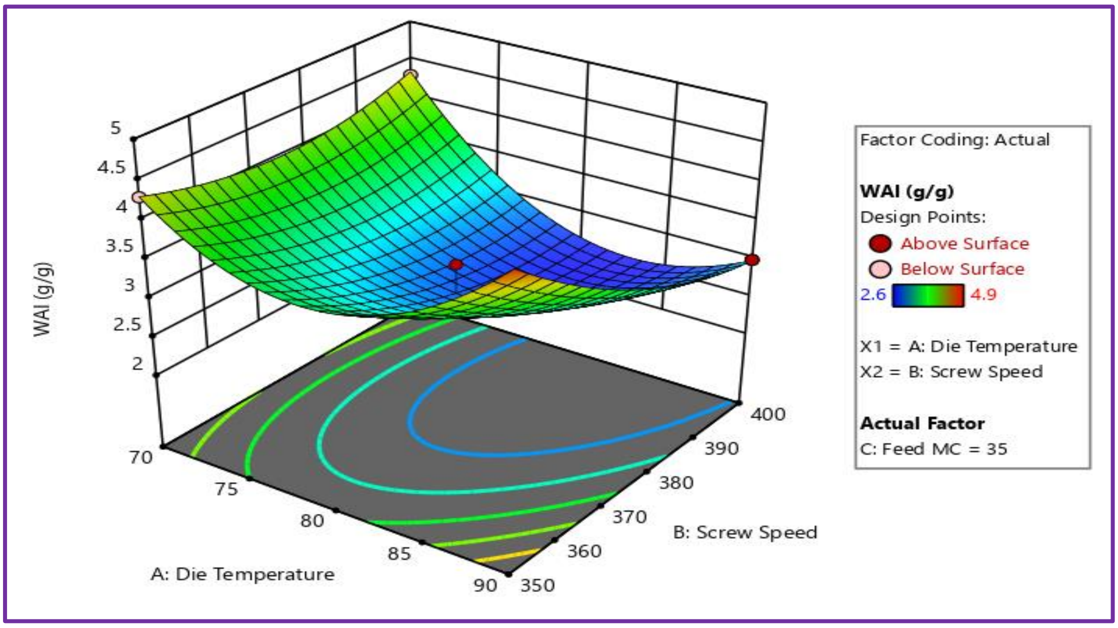
The water absorption index model F-value of 40.74 implies the model is significant. The model significantly ($p<0.0001$) predicts the variable (Table 4.4). The results demonstrated the high adequacy of the regression model to predict the response where the coefficient of determination (R^2) of the model was 0.9877, indicating that the model explained 98.77% of the variability in the response data fitted the model. The Predicted R^2 of 0.9431 is in reasonable agreement with the Adjusted R^2 of 0.9572; i.e. the difference is less than 0.2. The adequate precision of the model was 16.38 which indicates an adequate signal. For the model to be useful, adequate precision must have a signal-to-noise ratio larger than 4 (Table 4.4). On the other hand, the Lack of Fit F-value of 0.15 implies that the Lack of Fit is not significant compared to the pure error. The lack of Fit F-values of this magnitude is caused by noise with a 92.32% probability (Mark & Patrick, 2017). Non-significant lack of fit is good because the model needs to fit (Table 4.8). Figure 4.4 shows the 3-D surface plots describing sufficient trends of WAI of extrudates.

Water Absorption Index (WAI) is calculated as the weight of gel produced after mixing one gram of dry flour food sample with a suitable (yet measured) amount of water. In the existence of an excess of water, starch granules swelled, absorbing a large amount of water and remaining distributed while retaining the integrity of their granules (Godswill *et al*., 2019). The WAI of extrudates ranged from 2.6 - 4.9 g/g. The results are slightly below the value (5.9 - 6.32 g/g) reported by Naseer *et al*. (2021) for functional snack food from almond press cake and pearl millet flour and the value (5.78 - 6.4 g/g) reported by Yadav *et al*. (2021) for expanded extrudates but confirm well with the results (2.45 - 9.02 g/g) reported by Patil *et al*. (2021) for extruded corn flour and are in agreement with the results (3.63 - 5.42 g/g) reported by Molla and Zegeye (2020) for corn-peanut flakes. WAI was shown to be dependent on two factors: (a) the availability of hydrophilic groups to bind water molecules and (b) the gel-forming capability of macromolecules contained in flour (Sompong *et al*., 2011). The following polynomial (equation 4) with significant and non-significant terms shows the effect of independent variables on the WAI of extrudates:

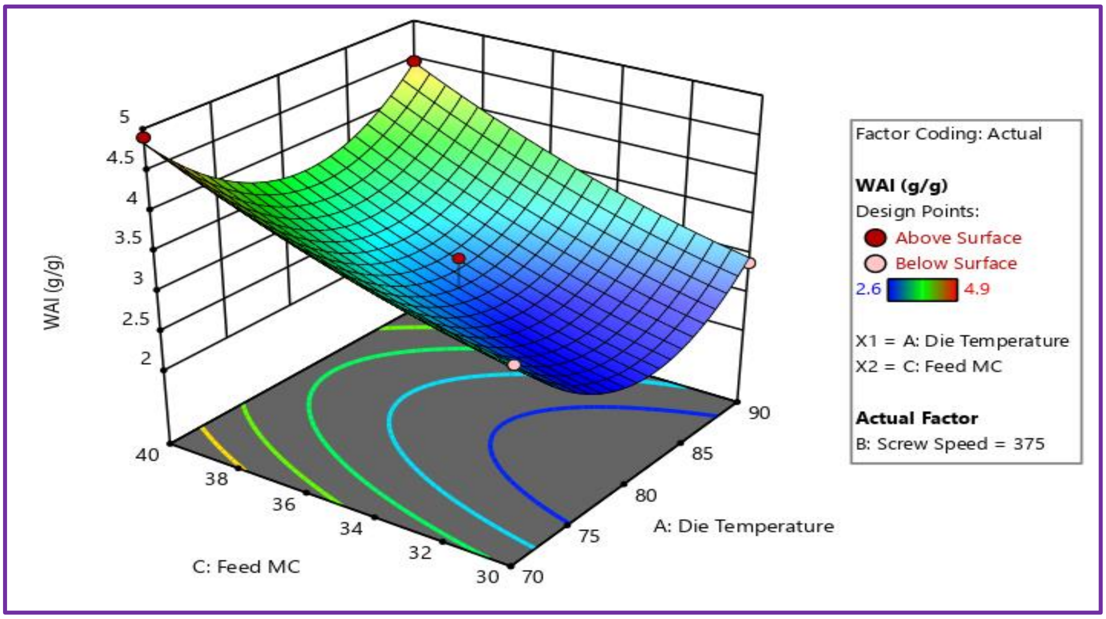
WAI = $2.82 - 0.23\ X_1 - 0.433\ X_2 + 0.71\ X_3 + 0.964\ X^2_1 + 0.314\ X^2_2 + 0.154\ X^2_3 - 0.45\ X_1X_2 + 0.04\ X_1X_3 - 0.44\ X_2X_3$ ………………………...………………………………….Equation 4

Where, X_1=Die Temperature, X_2=Screw Speed, X_3=Feed MC

WAI may also be employed as an indicator of gelatinization (Ding *et al*., 2006) because it assisted assess the quantity of water absorbed by starch; disrupted starch was thought to bind more water. Hashimoto and Grossmann (2003) discovered that increasing the fibre content and lowering the starch content improved the WAI of extrudates. The estimated regression coefficients indicated that the linear terms of die temperature and screw speed had a negative significant ($p<0.05$) effect on the WAI of extrudates. Therefore, the minimum WAI may be achieved by increasing linear terms of die temperature and screw speed. Molla and Zegeye (2020) reported that WAI drops with increasing extrusion temperature. Furthermore, Dalbhagat and Mishra (2019) reported the same observations where increasing the linear terms of die temperature and main screw speed reduced the WAI of extruded fortified rice kernels.



(a)



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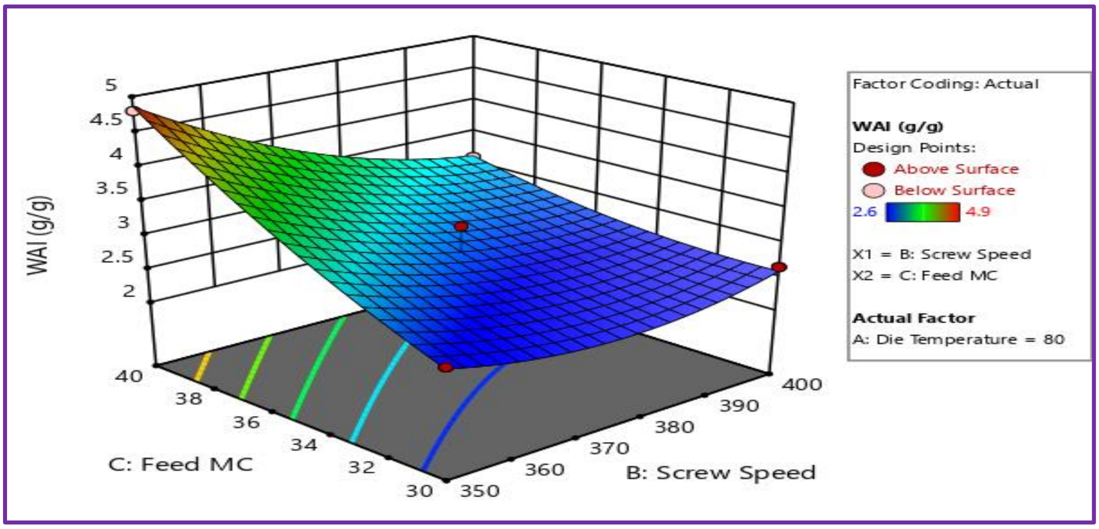


(c)

Figure 4. 4 The 3-D graphs representing the effect of die temperature and screw speed (a), die temperature and feed moisture content (b), and screw speed and feed moisture content (c) on water absorption index

The linear terms of feed moisture content and quadratic terms of die temperature and screw speed had a positive significant ($p<0.05$) effect on the WAI of extrudates. High WAI may be obtained by increasing linear terms of feed moisture content and quadratic terms of die temperature and screw speed. Dalbhagat and Mishra (2019) reported the same observations where increasing the linear terms of moisture content, and quadratic terms of die temperature increased the WAI of extruded fortified rice kernels.

WAI might be used to determine (a) the characteristics of starch in extrudates and (b) the water resistance of starch–bran composites. Thereby, within certain conditions like high temperature, high extrusion pressure, cooking, and shear stress, the hydrogen bonds between starch granules are disrupted. This leads to the destruction of the granular structure of starch and the exposure of more hydroxyl groups for the binding of water molecules. WAI is also a helpful parameter for determining the quality of flours, particularly for extrudates (Kaushik *et al*., 2021).

4.3.6 Effect of Extrusion Cooking Variables on Water Solubility Index (WSI) of Extruded Ready-To-Eat Food

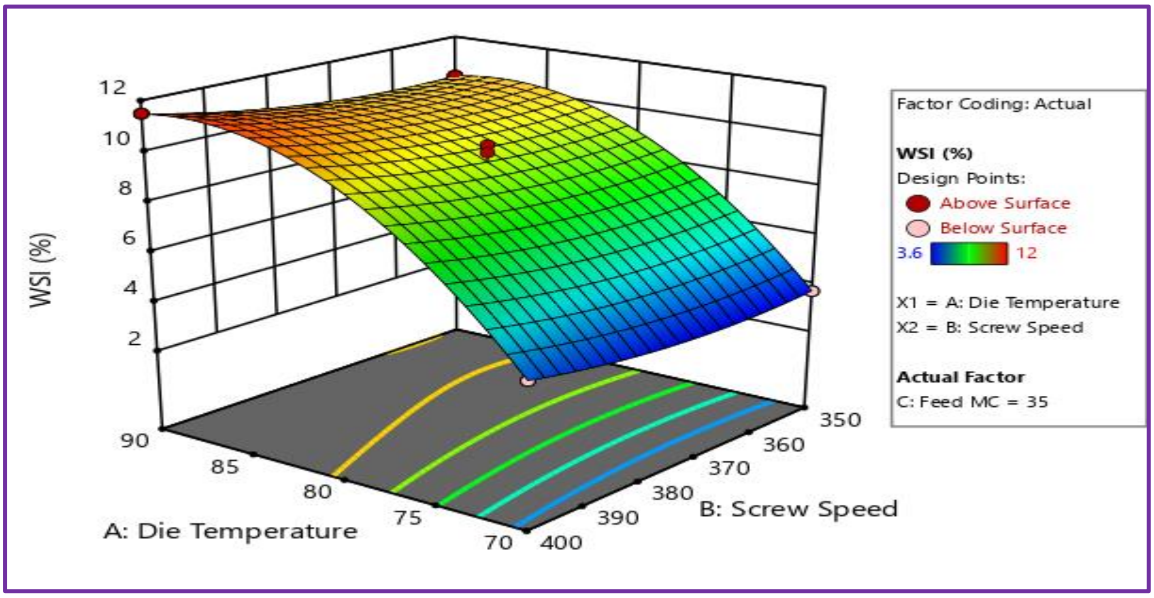
The water solubility index model F-value of 75.35 implies the model is significant. The model significantly ($p<0.0001$) predicts the variable (Table 4.4). The results demonstrated the high adequacy of the regression model to predict the response where the coefficient of determination (R^2) of the model was 0.9898, indicating that the model explained 98.98% of the variability in the response data fitted the model. The Predicted R^2 of 0.9662 is in reasonable agreement with the Adjusted R^2 of 0.9766; i.e. the difference is less than 0.2. The adequate precision of the model was 25.89 which indicates an adequate signal. For the model to be useful, adequate precision must have a signal-to-noise ratio larger than 4 (Table 4.8). On the other hand, the Lack of Fit F-value of 0.18 implies the Lack of Fit is not significant compared to the pure error. The lack of Fit F-values of this magnitude is caused by noise with a 90.24% probability (Mark & Patrick, 2017). Non-significant lack of fit is good because the model needs to fit (Table 4.4). Figure 4.5 shows the 3-D surface plots describing sufficient trends of WSI of extrudates.

The water solubility index (WSI) indicates the existence of soluble molecules and is an indicator of starch breakdown (Feyera *et al*., 2021). The WSI ranged from 3.6 to 12%. These results fall within the range (8.6 - 15.9%) reported by Naseer *et al*. (2021) for functional snack food from almond press cake and pearl millet flour and confirm well with the value (6.9 - 7.6%) reported by Yadav *et al*. (2021) for expanded extrudates. The quantity of soluble polysaccharides produced from fibre components during extrusion is indicated by WSI, which is frequently employed as an indicator for the breakdown of molecular components. It is primarily determined by the amount of solvent used (Tabibloghmany *et al*., 2020). The following polynomial (equation 5) with significant and non-significant terms shows the effect of independent variables on the WSI of extrudates:

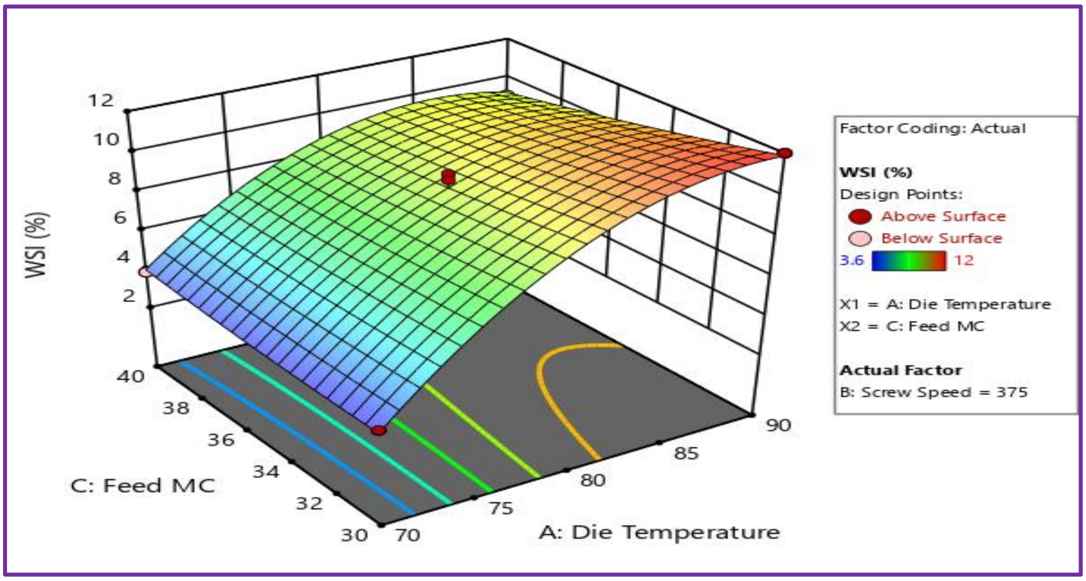
WSI = $9.32 + 3.41\ X_1 + 0.48\ X_2 - 0.513\ X_3 - 2.29\ X^2_1 + 0.44\ X^2_2 + 0.12\ X^2_3 + 0.13\ X_1X_2 - 0.80\ X_1X_3 + 0.03\ X_2X_3$ ……………………………………..…………………………Equation 5

Where, X_1=Die Temperature, X_2=Screw Speed, X_3=Feed MC

It is evident from the quadratic equation of WSI that the positive coefficients of linear terms of die temperature and screw speed had a significant ($p<0.05$) positive contribution to the WSI of extrudates. High WSI of extrudates may be achieved by increasing die temperature and screw speed. Dalbhagat and Mishra (2019) reported the same observations where increasing the linear terms of die temperature and main screw speed, quadratic terms of die temperature and main screw speed, and interaction terms of feed moisture content and die temperature increased the WSI of extruded fortified rice kernels. In addition, the barrel temperature and screw speed have a favourable effect on the water solubility index (WSI) of rice-based extrudates but feed moisture content has a negative effect on this WSI (Dalbhagat *et al*., 2019). An increase in screw speed leads to an increase in shear force, which increases the quantity of soluble dietary fibre and hence raises the solubility index (Tabibloghmany *et al*., 2020).



(a)



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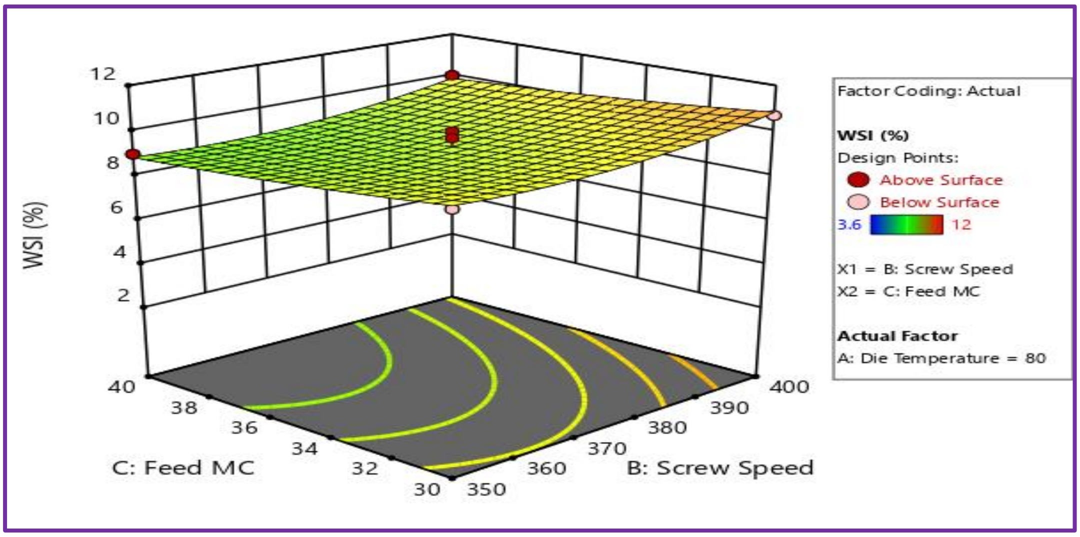


(c)

Figure 4. 5 The 3-D graphs representing the effect of die temperature and screw speed (a), die temperature and feed moisture content (b), and screw speed and feed moisture content (c) on water solubility index

In addition, at high screw speed, the breakdown rate of cereal starch granules is enhanced; such that, the longer starch chains are broken into shorter chains. As a result, these latter structures are more soluble than longer chains, which explains why WSI values at high screw speed values are greater (Sandrin *et al*., 2018). The reduction in WSI of extrudates may be observed by increasing the linear terms of feed moisture content, interaction terms of die temperature and feed moisture content as well as quadratic terms of die temperature. Dalbhagat and Mishra (2019) reported the same observations where increasing the linear terms of feed moisture content reduced the WSI of extruded fortified rice kernels.

The WSI is a starch destruction measurement. The higher the moisture level, the less starch breakdown occurs, and hence the lower the WSI (Li *et al*., 2007). The drop in WSI with increased moisture content might be attributed to the fact that increased moisture content can reduce protein denaturation, which reduces WSI values (Pathania *et al*., 2013). In the instance of cereal-based extrusion, Yuliani *et al*. (2006) and Sawant *et al*. (2013) both found a reduction in the WSI of extrudate with increasing moisture content.

4.3.7 Effect of Extrusion Cooking Variables on the Swelling Capacity of Extruded Ready-To-Eat Food

The swelling capacity model F-value of 66.60 implies the model is significant. The model significantly ($p<0.0001$) predicts the variable (Table 4.4). The results demonstrated the high adequacy of the regression model to predict the response where the coefficient of determination (R^2) of the model was 0.9885, indicating that the model explained 98.85% of the variability in the response data fitted the model. The Predicted R^2 of 0.9565 is in reasonable agreement with the Adjusted R^2 of 0.9736; i.e. the difference is less than 0.2. The adequate precision of the model was 28.38 which indicates an adequate signal. For the model to be useful, adequate precision must have a signal-to-noise ratio larger than 4 (Table 4.8). On the other hand, the Lack of Fit F-value of 0.24 implies the Lack of Fit is not significant compared to the pure error. The lack of Fit F-values of this magnitude is caused by noise with 86.41% probability (Mark & Patrick, 2017). Non-significant lack of fit is good because the model needs to fit (Table 4.4). Figure 4.6 shows the 3-D surface plots describing sufficient trends of SC of extrudates.

The swelling capacity is an essential element in predicting how much water food samples will absorb and how much swelling will occur in a given length of time (Ijarotimi & Keshinro, 2012). The swelling index (SI), also known as the swelling capacity (SC), is the volume in millilitres occupied by the swelling of one gram of food product under certain conditions. It is determined by adding water or a swelling agent as specified in the test method for each specific food product (whole, pulverized, or cut). The swelling capacity of starch is a measure of its ability to absorb water and swell, including the degree of associate forces in the starch granules. In some food items, such as bread products, swelling capacity (index) is considered a quality parameter. It is an indicator of non-covalent interaction between starch granule molecules and one of the variables influencing the -amylose and amylopectin ratios

(Iwe *et al*., 2016). The attraction forces responsible for linking water with water-loving moieties including such starch granules were attributed to the swelling capacity (SC) of flour, a measure of the ability of flour to absorb and swell (Adegunwa *et al*., 2014).

The SC ranged from 2.4 to 6.2 ml/g. The results are above the results (2.02 - 2.72 ml/g) reported by Liu *et al.* (2020) for rice bran dietary fibre modified by cellulase treatment and within the results (0.67 - 4.22 ml/g) reported by Ijarotimi and Keshinro (2012) for infant formula but below to the results (5.80 - 8.32 ml/g) reported by Song *et al*. (2018) for extrusion-cellulase treatment of bamboo shoots. The results are in the agreement with the results (4.65 - 5.79 ml/g) reported by Zhu *et al*. (2010) for wheat bran dietary fibre. The following polynomial (equation 6) with significant and non-significant terms shows the effect of independent variables on the SC of extrudates:

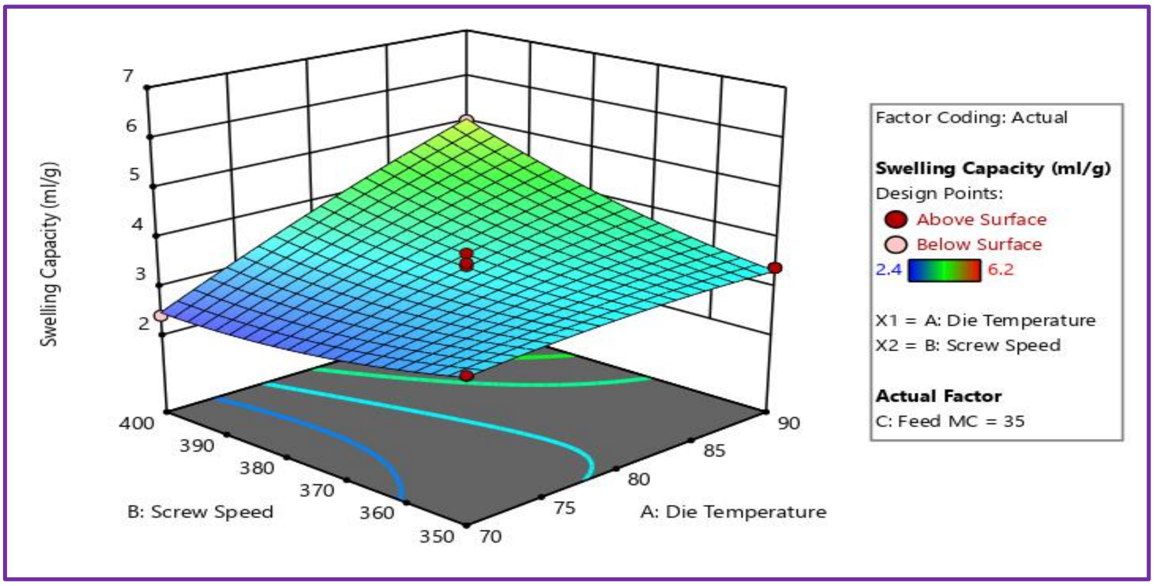
SC = $3.41 + 0.70\ X_1 + 0.26\ X_2 + 0.8413\ X_3 – 0.110\ X^2{}_1 + 0.21\ X^2{}_2 + 1.03\ X^2{}_3 + 0.61\ X_1X_2 + 0.33\ X_1X_3 – 0.458\ X_2X_3$ ……………………………………………………………Equation 6

Where, X_1=Die Temperature, X_2=Screw Speed, X_3=Feed MC

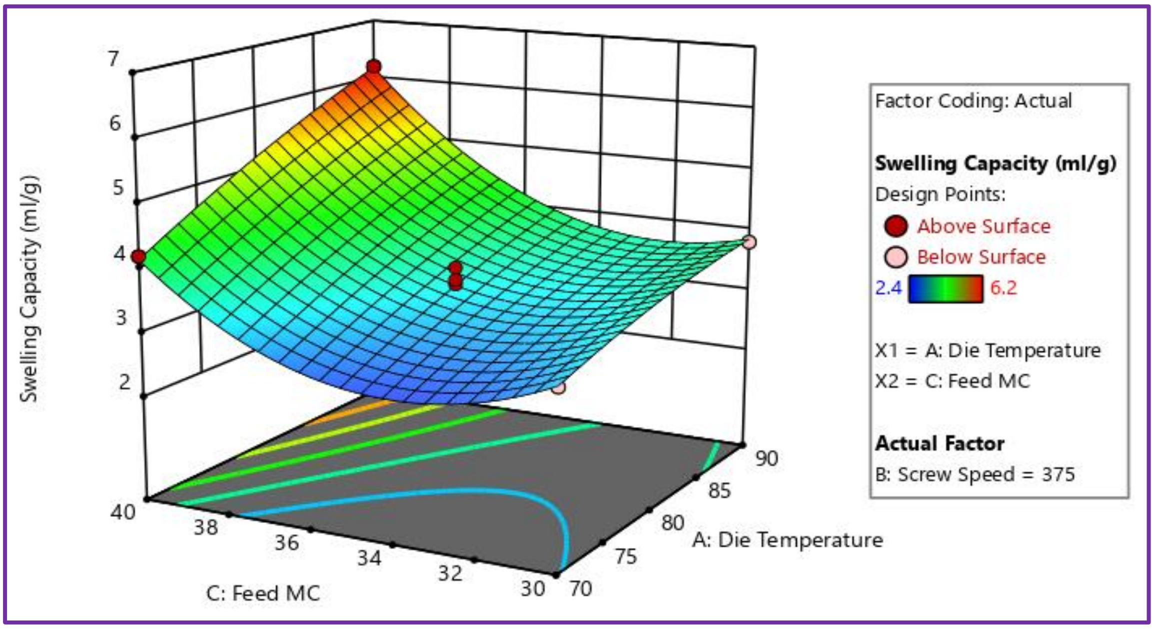
The increase in SC of extrudates may be observed with increased linear terms of die temperature, screw speed and feed moisture content, interaction terms of die temperature and feed moisture content, die temperature and screw speed as well as quadratic terms of feed moisture content and screw speed. These observations confirm well with the study of Tabibloghmany *et al*. (2020) who reported an increase in SC may be the result of increasing linear terms of feed moisture content and screw speed.

On the other hand, the reduction of SC in this study may be achieved by increasing the interaction terms of screw speed and feed moisture content as well as quadratic terms of die temperature. Tabibloghmany *et al*. (2020) noted a reduction in SC as the result of increasing quadratic terms of barrel temperature, screw speed, and feed moisture content. Because flours have a high starch content, the SC of food products produced from them is also high. As a result, flours are chosen depending on their SC value, and particularly for bakery goods (Kaushik *et al*., 2021). The swelling capacity (index) of flours is affected by particle size, species diversity, and processing technique or unit activities (Chandra & Samsher, 2013). A high starch content raises the swelling capacity (index) of food products and flours, particularly those containing a high proportion of branching amylopectin. Starch is made up of two chains of glucose units: amylose (linear chain) and amylopectin (branched chain). Granules of starch are very tiny packets of starch. The amount and percentage of amylose and amylopectin in starch differ depending on the plant source. This explains why the swelling capabilities of flours derived from various (plant) sources and species vary (Godswill *et al.*, 2019).

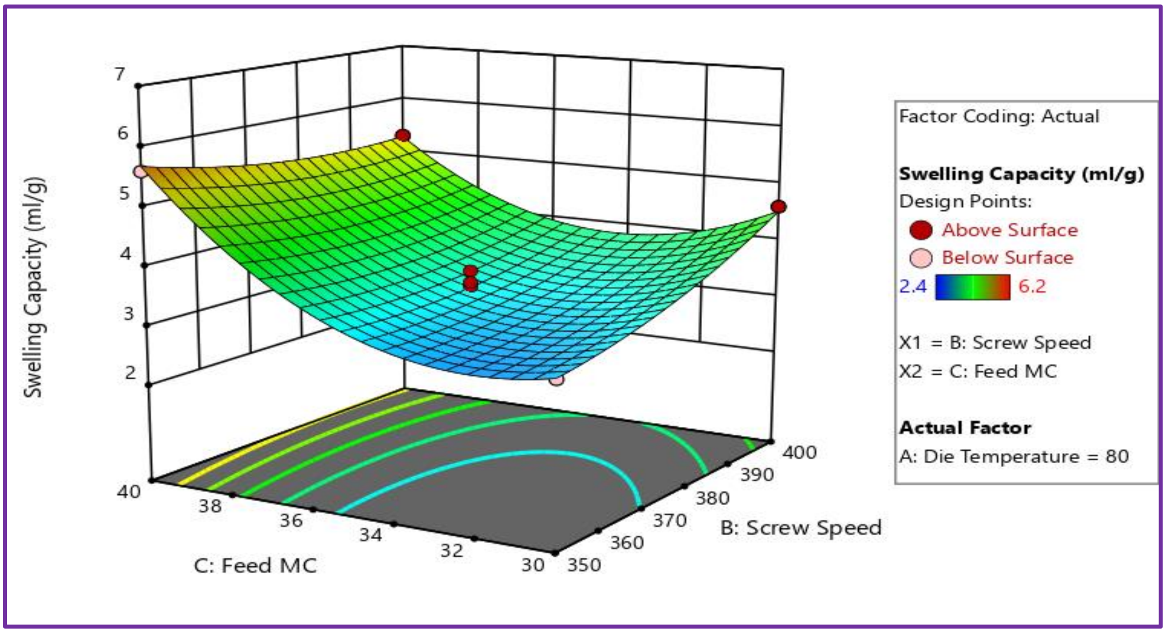
Extrusion cooking changes the three-dimensional fibre structure, increases the quantity of soluble dietary fibre, particularly pseudo-pectin polysaccharides, and decreases the degree of cellulose crystallization in the product due to the simultaneous application of high temperature and shear stress. Increasing the quantity of short-chain soluble molecules, also changes the fibre structure and significantly increases the amount of contained water within the porous fibre structure (Huang & Ma, 2016).



(a)



(b)



(c)

Figure 4. 6 The 3-D graphs representing the effect of die temperature and screw speed (a), die temperature and feed moisture content (b), and screw speed and feed moisture content (c) on swelling capacity

4.3.8 Effect of Extrusion Cooking Variables on the Oil Absorption Capacity of Extruded Ready-To-Eat Food

The oil absorption capacity model F-value of 44.44 implies the model is significant. The model significantly ($p<0.05$) predicts the variable (Table 4.4). The results demonstrated the high adequacy of the regression model to predict the response where the coefficient of determination (R^2) of the model was 0.9828, indicating that the model explained 98.28% of the variability in the response data fitted the model. The Predicted R^2 of 0.9629 is in reasonable agreement with the Adjusted R^2 of 0.9607; i.e. the difference is less than 0.2. The adequate precision of the model was 17.83 which indicates an adequate signal. For the model to be useful, adequate precision must have a signal-to-noise ratio larger than 4 (Table 4.8). On the other hand, the Lack of Fit F-value of 0.06 implies the Lack of Fit is not significant compared to the pure error. The lack of Fit F-values of this magnitude is caused by noise with a 97.97% probability (Mark & Patrick, 2017). Non-significant lack of fit is good because the model needs to fit (Table 4.4). Figure 4.7 shows the 3-D surface plots describing sufficient trends of OAC of extrudates.

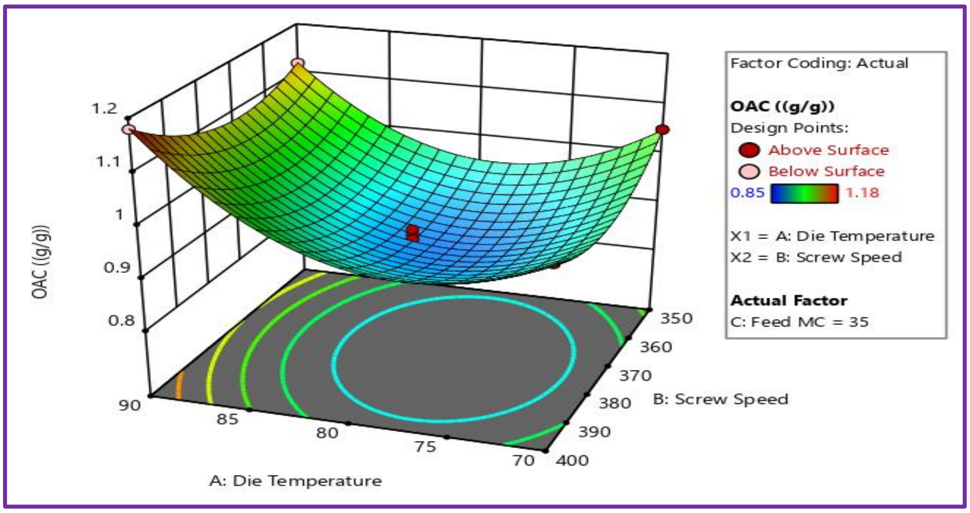
Oil absorption capacity (OAC), also known as water absorption, is the ability of proteins to bind fat via their non-polar side chains. Oil absorption capacity is an important functional feature that leads to improving mouth feel while maintaining the flavour of food products (Iwe *et al*., 2016). The oil absorption capacity (OAC) of flour was defined as its ability to absorb or physically bind oil or fat by attraction via capillary forces (Obadina *et al*., 2016). When combined with oilseeds, high OAC flours are more suitable for confectionary applications that require oil emulsification as well as food that need to keep oil well. It should be remembered that to establish if a protein is appropriate as a complete or partial substitution in food, its OAC, and WAC levels should be determined (Kaushik *et al*., 2021).

The OAC ranged from 0.85 to 1.18 g/g. The highest value (1.18 g/g) was observed at high temperature, screw speed, and intermediate feed moisture content. The results are in agreement with the range (1.23 - 1.57 g/g) reported by Pawase *et al*. (2021) for different millet flour as well as (0.70 - 1.122 g/g) reported by Nguyen *et al*. (2015) for swell-dried soybean powder. The results confirm well with the results (1.11 to 3.9 g/g) reported by Tabibloghmany *et al*. (2020) for extruded soybean hull. The following polynomial (equation 7) with significant and non-significant terms shows the effect of independent variables on the OAC of extrudates:

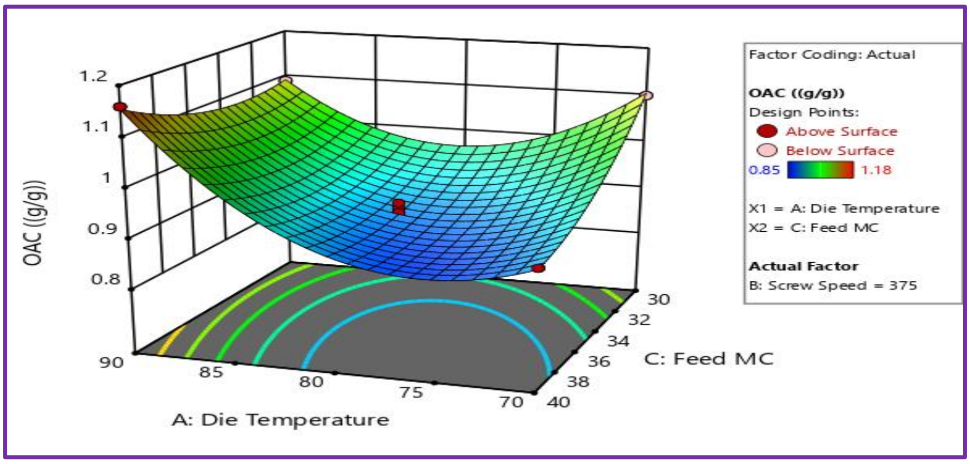
$$\textbf{OAC} = 0.90 + 0.06\ X_1 + 0.01\ X_2 - 0.034\ X_3 + 0.132\ X^2_1 + 0.065\ X^2_2 + 0.05\ X^2_3 + 0.023\ X_1X_2 + 0.07\ X_1X_3 - 0.06\ X_2X_3 \quad \text{.......Equation 7}$$

Where, X_1=Die Temperature, X_2=Screw Speed, X_3=Feed MC

Maximum OAC of extrudates may be observed by increasing linear terms of die temperature, interaction terms of die temperature and feed moisture content, and quadratic terms of die temperature, screw speed and feed moisture content. Tabibloghmany *et al*. (2020) reported similar findings, stating that an increase in interaction terms of feed moisture content and screw speed, moisture content and die temperature, screw speed and die temperature, and quadratic terms of feed moisture content, screw speed, and die temperature increased significantly ($p<0.05$) OAI of extrudates from soybean hull. Furthermore, Lazou and Krokida (2010) investigated the functional characteristics of corn and corn–lentil extrudates and discovered that increasing the extrusion temperature improved the OAI of maize and corn–lentil extrudates. On the other hand, increasing the linear terms of feed moisture content, interaction terms of screw speed, and feed moisture content may significantly ($p<0.05$) reduce the OAC of extrudates. Tabibloghmany *et al*. (2020) observed similar findings, namely that raising the feed moisture content and screw speed reduced OAI for extrudates derived from soybean hull. In addition, Lazou and Krokida (2010) noted that an increase in feed moisture content reduced the OAI of extrudates.



(a)



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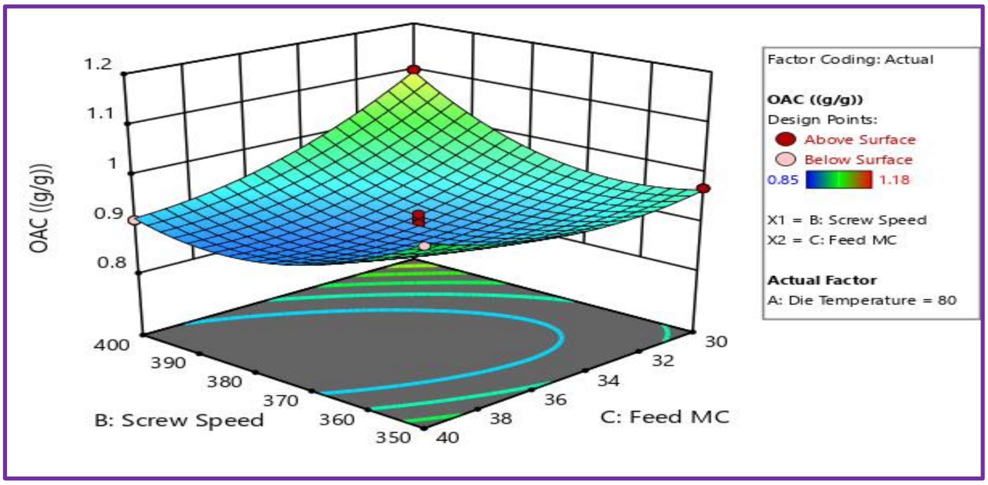

(c)

Figure 4. 7 The 3-D graphs representing the effect of die temperature and screw speed (a), die temperature and feed moisture content (b), and screw speed and feed moisture content (c) on the oil absorption capacity

The capacity of flours to bond with oil makes them helpful in food applications where maximum oil absorption is needed, making flours potentially functional in foods such as the manufacturing of pastries and sausage. The ability of flour to absorb oil also makes it excellent for enhancing taste and mouth feel when employed in food preparation (Chandra & Samsher, 2013). The ability of the dietary protein to absorb oil is determined by intrinsic variables such as amino acid content, protein structure, and surface polarity or hydrophobicity. Because the proteins in powders can interact with oil, they are helpful in food systems where maximum oil absorption is required (Chaudhary *et al*., 2020).

4.3.9 Optimization of Extrusion Cooking Variables and Predicted Response Variables

The optimum extrusion cooking variables (die temperature, screw speed, and feed moisture content) with predicted response variables (lateral expansion, bulk density, water hydration capacity, oil absorption capacity, water absorption index, water solubility index, and swelling capacity) are shown in Figure 4.8 – 4.11. The extrusion cooking conditions and response variables were set at different goal criteria (in range, maximum, and minimum) where, die temperature, screw speed, and feed moisture content were kept in range together with WAI and WSI. Lateral expansion, OAC, swelling capacity, and WHC were set at maximum levels while bulk density was minimized. All variables were set equally by importance 3 (Table 4.9).

Table 4. 9 Constraints with goals during the optimization process

Name	Goal	Lower Limit	Upper Limit	Importance
A: Die Temperature	In range	70	90	3
B: Screw Speed	In range	350	400	3
C: Feed MC	In range	30	40	3
Lateral Expansion	Maximize	85	125	3
Oil Absorption Capacity	Maximize	0.85	1.18	3
Water Hydration Capacity	Maximize	4.2	6.17	3
Swelling Capacity	Maximize	2.4	6.2	3
Water Absorption Index	In range	2.6	4.9	3
Water Solubility Index	In range	3.6	12	3
Bulk Density	Minimize	0.13	0.5	3

After numerical optimization of variables, it was found that optimum extrusion cooking variables were 90°C, 400 rpm, and 35% for die temperature, screw speed, and feed moisture content, respectively. The predicted values were 125%, 0.130 g/cm^3, 6.22 g/g, 1.182 g/g, 2.987 g/g, 11.512%, and 5.051 ml/g for lateral expansion, bulk density, water hydration capacity, oil absorption capacity, water absorption index, water solubility index and swelling capacity, respectively with the overall desirability of 0.930. Desirability is a utility function that ranges from zero (not acceptable) to one (ideal), which makes it possible to optimize multiple responses simultaneously via numerical methods (Mark & Patrick, 2017).

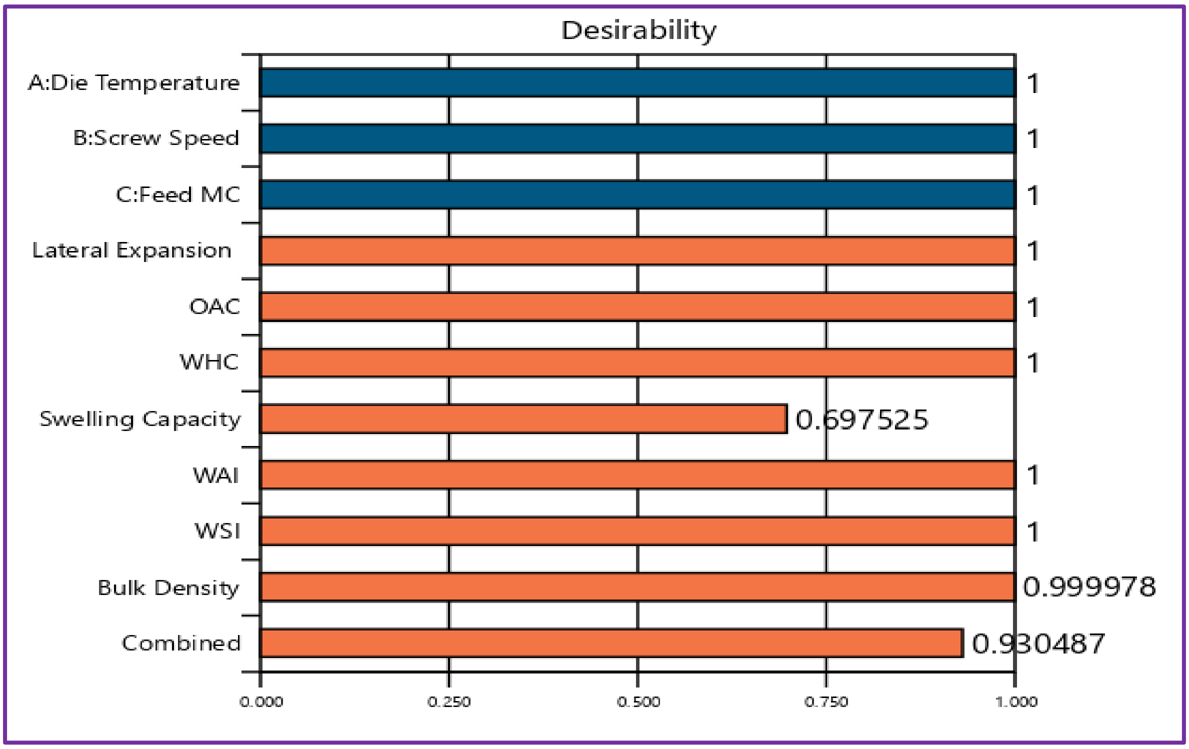

Figure 4. 8 Bar graph of coefficient of determination (desirability) of all tested variables, WHC: Water hydration capacity, WAI= Water absorption index, WSI= Water solubility index, OAC= Oil absorption capacity

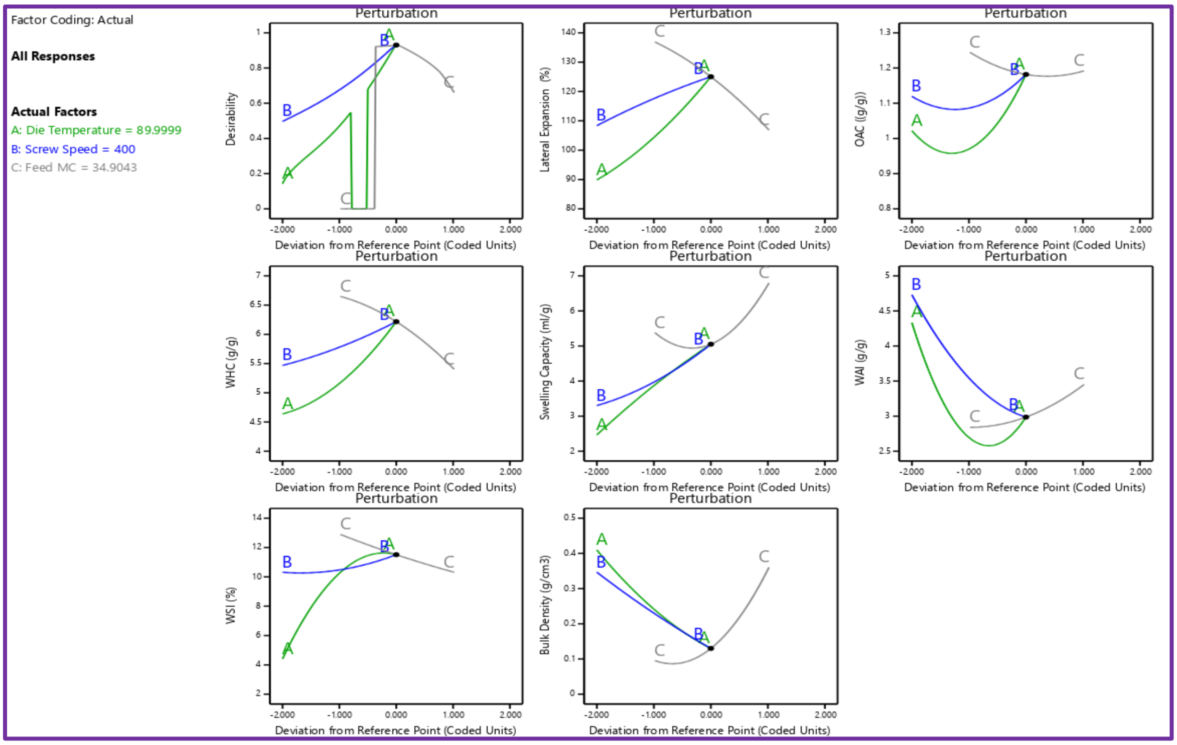

Figure 4. 9 Perturbation of variables showing deviation from a reference point (coded unit), WHC: Water hydration capacity, WAI= Water absorption index, WSI= Water solubility index, OAC= Oil absorption capacity

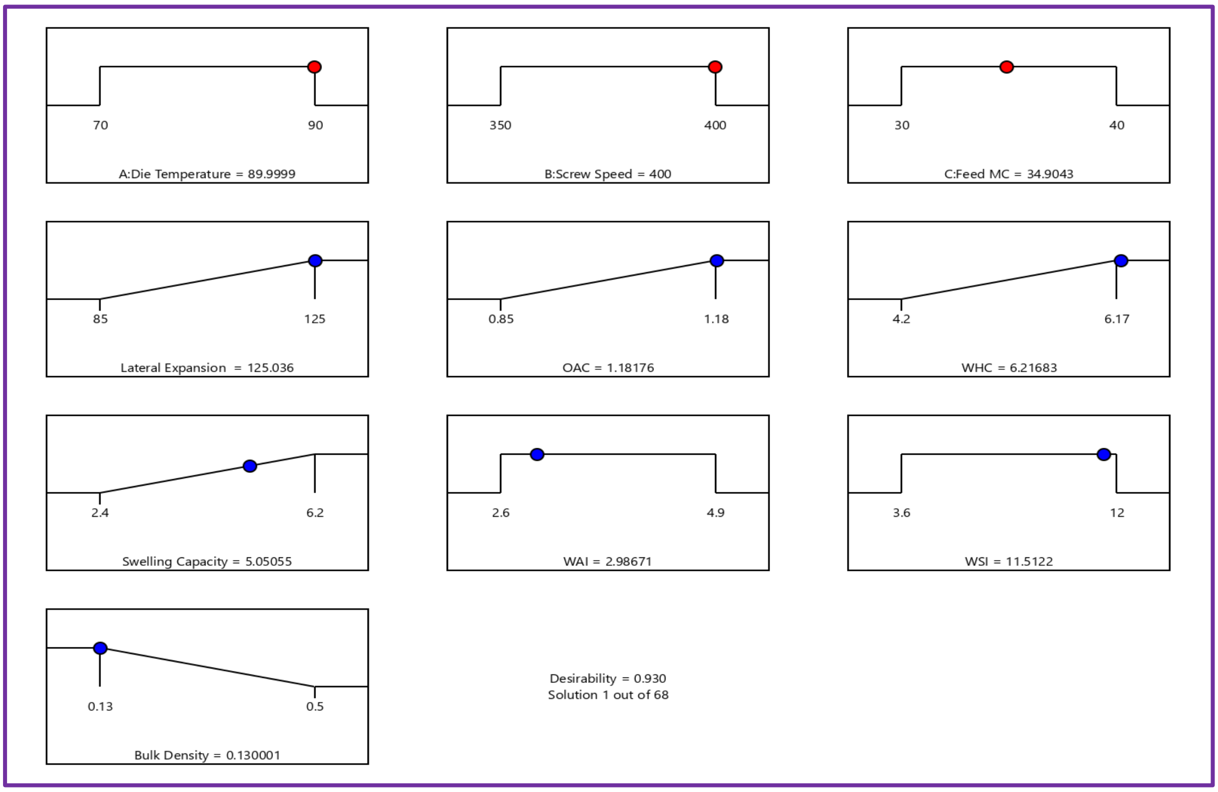

Figure 4. 10 Ramp graphs of all optimum variables, WHC: Water hydration capacity, WAI= Water absorption index, WSI= Water solubility index, OAC= Oil absorption capacity

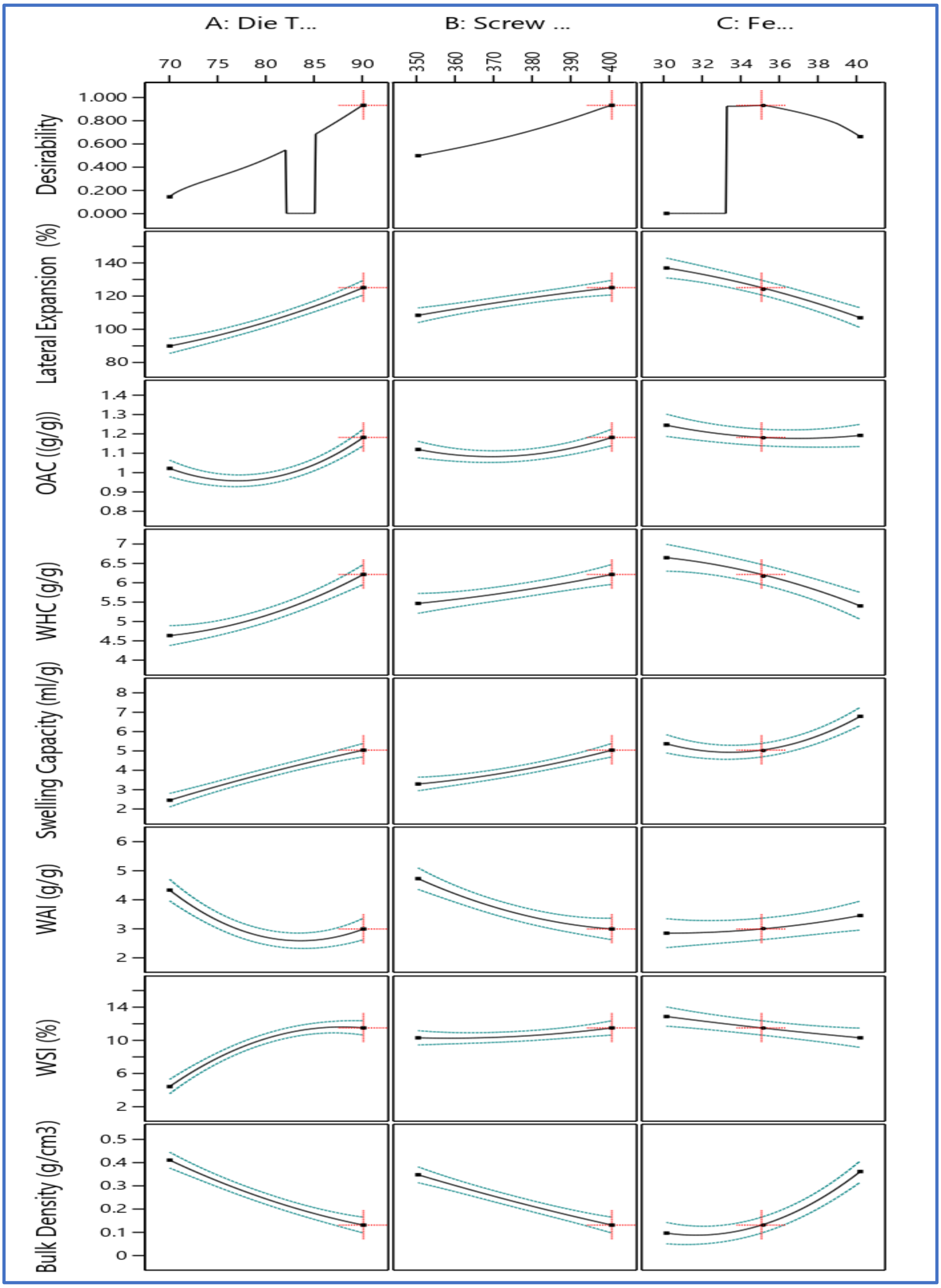

Figure 4. 11 Numerical optimization graphs of extrusion cooking conditions (die temperature, screw speed, feed moisture content) with predicted response parameters, WHC: Water hydration capacity, WAI= Water absorption index, WSI= Water solubility index, OAC= Oil absorption capacity.

4.4 Conclusion

Extrusion cooking process parameters namely, die temperature, screw speed, and feed moisture content significantly affect the functional characteristics of extruded ready-to-eat foods from orange-fleshed sweet potatoes flour. Linear, quadratic, and interaction terms of extrusion cooking variables significantly affect the functional characteristics of extruded ready-to-eat foods. Die temperature and screw speed are the most key extrusion cooking variables that affect the functional characteristics of extruded ready-to-eat foods positively. On the other hand, feed moisture content has a significant negative effect on the functional characteristics of extruded ready-to-eat foods. The generated optimum extrusion cooking conditions with the corresponding predicted functional characteristics have promising information that could be used by orange-fleshed-potato processors to predict the desired quality of extruded RTE foods. The produced ready-to-eat foods could increase profitability for farmers, and generate income and employment if the produced RTE extrudates are standardized and commercialized.

CHAPTER FIVE

COMPOSITE ANALYSIS AND SENSORY QUALITIES OF EXTRUDED READY-TO-EAT BABY FOODS FROM ORANGE-FLESHED SWEET POTATOES ENRICHED WITH AMARANTH SEEDS, AND SOYBEANS FLOUR

Abstract

Adequate nutrition is critical during infancy and childhood because life phases are marked by significant physical, mental, and changes in behaviour, such as fast growth, weight gain, the development of cognitive and psychomotor abilities, and a change in dietary preferences. This study was carried out to investigate the effect of extrusion cooking and blend proportions on the nutritional qualities of extruded baby foods. Different blends of orange-fleshed sweet potatoes, amaranth seeds, and soybeans flour were used to formulate foods and analyzed for proximate, minerals, vitamin A content, anti-nutrient content, and sensory qualities. Extrusion cooking was conducted at a temperature of 90^{o}C, screw speed of 400 rpm, and feed moisture content of 35%. The results reveal that extruded baby foods had a high protein content of 15.72%, total minerals (5.39%), carbohydrate content (80.58%), crude fibre content (5.04%), fat content (6.05%), energy value (380.84 kcal/100g), energy-to-protein ratio (128.67 kcal/g of protein) and vitamin A content (1044.70 REA µg/100g). The micronutrients of extruded baby foods resulted in high iron content of 3.10 mg/100g, zinc content (0.64 mg/100g), manganese content (0.90 mg/100g), copper content (0.97 mg/100g), magnesium content (81.70 mg/100g), calcium content (61.22 mg/100g), potassium content (68.18 mg/100g) and sodium content (41.44 mg/100g). The produced ready-to-eat baby foods showed a reduction in anti-nutrients as well as acceptable levels of phytate content which ranged from 0.47-1.79 mg/100g, oxalate content (0.16 – 0.50 mg/100g), and saponin content (0.20 – 0.48 mg/100g). All produced foods were highly accepted through sensory evaluation. These important findings confirm that extrusion cooking is useful in the production of nutrient-dense baby foods. In addition, the most significant observation of this study is that the produced foods could be used for under-five years children who suffer from protein-energy malnutrition and micronutrient deficiencies. These findings will also contribute to food and nutrition security.

5.1 Introduction

Adequate nutrition is required for newborns and children to reach their maximum potential in terms of growth, health, and development. Nutritional deficiencies render the body vulnerable to sickness (Rajesh *et al*., 2020). Poverty, food insecurity, child malnutrition, and unaffordability of healthy foods continue to be a challenge, particularly in Africa and Asia in vulnerable groups including children (FAO *et al*., 2021). Malnutrition is caused by poverty, inadequate nutritional quality of traditional complementary food, inappropriate complementary feeding practices, high disease burden, limited access to nutritious foods, low level of mother's education, low access to safe water and basic drugs, sanitation, and hygiene, and health services, and inadequate care practices and the increased cost of protein-based complementary foods (Eka *et al*., 2010; WFP, 2018). During the COVID-19 pandemic, interruptions in important nutrition measures and unfavourable impacts on food patterns hampered efforts to eradicate malnutrition in all of its manifestations (FAO *et al*., 2021). Poor weaning is one of the most common causes of child malnutrition, which raises the risk of baby morbidity and mortality (Jeelani *et al*., 2020).

According to the most current figures, an unacceptable number of people are still suffering from poverty, hunger as well as malnutrition (Global Nutrition Report, 2021). A range of biological, environmental, and social variables influence the growth of a child. The growth of children is inhibited when they are malnourished throughout their first two years of life. Undernutrition during the first 1000 days of life is connected to a reduced survival rate and a higher frequency of acute and chronic disorders in adulthood (Rajesh *et al*., 2020). According to the latest FAO *et al*. (2022) report, most East African Community countries still have high rates of stunted and wasted children.

On the other hand, anaemia is still high, rising, and prevalent among girls and women of reproductive age, with 570.8 million (29.9%) girls and women of reproductive age (15–49 years) suffering from anaemia (Global Nutrition Report, 2021). The findings show striking geographical differences: anaemia impacted more than 30% of women in Africa and Asia, compared to only 14.6% of women in Northern America and Europe (FAO *et al*., 2021). Likewise, in low- and middle-income countries, approximately 60% of children under the age of five are anaemic (with greater rates among those aged 6–24 months), with no improvement over the last decade (Zlotkin & Dewey, 2021).

Data on micronutrient deficiencies (vitamin A deficiency) in children suggests that the problem is still prevalent throughout Africa and South Asia. In the few countries where data exists, zinc deficiency affects nearly half of all children (Victora *et al*., 2021). A sustainable solution is thus needed to address food and nutrition insecurity. The simplest and least expensive method to reduce the burden of many diseases and the predisposing factors with them is adopting a proper diet (Rajesh *et al*., 2020).

A potential solution to this difficult problem could be the blending of locally available food crops to produce affordable, sustainable, and healthy diets and the utilization of low-cost processing techniques. The amaranth species are crops with a lot of potential for reducing hunger, malnutrition, and poverty and not only reducing illnesses that may associate with them but also achieving food and nutrition security. Amaranth is a high-yielding crop and can withstand a lot of stress conditions. Amaranth seed is high in nutritional value and bioactive components, and it has a wide range of applications in both household and industrial applications (Aderibigbe *et al*., 2020). Therefore, orange-fleshed sweet potatoes enriched with soybeans and amaranth seeds can boost nutrients specifically micronutrients (vitamin A and minerals) and macronutrients such as protein, fat, and carbohydrate.

On the other hand, antinutritional factors are substances or compounds of natural or synthetic origin that hinder nutrient uptake, digestion, and utilization in addition to having other detrimental consequences. On the other hand, such chemical substances can be advantageous to humanity when used appropriately. In actuality, plants predominantly use antinutrients as a form of defence. Antinutrients are typically removed from products through thermal processing, including extrusion, autoclaving, hydro techniques, enzymatic, and harvest treatments, among others (Awulachew, 2022).

Extrusion cooking is still an area of active research and this is a developing technology that reduces the problems found in traditional cooking methods. Therefore, this research was carried out to determine the effect of blend proportions and extrusion temperature on the nutritional and sensory qualities of extruded ready-to-eat (RTE) baby foods produced from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour blends.

5.2 Materials and Methods

5.2.1 Materials

Raw materials used in this study were purchased as described in section 3.2.1 of Chapter Three.

5.2.2 Production of Composite Flour from Orange-Fleshed Sweet Potatoes, Amaranth Seeds, and Soybeans

Orange-fleshed sweet potatoes flour, amaranth seeds flour, and soybeans flour were processed as described in sections 3.2.2, 3.2.3, and 3.2.4 of Chapter Three, respectively.

5.2.3 Blend Formulations and Extrusion Cooking Process

The blends used in this work were identified from the trial run and the available literature. The flour (100 g), sugar (15%), salt (1.5%), baking fat (1.5%), and vanilla essence (1 ml) were gently combined to produce a dough. Distilled water (35 ml) was progressively added while mixing until a well-textured, relatively hard dough was achieved. The dough was kneaded on a clean flat surface. Extrusion cooking process was employed as described in section 4.2.2 of Chapter 4. The firm dough was fed on the hopper of an extruder and extrudates were collected at the end zone of the extruder (die section), dried at 55±5^{o}C, cooled, and sealed in plastic polyethylene bags of 26.8 cm by 27.3 cm, and stored them at room temperature (24±4^{o}C) prior for physicochemical analysis.

5.2.4 Experimental Design

A Factorial Experimental Design with two variables (blend proportions at 5 levels and extrusion cooking temperature at two levels) was used in this study. Blends proportion: C0 (Control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: soybean flour, respectively, and extrusion cooking end barrel temperature (70-90^{o}C) were investigated. The effect of blend proportions and extrusion cooking temperature on the physicochemical quality of extruded ready-to-eat baby foods was studied. The screw speed, feed moisture content and die temperature used in this experiment were identified during the preliminary trial stage. The following model was used:

$$\mathbf{Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}}$$

Where, Y_{ijk}= observation k in level i of factor A and level j of factor B**,** μ = The grand mean
α_i= Effect due to blending, β_j= Effect due to extrusion temperature,
$\alpha\beta_{ij}$= Interaction between blends proportion and temperature, and ε_{ijk} = Random error

5.2.5 Determination of Moisture and Dry Matter Content in Ready-To-Eat Baby Extrudates

Dry matter content was determined by the oven (AOAC, 2000), method 934.01 in which 2 g of sample was measured, placed on a drying dish, capped and maintained at 95-100^{o}C under pressure ≤100 mm Hg (ca. 5 h). The moisture content was estimated using the loss on drying (LOD) (Equation 1). The dry matter content was calculated from equation 2.

$$\text{LOD}\ (\%) = \text{Moisture}\ (\%) = 100 \times \frac{\text{Weight loss on drying (g)}}{\text{Weight of sample (g)}} \qquad (1)$$

$$\text{Dry matter (\%)} = 100 - \%\ \text{LOD} \quad (2)$$

5.2.6 Determination of Crude Protein in Ready-To-Eat Baby Extrudates

The crude protein content was determined by the micro-Kjeldahl (AOAC, 2010), method 920.53. Two grams of samples were placed in the Kjeldahl flask. Anhydrous sodium sulphate (5 g of Kjeldahl catalyst) was added to the flask. Concentrated H_2SO_4 (25 ml) was added with a few boiling samples. The flask was heated in the fume chamber until the sample solution became clear. The sample solution was allowed to cool to room temperature, then transferred into a 25 ml volumetric flask and made up to volume with distilled water. The distillation unit was cleaned, and the apparatus was set up. A solution of 2% boric acid (5 ml) with a few drops of methyl red indicator was introduced into a distillate collector (100 ml conical flask). The conical flask was placed under the condenser. Then 25 ml of sample digest was pipetted into the apparatus and washed down with distilled water. A solution of 60% sodium hydroxide (5 ml) was added to the digest. The sample was heated until 100 ml of distillate was collected in the receiving flask. The content of the receiving flask was titrated with 0.049M H_2SO_4 to a pink-coloured endpoint. A blank with filter paper was subjected to the same procedure and the per cent total nitrogen and crude protein were calculated from Equations 3 and 4.

$$\text{Total Nitrogen (\%)} = \frac{(\text{Titer value} - \text{Blank})}{\text{Weight of sample}} \times \text{Normality of acid} \times \mathrm{N}_2 \quad (3)$$

$$\text{Crude Protein (\%)} = \%\ \text{Total Nitrogen} \times 6.25 \quad (4)$$

Nitrogen factor = 6.25

5.2.7 Determination of Crude Fat Content in Ready-To-Eat Baby Extrudates

This was determined using the Soxhlet extraction with petroleum ether (AOAC, 2010), method 920.39. A 500 ml capacity round bottom flask was filled with 300 ml petroleum ether and fixed to the 121hermos extractor. Two grams of sample were placed in a labelled thimble. The extractor thimble was sealed with cotton wool. The heat was applied to reflux the apparatus for 6 h. The thimble was removed with care. The petroleum ether was recovered for reuse. When the flask was free of ether, it was removed and dried at 105^{o}C for one hr in an oven (Fulton, Model NYC-101 oven). The flask was cooled in desiccators and weighed. The fat content was calculated from Equation 5.

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100 \quad (5)$$

5.2.8 Determination of Total Ash Content in Ready-To-Eat Baby Extrudates

The ash content was determined by muffle furnace at 550 °C for 12 h (AOAC, 2010), method 923.03. Two grams of samples were placed in a silica dish which was ignited, cooled, and weighed. The dish and the sample were ignited first gently and then at 550°C in a muffle furnace (Heraeus, Elisters 2000 Limited, Netherland) until white or grey ash was obtained. The dish and content were cooled in a desiccator and weighed. The total ash content was calculated from Equation 6.

$$\text{Total Ash Content } (\%) = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \qquad (6)$$

Where W_1= weight of the dish, W_2= weight of dish + sample before ashing

W_3= weight of dish + sample after ashing

5.2.9 Determination of Crude Fibre in Ready-To-Eat Baby Extrudates

The crude fibre of ready-to-eat baby foods was determined by the method described by Abifarin *et al*. (2020). Briefly, 1 g of the powdered sample was dissolved in 100 ml of 1.25 % H_2SO_4, boiled for 30 min, filtered under pressure, and the residue was washed with boiling water. This residue was then dissolved in 100 ml of 1.25 % NaOH solution and boiled. The remaining residue was then dried at 100°C and measured after cooling in a desiccator. After that, the final residue was incinerated for 5 hrs in a muffle furnace at 550°C, cooled in a desiccator, and measured again. This was expressed as a percentage weight of the original sample taken for analysis. The crude fibre content was estimated from Equation 7.

$$\text{Crude fibre } (\%) = \frac{W_1 - W_2}{W_3} \times 100 \qquad (7)$$

where W_1 is the oven-dried sample, W_2 is the weight of sample incineration, W_3 is the weight of the sample taken

5.2.10 Determination of Carbohydrates in Ready-To-Eat Baby Extrudates

The total carbohydrate content was determined by the difference method described by Gbadebo and Ahmed (2021).

5.2.11 Determination of Energy Value and Energy-To-Protein Ratio in Ready-To-Eat Baby Extrudates

The calorific value and energy-to-protein ratio were determined using the method of Menezes *et al*. (2015).

5.2.12 Determination of Energy and Nutrient Density in Ready-To-Eat Baby Extrudates

The energy and nutrient contents of extruded ready-to-eat foods were transformed into energy density (kcal/g) and nutrient density (g/100 kcal) as described in the WHO/UNICEF guideline (1998) and Marcel *et al*. (2021). Energy density was calculated by dividing the energy contents of the ready-to-eat baby foods by 100 while nutrient density was determined by dividing the targeted nutrient content of the ready-to-eat baby foods by its energy content and then multiplying by 100.

5.2.13 Determination of Vitamin A Content in Ready-To-Eat Baby Extrudates

The beta-carotene was determined by UV/Visible spectrophotometer according to Rodriguez-Amaya and Kimura (2004) method with modification. Vitamin A in retinol activity equivalent was converted following the method of Trumbo *et al*. (2003).

5.2.14 Determination of Mineral Content in Ready-To-Eat Baby Extrudates

Determination of Minerals (Ca, Fe, Mg, Mn, Zn, and Cu) were carried out by Atomic Absorption Spectrophotometry (Model AA-6300, Serial No A30524300916 SA, Shimadzu Corporation, Japan) (AACC International, 2010), Method 40 – 70.01 and Method 40 – 71.01 for K and Na, respectively

5.2.15 Determination of Phytate Content in Ready-To-Eat Baby Extrudates

Phytates were determined using an available commercial assay kit, K-PHYT 05/2019 (Megazyme International, Ireland) with modifications. A sample of 5 g was extracted for 30 min with 100 ml 0.5N HCl using a magnetic stirrer. The aliquot (10 ml) of the supernatant was transferred to a 40-ml conical centrifuge tube after centrifugation at 3000×g for 10 min. The phytic acid in the samples was precipitated quickly with 4 ml $FeCl_3$ into an aliquot in the centrifuge tubes, and the contents were heated in a boiling water bath for 45 min, yielding a clear supernatant after 30 min. The contents were centrifuged at 3000×g for 10 – 15 min, and the clear supernatant was carefully decanted. The precipitate was washed twice, first in 20 to 25 ml HCl, then in boiling water for 5 to 10 min before centrifugation (3000×g 10 min). The precipitate was washed once again with distilled water. The precipitate was mixed with 2 ml of 2 % NaOH and dispersed in a few millilitre of water. The volume was reduced to around 30 ml with distilled water and then cooked for 30 min in boiling water. Filtration was done on fairly retentive paper when it was hot (quantitatively). The filtrate was discarded after washing the precipitate with 60 to 70 ml of hot distilled water. The precipitate on the filter paper was transferred and dissolved into a 100 ml volumetric flask containing an acid combination of equal amounts (1 ml) of conc. H_2SO_4 and 65% $HClO_4$ solution /0.6 ml of ascorbic acid molybdenum blue. The paper was rinsed with distilled water, the contents were cooled to room temperature, and the volume was increased to 100 ml. A sample of 5 ml was transferred to a new 100-mL volumetric flask and diluted with distilled water to about 70 ml. With distilled water, the volume finally reached 100 ml. Using a spectrophotometer and distilled water as a blank, the absorbance of the sample and standard was measured quickly (within 1 min) at 520 nm. The phytate content was calculated using a potassium dihydrogen phosphate standard curve, and the result was expressed in mg/100g.

5.2.16 Determination of Oxalate Content in Ready-To-Eat Baby Extrudates

Oxalate content was determined by the method described by Fategbe *et al*. (2021) with slight modifications, where 5 g of the sample was added to a flask of 300 ml and 60 ml of 30% HCl and allowed to digest for 1 h, then filtered. Before heating to 90°C in a water bath, the pH of the mixture was adjusted by adding concentrated NH_4OH solution until the colour of the test solutions changed from salmon pink to a pale yellow. After that, it was filtered and cooled (to remove ferrous ion precipitates). The filtrate was heated to 90°C again, and 10 ml of 5% $CaCl_2$ solution was added with steady stirring, and then allowed to cool before being kept overnight at 5°C in the refrigerator. Solutions were then centrifuged at 3000×g for 5 min, supernatants removed, and precipitates dissolved in 10 ml of 20% H_2SO_4 solution and diluted to a total volume of 100 ml. Twenty-five ml of each filtrate was heated until close to the boiling point, then titrated against 0.05 M standardized $KMnO_4$ until a faint pink colour developed and remained for 30 s. The oxalate content was estimated by considering that 1 ml of 0.05M $KMnO_4$ solution correspondent to 0.00225 g anhydrous oxalic acid (Equation 8).

$$\text{Oxalate (mg/g)} = \frac{\text{Title Value} \times 100 \times 0.00225}{\text{Weight of Sample}} \qquad (8)$$

5.2.17 Determination of Saponins Content in Ready-To-Eat Baby Extrudates

Saponin content was determined by the method described by Abifarin *et al*. (2020), in which 5 g of the sample was measured into a beaker containing 200 ml of 20% ethanol. The solution was heated in a hot water bath for 4 hr at 55°C and filtered with Whatman filter paper, and the residue was re-extracted with another 50 ml of 20% ethanol. The filtrates were mixed and concentrated to 20 ml over the hot water bath at 90°C. The solution obtained was transferred into a 250 ml separating funnel containing 20 ml of diethyl ether. The aqueous layer was collected; 20 ml of n-butanol was added to it and then washed thrice with 10 ml of 5%

sodium chloride while the ether layer was thrown away. The mixture was oven dried to constant weight, and the percentage saponin content of the sample was calculated from equation 9:

$$\text{Saponin (\%)} = \frac{\text{A} - \text{B}}{\text{Sw}} \times 100 \tag{9}$$

where A = Mass of flask and extract, B = Mass of the empty flask and Sw = Sample weight

5.2.18 Determination of Expansion Ratio in Ready-To-Eat Baby Extrudates

Extrudates were measured using a digital electronic Vernier calliper (Mitutoyo Digital Calliper, Japan) with a 0.01- mm accuracy. Fifteen measurements were taken for each sample, and the average diameter was calculated. To determine the expansion ratio (ER), the diameter of the extrudate was divided by the die diameter as shown in equation 10 (Nagaraju *et al.*, 2021).

$$\text{ER} = \frac{\text{Extrudate Diameter}}{\text{Die Diameter}} \tag{10}$$

The sectional expansion ratio (SER) for the extrudates may also be calculated by dividing the square of extrudate diameter (Ed) by the square of die diameter (Dd) as presented in equation 11.

$$\text{SER} = \frac{\text{Ed}^2}{\text{Dd}^2} \tag{11}$$

5.2.19 Determination of Bulk Density in Ready-To-Eat Baby Extrudates

The bulk density of extrudate was calculated using the method described by Molla and Zegeye (2020).

5.2.20 Sensory Evaluation of Ready-To-Eat Baby Extrudates

Consumer acceptability test was used where sixty-five untrained panellists were used to assess the sensory quality of extruded ready-to-eat baby foods. The breastfeeding mothers and mothers who have children under under-five years of age evaluated RTE baby foods individually in testing booths under controlled conditions and without distraction. Mothers who have under-five year children were chosen to carry out the sensory evaluation of extruded ready-to-eat baby foods because under-five years children are verbal scale limited. Furthermore, smiling scale could have used for sensory evaluation but it has immense disadvantages such as lack of detailed information, subjective interpretation, limited discrimination ability, cultural and individual biases, insufficient granularity, limited versatility and over-reliance on emotional aspect. The panellists were reminded not participate in this study if they are sick, pregnant, or not breastfeeding, if they do not have a child under the age of five, or if they do not consume one of the ingredients used to make these baby foods (OFSP, Soybean and Amaranth seeds). The panellists were individually given a consent form and a hedonic assessment form with instructions as in Appendix E and F, and the attributes that were assessed, were based on appearance, colour, aroma, taste, texture, and overall acceptability using a 5-point hedonic scale (Singh-Ackbarali & Maharaj, 2014).

5.2.21 Data Analysis

The data for proximate composition, minerals, vitamin A content, anti-nutrients, physical properties, and sensory qualities were statistically analyzed using SAS version 9.4 TS Level 1M6 (SAS Institute Inc.). Basic statistical measures and goodness-of-Fit tests were carried out for the normal distribution of data using PROC UNIVARIATE while the LEVENE test was conducted for standard homogeneity of variance using HOVTEST=LEVENE. Data were presented as mean ± standard deviation of triplicate determination. Mean values were analysed using the analysis of variance (ANOVA), and the effect of blend proportions and extrusion cooking temperature was carried out using PROC GLM and mean separations were tested using Tukey's Studentized Range (HSD) Test at a 5% significance level.

5.3 Results and Discussion

5.3.1 The Nutrient Density of Ready-To-Eat Baby Foods

After statistical analysis, it was discovered that all tested parameters differ significantly ($p<0.05$) among samples. The nutrient density of the extruded ready-to-eat baby foods is shown in Table 5.1 and its graphical presentation is illustrated in Figure 5.1. The protein density of extruded ready-to-eat baby foods varied from 0.78 to 4.26 g/100 kcal.

This falls within the results (3.50–4.79 g/100kcal) reported by Tenagashaw *et al*. (2017) for complementary foods formulated from a blend of teff, soybean, and orange-fleshed sweet potato but also confirms well with the level (<5.5 g/100 kcal) recommended for young children (1–3 years old) according to Codex standard. When the food surpasses the recommended protein-energy (PE) ratio, protein nutrition difficulties will be caused by insufficient food rather than a lack of protein. PE ratios in most typical foods range between 10% and 15%. Human breast milk has a PE ratio of around 7%, which is sufficient for fast development in the early months of childhood.

Table 5. 1 Nutrient and Energy Density of extruded ready-to-eat baby foods

Blends Proportions	ET (ºC)	Protein Density (g/100Kcal)	Fat Density (g/100Kcal)	Carbohydrate Density (g/100Kcal)	Energy Density (Kcal/g)	Vitamin A Density (RAE µg/100Kcal)
C0	70	0.79 ± 0.00^{g}	0.82 ± 0.00^{e}	21.88 ± 0.01^{a}	3.68 ± 0.00^{cd}	284.13 ± 0.17^{a}
C1	70	4.22 ± 0.00^{b}	1.63 ± 0.00^{a}	16.45 ± 0.01^{g}	3.72 ± 0.00^{b}	190.91 ± 0.30^{e}
C2	70	3.85 ± 0.01^{e}	1.36 ± 0.01^{cd}	17.41 ± 0.01^{bc}	3.69 ± 0.01^{c}	195.87 ± 0.31^{cd}
C3	70	4.26 ± 0.02^{a}	1.48 ± 0.01^{b}	16.77 ± 0.02^{f}	3.67 ± 0.01^{d}	196.82 ± 0.39^{c}
C4	70	3.89 ± 0.01^{d}	1.41 ± 0.03^{c}	17.29 ± 0.06^{cd}	3.73 ± 0.01^{b}	195.49 ± 0.15^{d}
C0	90	0.78 ± 0.00^{g}	0.79 ± 0.01^{e}	21.97 ± 0.02^{a}	3.67 ± 0.00^{d}	273.86 ± 0.43^{b}
C1	90	3.94 ± 0.01^{c}	1.50 ± 0.06^{b}	17.06 ± 0.12^{e}	3.81 ± 0.01^{a}	182.63 ± 0.43^{g}
C2	90	3.84 ± 0.00^{e}	1.34 ± 0.01^{d}	17.46 ± 0.03^{b}	3.63 ± 0.00^{e}	190.57 ± 0.32^{e}
C3	90	3.94 ± 0.02^{c}	1.41 ± 0.01^{c}	17.27 ± 0.02^{d}	3.81 ± 0.01^{a}	178.30 ± 0.69^{g}
C4	90	3.75 ± 0.01^{f}	1.37 ± 0.01^{cd}	17.54 ± 0.02^{b}	3.74 ± 0.01^{b}	176.57 ± 0.22^{h}
Codex Standard		<5.5	4.4-6.0	9.0-14.0	>0.8	60-180

Data are indicated in triplicate values as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ($p \leq 0.05$). Where BP is the blend proportions, ET: Extrusion temperature; C0 (Control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: Soybean flour, respectively.

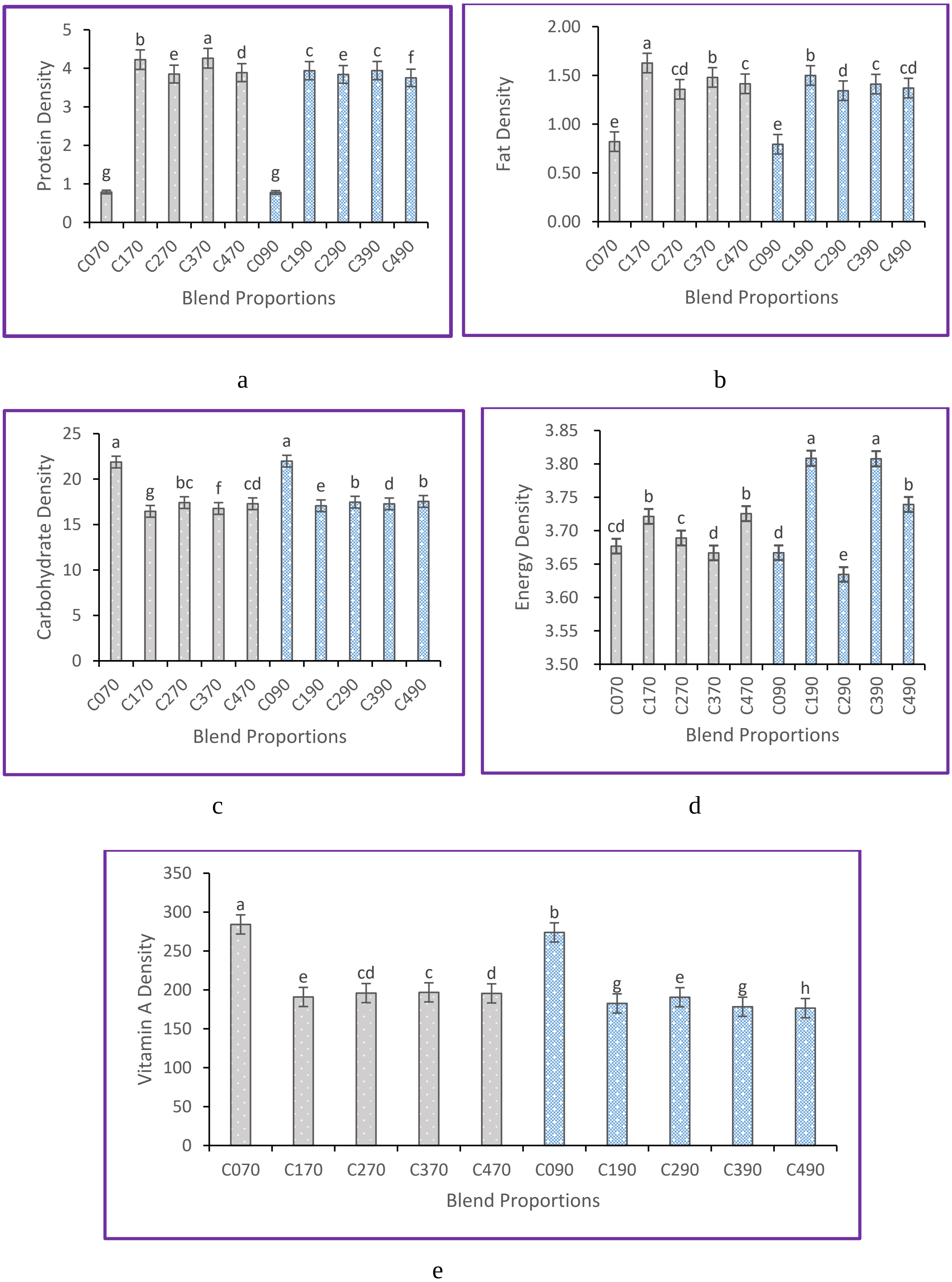

Figure 5. 1 Graph representing protein (a), fat (b), carbohydrate (c), energy (d), and vitamin A density of extruded ready-to-eat baby foods. These means bars having different letters are significantly different at $p < 0.05$. C070–C470, are blends proportions extruded at 70°C while C090–C490, are blends proportions extruded at 90°C.

The total fat density ranged from 0.79 to 1.63 g/100 kcal. This is in the agreement with the range 1.26–1.66 g/100 kcal reported by Tenagashaw *et al*. (2017) for complementary foods formulated from a blend of teff, soybean, and orange-fleshed sweet potato. This is below the maximum level of lipid (3.3 g/100 kcal) required from processed cereal-based foods for young children by the Codex Alimentarius standard. The codex also recommends a minimum of 4.4 g/100kcal and a maximum of 6.0 g/100 kcal of total fat density for infant formulas with no guidance on upper levels.

The carbohydrate density ranged from 16.45 to 21.97 g/100 kcal. Similar findings (18.22–20.05 g/100 kcal were reported by Tenagashaw *et al*. (2017) for complementary foods formulated from a blend of teff, soybean, and orange-fleshed sweet potato. There is no recommended level of carbohydrate density for processed young children's foods by the Codex Alimentarius Commission. However, it recommends carbohydrate density for infant formulas to fall within 9.0–14.0 g/100 kcal though there is no guidance on upper levels for carbohydrate density. The recommended dietary allowance of carbohydrates is 80-95 g/day for infants, and 130 g/day for children (Whitney & Rolfes, 2019).

The energy density ranged from 3.63 to 3.81 kcal/g. the codex recommends that the energy density of cereal-based foods should not be less than 0.8 kcal/g for infant and young children's formulas. The vitamin A density ranged from 176.57 to 284.13 RAE µg/100 kcal. These are within and above the range of 60–180 RAE µg/100 kcal of vitamin A recommended by codex standard for young children. There is no guidance on upper levels of vitamin A provided by the Codex Alimentarius Commission for young children but a tolerable upper intake level (UL) of 3000 µg/day of preformed vitamin A has been established for pregnancy and lactating mothers, 600 µg/day for infants and children aged 1–3 years old, 900 µg/day for children aged 4–8 years old and 1700 µg/day for children aged 9–13 years old (Gropper *et al*., 2022).

5.3.2 Nutritional Contents of Ready-To-Eat Baby Foods

The results from nutritional contents of ready-to-eat baby foods are given in Table 5.2 and significant differences ($p<0.05$) among samples were observed. In addition, blend proportions and extrusion cooking temperature significantly ($p<0.05$) affected the nutritional composition of ready-to-eat baby foods. The dry matter content, crude protein content, crude fibre content, total ash content, crude fat content, carbohydrate content, energy value, energy-to-protein value and vitamin A content of ready-to-eat baby foods as shown in Table 5.2, ranged from 91.87 to 95.47%, (2.85% – 15.72%), (3.39% - 5.04%), (4.60% – 5.39%), (2.91%

– 6.05%), (61.23% – 80.58%), (363.44 – 380.84 kcal/100g), (23.47 – 128.67 kcal/g of protein), (660.23 – 1044.70 RAE μg/100g), respectively.

5.3.3 Dry Matter Content and Moisture Content of Ready-To-Eat Baby Foods

The dry matter content of extruded ready-to-eat baby foods ranged from 91.87 to 95.47% which indicates 4.53% to 8.13% of moisture content. The moisture content is a measure of the water content available in food and is an index of the storage stability of the noodles and also an indication of the dry matter in that food (Adegunwa *et al*., 2014). The results are slightly above the results (1.86% to 5.2%) reported by Oke *et al*. (2022) for noodles from wheat-tigernut pomace flour blends and 5.9%-6% reported by Osibanjo *et al*. (2022) for extrudates from maize/soybean blends but confirm well with the results (3.10± 0.001 to 7.02± 0.003%) reported by Umoh and Iwe (2022), for the extruded aerial yam-soy bean flour blends. The moisture content of extruded ready-to-eat baby foods was typically lower than the 14% permitted by the Codex Alimentarius Commission (CAC) for non-fried noodles and less than 10% for fried noodles (CAC, 2019), which makes it appealing for long-term storage. The low moisture content of extruded ready-to-eat baby foods makes them less susceptible to microbial attack and would have longer shelf-life stability as well as a decreased risk of physical and chemical reactions that might deteriorate food quality.

5.3.4 Protein Contents of Ready-To-Eat Baby Foods

Proteins are a common class of biomolecules that are essential to the food industry as elements to give food products flavour, texture, and taste as well as nutritional and functional qualities (Aryee *et al*., 2018). The protein content of extruded ready-to-eat baby foods ranged from 2.85% – 15.72%. The results are close to the values (12.5% – 21.24%) reported by Osibanjo *et al*. (2022) for extrudates from maize/soybean blends and slightly below the results (16.38% – 33.46%) obtained by Sobowale *et al*. (2021) for extruded cocoyam noodles. The findings are also above the trends (2.91% to 4.28%) reported by Oke *et al*. (2022) for noodles from wheat-tiger nut pomace flour blends and slightly above the results (4.38±1.24% – 13.13±1.24%) reported by Maximus (2019) for ready-to-eat breakfast cereals from blends of sorghum, pigeon pea, and mango flour. The lowest protein content (2.85%) was observed in the control sample while blends C1 and C3 yielded high protein content of 15.72% and 15.62%, respectively. This may be the result of the incorporation of amaranth seeds and soybean flour which have a high quantity of protein at 19.80% and 31.53%, respectively, as observed from the previous research.

Table 5. 2 Nutritional compositions of ready-to-eat baby foods

BP	ET (ºC)	DM (%)	Crude Protein (%)	Crude Fibre (%)	Crude Ash (%)	Crude Fat (%)	CHO (%)	EV (kcal/100g)	ETPR (Kcal/g of Protein)	Vitamin A (RAE µg/100g)
C0	70	94.55±0.15^{b}	2.90±0.01^{g}	3.61±0.06^{e}	4.60±0.04^{e}	3.02±0.01^{g}	80.43±0.08^{a}	367.68±0.41^{cd}	126.93±0.14^{b}	1044.70±0.55^{a}
C1	70	92.68±0.10^{d}	15.72±0.03^{a}	4.93±0.01^{ab}	4.75±0.01^{d}	6.05±0.02^{a}	61.23±0.09^{f}	372.13±0.42^{b}	23.67±0.02^{f}	710.45±0.32^{e}
C2	70	93.29±0.20^{c}	14.20±0.06^{d}	5.04±0.03^{a}	4.80±0.01^{cd}	5.01±0.02^{ef}	64.24±0.17^{d}	368.91±0.69^{c}	25.97±0.08^{d}	722.59±0.32^{d}
C3	70	95.47±0.26^{a}	15.62±0.05^{b}	4.71±0.06^{d}	4.63±0.01^{e}	5.42±0.04^{c}	61.49±0.21^{e}	366.67±0.86^{d}	23.47±0.13^{f}	721.67±0.32^{d}
C4	70	94.15±0.14^{b}	14.48±0.04^{d}	4.78±0.05$^{\mathbf{bcd}}$	4.66±0.01^{e}	5.27±0.10^{cd}	64.41±0.28^{cd}	372.55±0.28^{b}	25.72±0.05^{de}	728.29±0.32^{c}
C0	90	94.58±0.07^{b}	2.85±0.01^{g}	3.39±0.05^{f}	4.85±0.02^{c}	2.91±0.03^{g}	80.58±0.05^{a}	366.70±0.37^{d}	128.67±0.58^{a}	1004.23±0.84^{b}
C1	90	95.41±0.30^{a}	15.00±0.03^{c}	4.75±0.12^{cd}	4.96±0.02^{b}	5.71±0.23^{b}	64.99±0.40^{c}	380.84±0.78^{a}	25.39±0.08^{e}	695.55±0.32^{f}
C2	90	92.61±0.06^{d}	13.96±0.01^{f}	4.92±0.04^{abc}	5.39±0.07^{a}	4.88±0.05^{f}	63.47±0.10^{e}	363.45±0.50^{e}	26.04±0.03^{d}	692.60±0.55^{g}
C3	90	91.87±0.13^{e}	15.00±0.02^{c}	4.66±0.04^{d}	4.66±0.01^{e}	5.37±0.05^{c}	65.78±0.22^{b}	380.77±1.21^{a}	25.38±0.11^{e}	678.92±0.55^{h}
C4	90	93.61±0.17^{c}	14.03±0.01^{f}	4.65±0.05^{d}	4.75±0.02^{d}	5.12±0.03^{de}	65.60±0.15^{b}	373.92±0.62^{b}	26.65±0.06^{c}	660.23±0.32^{i}

Data are indicated in triplicate values as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ($p \leq 0.05$). Where BP is the blend proportions. ET: Extrusion temperature, CHO: carbohydrate, EV: energy value, ETPR: Energy-to-protein ratio, C0: Orange-fleshed sweet potatoes (OFSP) as Control; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; C4:54.5:26.5:19 for OFSP: Amaranth seeds: soybeans, respectively.

These high values of protein in extruded ready-to-eat baby foods indicate that they could be a potentially rich source of protein required by humans for proper growth and development. All the extruded ready-to-eat baby foods meet the recommended dietary allowance of protein (13 g/day) for children aged 1–3 years except the control sample (Stathers *et al*., 2018). The extrusion cooking of plant-based proteins improves protein digestibility which is primarily linked to the inactivation and destruction of anti-nutrients compounds present in foods and the thermal denaturation of protein. Anti-nutrients may have a detrimental impact on human health by lowering mineral and protein bioavailability and protein digestibility (Ndidi *et al*., 2014). In addition, further changes in protein quality (amino acid profile) and protein functionality (texturization and solubility) may occur in various extrusion cooking conditions (Gulati *et al*., 2020).

5.3.5 Fat Contents of Ready-To-Eat Baby Foods

Fats have an important part in providing flavour, lubricity, and texture to foods, as well as contributing to the perception of satiety after consumption (Türkay & Şahin-Yeşilçubuk, 2017). The fat content of extruded ready-to-eat baby foods ranged from 2.91% – 6.05% and fell within the results (2.47%–8.98%) reported by Sobowale *et al*. (2021) for extruded cocoyam noodles, 1.24% to 5.14% reported by Oke *et al*. (2022) for noodles from wheat-tiger nut pomace flour blends, and compared well to the results (3.08±1.24% to 5.88±0.00%) found by Maximus (2019) for ready-to-eat breakfast cereals from blends of sorghum, pigeon pea, and mango flour. During extrusion cooking, low quantities of lipids as low as 5% tend to stabilize Specific Mechanical Energy (SME) and minimize glassiness in extrudates. However, high lipid concentrations reduce SME by increasing lubricity inside the extruder barrel and reducing expansion (Singh *et al*., 2007). From a nutritional perspective, lipids tend to react with starch and protein which results in the formation of starch-lipid complexes and lipid-protein complexes, respectively. Lipids act as a plasticizer and also impart adhesive texture. Higher temperatures would inactivate lipase and lipoxygenase activity and thus reduce the development and oxidation of fatty acids (Mathad *et al*., 2022).

5.3.6 Total Ash Contents of Ready-To-Eat Baby Foods

The ash content of extruded ready-to-eat baby foods ranged from 4.60% – 5.39%. The results are above the results (1% to 1.75%) reported by Oke *et al*. (2022) for noodles from wheat-tiger nut pomace flour blends and 2.15±0.35% to 4.15±0.35% found by Maximus (2019) for ready-to-eat breakfast cereals from blends of sorghum, pigeon pea, and mango flour but confirm well with the range (4.03±0.002 to 5.90±0.002%) reported by Umoh and Iwe (2022), for the extruded aerial yam-soy bean flour blends. The high values for ash content are an indication that extruded ready-to-eat baby foods from OFSP, amaranth seeds, and soybean flour blends have a potentially rich source of minerals.

5.3.7 Fibre Contents of Ready-To-Eat Baby Foods

The crude fibre content of extruded ready-to-eat baby foods ranged from 3.39% - 5.04%. The results are below the trends (5.78% to 14.81%) reported by Oke *et al*. (2022) for noodles from wheat-tiger nut pomace flour blends but fall within the results (3.80±1.25% to 13.50±0.25%) reported by Maximus (2019) for ready-to-eat breakfast cereals from blends of sorghum, pigeon pea, and mango flour, and confirm well with the trends (2.70±0.001 to 4.67±0.003%) reported by Umoh and Iwe (2022), for the extruded aerial yam-soy bean flour blends. The high values of fibre content is an indication that the extruded ready-to-eat baby foods from OFSP, amaranth seeds, and soybean flour blends are a potentially rich source of dietary fibre, which is important for the proper flow of food through the digestive tract and aid in the prevention of obesity, diabetes, colon cancer, and other conditions affecting the human gastrointestinal tract.

5.3.8 Carbohydrate Contents of Ready-To-Eat Baby Foods

The carbohydrate content of extruded ready-to-eat baby foods ranged from 61.23% - 80.58%. The results confirm well with the trends (73.23% to 83.9%) reported by Oke *et al*. (2022) for noodles from wheat-tiger nut pomace flour blends and fall within the values (50.65±0.34% to 77.13±1.8%) obtained by Maximus (2019) for ready-to-eat breakfast cereals from blends of sorghum, pigeon pea, and mango flour. The results compared well to the results (68.95%–77.61%) reported by Osibanjo *et al*. (2022) for extrudates from maize/soybean blends. The high range of values for carbohydrate content is an indication that extruded ready-to-eat baby foods from OFSP, amaranth seeds, and soybean flour blends is a potentially rich source of energy. The high value of the carbohydrate contents of extruded ready-to-eat baby foods may be attributed to the OFSP substitution. A few changes occur during the extrusion process, including the degradation of granular structure, the disruption of glycosidic bonding, and the creation of new molecular interactions (Agarwal & Chauhan, 2019).

5.3.9 Energy Contents of Ready-To-Eat Baby Foods

Energy values of extruded ready-to-eat baby foods ranged from 363.44 – 380.84 kcal/100g. the results are in agreement with the values (360.69-391.88 kcal/100g) reported by Awofadeju *et al*. (2021) for extruded ready-to-eat breakfast snacks and 362.59 to 371.50 kcal/100g reported by Edima-Nyah *et al*. (2020) for breakfast cereals produced with yellow maize, soybean, and banana blends. In addition, the results are slightly below the value (387.89 kcal/100g) reported by Naseer *et al*. (2021), for extrudate almond- pearl millet (APM) based snack but close to the range (383.58 to 409.12 kcal/100 g) reported by Gemede (2020) for complementary foods formulated from maize, pea, and anchiote flours. The high energy content observed in this study may be the result of the carbohydrate, fat, and protein content of extruded ready-to-eat baby foods. Energy is necessary to maintain the body's different processes, such as breathing, circulation, physical work, and protein synthesis.

5.3.10 Energy-To-Protein Ratio of Ready-To-Eat Baby Foods

Energy-to-protein ratio ranged from 23.47 – 128.67 kcal/g of protein. Studies have demonstrated the importance of the energy-to-protein ratio in broiler growth to maximize the use of amino acids and energy. Lean muscle can result from a diet that has a lower energy-to-protein ratio and higher feed intake, whereas a diet with a higher energy-to-protein ratio can cause lower feed intake and higher energy retention as fat (Musigwa *et al*., 2021). Energy-to-protein ratio of extruded ready-to-eat baby foods varied from 23.47 – 26.65 kcal/g of protein except for 128.67 kcal/g of protein from the control sample. The highest energy-to-protein ratio observed in this study could be the result of the low protein content of the control sample.

5.3.11 Vitamin A Contents of Ready-To-Eat Baby Foods

Vitamin A content of extruded ready-to-eat baby foods ranged from 660.23 – 1044.70 RAE μg/100g and a significant ($p<0.05$) difference was observed among samples. The results obtained from this study are in agreement with the results (53.03 – 8193 RAE μg/100 g reported by Kure *et al*. (2021) for bread from wheat and orange-fleshed sweet potato (flour, starch, and non-starch residue flour) blends. Furthermore, the results are within the range (370–2064±17 RAE μg/100g) reported by Adetola *et al*. (2020), for complementary foods from OFSP, soybean, and carrot flour blends. The highest value of vitamin A was observed in the control sample. This may be due to the high content of vitamin A present in OFSP flour.

Vitamins are a minor but necessary component of the diet. Deficiencies in these micronutrients can disrupt normal human growth, maintenance, and functioning, leading to various diseased situations. Inadequate vitamin intake might result from an imbalanced diet or vitamin losses caused by processing (Gulati *et al*., 2020). A lack of vitamin A has been linked to a decrease in lymphocytes, natural killer cells, and antigen-specific immunoglobulin responses (Institute of Medicine, 2001). Vitamin A is an important nutrient that is necessary for immunological function. It is commonly referred to as retinol since it releases pigment in the retina of the eye (Kure *et al*., 2021). Vitamin A is necessary for appropriate vision, gene expression, cellular differentiation, morphogenesis, growth, and immunological function (Institute of Medicine, 2001).

One of the most promising plant sources of beta-carotene, pro-vitamin A, has been discovered to be orange-fleshed sweet potatoes. For young children, a 100-150 g portion of boiling orange-fleshed sweet potato tubers can meet their daily vitamin A needs and prevent blindness (Mitra, 2012). Apart from infants and young children, the extruded ready-to-eat baby foods meet the recommended daily intake (770 RAE µg/day) of vitamin A for pregnant women. The recommended dietary allowances for vitamin A are 400–500 RAE RAE µg/day for infants, 300-400 for children, and 1300 RAE µg/day for lactating mothers (Gropper *et al*., 2022). The stability of vitamins during extrusion cooking is dependent on (a) processing factors such as temperature, residence time, screw speed, pressure, feed rate, and screw configuration, and (b) the source of the vitamin, chemical structure, and interactions with other flour components (Riaz *et al*., 2009).

5.3.12 Effect of Blends Proportions and Extrusion Temperature on the Nutritional Composition of Ready-To-Eat Baby Foods

The extrusion cooking temperature and blend proportions significantly ($p<0.05$) affected the nutritional composition of extruded ready-to-eat baby foods. Considering extrusion cooking at 70^{o}C, the moisture content, carbohydrate content, energy value, energy-to-protein value, and vitamin A content of extruded ready-to-eat baby foods were significantly ($p<0.05$) affected by extrusion cooking and blend proportions except for blends C1 and C2 in terms of energy-to-protein value (Table 5.3 and Table 5.4). The crude protein content, fibre content, ash, and fat content were not significantly ($p>0.05$) affected by extrusion cooking and blends proportions though changes occurred.

Table 5. 3 Effect of blend proportions and extrusion temperature on the nutritional composition of ready-to-eat baby foods

Data for ready-to-eat baby foods extruded at 70°C										
BP	**Statistics**	**MC**	**Protein**	**Fibre**	**Ash**	**Fat**	**CHO**	**EV**	**ETPV**	**Vitamin A**
C0	Non-Extruded	10.00±0.00	2.95±0.04	3.66±0.13	4.60±0.19	3.05±0.28	75.73±0.53	349.53±0.65	118.37±1.69	1,162.50±0.17
	Extruded	5.45±0.15	2.90±0.01	3.61±0.06	4.60±0.04	3.02±0.01	80.43±0.08	367.68±0.41	126.93±0.14	1,044.70±0.55
	t-value	51.97	2.40	0.71	0.03	0.22	-15.26	-41.07	-8.75	352.74
	p-value	<0.0001****	0.133^{ns}	0.54^{ns}	0.98^{ns}	0.84^{ns}	0.004**	<0.0001****	0.012*	<0.0001****
	Change (%)	45.53^{a}	1.92^{a}	1.55^{a}	0.07^{a}	1.22^{a}	5.85^{b}	4.94^{b}	6.75^{b}	11.28^{a}
C1	Non-Extruded	11.33±0.58	15.83±0.31	4.94±0.81	4.75±0.14	6.16±0.19	56.99±0.47	356.56±0.89	22.53±0.49	780.17±0.38
	Extruded	5.45±0.15	15.72±0.03	4.93±0.01	4.75±0.01	6.05±0.02	61.23±0.09	372.13±0.42	23.67±0.02	710.45±0.32
	t-value	11.88	0.64	0.021	0.042	0.932	-15.48	-27.29	-4.083	244.033
	p-value	0.006**	0.59^{ns}	0.99^{ns}	0.97^{ns}	0.449^{ns}	0.003**	0.0002***	0.055^{ns}	<0.0001****
	Change (%)	35.41^{a}	0.72^{a}	0.20^{a}	0.07^{a}	1.71^{b}	6.93^{b}	4.18^{b}	4.84^{b}	9.81^{a}
C2	Non-Extruded	11.10±0.17	14.50±0.50	5.00±0.10	4.83±0.06	5.07±0.12	59.50±0.43	351.61±0.70	24.27±0.89	800.00±0.17
	Extruded	6.71±0.20	14.20±0.06	5.04±0.03	4.80±0.01	5.01±0.02	64.24±0.17	368.91±0.69	25.97±0.08	722.59±0.32

	t-value	28.834	1.021	0.62	0.843	0.883	-17.69	-30.484	-3.321	369.71
	p-value	<0.0001****	0.412^{ns}	0.594^{ns}	0.484^{ns}	0.465^{ns}	0.001^{***}	<0.0001****	0.078^{ns}	<0.0001****
	Change (%)	39.52^{a}	2.09^{a}	0.73^{b}	0.62^{a}	1.20^{a}	7.37^{b}	4.69^{b}	6.56^{b}	10.71^{a}
C3	Non-Extruded	10.34±0.30	15.77±0.25	4.70±0.18	4.64±0.06	5.77±0.25	58.78±0.48	359.49±0.34	22.80±0.38	785.03±0.15
	Extruded	8.13±0.13	15.62±0.05	4.71±0.06	4.63±0.01	5.42±0.04	61.49±0.21	366.67±0.86	23.47±0.13	721.67±0.32
	t-value	11.89	0.967	-0.062	0.306	2.331	-8.983	-13.41	-2.865	310.593
	p-value	0.0003^{***}	0.388^{ns}	0.954^{ns}	0.775^{ns}	0.080^{ns}	0.001^{***}	0.0002^{***}	0.046^{*}	<0.0001****
	Change (%)	21.43^{a}	0.92^{a}	0.14^{b}	0.22^{a}	6.33^{a}	4.40^{b}	1.96^{b}	2.84^{b}	8.78^{a}
C4	Non-Extruded	10.33±0.58	14.68±0.35	4.79±0.01	4.65±0.05	5.38±0.42	60.16±1.04	357.36±0.64	24.35±0.63	801.03±0.15
	Extruded	6.39±0.17	14.48±0.04	4.78±0.05	4.66±0.01	5.27±0.10	64.41±0.28	372.55±0.28	25.72±0.05	728.29±0.32
	t-value	11.325	0.97	0.342	-0.450	0.441	-6.814	-37.644	-3.753	356.56
	p-value	0.0003^{***}	0.387^{ns}	0.750^{ns}	0.676^{ns}	0.682^{ns}	0.002^{**}	<0.0001****	0.020^{*}	<0.0001****
	Change (%)	38.16^{a}	1.38^{a}	0.21^{a}	0.29^{b}	2.09^{a}	6.60^{b}	4.08^{b}	5.35^{b}	9.99^{a}

Where C0 (Control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: Soybean flour, respectively. *, **, ***, ****, ns, a, b = Significant at $p \le 0.05$, $p \le 0.01$, $p \le 0.001$, $p \le 0.0001$, Not significant, Loss, Retention, respectively.

The extrusion cooking at 90°C, on the other hand, significantly ($p<0.05$) affected the moisture content, crude protein content, carbohydrate content, energy value, energy-to-protein value, and vitamin A content of extruded ready-to-eat baby foods in all tested samples (Table 5.4). However, only fat content was not significantly ($p>0.05$) affected by extrusion cooking and blend proportions in all samples. Blends C2 and C4 were significantly ($p<0.05$) affected by extrusion cooking and blend proportions in terms of total ash content. The extrusion cooking significantly ($p<0.05$) reduced vitamin A content by 21.33%, moisture content (59.47%), crude protein content (5.56%), and crude fibre content (3.06%) of extruded ready-to-eat baby foods in all tested samples while an increase of total minerals by 10.44%, carbohydrate content (12.31%), energy value (6.38%), energy-to-protein value (11.28%) were significantly ($p<0.05$) observed in all tested samples. Jozinovi´c *et al*. (2021) reported similar observations where extrusion cooking causes a loss in protein content and an increase in the total ash content of value-added corn snack products.

In addition, Iwe *et al*. (2001) reported an increase in carbohydrate content and energy value of extruded soy and sweet potato flours upon extrusion cooking. Extrusion cooking and post-extrusion process have been known to reduce heat-labile nutrients, anti-nutrients, and microbial elimination and increase product shelf-life by lowering the moisture content of extrudates. Thermal treatment of food containing protein can result in the breaking of disulphide bonds, unfolding, aggregation, and production of dimers and bigger oligomers of proteins (Aryee *et al*., 2018). On the other hand, the degree of the changes is influenced by moisture content, pressure, temperature, and shearing force (Agarwal & Chauhan, 2019). The increase in the carbohydrate contents of extruded ready-to-eat baby foods could be the result of high temperature, shear, and pressure involved during the extrusion cooking. When starch is extruded, it goes through several structural changes, including melting, gelatinization, and fragmentation (Agarwal & Chauhan, 2019).

Table 5. 4 Effect of blend proportions and extrusion temperature on the nutritional composition of ready-to-eat baby foods

Data for ready-to-eat baby foods extruded at 90ºC										
BP	**Statistics**	**MC**	**Protein**	**Fibre**	**Ash**	**Fat**	**CHO**	**EV**	**ETPV**	**Vitamin A**
C0	Extruded	5.42±0.07	2.85±0.01	3.39±0.05	4.85±0.02	2.91±0.03	80.58±0.05	366.70±0.37	128.67±0.58	1,004.23±0.84
	t-value	113.326	4.299	3.441	-2.166	0.870	-15.865	-39.856	-9.984	318.528
	p-value	<0.0001****	0.013*	0.026*	0.096ns	0.433ns	<0.0001****	<0.0001****	0.001***	<0.0001****
	Change (%)	45.80a	3.63a	7.37a	5.02b	4.93a	6.02b	4.68b	8.01b	15.76a
C1	Extruded	4.59±0.30	15.00±0.03	4.75±0.12	4.96±0.02	5.71±0.23	64.99±0.40	380.84±0.78	25.39±0.08	695.55±0.32
	t-value	17.984	4.71	0.387	-2.551	2.616	-22.547	-35.449	-10.056	296.190
	p-value	<0.0001****	0.009**	0.718ns	0.063ns	0.059ns	<0.0001****	<0.0001****	0.001***	<0.0001****
	Change (%)	59.47a	5.56a	3.71a	4.10b	7.82a	12.31b	6.38b	11.28b	12.17a
C2	Extruded	7.39±0.06	13.96±0.01	4.92±0.04	5.39±0.07	4.88±0.05	63.47±0.10	363.45±0.50	26.04±0.03	692.60±0.55
	t-value	35.387	1.882	1.338	-10.819	2.569	-15.539	-23.927	-3.464	321.591
	p-value	<0.0001****	0.133ns	0.252ns	0.0004***	0.062ns	<0.0001****	<0.0001****	0.026*	<0.0001****
	Change (%)	33.45a	3.89a	1.67a	10.44b	3.83a	6.24b	3.26b	6.80b	15.51a
C3	Extruded	4.53±0.26	15.00±0.02	4.66±0.01	4.66±0.04	5.37±0.05	65.78±0.22	380.77±1.21	25.38±0.11	678.92±0.55
	t-value	25.41	5.236	0.417	-0.612	2.678	-22.983	-29.211	-11.281	320.971

	p-value	<0.0001****	0.006**	0.698ns	0.573ns	0.055ns	<0.0001****	<0.0001****	0.0004***	<0.0001****
	Change (%)	56.20a	5.09a	0.92a	0.43b	7.39a	10.64b	5.59b	10.15b	15.63a
C4	Extruded	5.85±0.14	14.03±0.01	4.65±0.05	4.75±0.02	5.12±0.03	65.60±0.15	373.92±0.62	26.65±0.06	660.23±0.32
	t-value	13.071	3.189	4.919	-3.216	1.042	-8.938	-32.318	-6.284	690.20
	p-value	0.0002***	0.033*	0.008**	0.032*	0.356ns	0.001***	<0.0001****	0.023*	<0.0001****
	Change (%)	43.39a	4.66a	3.06a	2.11b	4.94a	8.29b	4.43b	8.65b	21.33a

Where C0 (Control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: Soybean flour, respectively. *, **, ***, ****, ns, a, b = Significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, $p \leq 0.0001$, Not significant, Loss, Retention, respectively.

5.3.13 Mineral Composition of Ready-To-Eat Baby Foods

The mineral composition of extruded ready-to-eat baby foods significantly ($p<0.05$) differs among the tested samples (Table 5.4). The iron content of extruded ready-to-eat baby foods varied from 1.18–3.10 mg/100g, zinc content (0.45–0.64 mg/100g), manganese content (0.31–0.90 mg/100g), Copper content (0.60–0.97 mg/100g), calcium content (30.41–61.22 mg/100g), magnesium content (40.52–81.70 mg/100g), sodium content (31.38–41.44 mg/100g) and potassium content (59.06–68.18 mg/100g). Extrusion cooking affects the bioavailability of minerals instead of their stability, contrary to vitamins. The quantity of a nutrient that is consumed and absorbed such that it is available for physiological processes is known as bioavailability.

Extrusion-based research has primarily employed *in vitro* methods to assess bioavailability since bioavailability is a complex process that is expensive and time-consuming to measure. The outcomes of these methods are sometimes referred to as "bioaccessibility," which is defined as the quantity of an element that can be absorbed and used for physiological processes (Etcheverry *et al*., 2012). Extrusion cooking parameters affect the mineral-binding elements contained in legumes and grains, such as phytic acid, phenolic compounds, dietary fibres, and proteins, which is the main cause of the variations in mineral bioavailability (Raes *et al*., 2014).

Extrusion cooking in this study increased Fe, Zn, Cu, Ca, and Mg. This may be the result of the destruction of anti-nutrients mainly phytate and oxalate in extrusion cooking which are major mineral binding compounds and hence improved mineral bioavailability. Oxalate forms close bonds between oxalic acid and other minerals including calcium, magnesium, sodium, and potassium, thereby forming oxalate salts that block nutrients from being available in the body (Nachbar *et al*., 2000). Phytate acts as a largely negative ion in a large pH range and its existence in food also has a negative effect on the bioavailability of divalent and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+} in the body (Mueller, 2001).

The poly-anionic phosphate groups of phytic acid make it a potent chelating agent. It has been demonstrated that phytic acid tightly binds Fe and Zn to create insoluble complexes. Because they are insoluble, phytic acid chelates do not absorb well from the digestive system. Extrusion results in the dephosphorylation of phytic acid and liberate chelated metals. Similar to this, many phenolic compounds have several anionic groups that can bind to important mineral elements and lower bioavailability.

Table 5. 5 Mineral composition (mg/100g) of ready-to-eat baby foods

BP	ET (ºC)	Fe	Zn	Cu	Mn	Ca	Mg	Na	K
C0	70	1.18 ± 0.00^{g}	0.61 ± 0.00^{b}	0.71 ± 0.01^{c}	0.90 ± 0.01^{a}	30.41 ± 0.37^{h}	40.52 ± 0.04^{e}	41.44 ± 0.15^{a}	68.18 ± 0.12^{a}
C1	70	2.83 ± 0.01^{c}	0.56 ± 0.02^{cd}	0.60 ± 0.00^{f}	0.40 ± 0.00^{c}	59.22 ± 0.07^{b}	80.89 ± 0.01^{ab}	34.44 ± 0.12^{e}	64.51 ± 0.16^{b}
C2	70	1.88 ± 0.00^{e}	0.45 ± 0.00^{g}	0.64 ± 0.01^{de}	0.32 ± 0.00^{de}	50.93 ± 0.32^{d}	78.74 ± 0.20^{c}	40.22 ± 0.02^{c}	61.43 ± 0.01^{c}
C3	70	2.79 ± 0.02^{c}	0.52 ± 0.01^{e}	0.62 ± 0.00^{e}	0.26 ± 0.01^{f}	52.69 ± 0.16^{c}	80.50 ± 0.00^{b}	31.51 ± 0.01^{f}	61.08 ± 0.02^{d}
C4	70	1.82 ± 0.00^{f}	0.46 ± 0.00^{fg}	0.95 ± 0.00^{b}	0.33 ± 0.01^{d}	33.28 ± 0.07^{f}	80.37 ± 0.02^{b}	38.64 ± 0.00^{d}	59.26 ± 0.02^{e}
C0	90	1.19 ± 0.01^{g}	0.64 ± 0.00^{a}	0.72 ± 0.01^{c}	0.87 ± 0.01^{b}	31.67 ± 0.43^{g}	41.85 ± 0.55^{d}	41.04 ± 0.03^{b}	68.03 ± 0.01^{a}
C1	90	2.98 ± 0.01^{b}	0.56 ± 0.00^{cd}	0.64 ± 0.01^{d}	0.39 ± 0.01^{c}	61.22 ± 0.07^{a}	81.55 ± 0.49^{a}	34.44 ± 0.12^{e}	64.35 ± 0.06^{b}
C2	90	2.21 ± 0.01^{d}	0.46 ± 0.00^{fg}	0.65 ± 0.00^{d}	0.31 ± 0.01^{e}	51.59 ± 0.26^{d}	79.41 ± 0.37^{c}	40.24 ± 0.12^{c}	61.41 ± 0.01^{c}
C3	90	3.01 ± 0.01^{b}	0.54 ± 0.00^{de}	0.64 ± 0.01^{de}	0.26 ± 0.00^{f}	53.36 ± 0.67^{c}	81.50 ± 0.00^{a}	31.38 ± 0.06^{f}	61.02 ± 0.02^{d}
C4	90	3.10 ± 0.06^{a}	0.47 ± 0.01^{d}	0.97 ± 0.01^{a}	0.34 ± 0.00^{d}	34.62 ± 0.55^{e}	81.70 ± 0.56^{a}	38.63 ± 0.02^{d}	59.06 ± 0.02^{f}
RDA (mg/day)		0.27-11*; 7-10**; 9***; 27****	2-3*; 3-5**; 12***; 11****	0.2-0.22*; 0.34 -0.44**; 1.3***; 1****	0.003-0.6*; 1.2-1.5**; 2.6***; ...	200-260*; 700-1000**; 1000***;****	30-75*; 80-130**; 310-320***; 350-360****	120-370*; 1000-1200**; 1500***;****	400-700*; 3000-3800**; 5100***; 4700****

Results are indicated in triplicate values as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ($p \leq 0.05$). Where BP is the blend proportions, ET: Extrusion temperature; C0 (Control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: Soybean flour, respectively. *Infant, **Children, ***Lactating Mothers, ****Pregnancy (Gropper *et al*., 2022).

During extrusion cooking, these phenolic compounds undergo degradation or polymerization, which diminishes their chelating capabilities (Sandberg *et al*., 1987).

5.3.14 Anti-nutrient Contents of Ready-To-Eat Baby Foods

The anti-nutritional contents of extruded ready-to-eat baby foods and their corresponding composite flour blend significantly ($p<0.05$) differ among samples (Table 5.6). The phytate content in the composite flour ranged from 7.49 mg/100g to 10.79 mg/100g) and the highest level was observed in the control sample (C0) which contains orange-fleshed sweet potato flour. The results are in agreement with the values (8.18–9.78 mg/100g) reported by Edima-Nyah *et al*. (2020) for breakfast cereals produced with yellow maize, soybean, and banana flour blends. The results are lower than the results (50 to 420 mg/100g) in SPK031 and Vitaa, respectively, for Kenyan sweet potato varieties in roots (Abong *et al*., 2020) and below the values (240±0.02 mg/100g), 300±0.11 mg/100g and 230±0.02 mg/100g for bread from Kabode, SPK and Yellow sweet potato varieties in Kenya, respectively (Abong *et al*., 2020) but confirm well with the results (10–12 mg/100g) reported by Olapade and Ogunade (2014) for cream flesh sweet potato and yellow flesh sweet potato flour. The depletion of phytate content during the drying process of raw materials may be the cause of low levels of phytate content in the orange-fleshed sweet potato flour.

The oxalate content in the composite flour ranged from 1.45-3.10 mg/100g and a significant difference ($p<0.05$) was observed among samples. The results are lower than the value (1130 mg/100g) reported by Airaodion *et al*. (2019) on raw cassava mash. In addition, Abong *et al*. (2020) reported 152.52±23.47 mg/100g of oxalate content in Kenspot 5 roots. The results are in agreement with the results (3.50–8.80 mg/100g) reported by Dako *et al*. (2016) for yellow, white, and orange-fleshed sweet potato varieties and very close to the values (4.23-4.66 mg/100g) reported by Fekadu (2014) for processed anchote tubers.

The saponin content in the composite flour ranged from 7.14-9.98 mg/100g and a significant difference ($p<0.05$) was observed among samples. The values are lower than the value of 222 mg/100g reported by Airaodion *et al*. (2019) for raw cassava mash but in agreement with the values (9.30–16.23 mg/100g) reported by Edima-Nyah *et al*. (2020) for breakfast cereals produced with yellow maize, soybean, and banana flour blends. On the other hand, the phytate content of extruded ready-to-eat baby foods varied from 0.47–1.79 mg/100g. The results are below the range (323.47 to 428.33 mg/100 g) reported by Tadesse *et al*. (2019) for sorghum-based extruded product supplemented with defatted soy meal flour.

Table 5. 6 Anti-nutrient composition (mg/100g) of ready-to-eat baby foods

BP (Product)	ET	Phytate	Oxalate	Saponin
C0	70	1.79±0.02^{a}	0.50±0.00^{a}	0.35±0.00^{d}
C1	70	1.51±0.07^{b}	0.39±0.01^{d}	0.48±0.01^{a}
C2	70	1.10±0.02^{d}	0.49±0.03^{ab}	0.32±0.00^{e}
C3	70	1.42±0.05^{c}	0.45±0.02^{bc}	0.37±0.00^{c}
C4	70	1.47±0.01^{bc}	0.41±0.00^{cd}	0.39±0.01^{b}
C0	90	0.96±0.01^{e}	0.20±0.01^{f}	0.21±0.00^{h}
C1	90	0.47±0.00^{g}	0.23±0.01^{ef}	0.29±0.01^{f}
C2	90	0.47±0.01^{g}	0.26±0.01^{e}	0.21±0.00^{h}
C3	90	0.80±0.01^{f}	0.16±0.01^{g}	0.24±0.00^{g}
C4	90	0.92±0.01^{e}	0.20±0.00^{f}	0.20±0.00^{h}
BP (Composite Flour)				
C0		10.79±0.03^{a}	1.45±0.00^{e}	7.14±0.01^{e}
C1		9.79±0.01^{b}	2.32±0.01^{b}	9.98±0.01^{a}
C2		7.49±0.02^{e}	3.10±0.00^{a}	7.47±0.01^{d}
C3		7.78±0.03^{d}	2.12±0.01^{c}	7.97±0.01^{b}
C4		8.80±0.01^{c}	1.71±0.00^{d}	7.86±0.01^{c}

Data are indicated in triplicate values as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ($p \leq 0.05$). Where BP is the blend proportions, ET: Extrusion temperature; C0 (Control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: Soybean flour, respectively.

Furthermore, the results are below to the range (64.74–72.15 mg/100g) reported by Gemede (2020) for complementary foods formulated from maize, pea, and anchote flours. The results are slightly above to the findings (0.20–0.32 mg/100g) reported by Awofadeju *et al*. (2021) for an extruded ready-to-eat breakfast snack. The results are lower than the range (18–37 mg/100g) reported by Fakolujo and Adelugba (2021) for extruded snacks made from acha, jack bean, and paw flour blends but in agreement with the results (0.55 mg/100g – 1.48 mg/100g) reported by Nkesiga and Okafor (2015) for extruded snacks from yellow maize, soybean and amaranth leaf flour blends.

Abubakar *et al*. (2010) reported very close values of 0.86 mg/100g for boiled sweet potato. Foods containing phytates have been reported to have beneficial impacts because they contain antioxidants that remove free radicals from the body (Olapade & Ogunade, 2014). The phytates can serve as prebiotics due to their capacity to bind enzymes such as amylases, causing a portion of the starch to enter the intestine undigested. Anti-nutrients may harm human health by limiting the digestibility and bioavailability of nutrients. However, the results from phytate content in extrudates are generally below the permissible limit of phytate in foods which is 250–500 mg/100g (Ndidi *et al*., 2014).

Oxalate content in the extruded ready-to-eat baby foods varied from 0.16-0.50 mg/100g and a significant ($p<0.05$) difference was observed among the samples. The results are below the values (37.45 to 44.82 mg/100g) reported by Gemede (2020) for complementary foods formulated from maize, pea, and anchote flours, and the values (66.61±1.13 mg/100g), 83.18±3.99 mg/100g and 77.13±2.61 mg/100g reported by Abong *et al*. (2020) for bread from Kabode, SPK and Yellow sweet potato varieties in Kenya, respectively, but above to the range (0.11–0.17 mg/100g) reported by Awofadeju *et al*. (2021) for an extruded ready-to-eat breakfast snack.

Oxalates can have harmful effects on human nutrition and health, such as reducing calcium absorption and aiding the creation of kidney stones. High intakes of soluble oxalate may cause calcium oxalate crystallization and the formation of kidney stones (nephrolithiasis) in the urinary tract (Irakli *et al*., 2021). Most urinary stones formed in humans are calcium oxalate stones; therefore, oxalate ingestion should not exceed 60 mg/day (Fategbe *et al*., 2021). The oxalate content of the extruded ready-to-eat baby foods is within the permissible limit which is between 0.3–0.5 mg/100g (Ndidi *et al*., 2014).

Saponin content of extruded ready-to-eat baby foods varied 0.20–0.48 mg/100 g. The results are below the range (35–73 mg/100g) reported by Fakolujo and Adelugba (2021) for extruded snacks made from acha, jack bean, and paw flour blends and lower than the value 90 mg/100g reported by Airaodion *et al*. (2019) for traditional *garri* but in agreement with the results (0.0123–0.40 mg/100g) reported by Kowalski *et al*. (2016) for selected quinoa extrudates The results are also below to the results (1.16 mg/100g – 6.57 mg/100 g) reported by Nkesiga and Okafor (2015) for extruded snacks from yellow maize, soybean, and amaranth leaf flour blends.

The majority of saponins are poisonous and bitter-tasting glycosidic chemicals made up of a steroid (C-27) or triterpenoid (C-30) nucleus with one or more carboxylate branches. The saponins are extremely heat-labile compounds and the concentrations of 3–7% are thought to be potent poisons, but concentrations of 1% are thought to be non-offensive; at 1.5%, certain biological activity on injured mucous membranes have been observed (Aletor & Oreoluwa, 2019). There are a variety of edible and toxic saponins (steroid or triterpene glycoside molecules). Bitter saponins can prevent nutrients from being absorbed by blocking digestive and metabolic enzymes and reacting to nutrients like zinc. They are harmful at large doses. Saponins are organic substances that have numerous biological impacts (Awulachew, 2022).

Saponins can bind proteins, enhancing protein stability against heat denaturation and decreasing the susceptibility of proteins to proteases. They may also cause gastrointestinal lesions, entering the bloodstream and haemolysing the red blood cells (Bissinger *et al*., 2014). Saponins have properties of precipitating and coagulating red blood cells and they also exhibit hypocholesterolemic properties (cholesterol binding properties) by forming insoluble complexes with cholesterol, resulting in slower absorption, formation of foams in aqueous solutions, and haemolytic activity (Fategbe *et al*., 2021). The shear and thermal energy present likely are enough to destroy the original structure of the saponins, leading to the creation of smaller chemical fragments (Kowalski *et al*., 2016).

Extrusion cooking resulted in a significant reduction of phytate content, oxalate content, and saponin content of ready-to-eat baby foods and ranging from 81.71 – 95.23%, 65.24 – 92.63%, and 95.06 – 97.43% reduction, respectively. The loss of these anti-nutrients during extrusion cooking might be attributed to the 149hermos-labile nature of these compounds. Similar observations were noted by many researchers. These anti-nutritional factors may be inactivated by processing methods involving heat generation (Ndidi *et al*., 2014). In plant foods, anti-nutritional factors are responsible for the deleterious effects of nutrient and micronutrient absorption. However, certain anti-nutrients may have beneficial health effects at low and medium levels, such as decreasing blood glucose and insulin reactions to starchy foods and/or plasma cholesterol and triglycerides, reducing cancer risks due to their antioxidant effect (phytate, oxalate, saponin) but in high concentration, they reduce the availability of nutrients and cause growth inhibition (Gemede & Ratta, 2014). In addition, the levels of phytate and oxalate in all extruded ready-to-eat baby foods fall within the safe permissible levels (0-5%) for infant foods (Oche *et al*., 2017).

5.3.15 Expansion Ratio and Bulk Density of Ready-To-Eat Baby Foods

The physical properties of ready-to-eat baby foods are shown in Table 5.7 and a significant difference ($p<0.05$) among samples was observed. The expansion ratio of extruded ready-to-eat baby foods ranged from 0.93–1.24 and a significant difference ($p<0.05$) was observed between the extrudates. The results are slightly below the range (2.941 to 3.559) reported by Naseer *et al*. (2021), for extrudate almond-pearl millet (APM) based snacks but close to the trends (2.42 to 3.30) reported by Sahu1 and Patel (2020) for RTE extruded products and 2.0 to 2.6 reported by Omwamba and Mahungu (2014) for protein-rich ready-to-eat extruded snack from a composite blend of rice, sorghum and soybean flour.

In addition, the results confirm well to the values (1.17±0.04 to 1.23±0.07) reported by Fang *et al*. (2019) for tuna meat-based extrudates. The sectional expansion ratio varied from 0.88-1.56 and falls within the range of 1.07 to 3.76 mm^2 sectional area of the extrudate to the sectional area of die, reported by Rweyemamu *et al*. (2015). Expansion is a crucial physical characteristic of extruded food and consumers like extrudates with high expansion ratio and low bulk density owing to their puffiness. The expansion occurring in a food material depends on the pressure differential between the die and the atmosphere (Azeez *et al*., 2015). The expansion ratio was high in the control sample and low in the other samples.

Table 5. 7 Physical properties of extruded ready-to-eat baby foods

BP (Product)	ET (ºC)	Expansion Ratio	Sectional Expansion	Bulk Density (g/cm^3)
C0	70	1.20 ± 0.07^{ab}	1.45 ± 0.17^{ab}	0.45 ± 0.05^{a}
C1	70	0.93 ± 0.09^{e}	0.88 ± 0.19^{e}	0.30 ± 0.04^{bc}
C2	70	1.05 ± 0.10^{cd}	1.12 ± 0.25^{cd}	0.36 ± 0.06^{b}
C3	70	0.96 ± 0.04^{de}	0.93 ± 0.09^{de}	0.27 ± 0.03^{c}
C4	70	1.02 ± 0.04^{de}	1.05 ± 0.09^{de}	0.34 ± 0.08^{bc}
C0	90	1.24 ± 0.13^{a}	1.56 ± 0.33^{a}	0.43 ± 0.07^{a}
C1	90	0.99 ± 0.04^{de}	0.98 ± 0.08^{de}	0.27 ± 0.06^{c}
C2	90	1.14 ± 0.07^{bc}	1.30 ± 0.16^{bc}	0.35 ± 0.04^{b}
C3	90	0.98 ± 0.09^{de}	0.97 ± 0.19^{de}	0.30 ± 0.05^{bc}
C4	90	1.16 ± 0.07^{ab}	1.35 ± 0.17^{ab}	0.33 ± 0.06^{bc}

Data are indicated in triplicate values as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ($p \leq 0.05$). Where BP is the blend proportions, ET: Extrusion temperature; C0 (Control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: Soybeans flour, respectively.

This may be an indication of the high carbohydrate and high protein content of extruded ready-to-eat baby foods. The extrudates expand more when there is more starch present, but they contract more when there is more protein. The expansion ratio of the extrudates is an essential factor related to the interaction between starch and protein. The viscosity and elasticity of the dough, which are influenced by the ratio of starch, protein, and fibre, may have a role in the expansion (Nagaraju *et al*., 2021). In addition, the expansion ratio of extruded food products depends on the atmospheric pressure, water vapour pressure, and the ability of the product to sustain expansion (Liu *et al*., 2021). Increasing in rotating speed of the screw and barrel temperature results in high expansion and low expansion owing to the increase in moisture content (Shelar & Gaikwad, 2019).

The bulk density of extruded ready-to-eat baby foods ranged from 0.27–0.45 g/cm^3 and a significant difference ($p<0.05$) was observed between the extrudates ($p<0.05$). The results are slightly above the range (0.107 to 0.156 g/cm^3) reported by Naseer *et al*. (2021), for extrudate almond- pearl millet (APM) based snack and 0.14–0.26 g/cm^3 reported by Sahu1 and Patel (2020) for RTE extruded products but within the values (0.41–0.59 g/cm^3) reported by Sobowale *et al*. (2021) for extruded snacks from whole pearl millet-based flour. The highest bulk density (0.45 g/cm^3) of extruded ready-to-eat baby foods was observed in the control sample which is OFSP only. This may be attributed to the highest carbohydrate content in OFSP. The higher the starch concentration, the greater the likelihood of a rise in bulk density. The high bulk density of flours implies that they are suitable for use in food preparation. Low bulk density, on the other hand, was found to be essential in complementary food formulations (Kaushik *et al*., 2021). The density of extruded food products is affected by the extrusion cooking conditions such as moisture content, temperature, and screw speed which is inverse to the expansion ratio (Shelar & Gaikwad, 2019).

5.3.16 Sensory Characteristics of Extruded Ready-To-Eat Baby Foods

The sensory evaluation was done using a 5-point hedonic scale after conducting the microbial analysis on day zero. All extruded ready-to-eat baby foods were free from microbial contamination. Microbial quality analysis is used to protect consumer health and assure product safety by identifying and managing microbial contamination. This, in turn, has a direct influence on sensory evaluation since microbial contamination can impair product sensory qualities, making it critical to maintain microbiological quality in order to provide a favourable sensory experience to consumers. For example, microbial spoilage can cause off-flavors, smells, and texture changes that impact the taste and overall sensory experience of the food. Results from the sensory evaluation of extruded ready-to-eat baby foods are shown in Table 5.8 and a significant difference ($p<0.05$) was observed among the samples.

This could be due to different ingredients used in formulations and also the composition of each extrudate. The sensory profile of all extruded ready-to-eat baby foods is graphically given in Figure 5.2. Among the 10 samples, the graph suggests that the samples with the best sensory scores in all the attributes are C170, C370 and C470 followed by C070, C490, C190, and C270, while the samples with the least favourable attribute are C290, C090, and C390. Taste was the attribute with the highest level of preference among the examined characteristics, followed by overall acceptability, appearance, and then texture, and aroma while colour had the lowest.

Table 5. 8 Sensory properties of extruded ready-to-eat baby foods

BP	Appearance	Colour	Aroma	Taste	Texture	OA
C070	4.62 ± 0.49^{bc}	4.20 ± 0.40^{c}	4.00 ± 00^{e}	4.95 ± 0.21^{a}	4.51 ± 0.50^{b}	4.51 ± 0.50^{cd}
C170	4.94 ± 0.24^{a}	4.83 ± 0.38^{a}	4.49 ± 0.50^{bc}	4.92 ± 0.27^{a}	4.57 ± 0.50^{b}	4.91 ± 0.29^{a}
C270	4.52 ± 0.50^{c}	3.98 ± 0.60^{cde}	4.37 ± 0.49^{bcd}	4.42 ± 0.50^{bc}	4.40 ± 0.49^{bc}	4.68 ± 0.47^{bc}
C370	4.74 ± 0.59^{abc}	4.52 ± 0.50^{b}	4.77 ± 0.42^{a}	4.98 ± 0.12^{a}	4.37 ± 0.49^{bcd}	4.82 ± 0.39^{ab}
C470	4.62 ± 0.49^{bc}	4.17 ± 0.38^{cd}	4.40 ± 0.49^{bcd}	4.83 ± 0.38^{a}	4.45 ± 0.56^{bc}	4.80 ± 0.40^{bc}
C090	3.92 ± 0.27^{d}	3.55 ± 0.50^{f}	4.52 ± 0.50^{bc}	4.09 ± 0.29^{d}	4.23 ± 0.42^{cd}	4.03 ± 0.17^{g}
C190	4.77 ± 0.42^{ab}	3.97 ± 0.17^{de}	4.31 ± 0.47^{cd}	4.51 ± 0.50^{b}	4.57 ± 0.50^{b}	4.28 ± 0.45^{ef}
C290	4.00 ± 0.25^{d}	3.89 ± 0.31^{e}	4.60 ± 0.49^{ab}	4.08 ± 0.27^{d}	4.48 ± 0.50^{bc}	4.31 ± 0.47^{de}
C390	3.85 ± 0.36^{d}	3.23 ± 0.42^{g}	4.22 ± 0.41^{de}	4.23 ± 0.42^{cd}	4.14 ± 0.35^{d}	4.06 ± 0.24^{fg}
C490	4.74 ± 0.44^{abc}	4.00 ± 00^{cde}	4.17 ± 0.38^{de}	4.48 ± 0.56^{b}	4.89 ± 0.31^{a}	4.37 ± 0.49^{de}

Like very much	Like moderately	Neither like nor dislike	Dislike moderately	Dislike very much
5	4	3	2	1

Data are indicated as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ($p \leq 0.05$). Where C070-C470, are blends proportions extruded at 70°C while C090-C490, are blends proportions extruded at 90°C. C070 and C090 are acting as the control (Orange-fleshed sweet potatoes).

The appearance of extruded ready-to-eat baby foods which was the third attribute preferred by panellists ranged from 3.85 to 4.94. There was no significant difference ($p>0.05$) between samples C170, C370, C190, and C490 although C170 and C370 were the most preferred products among all tested extrudates. In addition, there was no significant difference ($p>0.05$) between samples C070, C370, C470, C190, and C490. The overall appearance of a product or package is frequently the crucial factor that influences the choice to purchase or consume a product. It includes the colour, size, shape, clarity, and surface texture of food products. The colour of extruded ready-to-eat baby foods ranged from 3.23 to 4.83. There was no significant difference ($p>0.05$) observed between samples C070, C270, C470, and C490. Sample C170 scored more than other samples while C390 had the lowest score. Most samples extruded at 70°C scored higher than those extruded at 90°C in terms of colour even though there was no significant difference ($p>0.05$) between samples C270, C190, C290, and C490. The dark brown colour may be because extrusion cooking temperature and OFSP incorporation affected the colour of most food products. The aroma of extrudates which was the fifth-ranked among attributes evaluated ranged from 4.00 to 4.77. There was no significant difference ($p>0.05$) observed between samples C170, C270, C470, C090, and C290. Furthermore, there was no significant difference ($p>0.05$) observed between samples C170, C270, C470, C090, and C190. The taste of extrudates was the first attribute preferred by panellists and ranged from 4.08 to 4.98. There was no significant difference ($p>0.05$) observed between samples C070, C170, C370, and C470. In addition, samples C090, C290, and C390 revealed that there was no significant difference ($p>0.05$) observed between them in terms of taste. Taste (attestation) is properly defined as the reaction of the tongue to soluble, volatile substances (Kilcast, 2011).

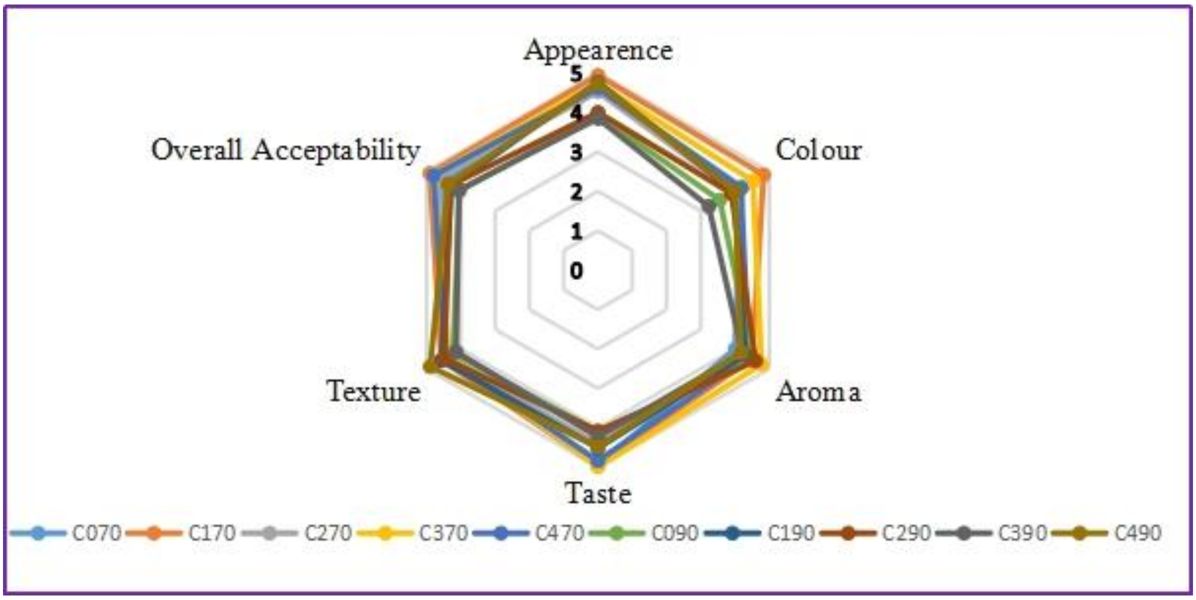

Figure 5. 2 Radar graph representing sensory qualities of extruded ready-to-eat baby foods. C070-C470, are blends proportions extruded at 70°C while C090-C490, are blends proportions extruded at 90°C.

The texture which was the fourth attribute liked ranged from 4.14 to 4.89. There are no significant differences ($p>0.05$) observed between samples C070, C170, C270, C370, C470, C190, and C290. This could be due to the fact that the panellists could not detect the difference among the extrudates regarding this sensory attribute. The sensation of touch detects texture, which is made up of two parts: somesthesis, a tactile surface reaction from the skin, and kinesthesis (or proprioception), a profound reaction from the tendons and muscles (Kilcast, 2011). The overall acceptability was the second attribute preferred by panellists and ranged from 4.03 to 4.91. There was no significant difference (p>0.05) observed between samples C070, C290, and C490. The acceptability of food products is crucial due to the distinct quality characteristics that attract consumers though there was no significant difference (p>0.05) observed between samples C270, C370, and C470. In addition, samples C190, C290, and C490 showed no significant difference (p>0.05) between them. On the other hand, the samples extruded at 70^{o}C scored more than the ones processed at 90^{o}C in terms of appearance, colour, taste, texture, and overall acceptability. This may be due to the brown colour of orange-fleshed sweet potatoes extrudates observed after extruding and drying extrudates which were not preferred by most of the panellists. There was no significant difference (p>0.05) between the control sample (C070) and other samples in terms of appearance and overall acceptability while C090 was significantly (p<0.05) different from other products in terms of appearance, colour, taste, and overall acceptability.

5.4 Conclusion

Extrusion cooking temperature and blend proportions significantly (p<0.05) affect the nutritional qualities, anti-nutrients, minerals, physical, and sensory properties of extruded ready-to-eat baby foods from orange-fleshed sweet potatoes, amaranth seeds, and soybeans flour blends. The ingredients enhanced macronutrients and micronutrient contents of extruded ready-to-eat baby foods. The nutrient contents of the produced extruded ready-to-eat baby foods comply with the recommended standards based on the targeted nutrients studied in this study. The extruded ready-to-eat baby foods have reliable findings that can be used not only for stunted and wasted children below five years of age but also people who suffer from inadequate nutrient intake in developing countries. The anti-nutrient contents are at safe permissible levels for consumption. The extrusion cooking could be used in the production of high-quality extruded ready-to-eat baby foods with highly acceptable sensory qualities. The utilization of extruded ready-to-eat foods could help in achieving food and nutrition security as well as poverty reduction in developing countries if food products are standardized and marketed.

CHAPTER SIX

PROTEIN QUALITY OF EXTRUDED READY-TO-EAT BABY FOODS FROM ORANGE-FLESHED SWEET POTATOES, AMARANTH SEEDS, AND SOYBEANS FLOUR BLENDS

Abstract

Protein quality refers to the total protein content, essential amino acid content, and digestibility of a protein. Source, bioavailability, food matrix, and processing conditions all have an impact on protein quality. Protein nutritional quality can be lost during food processing. This study was carried out to investigate the effect of extrusion cooking and blend proportions on the protein quality of extruded ready-to-eat baby foods. Different blends of orange-fleshed sweet potatoes, amaranth seed, and soybeans flour were used and analyzed for protein quality including *in vitro* protein digestibility (IVPD) of extruded ready-to-eat baby foods. In addition, nutrient damage due to heat or processing temperature was evaluated by analyzing available lysine in the end products to ensure the quality of extruded ready-to-eat baby foods. Extrusion cooking was carried out at a temperature of 90^{o}C, screw speed of 400 rpm, and feed moisture content of 35%. The results showed that IVPD ranged from 54.05 to 91.87%. The available lysine as a parameter to evaluate the nutritional damage due to thermal processing ranged from (1.69 to 2.79%). This research predicts the potential availability of highly digestible protein as well as the assurance of lysine availability once extrudates are consumed. Lysine can be retained during extrusion cooking by using low temperature, high screw speed, high feed moisture content, and high shear forces which lead to a short residence time.

6.1 Introduction

Proteins are enormous and complex containing nitrogen molecules created by amino acids that are essential for the structure, function, and control of body tissues. Proteins are required by the human body in several different ways, including antibodies, which attach to certain foreign particles to assist defend the body, enzymes, which perform nearly all of the chemical processes that occur in our cells, and structural components in muscle and other tissues (Hayes, 2019). Proteins play a variety of tasks and are essential to almost every aspect of all life processes in biological organisms. These functions can be divided into a few categories, such as the catalysis of metabolic processes, energy transfer, gene expression, solute transport across biological membranes, cellular communication, molecular recognition, defence, formation of intracellular and extracellular structures, and cell- and tissue-specific functions (Kessel & Ben-Tal, 2018).

Amino acids are important as both protein-building elements and metabolic intermediates. Twenty natural amino acids are contained within proteins, and their chemical characteristics dictate the biological functions of proteins (Hayes, 2019). Food proteins are a crucial nutrient and a dietary source of the amino acids required for the healthy growth and maintenance of the body (Gupta, 2020).

The availability of the amino acids and their composition, which are influenced by the protein-containing food‘s capacity to be digested, are key factors in determining the quality of protein (Hayes, 2019). Disulphide bond breaking, unfolding, protein aggregation, dimerization, and the development of bigger oligomers are all possible outcomes of thermal treatment. In addition to the protein alterations that take place during thermal treatments, other chemical processes such glycation, Maillard reactions, oxidation, and deamidation (caused by high-temperature acid treatment or enzymatic treatment with deamidase or transglutaminases) may also take place (Aryee *et al*., 2018).

When food is heated, the Maillard reaction occurs spontaneously, causing the reactive carbonyl groups of reducing sugars to combine with the nucleophilic amino group of amino acids, peptides, or proteins to produce a wide range of chemicals (Ruan *et al*., 2018). Thermal processing, particularly when food is being processed, can facilitate the Maillard reaction, a complicated series of chemical reactions. Maillard reaction products (MRPs), which are important for various aspects of food quality, including texture, flavour, and colour, are formed in greater quantity as a result of MR (Giannetti *et al*., 2021).

The detrimental effects of the Maillard reaction, which are mostly reflected in public health issues, were revealed by Parisi *et al*. (2019). Different Maillard reaction products (MRPs), such as acrylamide and 5-hydroxymethylfurfural, are under attention due to their potential for being mutagenic, cytotoxic, and carcinogenic. Advanced glycation end products (AGEs) can also exacerbate pre-existing diseases including diabetes and several cancers (Naik *et al*., 2022; Parisi *et al*., 2019).

Nevertheless, melanoidins, brownish polymers that are produced during the Maillard reaction, have been found to have beneficial impacts on human health in the form of antioxidant and/or antibacterial capabilities. The three chemical phases of the Maillard reaction—early, intermediate, and final stages—were succinctly described by Ruan *et al*. (2018). In the early stages of the Maillard reaction, sugar-amine condensation, the formation of a Schiff base, and the Amadori rearrangement products (ARPs) are all present. In the intermediate stage, sugar dehydration, fragmentation, and amino acid degradation are present, and in the final stage, reactive dicarbonyl and aldehyde intermediates are responsible for the formation of low- and high-molecular-weight heterocyclic compounds and polymers (Giannetti *et al*., 2021; Naik *et al*., 2022; Ruan *et al*., 2018). Cyclization, dehydration, and condensation are the three steps that make up the final phase of the Maillard reaction (Gancarz *et al*., 2021).

Bioactive peptides have a wide range of effects, including anti-inflammatory, anti-cancer, anti-oxidant, anti-diabetic, and anti-hypertensive ones. They also play a significant role in the management of anxiety, type 2 diabetes, obesity, and hypertension, as well as in the regulation of the immune system, and blood pressure, or as signalling molecules (Yada, 2018).

Extrusion cooking has mostly concentrated on the transitions that starches and proteins undergo during the process, but there is still untapped potential in terms of how starches, proteins, and other macro and micro-ingredients (phenolic chemicals) interact in the extruder (Shah *et al*., 2021). Lipids interact with amylase and form amylose–lipid complexes, the asparagine-carbohydrates (formation of acrylamide), lipid-protein complex, and protein-carbohydrate complex in the Maillard reactions that take place during extrusion cooking (Shah *et al*., 2021).

Therefore, there is a need to understand the interaction of protein-carbohydrate that occur during extrusion cooking of extruded ready-to-eat baby foods. On the other hand, malnutrition leads to poor cognitive development and weak human body self-defence against diseases. This lack of certain nutrients may cause adverse health effects and even associated diseases. Children and babies, on the other hand, require little but sufficient amounts of food to fulfil their daily basic needs for energy, protein, and micronutrients. This study aims at developing and analyse the protein quality of extruded ready-to-eat baby foods from blends of orange-fleshed sweet potatoes, amaranth seeds, and soybeans flour.

6.2 Materials and methods

6.2.1 Materials and Chemicals

Raw materials used in this study were purchased as described in section 3.2.1 of Chapter Three. The Acid Orange 12, 70% and the propionic anhydride were bought from Ipure Biology Co. Ltd, Jinhua, Zhejiang China, potassium dihydrogen phosphate anhydrous 98%, sodium acetate anhydrous 99%, and oxalic acid 99.0%, glacial acetic acid 99.7% were procured from LOBA Chemie PVT. Ltd, Mumbai, India.

6.2.2 Production of Flour from Orange-Fleshed Sweet Potatoes, Amaranth Seeds, and **Soyb*eans***

Orange-fleshed sweet potatoes flour, amaranth seeds flour, and soybeans flour were manufactured as described in sections 3.2.2, 3.2.3, and 3.2.4 of Chapter Three, respectively.

6.2.3 Blend Formulations and Extrusion Cooking Process

The blends used in this work were identified from the pilot experiments and the available literature. The formulations and extrusion cooking were done as described in section 5.2.3 of Chapter Five.

6.2.4 Experimental Design

A Completely randomized design (CRD) in a Factorial Experimental Design with two variables (blends proportions at 5 levels and extrusion cooking temperature at two levels) was used in this study. The model is described in section 5.2.4 of Chapter 5.

6.2.5 Determination of In Vitro Protein Digestibility

The *in vitro* protein digestibility (IVPD) of the ready-to-eat baby foods was evaluated using the pepsin and trypsin sequential digestion model according to the method of Manus *et al*. (2021) with modifications. Briefly, 5 g of the formulated samples were weighed into 5 ml centrifuge tubes and suspended in 20 ml of 0.1 N HCl. and the pH was measured and adjusted using the base. Then 0.02 g pepsin (CAS: 9001-75-6) was added and incubated in the water bath at 37^{o}C for 3.5 h while shaking the tubes using Lab Rotator (DSR-2800P, S/No: 14030067, Digisystem Laboratory Instruments Inc., Taiwan) at intervals of 10 - 15 min as the digestion goes on. After the digestion by the first enzyme is done, the pH was adjusted to 8.0 with 1.0 N NaOH and a mixture of 0.02 g trypsin (CAS: 9002-07-7, India), and 0.02 g of chymosin (CAS: 9001-98-3) was added into the tubes then incubated for a further 3.5 h at 37^{o}C, shaking the tubes at intervals of 10 - 15 min until the digestion is complete. The mixture was centrifuged using a centrifuge (Funke-Gerber, SuperVario-N, Germany) at 3500×g for 20 min and the liquid was decanted. The residue was dried at 95^{o}C in the oven to a constant dry weight and then analysed via Kjeldahl determination of the protein.

$$Protein\ Digestibility\ (\%) = \frac{(Total\ sample\ N\ - N\ in\ residue)}{Total\ sample\ N} \times 100$$

6.2.6 Preparation of Buffer Solution for Available Lysine Analysis

A 2 l volumetric flask was filled with 40 g of oxalic acid dehydrate 99.0% (CAS: 6153-56-6) and 6.8 g of potassium dihydrogen phosphate anhydrous 98% (CAS: 7778-77-0). The powder was partly dissolved by the addition of distilled water in a small amount. After that, 120 ml of glacial acetic acid 99.7% (CAS: 64-19-7) was poured into the flask. The distilled water was used to make a final volume of 2 l. The flask was set on a magnetic stirrer and stirred for roughly an hour to create a transparent, uniform solution. This produced 2 l of buffer solution which was enough to create the dye solution. The buffer solution was prepared every day before the analysis.

6.2.7 Preparation of the Dye Solution

The buffer solution was used to dilute the Acid Orange 12 dye (CAS: 1934-20-9) (0.27 g) to the desired final volume before being added to a 200 ml volumetric flask. This offered enough dye for the experiment.

6.2.8 Preparation of Sodium Acetate Solution

On a magnetic stirrer, 16.4 g of sodium acetate anhydrous 99% (CAS: 127-09-3) was dissolved with 100 g of distilled water (w/w) and mixed for around 30 mins. It was also made every day and is known as the solvent.

6.2.8 Determination of Available Lysine

The method of Aalaei *et al*. (2016) was used with slight modification. A sample of 0.2 g was weighed using an analytical balance and put into 100 ml Erlenmeyer glass flasks. The flask was then filled with 2 ml of sodium acetate solution as the solvent. All flasks were covered with Parafilm and shaken vigorously on an orbital shaker at 300 rpm for about 20 min to dissolve the powder. The flask was then filled with 0.2 ml propionic anhydride (the blocking agent) and shaken for another 20 mins. Lysine is propionylated to make it more stable and to make detection and quantification easier. The dye solution (3.89 mM) was added to all samples, covered with Parafilm, and shaken vigorously on an orbital shaker for 2 hrs at 300 rpm. During this time, the protein in the sample binds to the dye and forms a complex. Centrifugation was used to isolate the dye complex. The solution of 10 ml was poured into plastic tubes and centrifuged at 5000×*g* for 10 min, thoroughly separating the complex from the supernatant. The samples were diluted 100 times before the spectrophotometer could measure their absorbance. The oxalic acid–acetic acid phosphate buffer solution was used to dilute 1 ml of the supernatant to a final volume of 100 ml. After that, the absorbance was measured at 475 nm using UV-Visible Spectrophotometer (UV-1800, Shimadzu Corporation., Kyoto, Japan) with a buffer solution as a blank. The concentration of available lysine in the samples may be determined using the equation generated from the standard curve.

6.2.9 Data Analysis

The data for *in vitro* protein digestibility and available lysine were statistically analyzed using SAS version 9.4 TS Level 1M6 (SAS Institute Inc.). Basic statistical measures and goodness-of-Fit tests were conducted for normality of data using PROC UNIVARIATE while the LEVENE Test was carried out for standard homogeneity of variance using HOVTEST=LEVENE. The analysis of variance (ANOVA) was executed to examine the effect of blends proportions and extrusion cooking temperature on protein quality of extruded ready-to-eat baby foods while means separations were tested using Tukey's Studentized Range (HSD) Test at a 5% significance level.

6.3 Results and Discussion

The *in vitro* protein digestibility in extruded ready-to-eat baby foods varied from 54.05 to 91.87% and a significant difference ($p<0.05$) was noted among food samples (Table 6.1). The results fall within the values (80.46-86.44%) reported by Edima-Nyah *et al*. (2020) for breakfast cereals produced with yellow maize, soybean, and banana blends. Furthermore, the results are lower than the values (79-96%) reported by Kanu *et al*. (2009) for breakfast cereal-based porridge mixed with sesame and pigeon peas for adults. The results confirm well with the range (56.1-79.3%) reported by Elkonin *et al*. (2013) for grain sorghum. In addition, the results are in agreement with the values (88.53-92.97) reported by Wafula *et al*. (2020) for extrudates from rice, sorghum and bamboo shoots flour blends. The hydrolysis treatment of proteins can produce peptides with antihypertensive and antioxidant functions (Manus *et al*., 2021).

Protein availability for intestinal absorption is significantly influenced by protein digestibility. The widely used IVPD assay can be used to calculate many aspects of protein digestibility. By utilizing various proteolytic enzymes, the IVPD assay simulates conditions comparable to those of the human digestive tract (Kumar *et al*., 2021).

It was discovered that thermal treatment denatured native protein structure, altered the structure of protease inhibitors and storage proteins from legumes, and caused protein aggregation, rendering the proteins more vulnerable to digestive proteases during unfolding. Wet heating improves structural changes even more as gelatinization and crosslinking between proteins and starch take place. Thermal treatment boosts structural changes. Thermal treatment, however, has a considerable impact on non-polar interactions and intramolecular hydrogen bonds, changing the native structure (Ohanenye *et al*., 2022).

Table 6. 1 IVPD and available lysine of extruded ready-to-eat baby foods

BP	Temperature (°C)	IVPD (%)	Available lysine (g/ 100 g protein)
C0	70	54.05 ± 0.80^{h}	1.69 ± 0.09^{f}
C0	90	67.09 ± 0.72^{g}	1.87 ± 0.02^{ef}
C1	70	76.55 ± 0.30^{e}	1.91 ± 0.07^{e}
C1	90	89.37 ± 0.35^{c}	2.67 ± 0.18^{bc}
C2	70	71.60 ± 0.15^{f}	2.29 ± 0.05^{d}
C2	90	90.66 ± 0.58^{ab}	2.79 ± 0.05^{a}
C3	70	87.44 ± 0.28^{d}	2.35 ± 0.05^{cd}
C3	90	91.87 ± 0.09^{a}	2.56 ± 0.05^{abc}
C4	70	72.61 ± 0.17^{f}	2.02 ± 0.05^{e}
C4	90	90.15 ± 0.14^{bc}	2.47 ± 0.05^{bcd}

Data are indicated in triplicate values as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ($p \leq 0.05$). Where BP is blend proportions, C0 (Control): 100:0:0; C1:50:25:25; C2:54.5:24:21.5; C3:50:30:20; and C4:54.5:26.5:19 for OFSP: Amaranth Seeds: Soybean flour, respectively.

Extrusion cooking temperature and shear forces promote *in vitro* protein digestibility by causing protein denaturation by exposing the protein configuration to enzyme activities. The elimination of anti-nutrients may also contribute to an improvement of *in vitro* protein digestibility (Gulati, 2018). Food protein digestibility exposes the proteolysis vulnerability of the protein, which is dependent on factors such as the structure and amino acid composition of the protein, the pH and temperature of processing conditions, and the presence of certain secondary molecules such as antinutritional factors and emulsifiers (Jingyu *et al*., 2022).

The available lysine (Table 6.1) in extruded ready-to-eat baby foods varied from 1.69 to 2.79% and the standard curve used during the experiment is shown in Figure 6.1. The results are in agreement with the values 2.86%, 1.34%, 0.72%, 0.07%, and 0.52% in soybean meal, cottonseed meal, corn germ meal, corn grain, and wheat midds, respectively, reported by Li *et al*. (2019). The results are slightly above the values (0.95-1.28%) reported by Aalaei *et al*. (2019) in infant formulas but below the range (2.33-3.31%) reported by Aalaei *et al*. (2016) for skim milk powders. These high values may be due to the high protein content of skim milk powder compared to the low protein content present in extrudates from OFSP, amaranth seeds, and soybean flour blends.

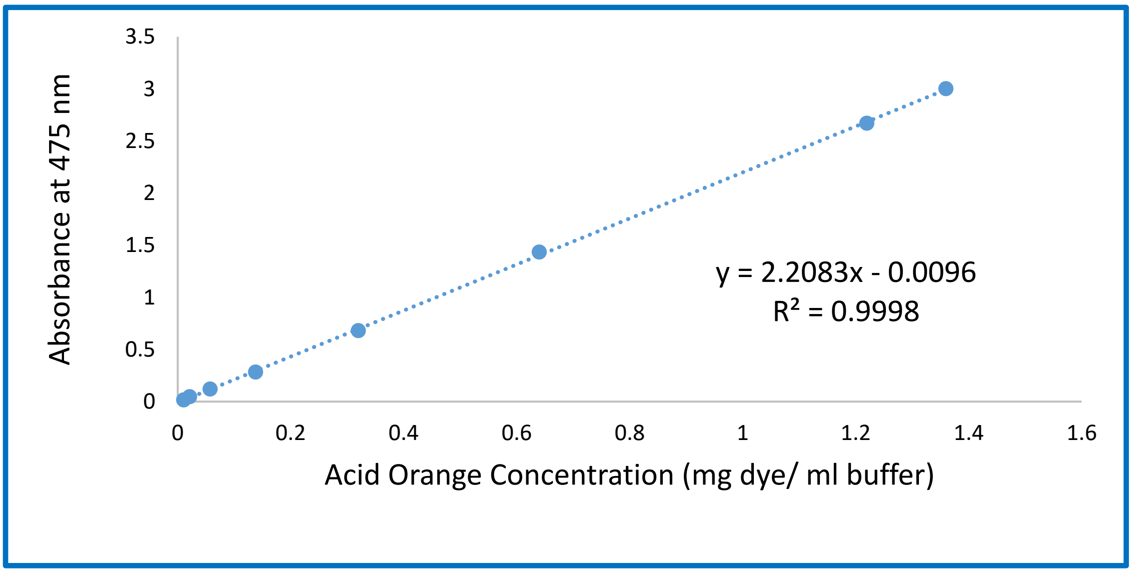

Figure 6. 1 Standard curve of available lysine

The available lysine of the extrudates was positively affected by blend proportions and extrusion cooking, probably due to the low temperature, low concentrations of sugar and high feed moisture content used during extrusion cooking. Similar observations were reported by Bjorck *et al*. (1983), and Brestenský *et al*. (2014) where the available lysine content did not change up to a process temperature of 144^{o}C but decreased 20-30% at temperatures around 150^{o}C. High screw speed and reduction in die diameter can substantially improve lysine retention during extrusion by reducing thermal exposure inside the barrel (Gulati *et al*., 2020). As the screw speed increases, shear increases, resulting in a more drastic breakdown of protein structure; the accompanying reduction in residence time (as a result of the increase in screw speed) reduces the duration of heat treatment, probably leading to high lysine retention. Additionally, a higher amount of sweet potato could enhance lysine retention, perhaps because of the lower levels of lysine in the sweet potato raw material. Losses are more pronounced at higher levels of soy component, given that it has a relatively higher lysine content (Gamlath *et al*., 2007). Furthermore, it is well-known that high nutrient digestibility is generally associated with its high availability. This notion supports the observation in the current study, where an increase of *in vitro* protein digestibility had a corresponding increase in the available lysine of extruded ready-to-eat baby foods (Table 6.1). It is generally understood that the primary reason for losses in available lysine can be associated with Millard-type reactions upon high extrusion temperature and low moisture content (Gulati *et al*., 2020). Therefore, some researchers such as Fellows. (2022) and Gamlath *et al*. (2007) suggest that to keep lysine losses within an acceptable range, it is necessary to avoid extrusion cooking above 180^{o}C at water contents below 15%, and/or avoid the presence of a higher amount of reducing sugars during the extrusion process.

Extrusion cooking is a technique of high temperatures processing that can affect the amino acid composition and enhance the nutritional and quality attributes of legume seed proteins, as evidenced by the observed rise in the phenylalanine content of kidney beans (Drulyte & Orlien, 2019). In a study carried out by Ohanenye *et al*. (2022), it was found that after extrusion at 142°C, the tryptophan content dropped while the contents of valine, phenylalanine, and lysine dropped in peas. In a reversible reaction known as the early Maillard reaction, extrusion at high temperatures can lead to the condensation of the free amino groups of amino acids (or proteins/peptides) with the carbonyl group of reducing carbohydrates. The advanced Maillard reaction is irreversible, though, once the condensation has progressed to cyclization and the aldose produced in the early stages is converted to ketone in an irreversible reaction (Ohanenye *et al*., 2022).

When food is heated, the Maillard reaction occurs spontaneously, forming a wide range of chemicals when the reactive carbonyl groups of reducing sugars combine with the nucleophilic amino group of amino acids, peptides, or proteins (Ruan *et al*., 2018). It is common to link the significance of Maillard reaction products to food and beverage processing as well as to related changes in foods, such as colours, flavours, and scent (Perisi *el al*., 2019). Nevertheless, the Maillard reactions, which result in the formation of protein-saccharide complexes that are unavailable to organisms, are the best-recognized interactions between lysine and reducing sugars. The ε-amino group of lysine attaches to molecules of reducing sugars during this reaction, lowering the availability of lysine and ileal digestibility. The only form of lysine that is present in living things is reactive lysine, which is not related to reducing sugars (Brestenský *et al*., 2014).

The availability and quantity of important amino acids determine the quality of the protein. A decline in protein quality results from numerous protein changes that occur during food processing, including cross-linking, racemization, breakdown, and the creation of complexes with sugars (Lalitha & Singh, 2020). Lysine shortage is an issue, especially among cereals, because of Maillard-type reactions that happen during food preparation and cause a loss of lysine that is readily available (Aggarwal & Bains, 2020). Lysine is an essential amino acid that is heat-labile. In heat-processed foods, the *ε*-amino group attaches to other groups, such as reducing sugars, and rendering it nutritionally unavailable (Lalitha & Singh, 2020).

It may be of interest to note also that, amaranth seed is a nutrient-rich pseudocereal that contains roughly the same amount of essential amino acids in its grains as is recommended by the FAO and the WHO standard. Furthermore, amaranth seed is a good source of polyphenols (phenolic acids and flavonoids) and fatty acids (Procopet & Oroian, 2022). The nine amino acids leucine, isoleucine, valine, phenylalanine, threonine, tryptophan, methionine, histidine, and lysine are considered to be essential. The body depends heavily on essential amino acids for boosting protein synthesis, human metabolism, regulation of several biological functions, body weight regulation, and energy balance (Drummen *et al*., 2018; Tashiro *et al*., 2020; Xiao & Guo, 2021).

6.4 Conclusion

The extrusion cooking temperature and blend proportions affect significantly ($p<0.05$) the protein quality of extruded ready-to-eat baby foods. The findings from this study suggest that the nutritional quality of extrudates was observed, controlled, and maintained, and hence, the products could be used as the potential source of protein by children as well as adult people in case of inadequate protein intake. Compositing soybean and amaranth seed with OFSP makes the extrudates a valuable source of nutrients and may be utilized in the preparation of many foods, particularly as fundamental components in enhancing other foods such as infant and baby foods and any other foods. The blend proportions and extrusion cooking have significant ($p<0.05$) positive effects on *in vitro* protein digestibility as well as available lysine in extruded ready-to-eat baby foods probably due to the relatively low concentrations of sugar, high screw speed and feed moisture content used during the experiment. The study found that extrudates have a high protein digestibility of 91.87% and available lysine of 2.79%, implying that high-quality protein is available in the body once extrudates are consumed.

CHAPTER SEVEN

MICROBIAL LOAD AND SHELF-LIFE ASSESSMENT OF READY-TO-EAT BABY FOODS FROM ORANGE-FLESHED SWEET POTATOES, SOYBEANS, AND AMARANTH SEEDS FLOUR BLENDS

Abstract

The microbiological load of any food product is a valuable indication of extruded food quality, as much as indicating the possible safety status of extrudates for human nutrition and storage. The shelf life of a food refers to the period during which the food product is safe for consumption and/or of an acceptable standard to customers. The microbial safety of food products serves as one of the most essential customer needs. The development of a child can be hampered by inadequate quality complementary foods, which can potentially cause illnesses and even child mortality. A critical step to be taken is post-extrusion processing to manage high-risk food safety concerns. The shelf-life study was carried out to find out how long extrudates could stay on a shelf while preserving their nutritional quality. Blend proportions namely C0 (Control): 100:0:0; C1:50:25:25; and C3:50:30:20 for OFSP: Amaranth Seeds: Soybean flour, respectively, extrusion cooking end barrel temperature (70-90^{o}C), die temperature of 90^{o}C and feed moisture content of 35% were used to formulate extruded ready-to-eat baby foods. The extrudates were collected in storage polyethylene bags and stored at 4 different accelerated conditions for microbial analysis for 28 days. Samples were aseptically taken at the interval of seven days for the determination of total viable count (TVC), total coliform count (TCC), yeasts, and moulds (Y&M). The latter was used as a quality index for an accelerated shelf-life study. The microbial counts of ready-to-eat baby foods ranged from 2.85 to 3.09 log_{10} cfu/g, 0.00 log_{10} cfu/g, and 1.93 to 2.06 log_{10} cfu/g for TVC, TCC, and Yeasts and moulds, respectively. The highest shelf life of extruded ready-to-eat baby foods was about 6 months while the least was 4 months. The extrusion technology has the potential to process products safely and provide them with a longer shelf life.

7.1 Introduction

Plant-based food products are a key growth sector in the food industry due to the increasing demand for healthful, and food sustainability. Ready-to-eat food products including cereal, roots, and tubers are a practical and economical approach to increasing accessibility to plant-based foods (Ströhla *et al*., 2022). The concept of complementary feeding involves giving babies additional food when breast milk is not enough to meet their nutritional demands as well as other foods and liquids are needed in combination with breast milk (Shewangzaw *et al*., 2021). Commercial baby food is highly expensive in developing nations, making it difficult for low-income people to purchase and access it. As a result, it may become less expensive and more accessible for the majority of people to produce supplementary foods at the local industrial scale. However, when consumed, this needs to be "convenient" and microbiological-safe (UNICEF, 2020).

New and complementary food processing methods are needed to meet consumer needs for healthy, high-quality, and safe food products that are also ready-to-eat foods and free of contamination and pathogens (Maharaj, 2019). The qualities of the ingredients and how they are handled during processing influence the food quality and shelf life. The contamination of food that happens throughout various phases of production, handling, and storage accelerates the deteriorative processes (Azad *et al*., 2019).

The accurate estimation of shelf life is crucial to ensuring sustainability across an entire life cycle of food products since it reduces the risk of food waste or brand damage due to under- or overrating the shelf life (Loey *et al*., 2018). Due to the demand to get their products on the market quickly, food companies frequently rely on their choices on shelf life on their business policies, and quality goals. The majority of techniques rely on inaccurate estimates or trial-and-error techniques (Nicoli, 2012). In the industry, shelf-life tests under accelerated environments are frequently used as a tool to make early observations regarding quality changes because ambient storage takes a long time to complete (Ströhla *et al*., 2022).

On the other hand, temperature appears to be the most often employed environmental component among all those that might potentially be utilized as an accelerated factor in Accelerated shelf-life testing (ASLT). This is because there is a theoretical foundation for the creation of a mathematical model of the temperature sensitivity of quality loss rates, which is one of the most important aspects impacting reaction kinetics in food and is among the most important influencing factors. It has been demonstrated empirically that the Arrhenius equation, which was derived theoretically from the molecular foundation for reversible chemical processes, stands true for a variety of complicated chemical, physical, and sensory changes happening in foods (Nicoli, 2012). The shelf-life of food is the period that food product will: (1) keep safe; (2) guarantee that it will maintain its desired sensory, chemical, physical, microbiological, and functional characteristics; and (3), as necessary, confirm to any label declaration of healthy food data when kept under the guidelines. Food quality and safety are undoubtedly the two key factors affecting food shelf life, and as food safety is both a moral imperative and a legal obligation, it must always come first (Subramaniam, 2016).

ASLT is subjective to any deteriorating process that may be quantitatively stated by a reliable model. This model may track changes in shelf life while expressing the importance of a deterioration indicator or the degree of product failure given a specific storage and handling history. The most popular technique for accelerated shelf-life testing is the kinetic model approach. The degrading mechanisms might be chemical, physical, biochemical, or microbial (Mizrahi, 2011). By deliberately raising the deterioration rate or the temperature during the experiment, the duration of the experiment is decreased. Instead of ideal temperature settings, samples for testing must be stored under actual conditions (Ströhla *et al.*, 2022).

Both composition and environmental factors during storage and distribution have an impact on the rate and level of deterioration. High temperatures are frequently used during accelerated shelf-life tests to achieve predictions of shelf-life at lower temperatures or to forecast shelf-life under variable time-temperature distributions (Fu & Labuza, 2012; Ströhla *et al.*, 2022). Temperatures are chosen following the anticipated commercial storage/distribution temperature range (Fu & Labuza, 2012). The ultimate goals of food processors are to reduce the microbiological load in food products and to end the global spread of food-borne disease outbreaks (Liu, 2021).

From "Farm to Fork," food-borne microbes can infiltrate the food supply at any point and lead to contamination and degradation. By using the Hazard Analysis and Critical Control Points (HACCP) method, food technologists and microbiologists are involved in both quality control and quality assurance during the manufacture of any food (Chauhan and Jindal, 2020). Microorganisms must initially be present in the food for them to induce food deterioration. The end customer, the farm, or any other location between them may introduce contaminants into food products (e.g. further processing, packaging, distribution). If a microbe is on food, its ability to grow relies on the environment, the food, and the microorganism itself (Steele, 2004). Each microbe has certain needs that must be addressed for it to flourish, including nutritional requirements, water activity, pH, temperature, oxygen availability, and the presence of antimicrobial agents both in the food itself and in the environment (Azad *et al.*, 2019). Numerous species of bacteria can develop and lead to food deterioration in a wide range of foods. Single-celled bacteria range in size from one to five microns. They proliferate by binary fission and can have a spherical, rod, or spiral form (Steele, 2004).

Packaging plays a big role in food such as protecting food products from external influences and damage, preserving the food, and providing ingredients and nutritional information to consumers (Coles, 2003). Traceability, convenience, and tamper indication are secondary functions of increasing importance. It is very important to choose good packaging material for food. Low-density polyethylene is flexible, strong, tough, easy to seal, and resistant to moisture (Marsh & Bugucu, 2007).

On the other hand, yeasts are a different kind of microbe that may degrade food and are employed in several fermentation processes. Single-celled fungi known as yeasts have a round or cylinder form and a size of three to five microns. Moulds are a different class of fungus with bigger (30 to 100 micron) cells that organize themselves into chains and branches. Moulds may be seen with the unaided eye as they establish their branching structure and appear in a wide variety of forms, sizes, and colours. Moulds can proliferate sexually or asexually by dispersing spores (Steele, 2004). Yeast and mould were used as the quality index in determining the shelf-life of extruded ready-to-eat baby foods to find out how long extrudates could stay on a shelf while preserving their nutritional qualities.

7.2 Materials and Methods

The samples were procured and prepared according to the procedure found in sections 3.2.1, 3.2.2, 3.2.3, and 3.2.4 of Chapter Three.

7.2.1 Extrusion Cooking

Extrusion was done as described in section 5.2.3 of Chapter Five.

7.2.2 Experimental Design

A completely randomized design (CRD) in a factorial experimental design was employed to find out the shelf-life of extruded ready-to-eat foods where the independent variables were the storage temperature (4 levels), storage time (4 levels), and blend proportions at 6 levels while microbial properties of extrudates were dependent variables.

$$Y_{ijkl} = \mu + \propto_i + \beta_j + \delta_k + \alpha\beta_{ij} + \alpha\delta_{ik} + \beta\delta_{jk} + \alpha\beta\delta_{ijk} + \varepsilon_{ijkl} ,$$

where Y_{ijkl}= observation, μ = The grand mean, α_i= The effect of blends, β_j= The effect of Storage Temperature δ_k= The effect of Period, $(\alpha\beta\delta)_{ijk}$= The effect of interaction between Blends, Storage Temperature, and Period, and ε_{ijkl} = Random error.

Furthermore, a multiple regression model with repeated measurement was employed to find out the relationship between storage temperature and storage time on response variables.

$$Y_{ijk} = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ij} X_i X_j + \varepsilon_{ijk} ,$$

where Y_{ijk} is the dependent variable, X_i is the storage temperature, X_j is the storage period, β_0 is the constant, β_i is the linear effects, β_{ij} is the interaction effects, and ε_{ijk} is the effect associated with random error.

7.2.3 Total Viable Counts (TVC)

The AOAC (2000), method 990.12 was used to determine total plate counts using plate count agar (M091, Himedia, Mumbai India). A sample of 25 g extruded products were aseptically weighed, transferred to 225 ml sterile buffered peptone water (RM001, Himedia, Mumbai India) thoroughly mixed then serially diluted. The sterilized Plate Count Agar (PCA) was prepared according to manufacturer's instructions and cooled to 45°C before pour plating. The media and the dilutions were mixed by swirling gently clockwise, anti-clockwise, to and from thrice while taking care that the contents do not touch the lid then allowed to set. The petri-dishes were incubated at 37°C inverted position for 48 hrs and the colonies produced were counted and expressed as colony-forming units per gram (cfu/g).

7.2.4 Yeast and Mould Counts

The AOAC (2000), method 997.02 was used to determine yeast and moulds count. A sample of 25 g extruded products were aseptically weighed, transferred to 225 ml sterile buffered peptone water (RM001, Himedia, Mumbai India) thoroughly mixed then serially diluted. The sample dilution weighing 0.1 ml was transferred from each dilution into corresponding plates and 15 ml sterile Potato Dextrose Agar (M096, Himedia, Mumbai India) media was poured and mixed thoroughly with the inoculum by rocking the plates. The media was cooled to about 45°C, mixed by swirling and allowed to solidify. The plates were incubated in inverted position for 25°C for 5 days, after which colonies were counted and expressed as colony-forming units per gram (cfu/g).

7.2.5 Total Coliform Counts (TCC)

The AOAC (2000), method 992.30 was used. Total coliform counts were analyzed by homogenizing 25 g the sample in 225 ml sterile buffered peptone water (RM001, Himedia, Mumbai India), serial diluted and each dilution transferred into each of the appropriately marked duplicate petri-dishes. About 20 ml of Mac Conkey Agar (M081, Himedia, Mumbai India) was poured into each plate, swirled then allowed to solidify. The petri-dishes were incubated (EU-DR-IN-001, Elisters 2000 Limited) in inverted position at 37°C for 24 hrs. After incubation, the number of colonies that appeared with dark red or pink centres was counted. This was expressed as a colony-forming unit per gram (cfu/g) (AOAC, 2000).

7.2.6 Determination of Accelerated Shelf-Life

In this study, samples were stored in airtight polyethylene bags of 17.7 cm by 20.3 cm and incubated in 4 batches namely 5ºC (Digital Inverter Technology, India), 24ºC (room), 35ºC (SK-50P, Carbolite), and 45ºC (7/98/1501, Elisters 2000 Limited). The quality index for shelf-life was microbial counts for each storage temperature. Microbial enumerations (total viable counts, total coliform counts, yeasts, and moulds) were determined according to AOAC (2000), official methods. Data were collected in duplicate at each stored temperature for analysis on days 0, 7, 14, 21, and 28. The loss of food quality was determined by measuring the quality index (A) at the level of <5.00 $\log_{10}$ cfu/g total viable Counts according to World Food Policy (2013) and <3.00 $\log_{10}$ cfu/g for yeasts and moulds according to the International Association of Microbiological Societies (1978). Data from each storage temperature were used to determine reaction order (n), and rate (k) and use the Arrhenius plot or the kinetic model for shelf-life prediction at the actual storage condition of 24ºC (Fu & Labuza, 2012).

The four phases that make up the fundamental procedure include (1) Choosing the preferred kinetically active elements to accelerate the degradation process (2) Conducting a kinetic analysis of the degradation process at accelerating factor levels when the rate of deterioration is sufficient. (3) Extrapolating the data to typical storage conditions while assessing the parameters of the kinetic model. (4) Calculate the shelf life at real storage conditions using extrapolation data or a kinetic model (Mizrahi, 2011). The five steps used for the shelf-life estimation include (1) To evaluate the kinetics order of response, data on yeasts and moulds were converted into $\log_{10}$ cfu/g and plotted against storage time for each of the 4 storage temperatures. (2) To determine the linear model equations concerning the following equation, the reaction order was chosen as the first order and a regression plot of [A]/[A0] against storage duration was made.

$$Ln\left(\frac{[A]}{[A_0]}\right) = kt,$$

where [A] is the yeasts and moulds count on a given storage period while [A_0] is the initial counts of day 0 and t is the storage time in days (Phimolsiripol and Suppakul, 2016). (3) The parameters of the Arrhenius equation may now be calculated using the estimated values of the first-order rate constants. To determine if the Arrhenius equation is acceptable to explain the relationship between the rate constant and temperature, a plot of the logarithmic values of reaction rate (ln k) vs the reciprocal value of the absolute temperature [1/T, measured in Kelvin (K)] is required. The following conversion of temperature data is required to use the re-parametrization process.

$$\text{Ln } k = \text{Ln}k_0 - \frac{E_a}{R}\left(\frac{1}{T}\right); \qquad \frac{1}{T} = \frac{1}{T^*} - \frac{1}{T_{ref}}$$

where k is the reaction rate constant; R is the universal gas constant (8.314 J/K mol), T is the absolute temperature in Kelvin degree (273 + ^oC); E_a is the activation energy (J/mol) and k_o is the pre-exponential factor; T_{ref}, corresponding to the average of the temperature range used during the experiment where in this case was taken to be 27.5^{o}C (300.65^{o}K) and T* is the temperature to which prediction of shelf life is done, in this case, it was 24^{o}C (297.15^{o}K) (Calligaris *et al*., 2019; Manzocco *et al*., 2016). (4) By using linear regression analysis, Arrhenius equation parameters may be calculated. As a result of using Arrhenius equations, a mathematical model is used to estimate reaction rate as a function of temperature:

$$K = K_{ref}\text{Exp}[-\frac{E_a}{R}(\frac{1}{T^*} - \frac{1}{T_{ref}})],$$

where K_{ref} and Ea must be substituted by the corresponding estimates. K_{ref} is the rate constant at the reference temperature, and T* is the temperature at which to predict shelf life (Katsouli *et al*., 2022), finally (5) It is feasible to estimate the values of k at the temperature of interest—in this case, 24°C, or 297^{o}K was derived from the Arrhenius equation. The Ea of the reaction in kJ/mol may also be determined from the equation's result, $k_{24°C}$. Using the information from the Arrhenius equation to determine shelf life, the formula below was used:

$$\text{Shelf life} = \frac{[\text{Ln } I_0] - [\text{Ln } I_{lim}]}{K_{24oC}}$$

$$\text{Shelf life} = \frac{[A_{lim}] - [A_0]}{K_{ref}\text{Exp}[-\frac{E_a}{R}(\frac{1}{T^*} - \frac{1}{T_{ref}})]}$$

where $[A_0]$ is the initial yeasts and moulds counts at time zero; E_a is the apparent activation energy (J/mol); T* is the temperature to which prediction of shelf life is done, in this case, it was 24^{o}C (297.15^{o}K); $[A_{lim}]$ is the standard acceptable limit for yeasts and moulds count in processed foods which is 3.00 $\log_{10}$ cfu/g; T_{ref}, corresponding to the average of the temperature range used during the experiment where in this case was taken to be 27.5^{o}C (300.65^{o}K) (Calligaris *et al*., 2019; Nicoli, 2012). If k_1 and k_2 are the rate constants at temperatures T_1 and T_2, respectively, then:

$$\text{Ln } \frac{k_2}{k_1} = \frac{E(T_2 - T_1)}{RT_1T_2}$$

The prediction of quality degradation as a function of temperature makes heavy use of the Arrhenius Law. The most significant relationship for the modelling temperature dependency of various quality changes in food is likely the Arrhenius relationship. The quantity known as Q_{10}, or the temperature quotient, is another method of expressing how sensitive the response is to temperature changes. Q_{10} is the ratio of the rate constant of a reaction to that of the same reaction at a temperature lower by 10°C (Berk, 2018). The activation energy and temperature quotient will have the following relationship after applying this definition to the equation above:

$$\mathrm{Ln}\ Q_{10} = \frac{10Ea}{RT_1(T_1 + 10)} = \frac{10Ea}{RT_1^2}$$

7.2.7 Data Analysis

The microbial counts were transformed by $\log_{10}$ for analysis. To fulfil the requirements of parametric data, data obtained were then tested through the normality distribution and standard homogeneity of variance tests. The analysis of variance (ANOVA) was carried out using statistical analysis software (SAS Version 9.4). The main and interaction effects of blend proportions, storage temperature, and storage time on the microbial load in extruded ready-to-eat baby foods were examined using the general linear model (GLM). Using yeasts and moulds as a measure of quality, a regression model was used to calculate the rate of chemical kinetics using the Arrhenius equation to estimate the shelf life. The regression model coefficient of determination (R^2) was used to evaluate how well the independent and dependent variables fit perfectly. Schwarz's Bayesian Criterion (BIC), Akaike's Information Criterion (AIC), and Conceptual Predictive criterion C(p) were used for the identification of the best model for the prediction of microbial load. Tukey's honest significant difference (HSD) was used for means separation, with a 5% level of significance.

7.3 Results and Discussion

The results revealed that all tested parameters were significantly ($p<0.05$) influenced by blends proportions, storage time, and storage temperature, and significant differences ($p<0.05$) were observed among microbial counts (Table 7. 1). The microbial counts of ready-to-eat baby foods ranged from 2.85 to 3.09 $\log_{10}$ cfu/g, 0.00 $\log_{10}$ cfu/g, and 1.93 to 2.06 $\log_{10}$ cfu/g for TVC, TCC, and Yeasts, and moulds, respectively by considering blend proportions.

The microbial results of extruded ready-to-eat baby foods are below the safe limit of <5 $\log_{10}$ cfu/g for TVC and <3 $\log_{10}$ cfu/g for yeasts and moulds (World Food Policy, 2013; International Commission on Microbiological Specifications for Foods, 1978) though the International Microbiological Standard recommends that the limit of bacteria contaminants for food should be less than 10^6 cfu/g (Mbaeyi-Nwaoha & Obetta, 2016). There is significant

($p<0.05$) difference among 6 samples (Table 7.1). The total coliforms were analysed on day 0 only and were not detected in extruded ready-to-eat baby foods.

The TVC results confirm well to the trends ($1.9 \times 10^4 – 4.8 \times 10^4$) reported by Mbaeyi-Nwaoha and Obetta (2016) for fermented unfermented pigeon pea flour, millet flour, breadfruit (*Artocarpus altilis*) leaf powder and the blends. The results are also close to the values (3.17-3.46 $\log_{10}$ cfu/g) reported by Mekuria *et al*. (2021) for complementary foods during storage. Yeasts and moulds results are below the range ($1 \times 10^3 – 3 \times 10^3$ cfu/g) of moulds reported by Mbaeyi-Nwaoha and Obetta. (2016) for fermented unfermented pigeon pea flour, millet flour, breadfruit (*Artocarpus altilis*) leaf powder, and the blends.

Table 7. 1 Microbial counts (Log_{10} cfu/g) and shelf-life (days) of extruded ready-to-eat baby foods

Comparison Factors	Y&M	TVC	TCC
Product			
C070	2.05 ± 0.64^{a}	2.85 ± 1.21^{c}	N.D
C090	2.06 ± 0.73^{a}	3.07 ± 1.22^{ab}	N.D
C170	2.00 ± 0.67^{ab}	3.02 ± 1.39^{ab}	N.D
C190	1.93 ± 0.70^{b}	3.00 ± 1.24^{b}	N.D
C370	1.93 ± 0.84^{b}	3.07 ± 1.27^{ab}	N.D
C390	1.96 ± 0.72^{b}	3.09 ± 1.23^{a}	N.D
Week			
1	1.07 ± 0.17^{d}	1.44 ± 0.20^{d}	
2	1.65 ± 0.18^{c}	2.39 ± 0.24^{c}	
3	2.31 ± 0.21^{b}	3.52 ± 0.27^{b}	
4	2.91 ± 0.12^{a}	4.71 ± 0.18^{a}	
Temperature (°C)			
5	2.03 ± 0.67^{a}	3.11 ± 1.23^{a}	
25	2.00 ± 0.74^{a}	3.05 ± 1.27^{a}	
35	1.98 ± 0.74^{ab}	3.01 ± 1.27^{ab}	
45	1.94 ± 0.73^{b}	2.89 ± 1.25^{c}	

Data are indicated in duplicate values as the mean ± standard deviation. Mean values with different superscript letters in the same column are significantly different ($p \leq 0.05$). Where N.D is not detected.

However, the results are in agreement with the results (2.0 log_{10} cfu/g – 2.60 log_{10} cfu/g) reported by Mekuria *et al*. (2021) for complementary foods during storage.

The microbiological load of any food product is a helpful indicator of the quality of extruded ready-to-eat baby foods as well as indicating if the extruded food products are potentially safe to eat and for storage (Mekuria *et al*., 2021). Particularly for ambient stable products, storage at elevated temperatures can be utilized as a strategy to accelerate the deteriorative processes of the food product (Calligaris *et al*., 2019). It has a significant benefit for the food industries since it may reduce research and manufacturing costs as well as time and resources (Ströhla *et al*., 2022).

Due to several diverse responses occurring simultaneously and interacting with one another at various reaction speeds, changes in food quality that affect the preferences of customers are excedingly complicated. Acceptance fluctuates depending on changes in food production and storage as well as how each consumer perceives food using their senses, which is dependent on their environment (Ströhla *et al*., 2022).

Temperature is one of the elements that influence the ability of an organism to grow. Microorganisms can grow quickly, slowly, cease developing, or even die depending on the temperature of storage (Steele, 2004). Microorganisms may develop at a range of different temperatures. The microbial growth temperature ranges from -34°C to 90°C. There is a minimum, ideal, and maximum growth temperature for each microbe. The term "cardinal temperatures" refers to them. To prevent food from spoiling and improve safety from pathogens, food bioprocessing, enumeration, and isolation of microorganisms from foods, exposure of bacteria beyond the maximum and lowest temperatures in foods is crucial. As the temperature rises over the optimum, enzymes and proteins get permanently denaturated and the cytoplasmic membrane breaks down, which causes the growth rate to fall much more abruptly. These alterations are enough to kill the bacterium at temperatures over the maximum (Erkmen & Bozoglu, 2016).

Depending on the temperature range in which they thrive, microorganisms may be divided into three primary types. Mesophiles are microorganisms that thrive between 30^{o}C and 40^{o}C, yet they may also flourish between 10^{o}C and 45°C. Psychrotrophs like cooler climates. They may grow at low temperatures as low as 7^{o}C, but 20^{o}C to 30°C is where they thrive. Thermophiles thrive in warmer climates and do best between 55^{o}C and 65^{o}C, while they can also thrive between 45^{o}C and 55^{o}C. When temperatures are above 60^{o}C, certain bacteria start to die (Erkmen & Bozoglu, 2016; Steele, 2004). Even greater temperatures will hasten the demise of microorganisms. Microbial development is influenced by the nutritional composition of food as well. Water, a carbon source for energy, a nitrogen supply, and certain vitamins and minerals are all necessities for microbes to develop. Moulds and yeasts have requirements that are very different from bacteria, which have a demand that is in the middle (Steele, 2004).

7.3.1 Multiple Regression for Yeasts and Moulds

The model shows the results of fitting a multiple linear regression model to describe the relationship between yeasts and moulds and 3 independent variables (product, storage time, and storage temperature). Since the P-value in the ANOVA Table 7. 2 is less than 0.05, there is a statistically significant relationship between the variables at the 95.0% confidence level. The R-Squared statistic indicates that the model as fitted explains 94.29% of the variability in yeasts and moulds. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 94.20%. The standard error of the estimate shows the standard deviation of the residuals to be 0 .0122. This value can be used to construct prediction limits for new observations.

The ANOVA shows that yeasts and moulds are significantly ($p<0.05$) influenced by the main factors and also their interactions except for product vs temperature (Table 7. 3). In determining the interactions effects of variables, the model revealed that the highest P-value on the independent variables is 0.0030, belonging to Temperature. Since the P-value is less than 0.05, that term is statistically significant at the 95.0% confidence level. The interaction effects of product and temperature did not significantly ($p>0.05$) affect yeasts and moulds.

Table 7. 2 Analysis of Variance for yeasts and moulds

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	96.18898379	1.01251562	83.09	<.0001
Error	96	1.16981912	0.01218562		
Corrected Total	191	97.35880291			
R^2			94.29		
Adjusted R^2			94.20		

The selection criteria are based on goodness of model fit which should have adjusted $R^2 \geq 0.80$, significant $p<0.05$, a small value of Schwarz's Bayesian Criterion (BIC), Akaike's Information Criterion (AIC), and Conceptual Predictive criterion C(p). The selection of the best model that predicts well the yeasts and moulds is shown in Table 7. 4. Up to 4 models in each subset of between 0 and 3 variables are shown in Table 7. 4.

Table 7. 3 Interaction of factors used in the accelerated shelf-life study for yeasts and moulds

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Product	5	0.52520439	0.10504088	8.62	<.0001
Time	3	91.81952875	30.60650958	2511.69	<.0001
Temperature	3	0.18134740	0.06044913	4.96	0.0030
Product*Time	15	1.35169945	0.09011330	7.40	<.0001
Product*Temperature	15	0.30812857	0.02054190	1.69	0.0666
Time*Temperature	9	0.91269003	0.10141000	8.32	<.0001
Product*Time*Temperature	45	1.09038519	0.02423078	1.99	0.0026

The best model contains 3 variables, product, temperature, and time for the best prediction of yeasts and moulds of extruded ready-to-eat baby foods followed product and time.

Table 7. 4 Selection of best model predicting yeasts and moulds

Model	C(p)	R^2	AIC	BIC	SSE	Variables in Model
3	4.00	0.9482	-690.6944	-688.5251	5.04532	Product Time Temperature
2	8.33	0.9464	-686.3381	-684.4103	5.21515	Product Time
2	16.53	0.9442	-678.4044	-676.7216	5.43516	Time Temperature
1	20.85	0.9424	-674.4970	-672.8328	5.60499	Time
2	3420.95	0.0057	-125.4926	-130.9660	96.79914	Product Temperature
1	3425.28	0.0040	-127.1560	-130.7366	96.96897	Product
1	3433.48	0.0017	-126.7209	-130.3024	97.18898	Temperature

7.3.2 Multiple Regression for TVC

The output shows the results of fitting a multiple linear regression model to describe the relationship between TVC and 3 independent variables. Since the P-value in the ANOVA

Table 7. 5 is less than 0.05, there is a statistically significant relationship between the variables at the 95.0% confidence level. The R-Squared statistic indicates that the model as fitted explains 97.17% of the variability in TVC. The adjusted R-squared statistic, which is more suitable for comparing models with different numbers of independent variables, is 97.12%.

Table 7. 5 Analysis of Variance for TVC

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	3.00	288.84	96.28	2,150.65	<.0001
Error	188.00	8.42	0.04		
Corrected Total	191.00	297.26			
R^2			97.17		
Adjusted R^2			97.12		

The results in Table 7. 5 show the models which give the largest adjusted R-Squared values. The adjusted R-Squared statistic measures the proportion of the variability in a product which is explained by the model. Larger values of adjusted R-Squared correspond to smaller values of the mean squared error (MSE). All main factors and their interactions significantly ($p<0.05$) affected the TVC of extruded ready-to-eat baby foods (Table 7. 6).

Table 7. 6 Interaction of factors used in the accelerated shelf-life study for TVC

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Product	5	1.2575531	0.2515106	18.86	<.0001
Time	3	287.8935276	95.9645092	7197.77	<.0001
Temperature	3	1.2079420	0.4026473	30.20	<.0001
Product*Time	15	1.9055638	0.1270376	9.53	<.0001
Product*Temperature	15	0.6568434	0.0437896	3.28	0.0002
Time*Temperature	9	0.4650879	0.0516764	3.88	0.0003
Product*Time*Temperature	45	2.5935334	0.0576341	4.32	<.0001

To determine which models are best according to these different criteria, the selection was carried out from Table 7. 6. This table shows the models which give the smallest values of Mallows' Cp statistic. Cp is a measure of the bias in a model, based on a comparison of the total mean squared error to the true error variance.

Unbiased models have an expected value of approximately p, where p is the number of coefficients in the fitted model including the constant. The results in Table 7. 7 sort the regression models according to the value of the Akaike Information Criterion (AIC), BIC, SSE, and R^2.

Table 7. 7 Selection of best model for TVC

Factors	**C(p)**	**R^2**	**AIC**	**BIC**	**SSE**	**Variables in Model**
3	4	0.9717	-592.44	-590.27	8.42	Product Time Temperature
2	16.41	0.9695	-580.26	-578.57	9.06	Time Temperature
2	25.17	0.9682	-572.13	-570.69	9.45	Product Time
1	37.59	0.966	-561.45	-560.09	10.10	Time
2	6416.37	0.0057	88.84	83.12	295.58	Product Temperature
1	6428.78	0.0035	87.25	83.48	296.22	Temperature
1	6437.54	0.0022	87.51	83.74	296.61	Product

The information criterion is based on the residual mean squared error with a penalty that grows as the number of model coefficients increases. The goal was to select a model with a small residual error and as few coefficients as possible, as well as a high coefficient of determination. The best model is the one that minimizes the information criterion. Often, the best model depends on the information criteria selected, which each uses a different formula for the penalty. In this case, up to 4 models in each subset of between 0 and 3 variables are shown. The best model contains 3 variables, product, temperature, and time for the best prediction of TVC of extruded ready-to-eat baby foods followed by time and temperature.

The best straight line fit across the experiment set of data, however, must also be found using the Arrhenius model. It is possible to predict the shelf life at a specific temperature by linearly extrapolating the produced straight line. The term ln (k) from reaction rate constants of first-order reactions (Table 7. 8) was taken for the appropriate accelerated storage temperature and plotted against the absolute inverse of temperature in Kelvin (1/T) to create the regression plot of the semi-logarithmic scale graph.

Table 7. 8 Regression equation of reaction order of extruded ready-to-eat baby foods based on yeasts and moulds

Food	ET (◦C)	ST (◦C)	Zero Order	R^2	First Order	R^2
C0	70	5	y = 0.4363x + 1.0291	91.67	y = 0.2032x - 0.209	95.16
C0	70	25	y = 0.3466x + 0.9065	94.97	y = 0.2214x - 0.0283	98
C0	70	35	y = 0.3782x + 0.8679	92.27	y = 0.235x - 0.048	96.46
C0	70	45	y = 0.364x + 0.8758	92.74	y = 0.2288x - 0.0445	97.05
C0	90	5	y = 0.452x + 1.0711	98.77	y = 0.2422x + 0.0076	99.74
C0	90	25	y = 0.4744x + 0.9651	92.86	y = 0.2594x - 0.0265	95.83
C0	90	35	y = 0.4824x + 0.9396	95.23	y = 0.2648x - 0.0218	98.21
C0	90	45	y = 0.4921x + 0.9743	98.67	y = 0.2723x + 0.0188	98.95
C1	70	5	y = 0.3321x + 0.9039	92.44	y = 0.2125x - 0.0261	98.1
C1	70	25	y = 0.381x + 0.8741	92.77	y = 0.2363x - 0.044	96.59
C1	70	35	y = 0.3927x + 0.8719	92.66	y = 0.2388x - 0.0363	98.41
C1	70	45	y = 0.4278x + 0.989	98.53	y = 0.2449x + 0.0049	99.81
C1	90	5	y = 0.3617x + 0.8941	92.26	y = 0.2251x - 0.0261	98.22
C1	90	25	y = 0.3986x + 0.8553	89.47	y = 0.2399x - 0.0429	97.07
C1	90	35	y = 0.3992x + 0.9501	91.67	y = 0.244x + 0.0011	94.36
C1	90	45	y = 0.4201x + 0.8238	88.83	y = 0.2529x - 0.0719	93.05
C3	70	5	y = 0.5022x + 0.7933	96.94	y = 0.3112x + 0.0527	97.18
C3	70	25	y = 0.5643x + 0.7175	93.71	y = 0.3297x - 0.0218	97.27
C3	70	35	y = 0.5575x + 0.7456	90.56	y = 0.3322x - 0.0015	94.49
C3	70	45	y = 0.5356x + 0.611	93.96	y = 0.3498x - 0.0112	97.31
C3	90	5	y = 0.4547x + 0.9674	95.03	y = 0.2498x - 0.0118	99.52
C3	90	25	y = 0.437x + 0.8712	94.27	y = 0.2564x - 0.0286	99.17
C3	90	35	y = 0.4551x + 0.8692	94.78	y = 0.2637x - 0.0281	99.08
C3	90	45	y = 0.4652x + 0.8303	94.59	y = 0.2735x - 0.0185	99.62

Where ET is extrusion temperature and ST is storage temperature

The obtained results were essentially in agreement with the relationship of the Arrhenius equations in Table 7. 9, which provides the best straight line that fits through the experimental data sets (Figure 7.1).

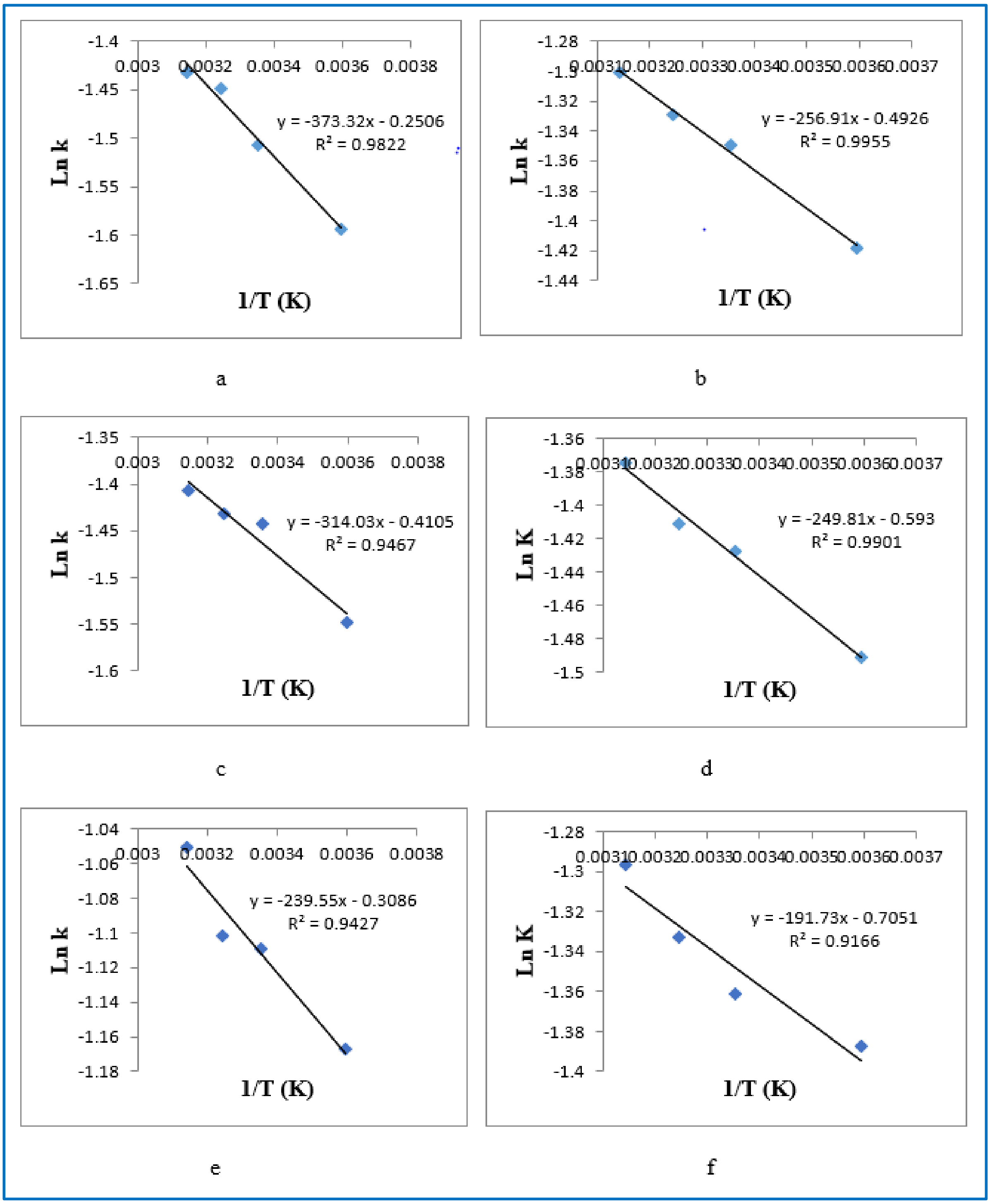

Figure 7. 1 Arrhenius graphs (a, b, c, d, e, and f) for shelf-life prediction of extruded ready-to-eat baby foods based on yeasts and moulds, where 1/T (K) is the absolute inverse of temperature in Kelvin.

The most significant uses of reaction kinetics in food engineering include (1) calculating thermal processing for the inhibition of enzymes and the destruction of microorganisms, (2) optimizing thermal processes for quality, (3) optimizing processes for cost, and (4) predicting the shelf life of foods as a function of storage conditions, (5) Calculating the refrigeration load required to store agricultural products that are still respiring, and (6) Creating time-temperature integrators (Berk, 2018).

On the other hand, it has been suggested that tests with accelerated temperatures be carried out at high temperatures. Significant variations in the rate of deterioration will be seen when the sample is exposed to higher temperatures. Estimating the deterioration behaviour in a short time becomes more practical as the temperature rises. Table 7. 8 shows the zero-order and first-order reactions of regression equations and coefficients of determination. The first-order reaction was used to create the Arrhenius equation from there. But when the temperature is lower, the process takes longer and the sample takes longer to deteriorate (Rashid *et al*., 2020). The activation energy, Q_{10}, and shelf life of extruded ready-to-eat baby foods at 24°C (297^{o}K) can be estimated by comparing the Arrhenius regression equation in Table 7.9. While the shelf life of extruded ready-to-eat baby foods may be determined by solving the regression equation with T* = 24°C, the activation energy can be determined from the slope of the line (the temperature to which the prediction of shelf life is done). The slope of the line is used to calculate the activation energy (divided by the gas constant R). Since a slight change in T results in a substantial change in rate, a steeper slope indicates that the reaction is more temperature sensitive (Calligaris *et al*., 2019).

Table 7.9 is a summary of the outcomes. With the data of activation energy, Q_{10} was also calculated. The activation energy ranged from 3.67 to 8.53 kJ/k/mol, shelf life ranged from 108 to 169 days while the Q10 ranged from 1.04 to 1.11 when yeasts and moulds were used as a quality index. The Q_{10} results are higher than the values (0.76-1.01) reported by Wafula *et al*. (2021) for extrudates from rice, sorghum, and bamboo shoots flour blends but within the values (1.154-1.202) reported by Tolve *et al*. (2022) for different chocolate spreads. The Q_{10} value of 2.0 was reported by Morsy *et al*. (2022) for oil oxidation and 2.8 was reported by Ditudompo *et al*. (2022) for salmon burgers. The Q_{10} results show how temperature affects reaction rate catalytically for all the extruded ready-to-eat baby foods under investigation. The Q_{10} coefficient shows how quickly a process speeds up when the storage temperature rises by 10°C. When the storage temperature is increased by 10^{o}C, the rate of chemical reactions often doubles or triples. The Q_{10} is used for different purposes, including estimating shelf life by changing chemical reaction rates in response to each 10°C increase in storage temperature (Ditudompo *et al*., 2022). The activation energy is a useful tool for predicting the reaction rate at the desired temperature. It varies according to the properties of food, as well as the preparation and storage conditions. The sensitivity of the reaction rate to temperature variations is truly represented by the activation energy (Calligaris *et al*., 2019).

Table 7.9 Shelf-life prediction at 24°C of extruded ready-to-eat baby foods based on yeasts and moulds as a quality index

Foods	ET (◦C)	ST (◦C)	Arrhenius Equation	R^2	Adjusted R^2	P-value	Ea (KJ/K/Mol)	SL_{24^oC}	Q_{10}
C0	70	5							1.11
C0	70	25	y = -373.32x - 0.2506	98.22	97.32	**	7.05	108	1.10
C0	70	35							1.09
C0	70	45							1.09
C0	90	5							1.08
C0	90	25	y = -256.91x - 0.4926	99.55	99.32	**	4.87	137	1.07
C0	90	35							1.06
C0	90	45							1.06
C1	70	5							1.09
C1	70	25	y = -314.03x - 0.4105	94.67	92.01	*	8.53	125	1.08
C1	70	35							1.08
C1	70	45							1.07
C1	90	5							1.07
C1	90	25	y = -249.81x - 0.593	99.01	98.51	**	4.78	151	1.06
C1	90	35							1.06
C1	90	45							1.06
C3	70	5							1.07
C3	70	25	y = -239.55x - 0.3086	94.27	91.40	*	6.16	113	1.06
C3	70	35							1.06

C3	70	45							1.05
C3	90	5							1.06
C3	90	25	y = -191.73x - 0.7051	91.66	87.49	*	3.67	169	1.05
C3	90	35							1.05
C3	90	45							1.04

Where, ET=Extrusion temperature, ST=Storage temperature, SL_{24^oC} is shelf-life pridicted at 24°C, and R^2=Coefficient of determination

*, ** = Significant at $p \leq 0.05$, $p \leq 0.01$, respectively.

The activation energy results obtained in this study are low compared to the values (11.31-14.48 kJ/k/mol) reported by Tolve *et al*. (2022) for different chocolate spreads and also low to the values (11.83-108.54 kJ/k/mol) as reported by Choosuk *et al*. (2022) for dried coconut chips. The results from this study however, fall within the range (0.63-66.60 kJ/k/mol) reported by Wafula *et al*. (2021) for extrudates from rice, sorghum, and bamboo shoot flour blends.

High Ea values indicate protein denaturation and microbial inactivation. The various compositional, processing, and packaging factors that are unique to the food product under consideration have a significant impact on the vast range of activation energies observed for the same alternative event (Calligaris *et al*., 2019). The accelerated storage test is one of the ways the Arrhenius model is used in food process engineering. It could take a while to investigate how food changes during normal storage. However, by storing the test samples at a higher temperature, the changes can be hastened. The rate of change at the normal storage temperature can be determined from the accelerated rate of change if the system has been proven to obey the Arrhenius Law and if the activation energy is recognized (Berk, 2018).

7.4 Conclusion

The study shows that extrusion cooking and post-extrusion operations have promising information that can be used to achieve a long shelf-life of extrudates. The shelf life of extrudates was estimated to be about 6 months and the lowest is 4 months. All ready-to-eat baby foods produced during the experiment were microbiologically safe for sensory evaluation. The blend proportions, storage period and temperature, and their interaction effects significantly ($p<0.05$) affect the yeasts and moulds, and TVC. The stability study shows that the extruded ready-to-eat baby foods can be stored for a long period while maintaining safety and quality and thus offering successful marketing and distribution operations.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

The general discussion is divided into six subsections. The first is the critical review of the main methodology employed in this study, the second is the identification of raw materials used in this study and the extrusion cooking technology in food processing, the third part reflects the major findings of this study, the fourth one is emphasizing on general conclusions for each specific objective of this study, the fifth part is concerning with recommendations for every specific objective while the last subsection is the area of further studies.

8.1 Critical Review of the Methodology

The study identified the potential of using different ingredients (OFSP, soybean, and amaranth seeds) for the production of nutrient-dense composite flour using extreme vertices of mixture design as the approach to optimization of ingredients. The mixture designs (MDs) are extensively used to optimize processing, development, or formulation conditions for new food products. The mixture design has found uses in both the academic community and industry in the fields of food, beverage, and pharmaceutical health (Galvan *et al*., 2021). In addition, the optimal extrusion cooking variables were established in this study using multivariate statistical analysis through using Box-Behnken design under response surface methodology. Several studies have been conducted to optimize the extrusion process parameters using various optimization strategies, with response surface methodology (RSM) being regarded as the most promising method for optimizing process parameters in the food industry (Srikanth *et al*., 2021). BoxBehnken design (BBD) and response surface methodology (RSM) are used to generate statistical models for fibre stability optimization and identification of stability zones (Nasser *et al*., 2021). This study also used the factorial design. Factorial design is an effective and frequently used experimental design approach in several disciplines, including psychology, sociology, engineering, and manufacturing. It entails altering many independent variables at the same time in order to explore their individual and interacting effects on a dependent variable (Madhyastha *et al*., 2020; Rigdon *et al*., 2022). Factorial designs are important in research and experimentation because they quickly examine several factors, capture interaction effects, improve external validity, increase statistical power, give flexibility, and enable practical applications in a variety of domains. Their significance derives from their capacity to give a thorough knowledge of complex situations while also supporting evidence-based decision-making.

On the other hand, kinetic modelling is significant in food processing and food quality because it allows for the quantitative description of food quality changes and their rates. It also elucidates fundamental response processes. Typical uses of kinetic modelling in food processing include microbial destruction kinetics, drying dynamics, cooking kinetics, kinetics of numerous processes occurring during processing (e.g., acrylamide generation during frying), enzymatic reaction kinetics, and fermentation kinetics (Gokhale *et al*., 2023). Microorganisms lose viability when exposed to temperatures over their optimum. Assuming that inactivating a single enzyme will inactivate the cell, the mortality rate, i.e., the rate of destruction, is proportional to the number of organisms in a suspension of microorganisms of a single species at a fixed temperature (Dash & Deka, 2021).

OFSP, amaranth seeds, and soybean flour were used for ingredients optimization and extruded for the production of ready-to-eat baby foods. The extruded ready-to-eat baby foods were analyzed for nutritional, anti-nutrients, microbial, sensory, and physical properties using standard methods of the American Association of Cereal Chemists (AACC) and the Association of Official Analytical Chemists (AOAC). In addition, protein quality including *in vitro* protein digestibility was determined using enzymatic methods and dye-binding methods for available lysine in the extruded ready-to-eat baby foods. Objective 1 used the extreme vertices method of mixture design for the ingredients optimization based on protein, total minerals, and vitamin A content of composite flour while objective 2 employed a Box-Behnken experimental design (BBD) of response surface methodology for optimization of extrusion cooking conditions. In objectives 3 and 4, a completely randomized design (CRD) in a factorial experimental arrangement was used then finally objective 5 employed a multiple regression with repeated measurement. The analysis of variance (ANOVA) and generalized linear model (GLM) was used in this study and mean separations were done using Tukey at a significance level of 5%. The standard homogeneity of variance was conducted using the LEVENE test, and the basic statistical measures and goodness-of-Fit tests were carried out for the normality distribution of data.

8.2 Identification of Raw Materials and Extrusion Cooking of Extruded Ready-To-Eat Baby Foods

The development of new products requires effective food formulation processes. Food formulation is important for creating new food products because of the increasing consumer demand for foods with important nutritional and health benefits. New food formulations and processing processes have been commercially adopted to improve nutrient retention, quality, and shelf life, as well as to assist sustainability. Increased use of high-protein, low-fat, low-calorie products and novel ingredients that promote excellent overall health has been driven by health-conscious customers (Pathania *et al*., 2021). As the second most significant source of carbohydrates for humans after cereals, root and tuber crops are essential for maintaining the world's largest food and nutrition security. In Africa, 20% of calories are consumed from roots and tubers. Cassava, yam, and potato crops are essential for women-owned small businesses and farmers in general, as well as for ensuring food security (FAO, 2021). Extruded weaning foods are prepared from a blend of cereals and legumes and supplemented with minerals and vitamins to provide an adequate amount of protein and energy for developing children. Another type of weaning food is RTE “rusk“ products, which resemble aerated cookies and are intended to dissolve in saliva when consumed (Fellows, 2022).

To boost the vitamin A content of baby food, OFSP has been utilized as an ingredient. Despite having low levels of protein and fat, OFSP should be combined with high levels of protein and fat, like soybean flour, to produce healthful foods (Amagloh & Coad, 2014). Value addition and innovative product diversification can help to improve the perception of amaranth seeds and OFSP and hence increase consumption of these products not only to children but also to urban populations and younger generations (Mazike *et al*., 2022). Considering this information, the diversification of diet through the inclusion of OFSP could help alleviate vitamin A deficiency (Emmambux *et al*., 2022).

Therefore, OFSP was blended with soybean and amaranth seeds flour for the production of extruded ready-to-eat baby foods not only that but also for vulnerable groups such as young children and pregnant and lactating mothers. The amaranth species are crops that have a great deal of potential for reducing hunger, malnutrition, and poverty as well as illnesses that may be linked to those conditions as well as attaining food and nutrition security (Aderibigbe *et al*., 2020). The flour of the amaranth seeds can be made into various products such as pancakes, flatbread, muffins, dumplings, crackers, tortillas, cookies, biscuits, puddings, pasta, breakfast cereal, soups, sauces, porridges, soufflés, beverages, and beer. The incorporation of amaranth flour in the manufacture of tortillas and crackers significantly increased the protein, ash, and fibre content of the formulations (Gebreil *et al*., 2020). The Amaranth seed has been described as a “super” food because it can produce gluten-free baked goods as compared to wheat (Aderibigbe *et al*., 2020).

On the other hand, fundamental issues in agri-food systems must be addressed through innovative food processing technology. Food processing alters the structure and physicochemical characteristics of proteins, modulating functioning by enhancing heat stability, protein-protein interaction, unfolding and aggregation, and changing interaction strength and bond types, among other factors (Aryee *et al*., 2018). The industrial adoption of innovative processing techniques is still in its early stages; yet, certain innovations have found commercial use (Pathania *et al*., 2021). Emerging food production technologies including extrusion cooking technology can turn diverse resource-intensive food systems into more affordable, sustainable, and healthy food than traditional food processing methods. Both commercial weaning foods and the emergency infant food that is provided by humanitarian organizations are produced using the extrusion cooking method. The products are guaranteed to be microbiologically safe by the high-temperature extrusion (Fellows, 2022).

One of the most cutting-edge technologies utilized in the food sector is extrusion cooking, which may create a wide variety of food products. Currently, texturized vegetable protein, confectionery foods, ready-to-eat snacks, meat analogues, infant foods, and other food products are all prepared using extrusion technology. Extrusion processing offers significant variety in creating extremely nutritious foods at a lower cost, which aids in displacing traditional techniques of making snacks that have a high processing cost and are less nutrient-dense (Mathad *et al*., 2022). Several benefits of extrusion cooking, notably reducing anti-nutritional compounds, including that it is reasonably affordable (Omosebi *et al*., 2018), starch gelatinization (Wang *et al*., 2017), increasing soluble dietary fibre (Zhong *et al*., 2019), inactivation of enzymes, reducing lipid oxidation, and lowering microorganism contamination (Shelar & Gaikwad, 2019) due to high temperature and low moisture content present in the food products during extrusion. It should be noted that the final extrudate quality is highly influenced by the types of extruders used and the processing environment. The most typical processing variables to take into account are the moisture content of feed materials, barrel temperature, screw speed, and feeding rate (Pichmony *et al*., 2020).

Extrusion cooking has been shown to generate food with a variety of extrudate features or characteristics, such as physical properties (such as expansion, density, and structure), nutritional content, sensory quality (such as texture), and microbial organisms are destroyed (Pichmony & Ganjyal, 2020). Additionally, because the raw materials are heated to high temperatures and the finished product is dried to low moisture content, the majority of conventional extruded food, such as snack foods, and breakfast cereals, are safe to consume and have a long shelf life (Mekuria *et al*., 2021). Over time, food processing has progressed from goals like transformation, elimination of pathogenic and spoilage bacteria, stability, and shelf-life extension to more sophisticated goals including modifying functioning and chemosensory qualities (taste, flavour, and texture) (Yada, 2018).

In this study, five hypotheses were investigated namely (i) The blends optimization has no significant effect on protein, total minerals, and vitamin A content of composite flour from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour, (ii) The extrusion process optimization by RSM has no significant effect on functional properties of extrudates, (iii) The blends and extrusion cooking have no significant effect on the physicochemical composition of ready-to-eat baby foods produced from orange-fleshed sweet potatoes, soybeans, and amaranth seed flour blends, (iv) The blends and extrusion cooking have no significant effect on protein quality of ready-to-eat baby foods produced from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour blends; and (v) The storage time and storage temperature have no significant effect on shelf-life of ready-to-eat foods produced from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour blends. The results are shown and discussed in chapters 3,4,5,6 and 7 of this study. Experimental Data support the rejection of null hypotheses.

8.3 Major Findings

The results in Chapter Three confirmed well the available literature where OFSP had very low protein content and fat content. Thus, blending it with amaranth seeds and soybean flour produced a composite flour rich in protein, total minerals, and vitamin A content. The carbohydrate and vitamin A content in the composite flour were increasing with the increase in OFSP incorporation. The mixture design helped to identify ingredients to be used in the production of extruded ready-to-eat baby foods. The composite flour had high protein content of 15.83±0.31%, total minerals (4.83±0.06%), fat content (6.16±0.19%), fibre content (5.18±0.15%), carbohydrate content (66.60±0.95%), and vitamin A content of 890.03±0.15 RAE μg/100g. The results are higher than the values found by Sanya *et al*. (2020) for cereal flours with baobab, orange-fleshed sweet potato and amaranth grain powders. This could be due to the various ingredients used and their proportions.

In Chapter Four, OFSP flour revealed good functional/physical properties and Box-Behnken experimental design of response surface methodology helped to achieve the optimum extrusion cooking conditions which give later the optimum conditions to be used in the processing of extruded ready-to-eat baby foods. The quadratic models were generated and their adaptation could produce products with desired quality attributes. Karun *et al*. (2023)

optimized process parameters at feed moisture content (8%-12%), screw speed (300–350 rpm), and barrel temperature (117°C–121°C) for multigrain ready-to-eat extruded snack. They found the optimized parameters of 120°C barrel temperature, 350 rpm screw speed, and 10% moisture content with desirability. Another study found the optimum extrusion conditions of die temperature 145.58°C, feed moisture content of 19.73% and cricket flour concentration of 31.89% in the mix with a desirability value of 78.7% which could be used to develop a snack with acceptable characteristics (Téllez-Morales *et al*., 2022). Kaur *et al*. (2022) investigated the impact of extrusion process factors such as screw speed (400-550 rpm), barrel temperature (125-175°C), and feed moisture (14-18%, on a dry basis) on the system and functional qualities of normal and high-quality protein maize snacks. Bobade *et al*. (2021) reported the optimum numerical optimization conditions of 16.50%, feed moisture content of 151.33°C extrusion temperature, and 12.83% honey level. On the other hand, optimizing extrusion cooking conditions is critical for assuring product quality, process efficiency, nutritional preservation, shelf stability, food safety, process flexibility, and consumer pleasure. It enables firms to generate improved food items while improving operational efficiency and addressing changing customer needs.

In Chapter Five, the blends with adequate protein content, total minerals, and vitamin A content were selected for the formulation of extruded ready-to-eat baby foods. Extrusion cooking significantly ($p<0.05$) increased the dry matter contents, total minerals, carbohydrate contents, energy value, and the energy-to-protein ratio of the extruded ready-to-eat baby foods while significantly ($p<0.05$) reduced the protein content, vitamin A content and anti-nutrients content of the extruded ready-to-eat baby foods. The produced extruded ready-to-eat baby foods were safe and complied with the recommended standards for children. The ingredients used in this study increased the macro and micronutrients of extruded ready-to-eat baby foods. Therefore, extruded ready-to-eat baby foods can be used for children but also vulnerable groups for food and nutrition security in developing countries. The RTE baby foods had high protein content of 15.72±0.03%, total minerals (5.39±0.07%), fat content (6.05±0.02%), fibre content (5.04±0.03%), carbohydrate content (80.58±0.05%), energy content (363.45±0.50 – 380.84±0.78 kcal/100g) and vitamin A content (660.23±0.32 – 1044.70±0.55 RAE μg/100g). The results are below to the values reported by Ubbor *et al*. (2022) for cookies from wheat, orange fleshed sweet potato and bambara nut flour blends and the values found by Oduro-Obeng and Plahar (2017) for snack foods prepared from wheat, rice, soybean and OFSP composite flours. Blending can involve the addition of enriched ingredients to enhance the nutrient content of the final product.

In Chapter Six, blend proportions and extrusion cooking have significant ($p<0.05$) positive effects on the protein quality of the extrudates in terms of *in vitro* protein digestibility and available lysine, probably due to the low temperature, low concentrations of sugar and high feed moisture content used during extrusion cooking. Studies revealed that high retention of available lysine may be achieved at the extrusion cooking below 144^{o}C and greater than 15% of feed moisture content, as well as high screw speed, shear forces, low resident time, and reduction of sugar content in food. The extrusion cooking above 180^{o}C favours the Maillard reaction and results in unstable conditions for available lysine of foods. Process temperature, feed moisture, and the presence of other sugars all have a significant impact on lysine loss during extrusion. Based on the processing parameters and ingredients content, the Maillard reaction is favoured by low moisture and high temperature, which results in a large loss of lysine (5%–40%) as well as other amino acids sulphur-containing (Fellows, 2022; Omwamba & Mahungu, 2014). The highest IVPD is 91.87±0.09% and available lysine of 2.79±0.05%. The IVPD results confirm well with the values obtained by Akande *et al*. (2017) for the formulations containing grain amaranth, groundnut, iron-rich beans, pumpkin, orange-fleshed sweet potato, carrot, and maize.

The primary reason for losses in available lysine can be associated with Millard-type reactions between the free amino group on lysine and reducing sugars naturally present in cereals upon high extrusion temperature and low moisture content (Gulati *et al*., 2020). Furthermore, the extrusion at low feed moisture has been shown to improve *in vitro* and *in vivo* protein digestibility of corn, rice, quinoa, sorghum, dry beans, pea, carob, and legumes (Gulati *et al*., 2017; Huang *et al*., 2018). The primary reason for the improvement in digestibility upon extrusion has been linked to the inactivation of antinutritional compounds and the thermal denaturation of proteins (Gulati *et al*., 2020).

In Chapter Seven, the shelf-life of extrudates was predicted using the Arrhenius equation. The products were stored at four different storage temperatures and microbial properties (yeast and moulds) were evaluated at the interval of 7 days starting from day one. The shelf-life of extruded ready-to-eat products was predicted at 24^{o}C and found to be 108-169 days. Due to the low water activity, the shelf life of extruded foods may exceed 12 months when packaged in moisture-proof and airtight packaging (Fellows, 2022).

8.4 Conclusions

i. The ingredients optimization significantly ($p<0.05$) affects the protein, total minerals, and vitamin A content of composite flour from orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour. The nutrient-dense composite flour from blends of orange-fleshed sweet potatoes, soybeans, and amaranth seeds flour can be used to make desired food for human nutrition.

ii. Extrusion cooking parameters optimization significantly ($p<0.05$) affect the functional properties of extrudates from OFSP. The main extrusion cooking factors (die temperature, screw speed, and feed moisture content) and their interaction effect have a considerable impact on the functional quality of extruded ready-to-eat foods. The optimum conditions can be used to make quality decisions on extrudates by food processors.

iii. Extrusion cooking temperature and blend proportions significantly ($p<0.05$) affect the nutritional qualities, anti-nutrients, minerals physical properties, and sensory properties of the extruded ready-to-eat baby foods. The amaranth seeds and soybean flour incorporation in OFSP flour significantly ($p<0.05$) increase the micro and macronutrient content of extruded ready-to-eat baby foods. The extruded ready-to-eat baby foods are safe for consumption in terms of microbial and anti-nutritional factors and meet the recommended standard for children, and lactating mothers in terms of targeted nutrients.

iv. Extrusion cooking temperature and blend proportions significantly ($p<0.05$) improve the *in vitro* protein digestibility while maintaining the quality of lysine content of extruded ready-to-eat baby foods.

v. The storage temperature and storage duration significantly ($p<0.05$) affect the shelf life of extruded ready-to-eat baby foods. The extrusion cooking and post-extrusion operation increase the shelf life of extruded ready-to-eat baby foods.

8.5 Recommendations

i. The composite flour from blends of amaranth seeds and soybean and OFSP flour can be used to process any food and serve as a nutrient booster. The optimum protein content, total minerals, and vitamin A content from their respective blends of amaranth seeds and soybean and OFSP flour can be used for the processing of any food and serve as a potential nutrient source for under-five children, and pregnant and lactating mothers.

ii. The extruder can be conveniently changed by the operators to provide enough control of different extrusion cooking variables to sufficiently monitor the behaviour of raw materials to achieve a desirable expansion, texture, and overall product quality including feed flow rate and application of different die sizes. The extrudates with functional properties are high-value-added products from OFSP. The optimum extrusion cooking parameters can be used by food processors for the production of diverse foods with the desired quality attributes.

iii. The processed nutrient-rich extrudates can be used for any people who lack adequate nutrient intake and they can serve as food and nutrition security in developed countries. The extrudates can be made into instant flour and served with milk. It can also be used in different ways such as a thickening agent.

iv. These foods can be stored under actual storage conditions to investigate the effect of storage as well as different processing methods on the Maillard reaction. Extrusion cooking at low temperatures, low sugar, high screw speed and feed moisture content can help to achieve high-quality protein foods.

v. Extrusion cooking and post-extrusion operations are key factors to consider for achieving the extended shelf life of extrudates as well as storage temperature and time.

8.6 Area for Further Research

i. It is also necessary to investigate the behaviour of various grain, amaranth, and tuber fractions to make better use of these materials. This will assist the food business in creating food products that consumers want in terms of both sensory qualities and nutritional benefits.

ii. To gain a better knowledge of how the various components of legumes, grains, and tubers interact with each other and with the extrusion processing parameters, more research is required.

iii. There should be more research done on the molecular and physical interactions between starches, proteins, and other macro- and micro-ingredients (phenolic compounds) in the extruder.

iv. More study is required to comprehend the nutritional qualities of different tubers, grains, legumes, and their constituent parts.

v. Further research on full-length storage for shelf-life testing of extruded ready-to-eat baby foods can be carried out.

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APPENDICES

Appendix A. Research Permit from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **244398**

Date of Issue: **28/October/2019**

RESEARCH LICENSE

This is to Certify that Mr.. Jackson NKESIGA of Egerton University, has been licensed to conduct research in Nakuru on the topic: DEVELOPMENT AND ANALYSIS OF EXTRUDED READY-TO-EAT BABY FOODS FROM ORANGE-FLESHED SWEET POTATO, SOYBEAN AND AMARANTH SEED FLOUR BLENDS for the period ending : 28/October/2020.

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Appendix B. Optimization of Protein, Total Minerals and Vitamin A Content of Orange-fleshed Sweet Potato, Amaranth Seed and soybean flour blends

Global Journal of Food Sciences and Nutrition

Research Article

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Optimization of Protein, Total Minerals and Vitamin A Content of Orange-fleshed Sweet Potato, Amaranth Seed and Soybean Flour Blends

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Abstract

Food and nutrition security remain a major challenge facing the world and especially the developing world. This situation could be mitigated by utilization and blending locally available food crops. In this study, the nutritional contents of orange-fleshed sweet potato (OFSP), amaranth seed and soybean composite flours were optimized by Extreme Vertices Mixture Design using Minitab Software. The software generated 11 experimental runs from the flour blends. Each blend was analyzed for proximate, minerals and vitamin A contents. The analysis of blends lead to some useful conclusions, most important of which yielded high protein content (15.83%) and fat (6.16%) for blend C1 (50:25:25 for OFSP, amaranth seed, and soybean flour respectively), fiber (5.18%) for blend C11 (60:15:25 for OFSP, amaranth seed, and soybean flour respectively), total minerals (4.83%) for blend C2 (54.5:24:21.5 for OFSP, amaranth seed, and soybean flour respectively), energy value (359.75 kcal/100g) for C3 (50:30:20 for OFSP, amaranth seed, and soybean flour respectively), while blend C6 (75:15:10 for OFSP, amaranth seed, and soybean flour respectively) was higher in carbohydrate (66.60%), energy-to-protein ratio (37.98 Kcal/g of Protein) and vitamin A content (890.03 RAE µg/100g) than others. Generally, blend C1 was the highest in iron content (2.64 mg/100g), Zinc (0.56 mg/100g), magnesium (81.25 mg/100g) and calcium (58.10 mg/100g). The blend C6 was higher in sodium content (41.63 mg/100g) and potassium (65.18 mg/100g) than others, while blend C11 was high in manganese content (0.59 mg/100g) and the highest copper content (0.95 mg/100g) was observed in blend C8 (54.5: 26.5:19 for OFSP, amaranth seed, and soybean flour respectively). The most significant observation of this study is that the optimum blend was 57%, 24% and 19% of OFSP, amaranth seed, and soybean flour respectively for the production of protein (14%), total minerals (4.7%) and vitamin A content (813.6 RAE µg/100g). These findings could be applicable in cases of processing of nutritious foods for people in need in an economical way and promote the utilization of orange fleshed sweet potatoes.

Keywords: *Optimization; Mixture Design; Orange-fleshed Sweet Potato; Soybean; Amaranth Seed; Nutritional Content.*

Introduction

affordability of healthy, sustainably produced food

Appendix C. Optimization of Extrusion Cooking Parameters on Functional Properties of Ready-to-eat Extrudates

Journal of Food Science & Nutrition

SkepticMed Publishers
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DOI: 10.46715/jfsn2021.12.1000121

Research Article

Optimization of Extrusion Cooking Parameters on Functional Properties of Ready-to-eat Extrudates from Orange-fleshed Sweet Potato Flour

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Abstract

The ability to understand the functional aspects/properties of food can help to improve the use of orange-fleshed sweet potato flour (OFSP) into various food products. This study was carried out to investigate the effect of extrusion cooking parameters (die temperature, screw speed and feed moisture content) on physical (functional) properties of extruded ready-to-eat (RTE) foods from orange-fleshed sweet potato flour using response surface methodology (RSM). Box-Behnken experiment under Design Expert software was used and 17 randomized experimental runs with 5 centre points were generated. In order to ensure the quality of extruded ready-to-eat foods, lateral expansion, bulk density, water hydration capacity, water absorption index, oil absorption capacity, water solubility index, and swelling capacity were analyzed. Co-rotating twin screw extruder was set at constant barrel temperature of 65 ± 5°C for three heating zones. Extrusion cooking variables were at three levels for die temperature (70, 80 and 90°C), screw speed (350, 375 and 400 rpm), and the feed moisture content (30, 35 and 40%). Multiple regression and Analysis of Variance (ANOVA) at 5% significance level were carried out. The results showed that independent variables had significant effect ($p<0.05$) on functional properties of RTE foods. The lateral expansion of extruded ready-to-eat food ranged from 85 - 125%, bulk density (0.13 - 0.50 g/cm^3), water hydration capacity (4.2 - 6.17 g/g), oil absorption capacity (0.85 - 1.18 g/g), water absorption index (2.6 - 4.9 g/g), water solubility index (3.6-12%) and swelling capacity (2.4 - 6.2 ml/g). The numerical optimization generated the optimum extrusion process conditions of 90°C, 400 rpm and 35% for die temperature, screw speed and feed moisture content, respectively. The predicted values were 125%, 0.13 g/cm^3, 6.22 g/g, 1.18 g/g, 2.99 g/g, 11.51%, and 5.05 ml/g for lateral expansion, bulk density, water hydration capacity, oil absorption capacity, water absorption index, water solubility index and swelling capacity, respectively with the overall desirability (coefficient of determination) of 0.930. The optimum conditions and the generated models can be used for food processors and academic communities to predict the quality of extruded ready-to-eat foods from OFSP.

Keywords: Extrusion Cooking, Optimization, Functional Properties, Extruded, Ready-to-eat, Foods, Orange-fleshed

generated up at the end of the screw and the die (Pichmony & Ganjyal, 2020). Extrusion process variables also play a big

Appendix D. Nutritional and Sensory Qualities of Extruded Ready-To-Eat Baby Foods

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Full Length Research

Nutritional and sensory qualities of extruded Ready-To-Eat baby foods from orange-fleshed sweet potato enriched with amaranth seeds, and soybean flour

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ABSTRACT: Adequate nutrition is critical during infancy and childhood because life phases are marked by significant physical, mental, and changes in behaviour, such as fast growth, weight gain, the development of cognitive and psychomotor abilities, and a change in dietary preferences. This study was carried out to investigate the effect of extrusion cooking and blend proportions on the nutritional qualities of extruded ready-to-eat baby foods. Different blends of orange-fleshed sweet potato, amaranth seeds, and soybean flour were used to formulate foods and analyzed for proximate, minerals, vitamin A content, anti-nutrient content, physical properties, and sensory qualities. Extrusion cooking was conducted at a temperature of 90°C, screw speed of 400 rpm, and feed moisture content of 35%. The results reveal that extruded ready-to-eat baby foods had a high protein content of 15.72%, total minerals (5.39%), carbohydrate content (80.58%), crude fibre content (5.04%), fat content (6.05%), energy value (380.84 kcal/100g), energy-to-protein ratio (128.67 kcal/g of protein) and vitamin A content (1044.70 REA µg/100g). The micronutrients of extruded baby foods resulted in high iron content of 3.10 mg/100g, zinc content (0.64 mg/100g), manganese content (0.90 mg/100g), copper content (0.97 mg/100g), magnesium content (81.70 mg/100g), calcium content (61.22 mg/100g), potassium content (68.18 mg/100g) and sodium content (41.44 mg/100g). The produced ready-to-eat baby foods showed a reduction in anti-nutrients as well as acceptable levels of phytate content which ranged from 0.47-1.79 mg/100g, oxalate content (0.16 – 0.50 mg/100g) and saponin content (0.20 – 0.48 mg/100g). All produced foods were highly accepted through sensory evaluation. These important findings confirm that extrusion cooking is useful in the production of nutrient-dense baby foods. In addition, the most significant observation of this study is that the produced foods could be used for under-five years children who suffer from protein-energy malnutrition and micronutrient deficiencies. These findings will also contribute to food and nutrition security.

Keywords: Amaranth seeds, extrusion cooking, nutritional content, orange-fleshed sweet potato, ready-to-eat extrudates, sensory characteristics, soybean.

INTRODUCTION

Nutrition is the collection of physiological activities that include food intake, digestion, absorption, assimilation, and egestion of undigested food from the alimentary canal and also its impact on human growth and development (Gupta, 2020). Poverty, food insecurity, child malnutrition, and unaffordability of healthy foods continue to be a challenge, particularly in Africa and Asia in vulnerable groups including children (FAO *et al.*, 2021). Malnutrition is caused by poverty, inadequate nutritional quality of traditional complementary food, inappropriate complementary feeding practices, high disease burden, limited access to nutritious foods, low level of mother's education, low access to safe water and basic drugs, sanitation, and hygiene and health services, and inadequate care practices and the increased cost of protein-based complementary foods (Eka *et al.*, 2010,

Appendix E. Protein Quality of Extruded Ready-To-Eat Baby Foods

Protein Quality of Extruded Ready-to-Eat Baby Foods from Orange-Fleshed Sweet Potato, Amaranth Seeds, and Soybean Flour Blends

Jackson Nkesiga, Joseph O. Anyango, and Peninah N. Ngoda

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Protein Quality of Extruded Ready-to-Eat Baby Foods from Orange-Fleshed Sweet Potato, Amaranth Seeds, and Soybean Flour Blends

Jackson Nkesiga[1*], Joseph O. Anyango[1], and Peninah N. Ngoda[1]

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DOI: https://doi.org/10.47672/ajfsn.1287

Abstract

Purpose: Protein quality refers to the total protein content, essential amino acid content, and digestibility of a protein. Source, bioavailability, food matrix, and processing conditions all have an impact on protein quality. Protein quality can be lost during food processing. This study was carried out to investigate the effect of extrusion cooking and blend proportions on the protein quality of extruded ready-to-eat baby foods.

Methodology: Different blends of orange-fleshed sweet potato, amaranth seeds, and soybean flour were used and analyzed for protein quality including *in vitro* protein digestibility (IVPD) of extruded ready-to-eat baby foods. In addition, nutrient damage due to heat or processing temperature was evaluated by analyzing available lysine in the end products to ensure the quality of extruded ready-to-eat baby foods. Extrusion cooking was carried out at a temperature of 90°C, screw speed of 400 rpm, and feed moisture content of 35%.

Findings: The results showed that IVPD ranged from 54.05 to 91.87%. The available lysine as a parameter to evaluate the nutritional damage due to thermal processing ranged from (1.69 to 2.79%). This research predicts the potential availability of highly digestible protein as well as the assurance of lysine availability once extrudates are consumed. Achieving high lysine retention during extrusion cooking depends on a number of factors, including low temperature, high screw speed, high feed moisture content, and high shear forces that lead to a short residence time.

Recommendation: It is important to conduct more research on how extrusion cooking affects the molecular and physical interactions between starches, proteins, lipids, and phenolic compounds.

Keywords: *Extrusion cooking, protein quality, ready-to-eat extrudates, orange-fleshed sweet potato, amaranth seeds, and soybean flour*

Appendix F. Microbial Load and Shelf-Life Assessment of Ready-To-Eat Baby Foods

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Journal homepage:www.ModernScientificPress.com/Journals/IJFNS.aspx *Florida, USA*

Article

Microbial Load and Shelf-Life Assessment of Ready-To-Eat Baby Extrudates from Orange-Fleshed Sweet Potato, Soybean, and Amaranth Seeds Flour Blends

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Abstract: The microbiological load of any food product is a valuable indication of extruded food quality, as much as indicating the possible safety status of extrudates for human nutrition and storage. The shelf life of a food refers to the period during which the food product is safe for consumption and/or of an acceptable standard to customers. The microbial safety of food products serves as one of the most essential customer needs. The development of a child can be hampered by inadequate quality complementary foods, which can potentially cause illnesses and even child mortality. A critical step to be taken is post-extrusion processing to manage high-risk food safety concerns. The shelf-life study was carried out to find out how long extrudates could stay on a shelf while preserving their nutritional quality. Blend proportions namely C0 (control): 100:0:0; C1:50:25:25; and C3:50:30:20 for OFSP: Amaranth Seeds: Soybean flour, respectively, extrusion cooking end barrel temperature (70-90°C), die temperature of 90°C and feed moisture content of 35% were used to formulate extruded ready-to-eat baby foods. The extrudates were collected in storage polyethylene bags and stored at 4 different accelerated conditions for microbial analysis for 28 days. Samples were aseptically taken at the interval of seven days for the determination of total viable count (TVC), total coliform count (TCC), yeasts, and moulds (Y&M). The latter was used as a quality index for an accelerated shelf life study. The

Appendix G. International Conference

Optimization of Protein, Total Minerals and Vitamin A Content of Orange-fleshed Sweet Potato, Amaranth Seed and Soybean Flour Blends

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Department of Dairy, Food Science and Technology, Egerton University

Dr. Joseph O. Anyango
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Dr. Peninah N.Ngoda
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Keywords: Optimization, Mixture Design, Orange-fleshed Sweet Potato, Soybean, Amaranth Seed, Nutritional Content

Abstract

Background: Food and nutrition security remain a major challenge facing the world and especially the developing world. This situation could be mitigated by utilization and blending locally available food crops.

Methods: In this study the nutritional contents of orange-fleshed sweet potato (OFSP), amaranth seed and soybean composite flours were optimized by Extreme Vertices Mixture Design using Minitab Software. The software generated 11 experimental runs from the flour blends. Each blend was analyzed for proximate, minerals and vitamin A contents.

Results and Discussion: Soybean flour had higher protein content (31.53%), ash content which could be an indication of total minerals was 5.98%, fiber (6.29%) and energy value (389.19 kcal/100g) than amaranth seed and OFSP flour but low in carbohydrate (33.09%) and energy-to-protein ratio (12.35 kcal/g of protein). On the other hand, OFSP had low protein content (2.95%), fat (3.05%), and fiber (3.66%), energy value (349.53 kcal/100 g) but produced high carbohydrate content(75.73%) and energy-to-protein ratio (118.37 kcal/g of protein) among others. The analysis of blends lead to some useful conclusions, most important of which yielded high protein content (15.83%) and fat (6.16%) for blend C1 (50:25:25 for OFSP, amaranth seed, and soybean flour, respectively), fiber (5.18%) for blend

nferences.egerton.ac.ke/index.php/euc/.../3 seed, and soybean flour,

Published

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More Citation Formats

Issue

Egerton University 14th Biennial International Conference

Section

Transformative Agri-food Systems

Appendix H. Sensory Evaluation Consent Form

Study Title: Sensory analysis of extruded ready-to-eat baby foods from orange-fleshed sweet potatoes, soybeans, and amaranth seed flour blends

Researcher: Jackson Nkesiga, Dairy, Food Science and Technology Department, Egerton University

You have been asked to participate in a sensory evaluation research study conducted by Jackson Nkesiga. This form describes the research study and your role in it if you choose to conduct the sensory evaluation. Please read the form thoroughly and take as much time as you need. Request that the researcher explains whatever you don't understand. You can decide not to do the sensory. If you choose not to participate in the sensory evaluation of extruded ready-to-eat infant foods, there will be no penalty or advantage.

The purpose of this study is to examine the sensory properties of extruded ready-to-eat baby foods made from orange-fleshed sweet potatoes, soybeans, and amaranth seed flour blends. You are being invited to participate because we need sensory evaluation data to determine the complete acceptability of these products. Participating in the study will take about 10-20 mins of your time. You cannot participate in this study if you are sick, pregnant, or not breastfeeding, if you do not have a child under the age of five, or if you do not consume one of the ingredients used to make these baby foods. This study developed extruded ready-to-eat foods for children under the age of five to combat child malnutrition. We would like to use you as their mothers to evaluate the sensory acceptability of these extrudates because these children have verbal limitations. This study will include approximately 65 participants where the youngest is 22 years old and the oldest is 40 years old.

You will be asked to evaluate 10 samples of extruded ready-to-eat baby foods if you participate in the study. Before the evaluation, you will be requested to fill out and sign a consent form. You will complete a 5-point hedonic assessment form for each sample, ranking it based on how you enjoy the sensory characteristic. We will request that you spit the sample into the provided cuspidor and rinse your mouth with distilled water. The potential benefits of participating in this study include learning about the sensory properties of food. There are no immediate advantages to participating in this study. The potential risks of participating in this study include a high time commitment (no more than 20 mins). The information for this study is being collected in the form of confidential coded data (linked to a specific subject by a code, not by a direct identifier). All data analysis, storage, and dissemination will be kept strictly confidential. Nobody, including the researcher(s), will be able to link data to you.

You will incur no charges by participating in this study. This sensory evaluation is entirely optional on your side.

Your signature on this form indicates that:

i. You understand the information provided in this form;
ii. You have had the opportunity to ask the researcher questions and express any concerns.
iii. You believe you grasp the sensory evaluation qualities as well as the potential benefits and risks.

Statement of Consent

I voluntarily consent to participate in this sensory evaluation. This permission document will be copied for my records.

………………………………………… ……………………………

Signature of Participant Date

…………………………………………

Name of Participant

Appendix I. Hedonic Assessment Form

PRODUCT: EXTRUDED READY-TO-EAT BABY FOODS

DATE: …………………………….….

CODE: …………………………. NAME: …………………..………..……………….

AGE……………………………..

Please evaluate the attributes of the six coded food samples. Indicate how much you like or dislike each sample by scoring from 5-like very much to 1-dislike very much, and record each coded food sample on the left side of the following table 1A in the appropriate box. Please rinse your mouth with distilled water after tasting each sample.

Like very much	Like moderately	Neither like nor dislike	Dislike moderately	Dislike very much
5	4	3	2	1

Sample Codes	**Attributes**				
	Appearence	Aroma	Taste	Texture	Overall acceptability
C070					
C170					
C270					
C370					
C470					
C090					
C190					
C290					
C390					
C490					

Comment:

…………………………………………………………..………………………………......

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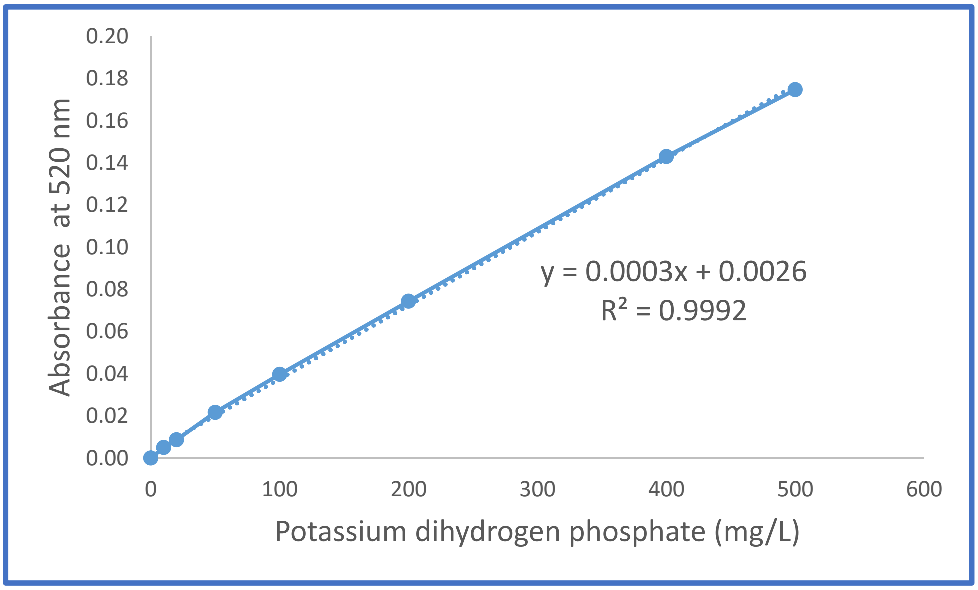
Appendix J. Phytate Standard Curve



Appendix K. Normality Distribution Test

Goodness-of-Fit Tests for Normal Distribution for Manganese

Test	Statistic		p Value	
Kolmogorov-Smirnov	D	0.08081503	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.03187425	Pr > W-Sq	>0.250
Anderson-Darling	A-Sq	0.22392477	Pr > A-Sq	>0.250

Goodness-of-Fit Tests for Normal Distribution for Zinc

Test	Statistic		p Value	
Kolmogorov-Smirnov	D	0.10786598	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.05220692	Pr > W-Sq	>0.250
Anderson-Darling	A-Sq	0.36497460	Pr > A-Sq	>0.250

Goodness-of-Fit Tests for Normal Distribution for Potassium

Test	Statistic		p Value	
Kolmogorov-Smirnov	D	0.10109840	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.04801532	Pr > W-Sq	>0.250
Anderson-Darling	A-Sq	0.30904365	Pr > A-Sq	>0.250

Goodness-of-Fit Tests for Normal Distribution for Dry Matter

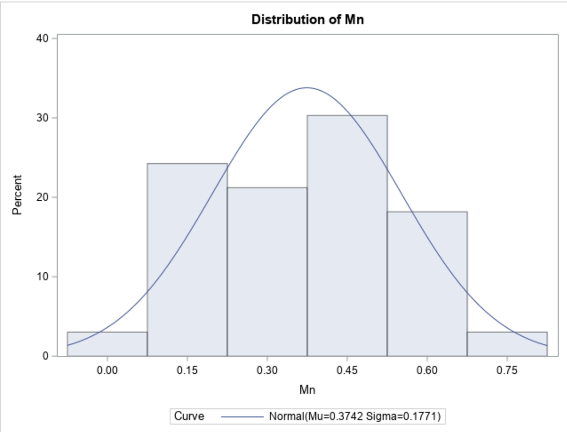
Test	Statistic		p Value	
Kolmogorov-Smirnov	D	0.11755686	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.05987451	Pr > W-Sq	>0.250
Anderson-Darling	A-Sq	0.40884648	Pr > A-Sq	>0.250

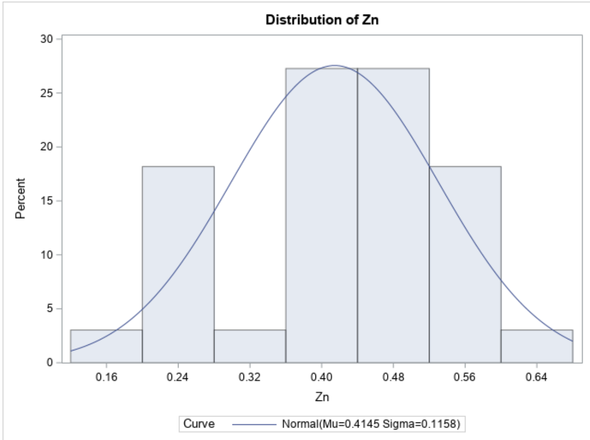
Goodness-of-Fit Tests for Normal Distribution for Available Lysine

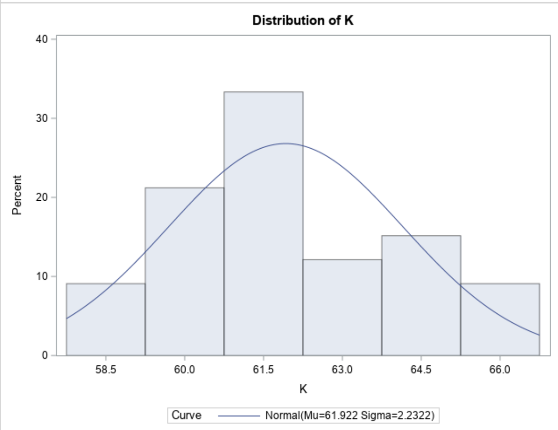
Test	Statistic		p Value	
Kolmogorov-Smirnov	D	0.11811392	Pr > D	>0.150
Cramer-von Mises	W-Sq	0.08640494	Pr > W-Sq	0.169
Anderson-Darling	A-Sq	0.50625649	Pr > A-Sq	0.195

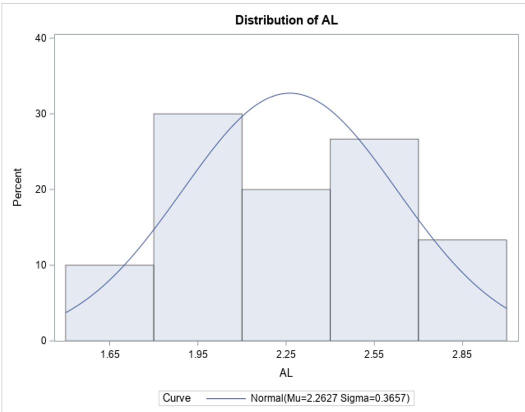
Goodness-of-Fit Tests for Normal Distribution for Expansion Ratio

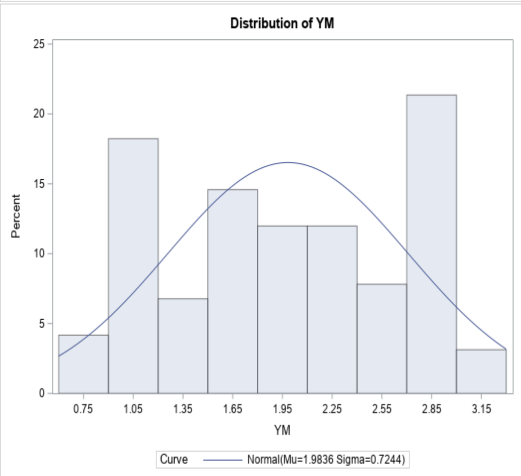
Test		**Statistic**		**p Value**
Kolmogorov-Smirnov	D	0.09100517	Pr > D	0.126
Cramer-von Mises	W-Sq	0.06043132	Pr > W-Sq	>0.250
Anderson-Darling	A-Sq	0.54646076	Pr > A-Sq	0.160

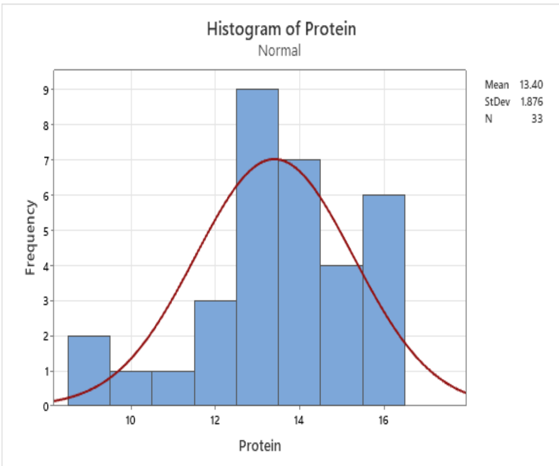


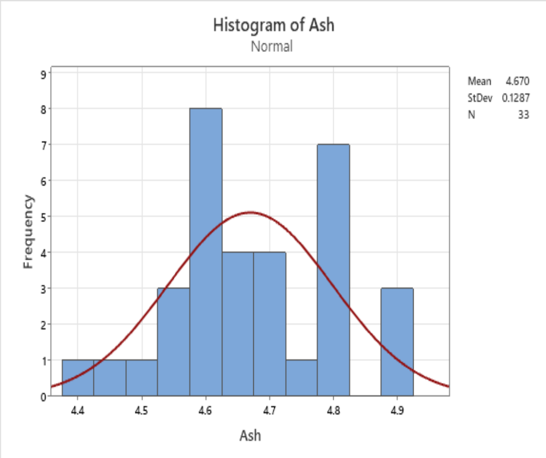


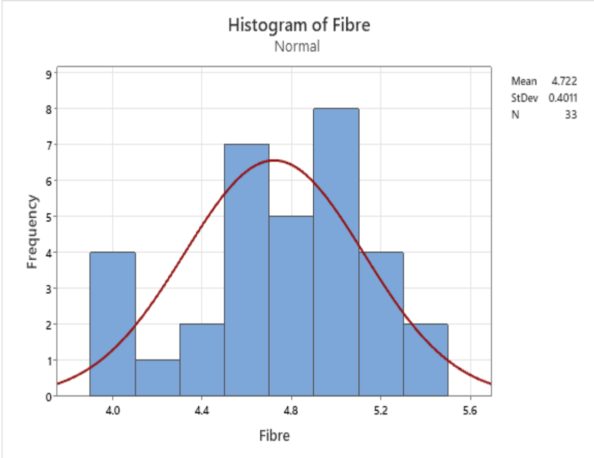


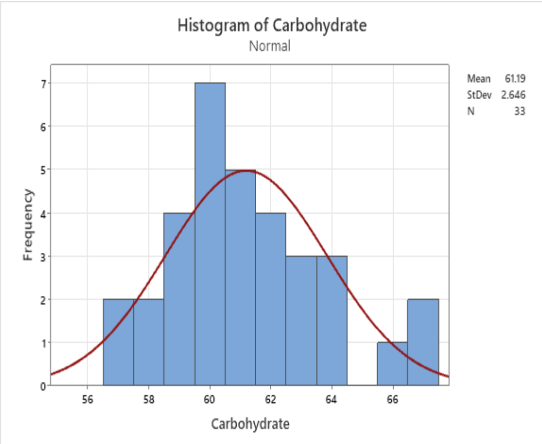


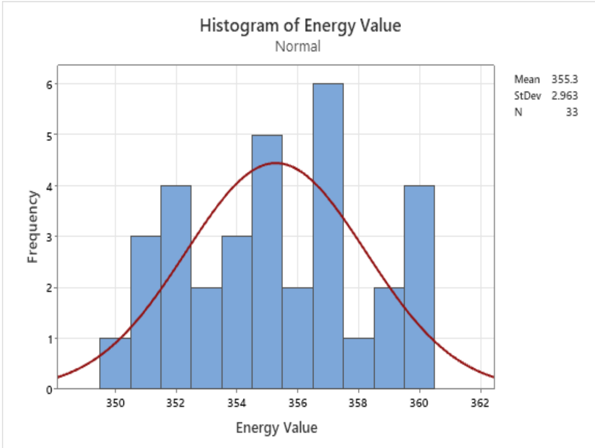


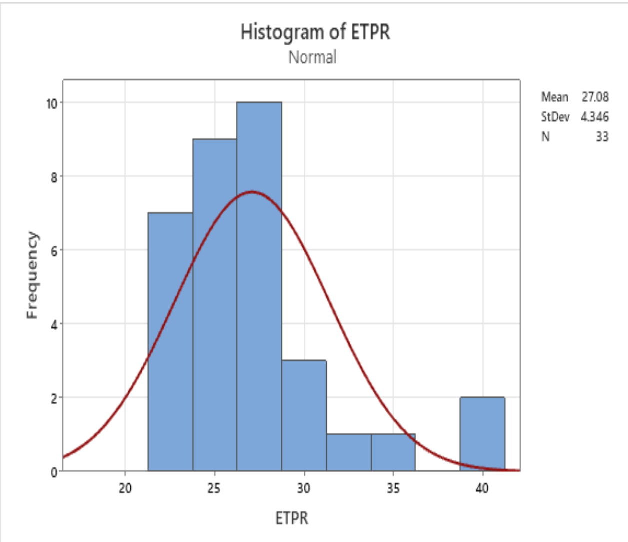



Histogram of Ash
Normal
Mean 4.670
StDev 0.1287
N 33
Frequency
Ash


Histogram of Fibre
Normal
Mean 4.722
StDev 0.4011
N 33
Frequency
Fibre


Histogram of Carbohydrate
Normal
Mean 61.19
StDev 2.646
N 33
Frequency
Carbohydrate


Histogram of Energy Value
Normal
Mean 355.3
StDev 2.963
N 33
Frequency
Energy Value


Histogram of ETPR
Normal
Mean 27.08
StDev 4.346
N 33
Frequency
ETPR

Appendix L. LEVENE Test for Standard Homogeneity of Variance

LEVENE Test for Nutritional Content of Extruded Ready-To-Eat Baby Foods

Levene's Test for Homogeneity of DM Variance ANOVA of Absolute Deviations from Group Means

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
BP	4	13.9680	3.4920	153.34	<.0001
Error	25	0.5693	0.0228		

Levene's Test for Homogeneity of Protein Variance ANOVA of Absolute Deviations from Group Means

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
BP	4	0.4507	0.1127	146.73	<.0001
Error	25	0.0192	0.000768		

Levene's Test for Homogeneity of Charbohydrate Variance ANOVA of Absolute Deviations from Group Means

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
BP	4	20.8895	5.2224	159.30	<.0001
Error	25	0.8196	0.0328		

Levene's Test for Homogeneity of Vitamin A Variance ANOVA of Absolute Deviations from Group Means

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
BP	4	2284.2	571.0	3195.88	<.0001
Error	25	4.4670	0.1787		

Levene's Test for Homogeneity of *In Vitro* Protein DigestibilityVariance ANOVA of Absolute Deviations from Group Means

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
BP	4	195.0	48.7555	324.58	<.0001
Error	25	3.7553	0.1502		

LEVENE Test for Anti-Nutrient Content of Extruded Ready-To-Eat Baby Foods

Levene's Test for Homogeneity of Phytate Variance ANOVA of Absolute Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
BP	4	1.1671	0.2918	20.98	<.0001
Error	25	0.3477	0.0139		

Levene's Test for Homogeneity of Saponins Variance ANOVA of Absolute Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
BP	4	0.7873	0.1968	247.98	<.0001
Error	25	0.0198	0.000794		

LEVENE Test for Mineral Content of Extruded Ready-To-Eat Baby Foods

Levene's Test for Homogeneity of Iron Variance ANOVA of Absolute Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
BP	4	1.5392	0.3848	1014.40	<.0001
Error	25	0.00948	0.000379		

Levene's Test for Homogeneity of Sodium Variance ANOVA of Absolute Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
BP	4	0.1244	0.0311	10.33	<.0001
Error	25	0.0753	0.00301		

LEVENE Test for Physical Properties of Extruded Ready-To-Eat Baby Foods

Levene's Test for Homogeneity of SER Variance ANOVA of Absolute Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
BP	4	0.3309	0.0827	5.71	0.0005
Error	70	1.0149	0.0145		

Appendix M. Pictures Taken During Experiments









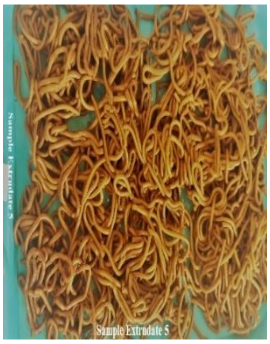






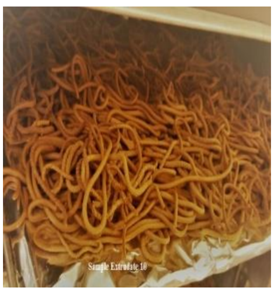






Sample Extrudate 5

Sample Extrudate 6

Sample Extrudate 7

Sample Extrudate 8


Sample Extrudate 11

Sample Extrudate 12