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MILK FAT CHANGES IN ACCELERATED SHELF LIFE TESTING OF READY-TO-FRY CHAPATI

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A thesis submitted to the Graduate School in partial fulfilment for the requirements of the Master of Science Degree in Food Science of Egerton University.

Egerton University.

2013/94602

April 2004.

DECLARATION AND RECOMMENDATION

This thesis my original work and has not been presented in any other university.

Signature.....................Date. 23/04/2004

MWANGOME GRACE CHITSAKA

This thesis has been presented with our approval as the supervisors:

Signature.....................Date. 23.04.2004

PROF. S. M. MAHUNGU,Ph.D.

Signature.....................Date. 29/4/04

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DEDICATION

To my lovely daughter Lynn Ndimu Mwongela, William and Lydia Mwangome, Sisters Nimwaka, Kaeni and Mbeyu and Brother Muye. You are an inspiration in my life.

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ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my Supervisors Dr. M. S. Ngari and Prof. S. M. Mahungu for their fruitful ideas, valuable advice and persistent encouragement during the period of my study.

Many thanks are extended to Dr. M. G. Kinyua, Centre Director, National Plant Breeding Research Centre-KARI. Njoro and all technical staff for their technical assistance. Particularly the staff of the Cereal Chemistry Laboratory are acknowledged for their cooperation. Mr. P. Kimani, Mr. Rotich, Mr. J. Ndungu and Mrs A. Muthangya, Mrs. M. Meso, Miss H. Akoth and the Section Head Miss E. Kimani are particularly thanked very much. Dr. A. Wangai also acknowledged for providing an incubator.

Special thanks go to Chairpersons of Animal Science, Chemistry, Botany and Horticulture Departments of Egerton University for allowing the use of their Departmental laboratories. I am specifically grateful to Mr.S. M. Kariuki, Mr.F. T. Mwanyika, Mr. E. K. Shakala Mr. K. Mwavishi, Mr. G. Ochieng and Mr. F. Ngumbu for their help in instrumental analysis of samples through out the study. All members of the department of Dairy and Food Science and Technology of Egerton University are thanked for their assistance. Dr. A. Islam is also acknowledged for his advice on statistical data analysis.

The Egerton University Research and Extension Division and the Higher Education Loans Board are gratefully acknowledged for financing my studies and this research project. Special gratitude goes to my friends Job Okoko Mapesa, Bernard Ouna, Margaret Mathenge, Franklin Nyabuga, Dr. P. L. Githua, Dr. Tonui, Dr. H. Rono and Dr. B. Lukoye for support and encouragement in addition to constructive criticism during this study.

Above all, I am greatly indebted to my parents William and Lydia Mwangome, sisters Mary, Martha and Nancy and brother James for their sacrifice, encouragement, patience, support and understanding during the course of my study. Finally, my heartfelt gratitude to Boniface Mwongela and my dear daughter Lynn Ndimu for their patience, encouragement and inspiration through out this study.

ABSTRACT

Chapati is a flat Indian bread made from whole-wheat flour, water, salt and edible fat or oil as the main ingredients. Although most edible fats or oils may be used to make chapati, milk fat gives the best product in terms of flavour and texture. Despite chapati being a common delicacy in most homes in the East African region, it is not available as a ready-to-fry product. To the best knowledge of the author, there is no reported work on the shelf life of ready-to-fry chapati especially using milk fat change as a quality index. Lipid oxidation and hydrolysis are a major cause of loss of shelf life, palatability, functionality and nutritional quality of foods. This study was therefore set up to establish an equation to be used for quick determination of shelf life of ready-to-fry chapati with respect to milk fat oxidation. Accelerated tests were used to cut down on the testing time, cost and subjectivity of most shelf life testing procedures. Chapati dough was prepared using an experimentally established method, sheeted, packaged and stored at 35°C, 40°C, 45°C and 50°C for 0, 24, 48, 72, 96, 120 and 144 hours. For each storage time and temperature the p-anisidine, % free fatty acid and Acid Values were determined and used to obtain the reaction rate constants at the studied temperatures. The determined rate constants k_1, k_2, k_3 and k_4 were then used to prepare an Arrhenius plot and to formulate a shelf life plot for quick determination of shelf life of the ready-to-fry chapati at desired storage temperatures based on milk fat oxidation. The results obtained showed that lipid oxidation of milk fat in ready-to-fry chapati follows pseudo-zero order kinetics and Arrhenius behaviour for temperature dependence. The mathematical equation obtained for shelf life estimation of ready-to-fry chapati based on milk fat oxidation $[\theta = e^{(-bT+c)}]$ where θ is shelf life, b is the slope of the shelf life plot, T is temperature in ^{0}C and c is a constant, was found to be quite a quick and objective method. It can therefore be recommended for application to other new food products and periodic shelf life determination of lipid containing foods. The findings of this research have contributed towards an important step in developing ready-to-fry chapati as a semi-convenience food to meet the growing demand in East African countries.

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LIST OF ABBREVIATIONS

A.R.	Analytical Reagent
ASLT	Accelerated Shelf Life Testing
BU	Brabender Units
^oC	Degree [Celcius]
DNA	Deoxyribonucleic Acid
E_A	Activation Energy
EDTA	Ethylene Diaminetetraacetic Acid
FFA	Free Fatty Acid
g	Grams
h	Hours
HCl	Hydrochloric Acid
H_2SO_4	Sulphuric Acid
^oK	Degrees [Kelvin]
KOH	Potassium Hydroxide
L	Litre
ln	Natural Logarithm
MRPs	Maillard's Reaction Products
N	Normality
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
ppm	Parts per million
s	Seconds
SCFA	Short Chain Fatty Acids
T	Absolute Temperature
TAG	Triacylglycerol
UFA	Unsaturated Fatty Acid
UV	Ultra violet

CHAPTER ONE

INTRODUCTION

Chapati is a form of Indian unleavened flat bread made from dough of whole-wheat flour, water, oil and salt as the main ingredients. Though most edible oils may be used, milk fat gives the best product in terms of flavour and texture. This is because milk fat contains mostly short chain fatty acids that act as emulsifiers and improve the texture of chapati by enhancing hydration of the gluten in chapati dough. Traditionally, the quantities of water, oil and salt added to the whole-wheat flour to make chapati dough was determined by trial and error until the optimum dough in terms of handling characteristics, texture and flavour was obtained. Chapati is a common delicacy in Eastern Africa especially Kenya, Uganda and Tanzania. There is an emerging market for chapati as a semi-convenience product owing to its relative difficulty in preparation.

Oxidative and hydrolytic changes in milk fat lead to quality deterioration in chapati. Chapati usually contains approximately 10% oil. When milk fat is used, a considerable amount of unsaturated fatty acids are introduced. This is because milk fat contains approximately 35% unsaturated fatty acids [Gunstone, 2002]. This makes the lipids in chapati highly vulnerable to oxidative degradation. Lipid oxidation is a major cause of oxidative rancidity that leads to loss of shelf life by altering flavour, texture, and palatability in addition to loss of nutritional quality of foods. This makes food less acceptable or even unacceptable to consumers [Min and Boff, 2002]. Secondary products from the autoxidation of lipids are typically low molecular weight compounds as well as off–flavour compounds especially aldehydes, ketones and alcohols that mask other flavour enhancing compounds in foods to produce a flavour 'fade' [Lee and Krochta, 2002].

Lipid oxidation is a natural process that takes place between molecular oxygen and unsaturated fatty acids in lipid containing foods. It occurs via a self-sustaining free radical chain mechanism consisting of basic steps of initiation, propagation and termination. The propagation stage is responsible for autocatalytic nature of the process [Shahidi and Wanasundara, 2002a]. Ghee is primarily milk fat with oleic acid as one of the major fatty acids. It is reported that oleic acid accounts for 28-34% of fatty acids in ghee [Gunstone, 2002] and its autoxidation produces both saturated and unsaturated aldehydes as secondary

products. These are mainly heptanal, octanal, nonanal, 2-nonenal, 2-decenal and 2-undecenal [Warner, 2002]. Propanal and pentanal have also been reported among dissolved volatiles in triolein, a triacylglycerol of oleic acid [Mahungu *et al,* 1998]. The rate of oxidation depends on temperature, oxygen availability and presence of pro- or anti-oxidants in the food matrix. The food type and the oil structure also affect the oxidation rate.

Hydrolytic rancidity in milk fat or foods containing milk fat is usually caused by accumulation of short chain fatty acids [SCFAs] resulting from hydrolysis of triacylglycerols in milk fat during storage. Lipolytic enzymes or water molecules present in the food especially at high temperatures may initiate hydrolysis. Hydrolysis produces monoacylglycerols, diacylglycerols and free fatty acids and glycerol. The amount of water in the food influences the extent of hydrolysis of fat. In addition, free fatty acids produced by lipolysis are more susceptible to oxidative changes than when esterified to the glycerol. This tends to increase the rate of oxidative degradation and accumulation of off-flavours in lipid containing foods during processing, handling and storage [Erickson, 2002].

Lipid oxidation and hydrolysis are associated with development of rancidity and off-flavours in lipid-containing foods. These phenomena represent a major cause of loss of food quality and nutritional value especially when the oxidation products interact with carbohydrates and amino acid groups of proteins. The oxidation products have also been found to alter functions of proteins, lipoproteins, biological membranes, enzymes and DNA in living cells causing a major health concern [Decker, 2002]. Therefore, it is necessary to develop accurate and reproducible methods of monitoring these processes in foods. This will assist in knowing the extent of fat decomposition in a particular food system, and new formulations, and possibly determine their shelf life in terms of oxidative stability [Finlay, 1993].

1.1 Statement of the problem

Information on shelf life testing of ready-to-fry chapati using milk fat change as a quality index, will contribute to an important step in the development of a ready-to-fry chapati as a semi-convenience food product.

1.2 Justification of the study

Accelerated Shelf Life Testing quickly estimates the approximate shelf life of a product and therefore substantially cuts down on the testing time and cost. It is also more objective at giving a clear-cut shelf life of a food compared to sensory shelf life tests [Reid *et al.*, 2003]. When using controlled temperature storage to extend shelf life of foods, microbiological deterioration may be restrained especially at refrigeration and freezing temperatures. However, lipid autoxidation has been reported at temperatures below –80°C making it one of the major quality deterioration mechanisms in lipid-containing foods following storage at refrigeration and freezing temperatures [Erickson, 2002].

Hydrolysis of fats during storage has been reported to produce free fatty acids that contribute to the increase in intensity of oily taste, bitterness and metallic taste in fat containing foods. In addition, lipid oxidation products have been shown to interact with other food constituents to lower nutritional value of foods, while others may alter the biological functions in living tissues thereby posing a health risk if ingested [Decker, 2002]. Thus, determining oxidative stability of new food formulations and food systems such as ready-to-fry chapati is of paramount importance to the growing food industry.

1.3 Objectives

1.3.1 Broad Objective

To determine the shelf life of ready-to-fry chapati using milk fat change as a quality index.

1.3.2 Specific Objectives

1. To determine the levels of free fatty acids and the p-anisidine values during the storage period of ready-to-fry chapati.

2. To determine the effect of temperature on p-anisidine values and free fatty acids concentrations during the storage period of ready-to-fry chapati.

3. To establish a mathematical equation for the quick determination of the shelf life of ready-to-fry chapati.

CHAPTER TWO

LITERATURE REVIEW

2.1 Traditional Chapati Making

Like bread in the West, chapati [unleavened baked flat bread], is the staple diet of majority of the people living in the Indian subcontinent [Prabhasankar and Manohar, 2002]. It is believed to be the first of all Indian breads to be known outside India since it is relatively easy to prepare when compared with other Indian breads. Typically, chapati dough is rolled thin and baked in such a way as to give a puffed ball. The doughs stretching ability depends on high gluten content of wheat. Therefore, gluten content of wheat makes it ideal for chapati making as opposed other cereals like maize that contain less gluten.

For chapati making in the household, whole-wheat flour [locally known as *atta*] is first made into dough of stiffer consistency than the bread dough. The dough is then rested for about 1hour then rolled with a wooden rolling pin into a circular sheet, which was smeared with refined vegetable oil or ghee, then folded into a semi-circle. Oil was smeared again before folding it into a quadrant and rounding the corners by squeezing in. The dough was then rolled out into as round a shape as possible and a thickness of about 2-3 *mm* and baked on a hot cast-iron griddle. Greasing the surface during each folding was done to facilitate the formation of discrete laminations during baking [Shurpalekar and Prabhavathi, 1976]. The quantity of water, salt and oil required for the preparation of *chapati* dough was determined only by trial and error. It was therefore fundamental to determine the recipe for optimum chapati dough to be used for subsequent experiments in this work to ensure reproducibility of the dough used.

2.2 Accelerated Shelf Life Testing [ASLT]

To attain knowledge of a food's expected shelf life, one must understand the microbiological, enzymatic and physicochemical reactions that simultaneously take place in any given food. One must also identify the mechanisms responsible for spoilage or loss of desirable characteristics such as flavour, odour or nutrients, and implement scientific models for estimating the period it will retain an acceptable level of eating quality from a safety and organoleptic perspective [Labuza, 2000]. The four critical factors in this endeavour are formulation, processing, packaging and storage conditions. Understanding the interplay

between these factors is key to shelf life estimation and testing. Appropriate shelf life testing is normally required to take into account the different scenarios brought about by this interplay.

Several approaches have been used to estimate shelf life including the use of published data, utilisation of known distribution times for similar products on the market, or use of consumer complaints as a basis to determine the end of shelf life [Labuza, 2000]. These methods have their downsides, which include the specificities of differently engineered foods, high costs and time consumed given large sample sizes and testing panels who may not even be representative of consumers let alone consumer segments [Fu and Labuza, 1997; Cardelli *et al.*, 2001]. This has therefore encouraged the use of alternative, more objective, quicker and less costly methods of shelf life determination such as Accelerated Shelf Life Testing [ASLT]. The objective is to store a finished product/package combination under elevated conditions and then periodically examining the product until the end of shelf life occurs. The data is then used to project shelf life under normal distribution conditions [Lee and Krochta, 2002]. Kinetic models of foods are usually characteristic for the studied food and set environmental conditions of the experiment and underlay the assumption that environmental conditions are constant [Taoukis *et al.*, 1997]. Since most foods are chemically or biologically active, they undergo changes, the rate of which is temperature-dependent [Peleg *et al.*, 2002]. As a result, the factor most often studied and considered is temperature.

The most common mathematical model to describe the effect of temperature on the rate of chemical and biochemical reactions is the Arrhenius equation since most food reactions described have been shown to follow Arrhenius behaviour [Peleg *et al.*, 2002]. It is an empirical fact that in many systems [at certain characteristic temperature range] the plot of $\ln k$ versus $\frac{1}{T}$ is a straight line or an approximately straight line as judged by statistical criteria. The Arrhenius model contains the activation energy constant, which is a measure of the temperature sensitivity of the reaction. That is, how much faster or slower the reaction goes if the temperature is raised or lowered [Taoukis *et al.*, 1997]. The commonly used parameter is Q_{10} in temperature dependent reaction rates.

Q_{10} is defined as the ratio of the reaction rates or rate constants at temperatures differing by 10. The model can therefore be used to describe how much faster a reaction will go if the product is held at some other temperature. It has been found that as long as the temperature range is not greater than 30 °C to 40 °C, this holds fairly well. A semi-log plot of shelf life versus temperature, which gives a straight line, best describes this relationship. By choosing the proper temperature range ASLT can be used to quickly estimate the shelf life of a product [Labuza, 2000].

2.3 Milk Fat Changes

Milk fat is fat derived from mammalian milk particularly dairy cows that mainly contain palmitic, oleic and myristic fatty acids. Fatty acids are organic acids that esterify on to a glycerol molecule to form acylglycerides. Milk fat also contains appreciable amounts of C4-C12 short chain fatty acids and small amounts of branched and odd-numbered acids. Cow's milk fat contains over 500 different types of fatty acids and 2-6% being trans fatty acids derived from bio hydrogenation of fatty acids in the rumen during microbial digestion. This also results in short chain fatty acids [SCFAs] ranging from C2:0 to C6:0 that ubiquitously occur in milk fat [Gunstone, 2000]. SCFAs are also known as volatile fatty acids and have higher solubility in water. The main lipid classes in milk fat are triacylglycerols, monoacylglycerols, 1,2-diacylglycerols, 1,3-diacylglycerols, free fatty acids and cholesterol esters [Al-Khalifah and Al-Kahtani, 1993]. Bovine milk fat has a mixture of triacylglycerols [TAGs] containing about 5-10% butyric acid and 3-5% caproic acid. Butyric acid is about 3.6% of the TAGs in butterfat [Table 1]. Generally, fats are of animal origin and are solids at room temperature while oils originate from plants and are liquid at room temperature.

Ghee is a concentrate of butterfat with more than 99% milk fat and less than 0.2% water. It has a shelf life of 6-8 months even at ambient tropical temperatures. Butter or cream is converted into ghee by controlled heating to reduce water content to below 0.2%. In other procedures, the aqueous fraction is allowed to separate and some of it is run off before residual moisture is removed by heating. Ghee has a cooked, caramelised flavour varying slightly with the method of preparation [Gunstone, 2002]. Ghee has been reported to contain 315-376 μg/100g vitamin A and 252-284 mg/100g cholesterol [Al-Khalifah and Al-Kahtani, 1993]. The level of unsaturation in ghee is typically 30-40% [28-31% monoenes

and 1-3% polyenes] making it susceptible to autoxidation and hydrolysis especially in high moisture food matrix [Al-Khalifah andAl-Kahtani, 1993; Gunstone, 2002]. **Table 1** shows the major fatty acids and their classes in cow's milk fat.

Table 1: Major fatty acids [%weight] in cow's milk fat.

Short Chain Fatty Acids	Content	Medium Chain Fatty Acids	Content
4:0#	3.6%	10:0	2.8%
6:0	2.2%	12:0	2.8%
8:0	1.2%	14:0	10.1%
		16:0	5.0%
Long Chain Fatty Acids	**Content**	**Others**	8.6%
18:0	2.1%		
18:1*	27.1%		
18:2	2.4%		
20:0	2.1%		

*Oleic acid is the dominant fatty acid in milk fat. # Butyric acid is the dominant Short Chain Fatty Acid. Source: [Gunstone, 2002]

2.3.1 Hydrolysis

Hydrolysis is the addition of a water molecule to an ester bond of a fatty acid and glycerol. Simple lipids may be hydrolysed to fatty acids and glycerol [**Figure 1**]. Hydrolysis may be catalysed by the presence of water in the system, high temperatures or lipolytic enzymes. Free fatty acids and low molecular weight acidic products of fat oxidation enhance hydrolysis especially in the presence of steam at high temperatures [Warner, 2002]. Hydrolytic breakdown of milk fat produces short chain free fatty acids that contribute directly to rancid aromas. Higher fatty acids produced are usually further oxidised into more flavour compounds. This is because they are more susceptible to oxidative changes than when esterified to the glycerol. Free fatty acids formed by lipolysis of triacylglycerols have

been shown to accelerate oxidation while those from phosphoglycerols limit oxidation [Erickson, 2002].

Lipases [Triacylglycerol acylhydrolases EC 3.1.1.3] are enzymes that catalyse the reversible hydrolysis of TAGs under natural conditions and are widely distributed in animals, plants and microbes. They differ from other esterases in that their activity is greatest against water insoluble substrates and is enhanced at substrate [oil]- water interface; that's why they exhibit "interfacial activation". Microbial lipases such as fungal and bacterial lipases are of importance especially during food storage periods when microbial growth occurs [Weete, 2002]. Lipases are more active in n-hexane and iso-octane than in other solvents. This is because hexane tends to promote acyl migration due to low solubility of free fatty acids and partial glycerides, which forces them into microaqueous region around the lipase. Other solvents such as diethyl ether more readily solubilize free fatty acids and partial glycerides therefore reducing the risk of acyl migration and the activity of lipases. Microbial lipases have demonstrated optimum activity at pH range 7-10 and temperature range 30-62°C, with mould lipases being more tolerant to low water content [Weete, 2002].

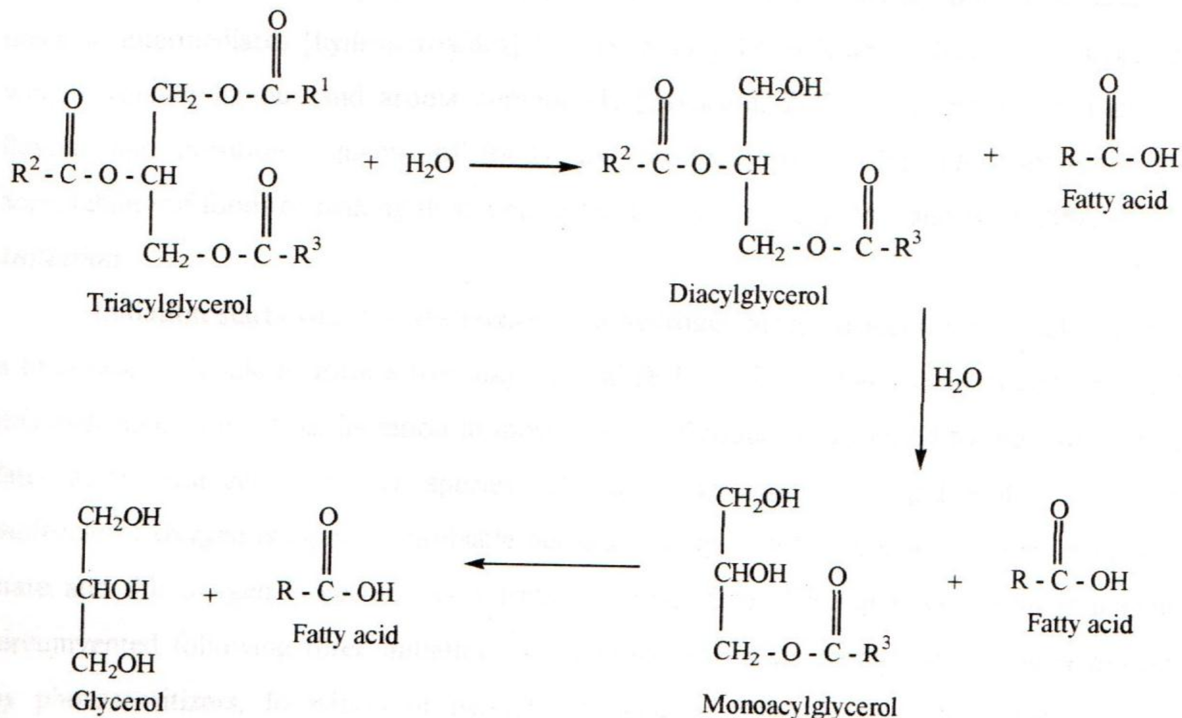
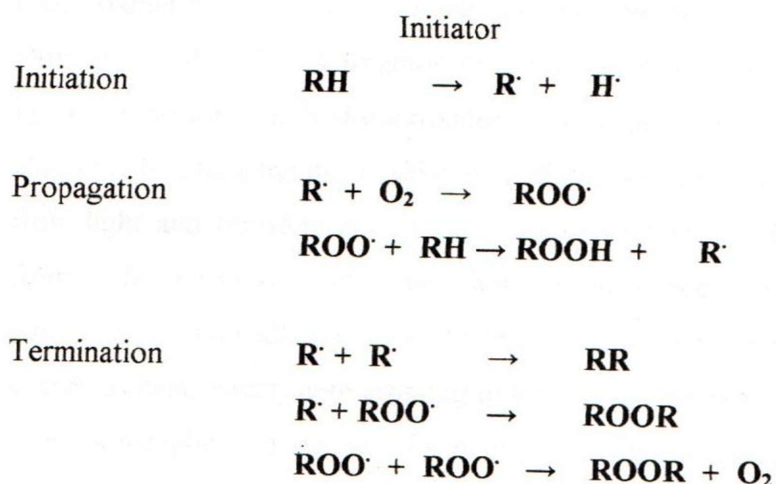


Figure 1: Hydrolysis of triacylglycerols to free fatty acids and glycerol [Warner, 2002].

2.3.2 Hydroperoxidation

These are chemical reactions that result in generation of hydroperoxides and their degradation products. Unsaturated fatty acids [UFAs] are mainly peroxidised by autoxidation. Autoxidation is a natural process that takes place between molecular oxygen and UFAs that occurs via a free radical chain mechanism consisting of initiation, propagation, branching and termination as the main steps [Shahidi and Wanasundara, 2002b]



Initiation $\mathbf{RH} \xrightarrow{\text{Initiator}} \mathbf{R^{\cdot} + H^{\cdot}}$

Propagation $\mathbf{R^{\cdot} + O_2 \rightarrow ROO^{\cdot}}$

$\mathbf{ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}}$

Termination $\mathbf{R^{\cdot} + R^{\cdot} \rightarrow RR}$

$\mathbf{R^{\cdot} + ROO^{\cdot} \rightarrow ROOR}$

$\mathbf{ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O_2}$

In this process, oxygen from the atmosphere is added to certain fatty acids creating unstable intermediates [hydroperoxides] that eventually breakdown to form low molecular weight volatile flavour and aroma compounds [Erickson, 2002]. Oxidation can alter the flavour and nutritional quality of foods and produce toxic compounds, lowering the acceptability of foods or making them unacceptable to consumers [Min and Boff, 2002].

Initiation

Initiation starts with the abstraction of a hydrogen atom adjacent to a double bond in a fatty acid molecule to form a free alkyl radical **[R$^{\cdot}$]**. While irradiation can directly abstract this hydrogen from lipids, initiation in most foods is frequently attributed to reaction of free fatty acids with active oxygen species. The direct reaction of a lipid molecule with a molecule of oxygen is highly improbable because the lipid molecule is in a singlet electronic state and the oxygen molecule has a triplet ground state. This spin restriction is usually circumvented following three initiation mechanisms. These are formation of singlet oxygen by photosensitizers, formation of partially reduced or activated oxygen species such as H_2O_2, superoxide anion or hydroxyl radicals, and/or formation of active oxygen–iron

complexes [ferryl iron or ferric-oxygen-ferrous complex], [Shahidi and Wanasundara, 2002b].

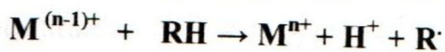
In addition, the action of enzyme systems may cause direct or indirect oxidation of fats. These include microsomal enzymes, peroxidase and dioxygenases such as lipoxygenases and cycloxygenases. Therefore, activated oxygen species are likely to be present in the food even before it is harvested, or may be formed during processing and storage [Erickson, 2002]. Singlet oxygen [$^1O_2$] oxidation is much faster than triplet oxygen [$^3O_2$] oxidation resulting in drastic increases in oxidation rates at very low temperatures [Min and Boff, 2002]. Formation of $^1O_2$ species may be chemically induced, enzymatic or by decomposition of hydroperoxides. It is mainly formed when photosensitizers such as chlorophyll, pheophytins, riboflavins and myoglobin in foods absorb energy very rapidly from light and transfers it to triplet oxygen to form singlet oxygen. On absorbing energy from light in picoseconds, the photosensitizer becomes unstable, excited singlet state molecule and immediately seeks to return to ground state by internal conversion to loose energy as heat, intersystem crossing to become an excited triplet state molecule or react with triplet atmospheric oxygen to form singlet oxygen and singlet state sensitizer via a triplet-triplet annihilation mechanism [Min and Boff, 2002]. This step has the minimal hydroperoxides formation rate.

Propagation

Propagation is fostered by lipid-lipid interaction whereby the free alkyl radicals formed in initiation step [$\mathbf{R^{\cdot}}$] react with atmospheric oxygen to form an unstable peroxy free radical [$\mathbf{ROO^{\cdot}}$]. The peroxy free radical may in turn abstract a hydrogen atom from an adjacent UFA molecule resulting in a lipid hydroperoxide [**ROOH**] and a new free radical [$\mathbf{R^{\cdot}}$], which initiates further oxidation.

Branching

Additional magnification of lipid oxidation however occurs through branching reactions [also called secondary initiation]. Metal ions interact directly with unsaturated fatty acids and lower the activation energy for the initiation step of autocatalysis to produce radicals that abstract hydrogen atoms from other unsaturated fatty acids.



$$\mathbf{M^{(n-1)+} + RH \rightarrow M^{n+} + H^+ + R^{\cdot}}$$

They also interact with hydroperoxides to prompt decomposition of the hydroperoxides.

$$M^{(n+1)+} + ROOH \rightarrow M^{n+} + H^{+} + ROO^{\cdot}$$

$$M^{n+} + ROOH \rightarrow Mn^{(n+1)+} OH^{-} + RO^{\cdot}$$

Termination

To break the repeating sequence of propagation step, two types of termination reactions are encountered. These are radical-radical coupling and radical-radical disproportionation in which two stable products are formed by an atom or group transfer process. In both cases non-radical products are formed. However, termination reactions are not always efficient [Min and Boff, 2002]. Although enzymatic and photogenic oxidation play a role, the most common and important process by which unsaturated fatty acids interact with oxygen is the free radical mechanism called autoxidation. The rate of oxidation is dependent on many factors including temperature, level of unsaturation, oxygen absorption, partial pressure, presence of pro- and anti oxidants and the concentration of decomposition products [Erickson, 2002].

2.3.3 Degradation of Hydroperoxides

Hydroperoxides are the primary products of lipid oxidation although they are not considered harmful to food quality since they are generally tasteless. However, they are further degraded into compounds such as hydrocarbons, alcohols, furans, aldehydes, ketones and acidic compounds that are responsible for off-flavours [Shahidi and Wanasundara, 2002b].

The main mechanism for the formation of aldehydes from lipid hydroperoxides is homolytic scission [β- cleavage] of two bonds on either side of the hydroperoxy group [**Figure 2**]. This reaction proceeds via the lipid alkoxy radical with two odd electrons produced on neighbouring atoms forming a carbonyl double bond. Aliphatic aldehydes are derived from the methyl terminus of the fatty acid chain while other aldehydes are formed from the part still bound to the parent lipid molecule. Since unsaturated aldehydes can be oxidised further, additional volatile compounds may be formed [Erickson, 2002]. The type of oxidative cleavage of double bonds in fatty acids and composition of hydroperoxides influence the type of volatile compounds produced from oxidation [Shahidi and Wanasundara, 2002b]. Some of the resulting compounds are extremely potent and can affect flavour at concentrations as low as 1ppm. **Table 2** shows flavour perception of some volatile compounds produced by lipid oxidation.

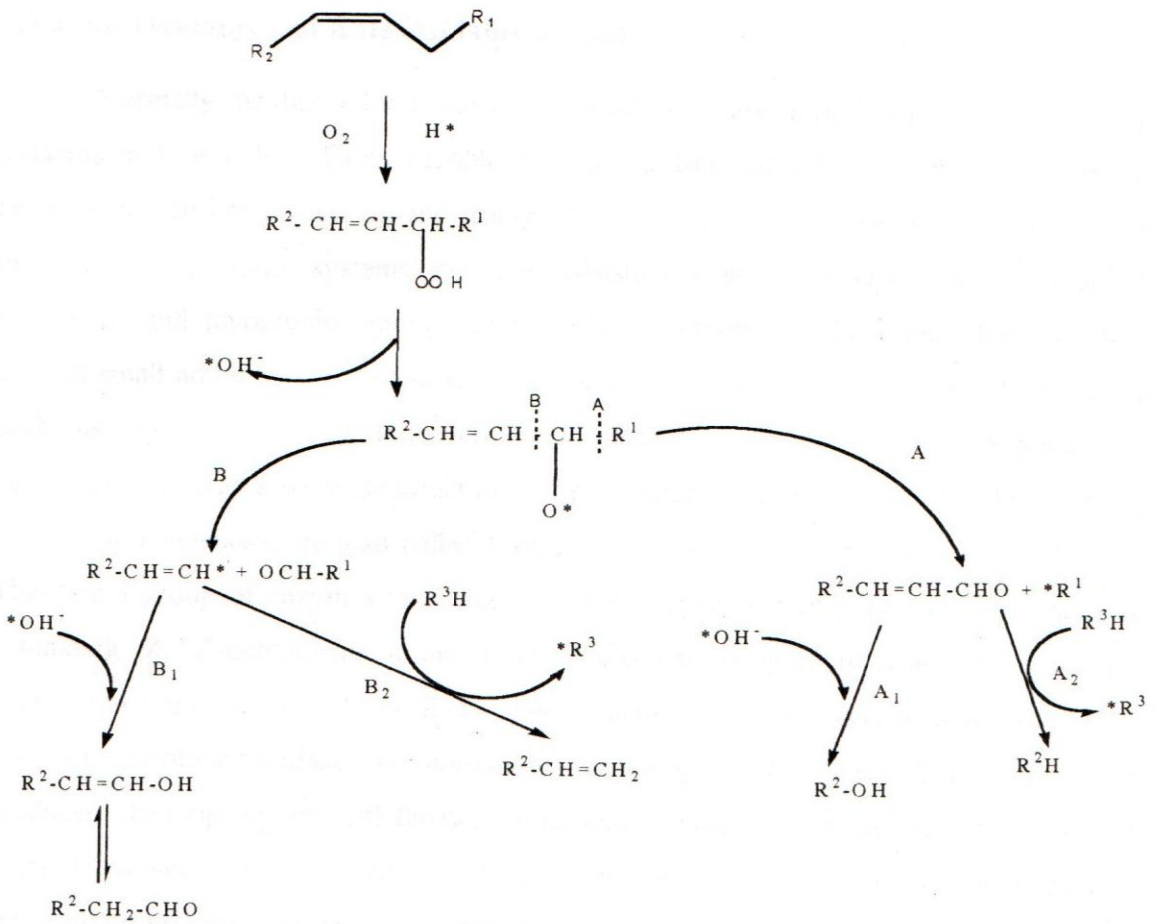


Figure 2: Decomposition of hydroperoxides to produce volatile compounds

Source: [Min and Boff, 2002]

Table 2: Flavour perceptions of some volatile compounds from lipid oxidation.

Flavour perception	Responsible compounds
Oily	Aldehydes
Painty	Pent-2-enal, aldehydes
Grassy	Trans-2-hexenal
Fatty	(E)-2-nonenal, (E, E)-2,4-decadienal
Malty	3-methylbutanal
Sour	Acetic acid
Metallic	(E)-4,5-epoxy- (E)-2-decenal

Adopted from Czerny and Schieberle. [2002].

2.3.4 Pro-Oxidants and Anti-Oxidants in foods

Normally, neither a food matrix nor food lipids are pure. They usually contain pro-oxidants and or anti-oxidants capable of forming free radicals or limiting lipid oxidation respectively, during processing and storage [Orlein *et al.*, 2000]. Among naturally occurring pro-oxidants in food systems are photosensitizers such as chlorophyll, pheophytins, riboflavins and myoglobin among others. Wheat contains 0.13mg/100g riboflavin. Metals even in small amounts can encourage lipid oxidation through secondary initiation. Enzymes such as microsomal enzymes, peroxidase and dioxygenases like lipoxygenases and cycloxygenases may also cause direct or indirect oxidation of fats in food [Erickson, 2002].

Lipoxygenases are also called Linoleate: Oxygen Oxidoreductases [EC 1.13.11.12]. They are a group of enzymes that catalyse the dioxygenation of polyunsaturated fatty acids containing Z, Z-pentadiene moieties by molecular oxygen resulting in enantiomeric hydroperoxy fatty acids with the Z, E –diene conjugation. They were formally known as fat oxidases, carotene oxidases or lipoxidases [Zhuang *et al.*, 2002]. The hydroperoxides produced decompose into off-flavour compounds. Most lipoxygenases act on free fatty acids. However, there are reports of lipoxygenases acting directly on phosphoglycerols hence hydrolysis may not always be required prior to lipoxygenase activity. In wheat, three lipoxygenase isoenzymes have been studied at biochemical level namely L-1, L-2 AND L-3 [Barone *et al.*, 2000]. Some added ingredients such as common salt [NaCl] added in foods at low concentrations have been shown to accelerate oxidation of lipids [Erickson, 2002].

Anti-oxidants either delay the onset of oxidation or slow the rate of its progress. They extend shelf life of foods; reduce raw material waste and nutritional losses [Reische *et al.*, 2002]. Anti-oxidants can occur naturally or be intentionally added during processing. It has also been reported that some compounds formed during processing may exhibit antioxidant characteristics [Mastrocola and Munari, 2000]. Some naturally occurring antioxidants are carotenoids, which quench singlet oxygen at low oxygen partial pressure. They deplete singlet oxygen of its excess energy and dissipate it in the form of heat. The rate of quenching depends on the number of conjugate bonds on the carotenoids and the amount present [Reische *et al.*, 2002]. Tocopherols belong to the tocotrienols group of compounds that are free radical scavengers through a mechanism involving charge transfer. They are found in palm oil, rice bran oil, cereals and legumes. Among α, β, γ and δ- tocopherols, α-

tocopherols have the highest quenching rate. They are the most abundant natural antioxidants [Min and Boff, 2002]. Spices, fruits, tea, coffee, seeds and grains contain phenolic compounds that have been shown to have antioxidant activity in olive oil.

Numerous amines, amino acids, peptides and protein hydrolysate have demonstrated antioxidant effect by chelating metal ions in a manner that decreases their activity [Reische *et al.*, 2002]. Wheat gluten has also been isolated and used as a natural antioxidant [Al-Neshawy and Al-Eid, 2000]. Metal chelators that may be added in foods include citric acid and its lipophilic monoglyceride ester, phosphoric acid and polyphosphate derivatives, EDTA among others.

In many heated foods, amines react with carbonyl compounds mainly reducing sugars leading to formation of a complex series of compounds called Maillards Reaction Products [MRPs]. These compounds have been shown to reduce lipid oxidation rate [Mastrocola and Munari, 2000]. It has also been demonstrated that some carbonyl compounds including lipid peroxidation products react with amino acid groups to form pigments having properties similar to melanoidins especially at lower temperatures [25 - 50°C] [Hildago *et al.*, 1999]. Oxidised lipid products also react with carbohydrates at higher temperatures [80 – 120°C] to form antioxidant compounds.

2.4 Kinetic Modelling of Shelf Life Deterioration

The pathways and relationships involved in lipid oxidation are complex. Consequently, there is need to have methods to quantitatively link product composition to oxidative stability. Mathematical modelling is a tool that allows the synthesis of data from one or many experiments into an integrated system from which quantitative changes in many components may be calculated [Erickson, 2002].

Kinetics is the study of the rates at which chemical reactions occur. The rate of a reaction and how this rate changes in response to different conditions is ultimately related to the path followed by the reaction and is therefore indicative of its reaction mechanism [Voet and Voet, 1990]. Aldehydes are the most important of volatile products of lipid hydroperoxidation of oleic acid [Mahungu *et al.*, 1998] and have been used to measure lipid oxidation in foods [Al-fawaz *et al.*, 1994]. The presence of free fatty acids in a lipid containing food indicates lipase activity and other hydrolytic reactions [Shahidi and Wanasundara, 2002b]. Being products of quality deterioration mechanisms, the

concentrations of aldehydes and free fatty acids may be experimentally monitored as a function of time. The data can be used to formulate mathematical models for shelf life determination of lipid containing foods with respect to oxidative stability. In kinetic experimental studies, it is impossible to measure the reaction rate itself. Instead, the concentration of "A" is measured directly or indirectly as a function of time [Taoukis *et al.*, 1997]. Where, "A" is the substrate or product of the reaction being monitored. Since most food quality deterioration has been found to fit either a zero or first order, a mathematical expression is fitted onto the data obtained to produce a straight line.

$$-\frac{dA}{dt} = k\,[A]^n$$

Here, k is the rate constant and $\boldsymbol{n}$ is the reaction order. If the environmental factors are held constant, $\boldsymbol{n}$ defines the shape of the deterioration curve [Fu and Labuza, 1997]. The reaction rate constants obtained are plotted on a semi log plot against inverse of absolute temperature $[\frac{1}{T}]$ to obtain a straight line. This is the Arrhenius plot used to describe how much faster or slower a reaction will go if held at some other temperature [i.e. the effect of temperature on k].

The linear model may also be used for either zero or first order reactions to extrapolate shelf life results from accelerated tests at higher temperatures to estimate shelf life results under lower temperature. The equation below is applied:

$$k_1 t_{s1} = k_2\, t_{s2}$$

Where: k_1 and k_2 are rate constants at temperatures T_1 and T_2 respectively, while t_{s1} and t_{s2} are estimated shelf life at T_1 and T_2 respectively [Lee and Krochta, 2002].

From the Arrhenius plot whose slope $\frac{E_A}{R}$ [k is the rate constant at different temperatures], E_A the activation energy [Joules/mole], R the gas constant [8.314 J K^{-1} mol^{-1}] and T the absolute temperature [°K= 273 + °C], [Lee and Krochta, 2002].

ASLT methods have been used to predict the shelf life of products such as commercially sterilised skim milk sweetened with aspartame [Bell *et al.*, 1994], frozen foods [Reid *et al.*, 2003] and peanuts coated with whey protein [proteins isolated from whey, Lee and Krochta, 2002]. Dehydrated potatoes are also among foods whose shelf life has been predicted using ASLT [Labuza, 1996].

CHAPTER THREE

MATERIALS AND METHODS

3.1Experiment 1: Dough Preparation

The study was carried out at the Cereal Chemistry Laboratory at the National Plant Breeding Research Centre [Kenya Agricultural Research Institute], Njoro and various Egerton University Departmental Laboratories [Botany, Animal Science, Horticulture and Chemistry Department].

3.1.1 Kneading Trials

Commercial grade whole-wheat flour, ghee and salt were used in the experiment. An analytical balance was used for taking weights. The water used was laboratory grade distilled water. Several preliminary kneading trials were carried out on the Brabender Farinograph [Model OHG, Duisburg, Germany] to arrive at the optimum levels of water addition and mixing time for the preparation of chapati dough of desired characteristics. Criteria like hand-feel [stiff or soft], stickiness and ease of rolling into a sheet formed the basis of evaluation of dough characteristics. The moisture content of the flour was determined using Brabender moisture determining equipment [Duisburg, Germany].

After thoroughly mixing the flour to achieve homogeneity, four samples, approximately 10g each, were carefully weighed in moisture dishes using an analytical balance and placed in a pre-warmed Brabender flour moisture determining equipment at 30°C for 3 hours. Based on that flour moisture content, the Brabender Farinograph [Duisburg, Germany] was used to establish water absorption and optimum-mixing time required to obtain desirable dough. Samples weighing 48.35g each of whole-wheat flour were placed in the mixing bowl. Distilled water at 30°C was added in different amounts ranging from 31.0-36.3ml until the resulting dough attained 500 ± 20 Brabender Units [BU]. Based on the water absorption of the optimum dough, the dough water content and the mixing time were calculated from the mixing curves obtained.

The Alveograph was used to determine the elasticity and strength of the dough, which is a function of gluten content of the flour used. Samples of whole-wheat flour weighing 60g each were mixed with 44.2ml of 2.5% [w/v] salt solution at 30°C for 8 minutes. The resultant dough was then sheeted and cut into small discs that were rested at

30°C for 20 minutes. The small discs were then set on the Alveograph, air blown into them to produce curves that indicate the stretchiness of the dough.

It was necessary to determine the level of salt and ghee, traditionally added in home-scale preparation for taste as well as desirable handling and rolling characteristics. Since commercial ghee was used in this study, its oxidative and hydrolytic history was unknown to the author. Doughs containing 0.5, 1.0 1.5, 2.0% salt and 5, 6,10% ghee were prepared and evaluated for their consistency by hand-feel and rolling behaviour

3.2 Experiment 2: Proximate Analysis

An analytical balance was used in the experiment. The moisture was determined by oven drying a weighed approximately 2g amount of sample at 105 ± 3°C for 8 hours to a constant weight, cooled in a dessicator and weighed [AOAC, 1996]. Ash content was determined by ignition of sample at 550°C for 4 hours, cooled in a dessicator and weighed [AOAC, 1996].

Lipid content was determined by extracting the dried ground samples weighing approximately 2g with petroleum ether [40-60°C] for 8 hours at 50 °C by semi-continuous liquid extraction using Soxhlet apparatus [**Plate A**] and the extract concentrated in *vacuo* by rotary evaporation before being cooled in a dessicator and weighed.

Crude protein was estimated using the micro-Kjeldahl method. Samples of approximately 0.2g were digested at 490 °C for 1 hour using concentrated H_2SO_4 and selenium tablets as catalysts. Distillation was done by receiving the gas in 20ml 0.1N HCl and the distillate titrated against 0.1N NaOH using a mixed indicator [0.1% methyl red, 0.2% bromocresol green and 0.5 % phenolphthalein] for each sample.

Crude fibre was obtained by digesting samples approximately 2g each, with 2.04N H_2SO_4 then with 1.78N KOH by boiling each of them for 30 minutes. The residue was ignited at 550°C overnight before being cooled in a dessicator and weighed. Nitrogen-free extracts, which include simple sugars and other soluble carbohydrates, were obtained by subtraction.

3.3 Experiment 3: Shelf Life Testing

3.3.1 Experimental Plan

The experiment was set up as a Split-plot in time [Repeated measures experiment] with the storage temperature being the main plot treatment and storage time the sub plot treatment. The sub-plot was assigned to the main plot in a completely randomised block design [RCBD]. The response variables were acid value, % free fatty acids and para-anisidine value.

Raising the test temperatures when conducting Accelerated Shelf Life Tests [ASLT] in food systems, the results of such a test can be modelled using temperature versus reaction rate relationship to predict shelf life at lower storage temperatures [Lee and Krochta, 2002]. In this study, the Arrhenius modelling was used to predict the shelf life of ready-to-fry chapati at normal storage temperatures with respect to oxidative stability. The temperatures of the study were chosen to lie within a range of 30-40^{0}C in which the Arrhenius model has been shown to apply [Labuza, 2000].

3.3.2 Dough Preparation and Storage Conditions

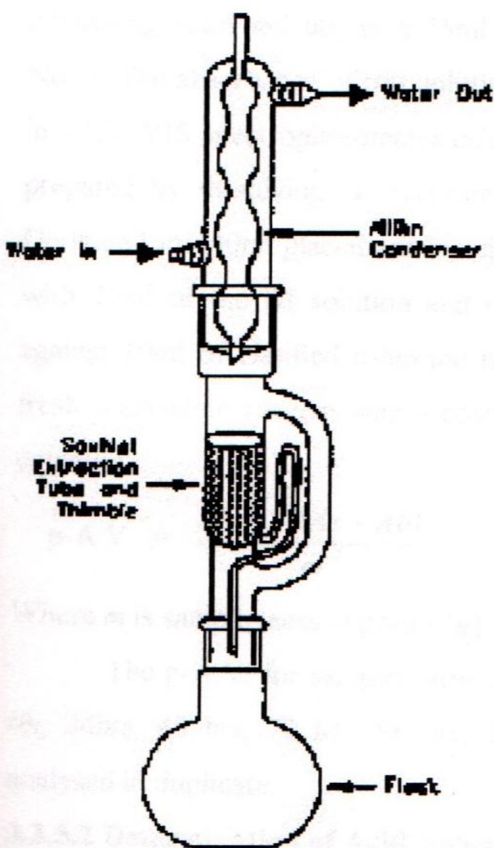
The dough used in this experiment was prepared using the established recipe and method. For each storage temperature, 700g of dough was prepared and divided into seven portions to make 7 chapati*s* of approximately 100g each. Each chapati was then greased with ghee, folded and rolled out into as round a shape as possible before being packed in transparent low-density polyethylene bags and stored at specified temperatures. Four different storage temperatures were included in the experiment; these were 35 °C, 40 °C, 45 °C and 50 °C and four identical doughs were made for storage at those temperatures. The chapati*s* were stored for 0 hour [immediately after mixing], 24, 48, 72, 96, 120 and 144 hours at the various storage temperatures.

3.3.3 Drying and Grinding of Samples

For each point in storage time, a single plastic bag was pulled out from each storage temperature and oven-dried at 100°C for at least 8 hours then ground using a hand mill No.3 [England]. The coarse powder obtained was carefully packed in Soxhlet extraction thimbles and extracted using petroleum ether [40-60°C] as a solvent.

3.3.4 Extraction of Fat for Analysis

This was done by semi-continuous liquid extraction with Soxhlet apparatus [**Plate A**] using petroleum ether [40-60°C] as a solvent at 50°C for at least 8 hours. The extract was concentrated *in vacuo* by rotary evaporation at a temperature of 50°C, and then left overnight in a dessicator to dry to a constant weight for further analyses. The extraction solvent was dried over anhydrous sodium sulphate and then distilled at 60°C before use.



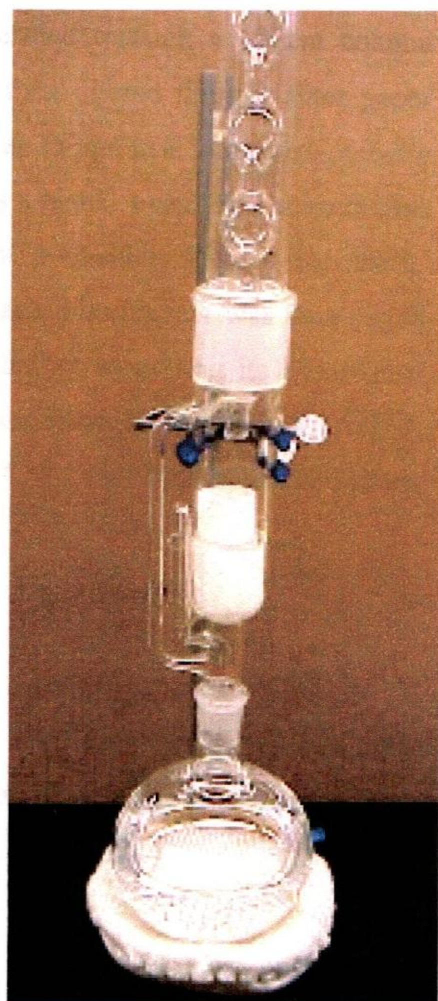


Plate A: Soxhlet Apparatus

3.3.5 Instrumental Analysis

3.3.5.1 Determination of p-anisidine Value [p-A.V.]

The method used is a modification of A.O.C.S. Official Method Cd 18-90 [A.O.C.S., 1990]. It is a measure of aldehydes principally 2-alkenals and dienals present in oils by reaction of the aldehydic compounds in a fat and the p-anisidine, in an acetic acid solution and then determination of absorbance at 350nm. It is often used to detect secondary oxidation products [Che Man *et al.*, 2003]. Approximately 1g of extracted milk fat was dissolved and diluted to volume with n-hexane [AR, clarified through a 100cm column containing silica gel 60] in a 25ml volumetric flask; and then filtered through filter paper No. 1. The absorbance of this solution [Ab] was measured at 350nm in a 1cm quartz cuvette in a UV-VIS spectrophotometer using clarified n-hexane as a blank. p-anisidine reagent was prepared by dissolving an accurately weighed 0.2503g of p-anisidine [Merck-Shuchardt, Germany] in 100ml glacial acetic acid [AR]. Two ml of p-anisidine reagent was then mixed with 10ml of the fat solution and rested for 20 minutes before measuring its absorbance against 10ml of clarified n-hexane mixed with 2ml p-anisidine reagent as a blank [As]. A fresh p-anisidine reagent was prepared before analysis. p-A.V. was then calculated using equation;

$$\text{p-A.V.} = \frac{25 \times [1.2As - Ab]}{m}$$

Where m is sample mass in grams [g]

The p-A.V. for samples stored at 35 °C, 40 °C, 45 °C and 50°C were determined after 0h, 24hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs and 144 hours of storage. Each sample was analysed in duplicate.

3.3.5.2 Determination of Acid Value [%FFA]

The acid value was used to measure of the extent to which lipolysis has occurred to produce free fatty acids. Approximately 1g of melted fat was dissolved in 20ml of ethanol/diethyl ether mixed in a 1:1 ratio and titrated with a standard solution of 0.1N NaOH using 1% phenolphthalein indicator until a faint pink colour persisted for 20-30 seconds. The phenolphthalein indicator was prepared using the ethanol/ diethyl ether solvent.

The titre obtained was used to compute the acid value [A.V.] and % free fatty acids [%FFA] using the given equations. Each titration was carried out in triplicate for samples stored at 35°C, 40°C, 45°C and 50oC for 0h, 24h. 48h, 72h, 96h, 120h and 144 hours.

$$\text{Acid Value} = \frac{Titre \times 5.61}{W} \qquad \%FFA = \frac{V \times M_1 \times M_2}{10 \times W}$$

Where;

V is the titre volume

M_1 is the concentration of NaOH (0.1N)

M_2 is the molecular weight of free fatty acid [AOCS standard is oleic acid (282)]

W is the sample weight [g]

3.3.6 Statistical Analysis

Differences between means in the results obtained from the shelf life tests were tested by analysis of variance [ANOVA]. Mean separation was done using Duncan's Multiple Range Test [DMRT] at $p<0.05$. Procedure general linear model [GLM] of SAS [Statistical Analysis System, 2000] was used for these computations.

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CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Method for Preparation of Experimental Chapatis

The flour Farinograph water absorption to reach 500 ± 20 BU and maximum extensibility was found to be 42% [**Plate B**]. A dough development time of 5 minutes was determined from the Farinograph curves.

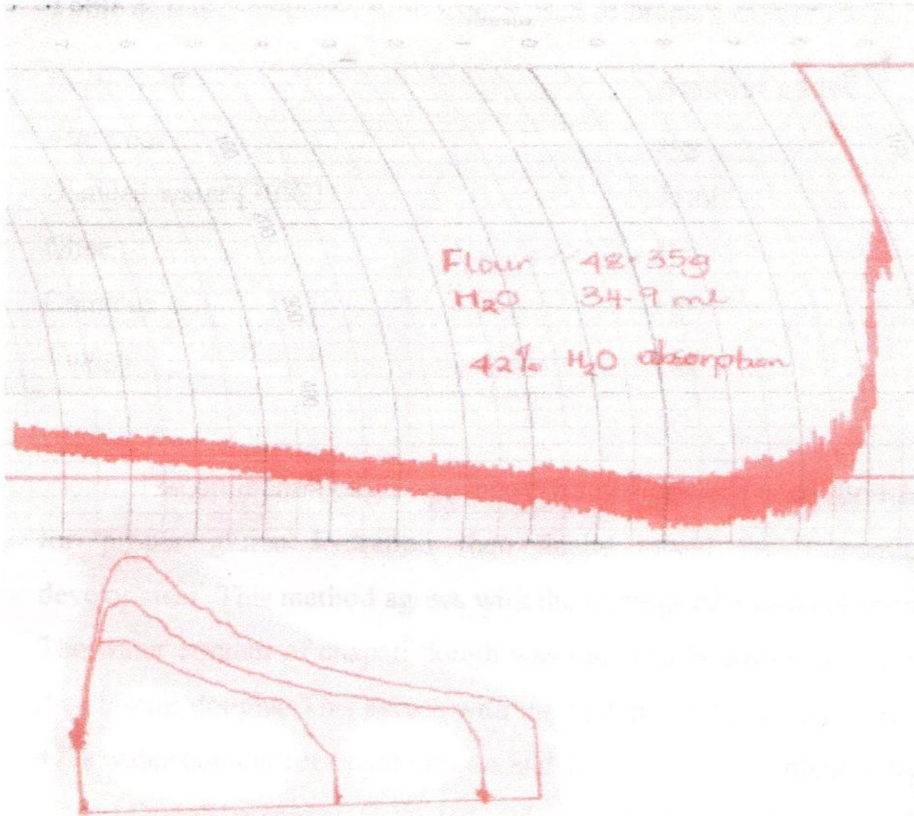


Plate B: Farinogram and Extensogram.

The findings from the Farinograph and Alveograph were used to generate the recipe used in the experiment [**Table 3**]. In addition, a small amount of vinegar was included in the dough to act as an antimicrobial agent. However, small quantities were applied to avoid a shift from the generally acceptable flavour of chapati. Vinegar was chosen as an antimicrobial agent due to its low cost, ease of availability and low toxicity. It was, however, observed that its addition might not have significantly inhibited microbial spoilage

of samples. The setting in of fermentation and evident surface mould growth in the stored samples manifested the spoilage, by the third day of storage. The spoilage may have set in due to the low concentration of acetic acid approximately 4.5% in the vinegar used. Acetic acid is the active antimicrobial agent in vinegar. Acetic acid is reported to be more effective at pH 4.5 or less. However, the samples had a pH range of 5.5-6.5 hence, ineffective and particularly more against moulds than yeasts [Davidson *et al.*, 2002].

Table 3: Ingredients and proportions used to prepare experimental chapati dough.

Ingredient	Amount added
Atta flour	56g
Distilled water [30°C]	31ml
Ghee	10g
Common salt	1g
Vinegar	1ml

The optimum dough was mixed for 5 minutes, rested for 1 hour at room temperature for proper gluten hydration then finally mixed for 1 minute for optimum gluten development. This method agrees with the findings of Shurpalekar and Prabhavathi [1976]. The water content of chapati dough was found to be lower than of bread doughs, but higher than biscuit doughs. This agrees with the findings of Papantoniou *et al.*, [2003] who reported 47% water content for bread doughs and 11-12% water content in biscuit doughs.

Dough prepared with 10% ghee and 1% salt had desirable handling and rolling characteristics. One percent salt content lies within recommended range by Shurpalekar and Prabhavathi, [1976]. Ten percent fat content for chapati dough was lower than 12% in biscuit dough [Papantoniou *et al.*, 2003].

4.2 Proximate Composition

The dried sample was lower in moisture content [**Table 4**] than the dough itself. Drying facilitated extraction of the fat contained therein for further analysis to determine the level of oxidation and lipolysis. However, the moisture content of freshly prepared dough

was slightly different from that of bread dough as reported in 1999 by Sidhu *et al.*, [39.29 ± 0.34%]. This may have resulted from the fact that the chapati dough is typically stiffer than bread dough.

Table 4: Proximate composition[a] of chapati samples

	Proximate composition %	
	Dried sample[b]	Dough sample[c]
Moisture	5.30 ± 0.37	38.27 ± 0.50
Crude protein (N X 6.25)	12.97 ± 0.40	11.78 ± 1.21
Crude fibre	12.27 ± 0.15	12.21 ± 0.76
Lipid content	24.28 ± 1.22	21.83 ± 0.85
Ash	2.56 ± 0.12	2.22 ± 0.15
N-free extracts[#]	42.60	13.70

[a] Mean values of triplicates ± Standard Error of the Mean.

[b] Chapati sample dried at 100°C for at least 8 hours. % composition on wet weight basis

[c] Freshly prepared chapati dough sample. % composition on dry weight basis.

[#] Nitrogen-free extracts [simple sugars and other soluble carbohydrates] values obtained by subtraction.

The crude protein content was similar to that reported by Sidhu *et al.*, [1999] for whole-wheat flour [12.69 ± 0.15%]. It was higher than that of white flour due to the inclusion of the germ, and some bran. The protein content was within the desirable range for chapati dough making [10-13%] [Shurpalekar and Prabhavathi, 1976]. The crude fibre content was also in the range reported by Sidhu *et al.*, 1999 [11.25%] in whole-wheat flour. The ash content was similar to that of bread [2.54%] while lipid content was notably higher than the reported values for bread [3.07%] [Sidhu *et al.*, 1999] and for whole-wheat flour [2-4%] [Papantoniou *et al.*, 2003]. This may have been due to the difference in formulation especially the fat added during dough mixing and greasing the surfaces of sheeted dough to facilitate formation of discrete laminations during baking that is unique to chapati.

4.3 Shelf Life Testing

The experiment was designed to determine the effect of storage temperature on the rate oxidation of milk fat in ready-to-fry chapatis during storage periods. The parameters of choice were the concentration of free fatty acids and the amount of aldehydes in the samples. The p-anisidine values were used as a quality index to formulate an equation for quick determination of shelf life of ready-to-fry chapati.

The initial content of free fatty acid [Al-Khalifah and Al-Kahtani, 1993] and aldehyde [Mahungu *et al.*, 1994] for all temperatures for all storage times were compared with reported values for refined oil. The values are shown in **Figure 3**. It was interesting to note that at 0h, both concentrations of aldehydes and free fatty acids were high suggesting that both the levels of hydrolysis and oxidation in the samples were high. High drying temperatures [100°C] and high moisture content [38%] of the chapati samples encouraged both hydrolysis and oxidation of milk fat.

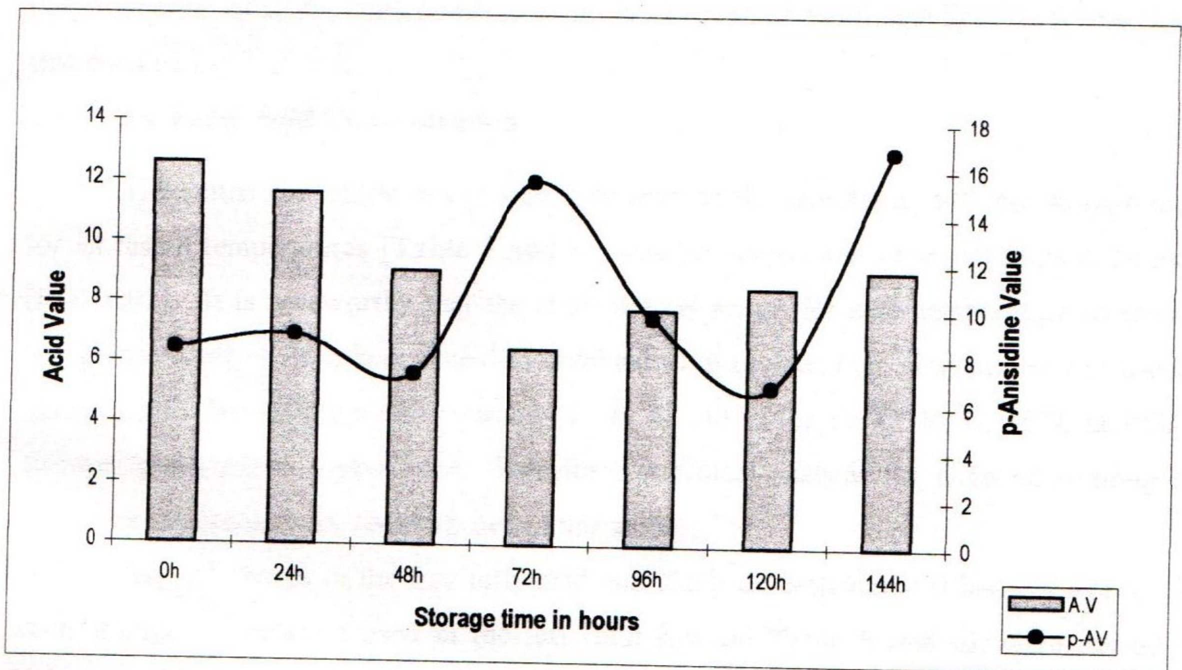


Figure 3: Average free fatty acid and aldehyde concentrations

However, during storage the free fatty acids content started declining to a minimum at 72h, while the aldehyde content reduced slightly then rose to a maximum at 72h. The decline in free fatty acid content may have been due to further oxidation of the free fatty

acids, which are more susceptible to oxidation than when esterified to the glycerol. They are quickly oxidised into aldehydes [Erickson, 2002]. Initially, the aldehyde content displayed some variations in their concentrations probably due to the formation of volatile aldehydes that were evaporating even as they were being formed. The aldehydes accumulated to reach a maximum after 72h. After 72 hours of storage, the aldehyde content decreased rapidly to a minimum at 120h before starting to rise again. This observation concurs with previous work that reported decrease in aldehyde concentrations with time due to their oxidation into acids and their further decomposition into lower molecular weight compounds as reported by [Mahungu *et al.*, 1994]. After 120 hours of storage, the anisidine values started increasing steadily suggesting the formation of more aldehydes especially the non-volatile oxo-compounds that remain attached to the rest of the triacylglycerol molecule with longer storage hours and high temperature. The concentration of free fatty acids steadily started rose after 72hours of storage. This could have been due to microbial deterioration that led to the production of acidic compounds in addition to bacterial and fungal lipases [Weete, 2002] that encouraged hydrolysis

4.3.1 Free Fatty Acid Concentration

The actual concentrations of free fatty acids in the samples at different storage times for all tested temperatures [**Table 5 and 6**] were compared and gave statistical differences [$P<0.0001$]. It is noteworthy that the experimental set up for each temperature of storage was independent of the others. The data obtained from each storage temperature was used to generate milk fat oxidation rate constants k_1, k_2, k_3 and k_4 for 35 ^{0}C, 40 ^{0}C, 45 ^{0}C and 50 ^{0}C storage temperatures, respectively. Therefore, statistical analysis for differences along the rows was unnecessary for the purposes of this study.

The differences in the free fatty acid and aldehyde contents at 0 hour of storage for each storage temperature used in the test [first row on **Table 5 and 6**], were caused by independent preparation of samples for storage at designated temperatures. Since this was done on different days, variations arose from the continuous decomposition of the ghee used during experimentation as a result of exposure to oxygen during extended periods of use. In addition, changes in experimental conditions such as room temperature during dough preparation, differences arising from the commercial ghee and whole-wheat flour may have greatly contributed to such a differences since the ingredients were not of the same batch.

Table 5: Effect of storage time on acid value [A.V.]# at different storage temperatures.

Storage time	Temperature [^{0}C]			
	35	40	45	50
0h	17.75^a±0.2	4.30^c ± 0.3	24.37^a ± 3.5	3.95^c ± 0.1
24h	17.72^a ±0.2	8.48^b ± 2.3	16.45^b ± 2.8	3.73^c ± 0.3
48h	6.35^b±0.5	8.90^b ± 1.4	11.71^{bc}± 4.2	9.25^a ± 0.6
72h	5.75^b±0.4	7.74^{bc} ±1.2	5.44^c ± 0.3	6.87^b ± 0.3
96h	7.17^b±2.9	9.91^b ± 0.3	9.99^b ± 0.4	4.12^c ± 0.2
120h	7.4^b ± 0.1	15.82^a ± 0.9	6.66^c ± 1.1	4.21^c ± 0.1
144h	16.11^a±0.1	8.98^b ± 0.5	7.06^c ± 1.4	4.31^c ± 1.3

Means in the same column with different superscripts are significantly different [α=0.05].
Mean of Acid Values ± Standard Error of the Mean; n=3.

Table 6: Effect of storage time on free fatty acids[%FFA]# at different storage temperatures.

Storage Time	Temperature [^{0}C]			
	30	35	40	45
0h	8.90^a ±0.7	2.16^c ± 0.1	12.25^a ±1.8	1.99^c ± 0.1
24h	8.89^a ±0.1	4.26^b ±1.2	8.27^b ±1.4	1.88^c ± 0.2
48h	3.19^b ±0.3	4.47^b ±0.7	5.89^{bc}±2.1	4.65^a ± 0.3
72h	2.88^b ±0.2	3.89^{bc} ±0.6	2.73^c ± 0.3	3.46^b ± 0.1
96h	3.60^b ±1.4	4.98^b ± 0.1	5.02^{bc}±0.2	2.07^c ± 0.1
120h	3.72^b ±0.0	7.95^a ±0.5	3.35^c ± 0.6	2.12^c ± 0.1
144h	8.08^a ±0.0	5.52^b ± 0.2	3.55^c ± 0.7	2.17^c ± 0.7

Means in the same column with different superscripts are significantly different [α=0.05].
Mean of % FFA Values ± Standard Error of the Mean; n=3.

The results in **Tables 5 and 6** indicate that under the storage conditions used in this experiment, the levels of free fatty acids varied significantly with storage times for all storage temperatures tested [$p<0.05$]. It should be noted that, the experimental procedure for determining %FFA and Acid Values was the same except for the final calculation. The

trends observed for these values were similar since the two are perfectly correlated [r = 1.000, p<0.0001]. The data was used to develop an equation for quick shelf life determination of ready-to-fry chapati. Therefore, the variations in aldehyde and free fatty acid content at 0h of storage confirm that regardless of the initial product quality the method is still applicable for shelf life testing of lipid containing food products. This also agrees with the observation that milk fat oxidation ready-to-fry chapati follows pseudo-zero order kinetic, where the initial concentration of the substrates does not affect the reaction rate [Walstra, 2003].

The average free fatty acid values obtained were generally higher [**Table 5 and 6**] than reported values at normal storage [0.5-1.0% at ~25°C] [Al-Khalifah and Al-Kahtani, 1993]. This may be due to the acceleration effect of raised testing temperatures and high water content that encouraged hydrolysis of the milk fat. Ghee in a food matrix interacts with other food components some of which may contain lipolytic agents such as wheat lipoxygenases [Barone *et al.*, 1999]. High drying temperatures [100°C] were used to dry samples for grinding and extraction of milk fat from chapati samples. This consequently enhanced hydrolysis prior to analysis since hydrolysis is a temperature dependent reaction [Warner, 2002].

From **Figure 4**, it is evident that samples stored for 0 hours at all temperatures demonstrated high free fatty acid concentrations with 45°C having the highest [**Table 5 and 6**]. However, these values reduced to the lowest levels being reported at 72 hours storage, rising again to 144 hours where samples stored at 35°C had the highest amounts. This trend may have been due to the setting in of yeast and mould growth noted after 72 hours of storage since the samples were stored before drying. During their proliferation, these fungi can elaborate lipases [Erickson, 2002]. Some of these lipases are heat stable and can withstand low water content after evaporation of water from foods. This is the case especially on long storage periods at high temperatures, causing increased rate of hydrolysis at later stages of storage. This increased lipolytic activity in the food matrix hence, a notable rise in free fatty acid content in the latter days of storage.

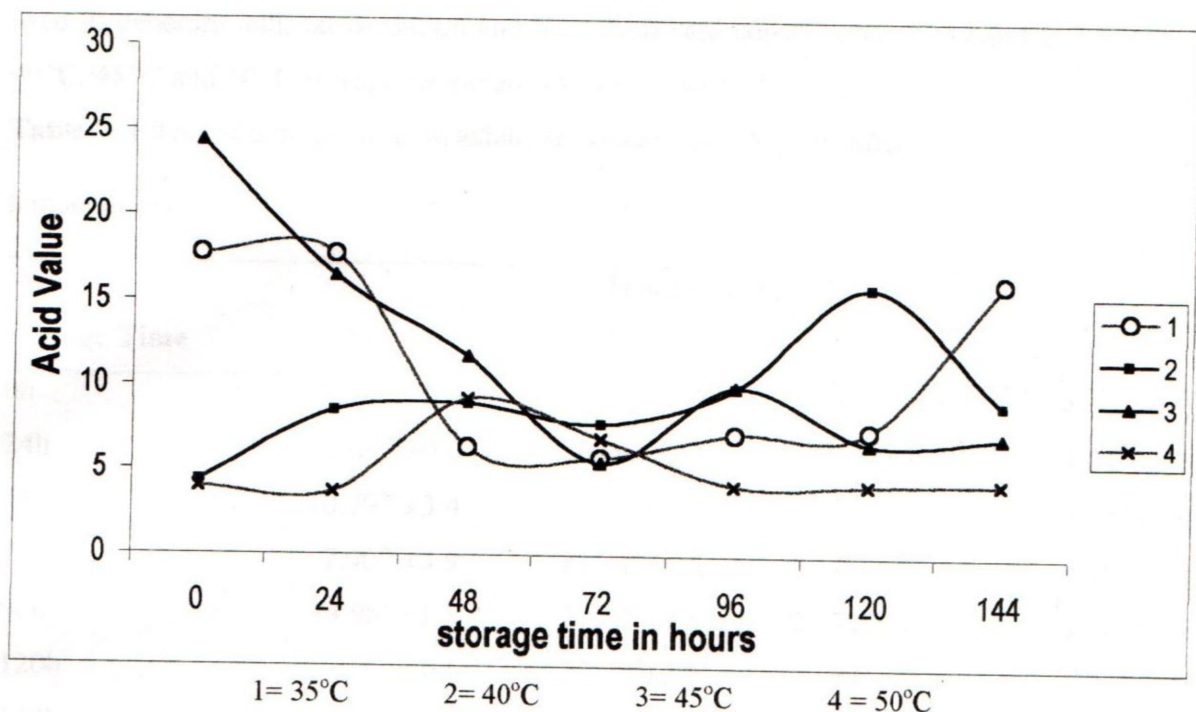


Figure 4: Development of free fatty acids with time temperature at different temperatures

Fermentation is another factor that could have contributed greatly to this rise in acidity after 72 hours of storage by producing some organic acids that added onto the acidity of the samples. It was observed that samples stored at 35°C demonstrated the highest free fatty acid content with some variations, while samples stored at 50°C had the lowest acid values. This observation may be attributed to denaturation of lipolytic enzymes at higher temperatures and volatilisation of some of the short chain fatty acids and acids formed by further aldehyde oxidation [Mahungu *et al.,* 1994, 1998] at higher temperatures compared to lower temperatures of 35°C. Generally, two maxima of the free fatty acid levels were observed suggesting the presence of two major lipid classes in milk fat. Those that hydrolyse first are likely to be saturates mainly palmitic acid while the unsaturates mainly oleic acid hydrolyses later during storage [Gunstone, 2002].

4.3.2 Aldehydes Concentration

Statistical analysis gave significant differences [$p<0.05$] in the levels of aldehydes in sample stored at 35°C. However, the samples stored at 40°C, 45°C and 50°C were not significantly different [$p>0.05$] [**Table** 7]. The experimental set up for each temperature of storage was independent of the others. The data obtained from each storage temperature was

used to generate milk fat oxidation and hydrolysis rate constants k_1, k_2, k_3 and k_4 for 35 °C, 40 °C, 45 °C and 50 °C storage temperatures, respectively.

Table 7: Effect of storage time on aldehyde content [p-A V.]* at different storage temperatures

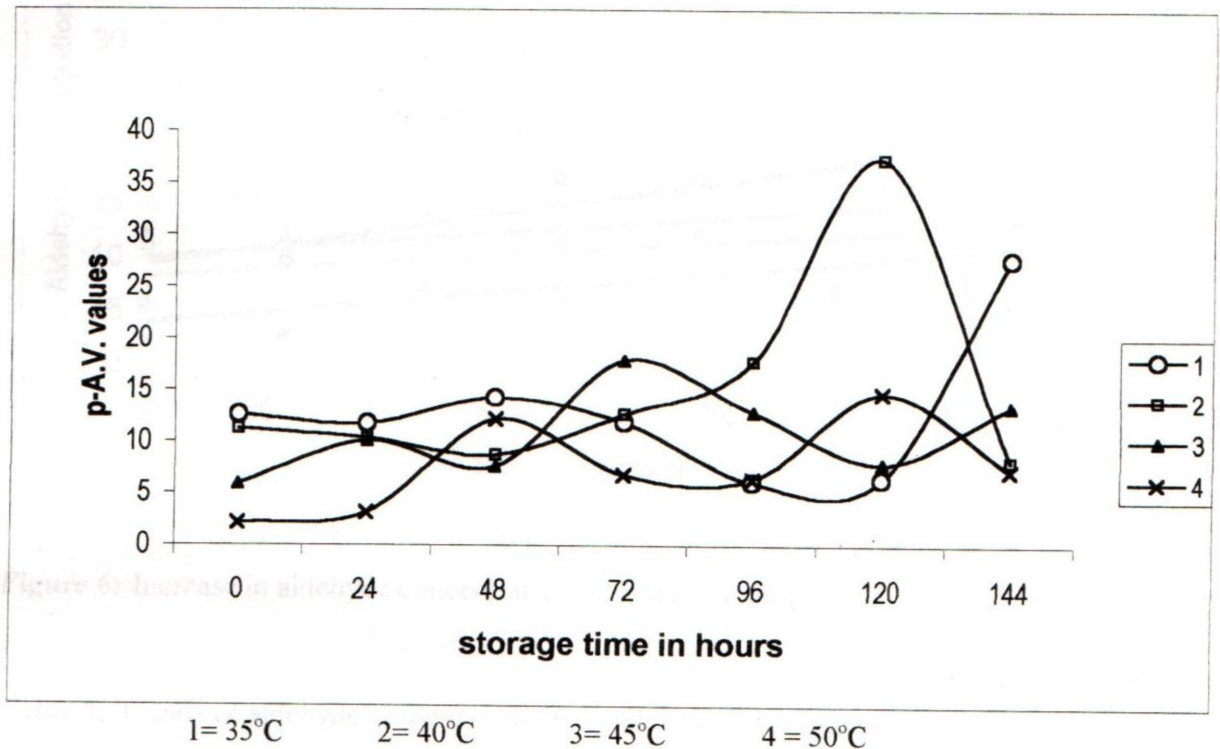
	Temperature [°C]			
Storage Time	35	40	45	50
0h	12.53^{b} ±0.0	6.98^{a} ±4.2	5.31^{a} ± 0.5	4.56^{a} ±2.0
24h	11.69^{bc} ±0.0	6.99^{a} ±3.3	16.12^{a} ± 6.1	13.04^{a} ±9.6
48h	10.79^{bc} ±3.4	6.05^{a}±2.7	7.39^{a} ±0.2	13.71^{a} ±7.8
72h	7.90^{bc} ±3.9	27.24^{a} ±1.5	12.00^{a} ± 5.8	8.48^{a} ±5.5
96h	4.98^{c} ±1.1	20.23^{a} ±2.5	23.72^{a}±10.0	4.42^{a} ±1.3
120h	7.50^{c} ±1.1	22.29^{a}±2.5	7.84^{a} ± 0.0	7.11^{a}±4.6
144h	26.72^{a} ± 0.8	8.09^{a}±0.0	18.45^{a} ±5.1	5.92^{a}±2.7

Means in the same column with different superscripts are significantly different [α=0.05].
*Mean p-anisidine Values ± Standard Error of the Mean n=2.

The change of aldehyde concentration with time at different storage temperatures is given in **Figure 5**. Generally, there were lower levels of aldehydes in the earlier storage times [0- 48hours] than in the latter times [72-144 hours]. The samples were dried at 100°C before extraction reducing variation of the samples with time. The heating of butter oil to produce the ghee may have produced Maillards Reaction Products [MRPs]. The MRPs have been shown to exhibit antioxidant activity during storage of foods heated prior to storage [Mastrocola and Munari, 2000]. These may have imparted an antioxidative effect on the samples during the later hours of storage.

It was observed that at each storage temperature there were two maxima in aldehyde concentration. This shows that there are generally two types of fatty acids that were getting oxidised in the milk fat. Those that oxidise rapidly [unsaturated] giving maxima between 48-72 hours of storage and those that oxidise slowly [saturated] producing high aldehyde concentrations at 120-144 hours **[Figure 5]**. The unsaturated fatty acids in milk fat mainly oleic acid [~ 27.1%] oxidise faster due to lowered activation energy in the initiation of free

radical formation. The saturated fatty acids mainly palmitic acid [~25.0%] have a higher activation energy for radical formation. Hence, they oxidise at later stages of storage [Min and Boff, 2002]. The decrease in aldehyde concentrations in between the maxima concurs with previous findings by Mahungu *et al.*, [1994] and may be attributed to their volatility and decomposition to lower molecular weight compounds especially the long chain aldehydes. Some saturated aldehydes have also been shown to oxidise into acids with storage [Mahungu *et al.*, 1994].



1= 35°C 2= 40°C 3= 45°C 4 = 50°C

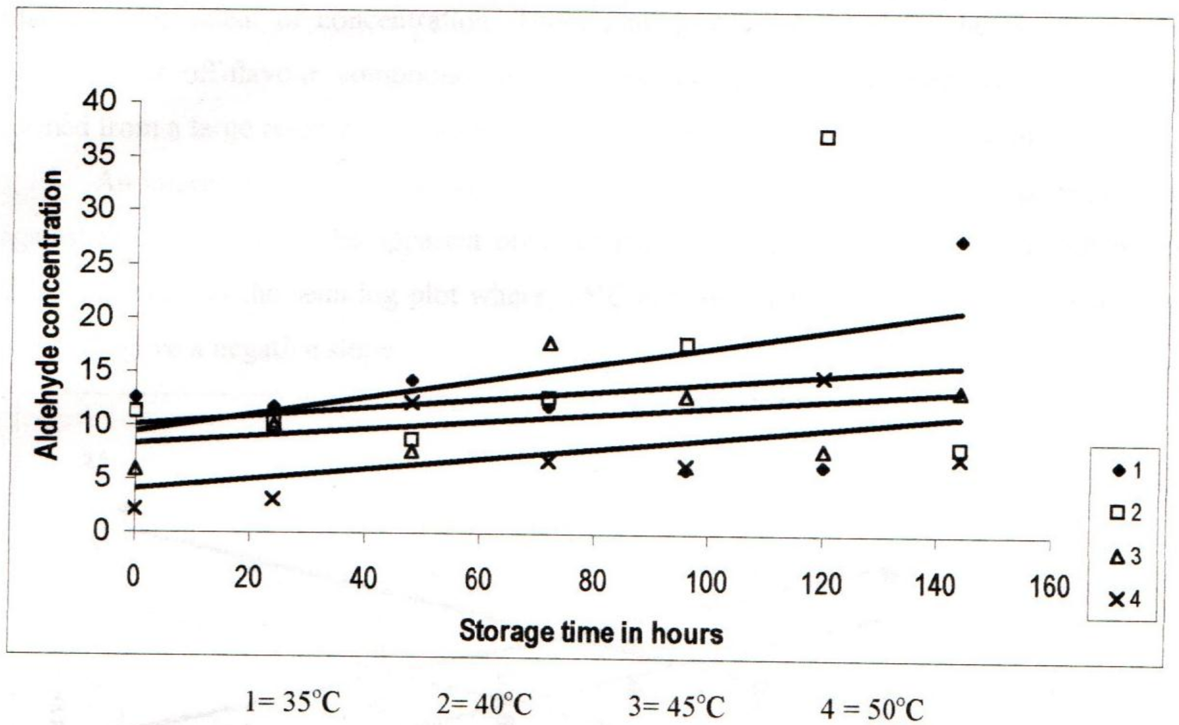
Figure 5: Change of aldehyde concentration with time at different temperatures

Storage was terminated at 144h since the data obtained was aimed at calculating the rate constant for lipid oxidation in ready-to-fry chapati and by the 144th hour the trend of aldehydes formation had been established. Due to microbial deterioration under the experimental condition, the storage could not be extended beyond 144 hours.

4.3.3 Reaction Rate Constant and Apparent Reaction Order

The general trends in aldehydes formation [**Figure 5**] in the samples were established by linear regression to obtain the rate constants for each storage temperature tested. Even though some data points seem out of the way, it is to be noted that despite the

periodic increases and decreases in aldehyde contents the general trend was of much more interest *i.e.* were the aldehydes increasing or decreasing with time. Therefore, the regression/trend lines [**Figure 6**] demonstrated the trends more clearly than the actual periodic fluctuations.



1= 35°C 2= 40°C 3= 45°C 4 = 50°C

Figure 6: Increase in aldehyde concentration with storage time

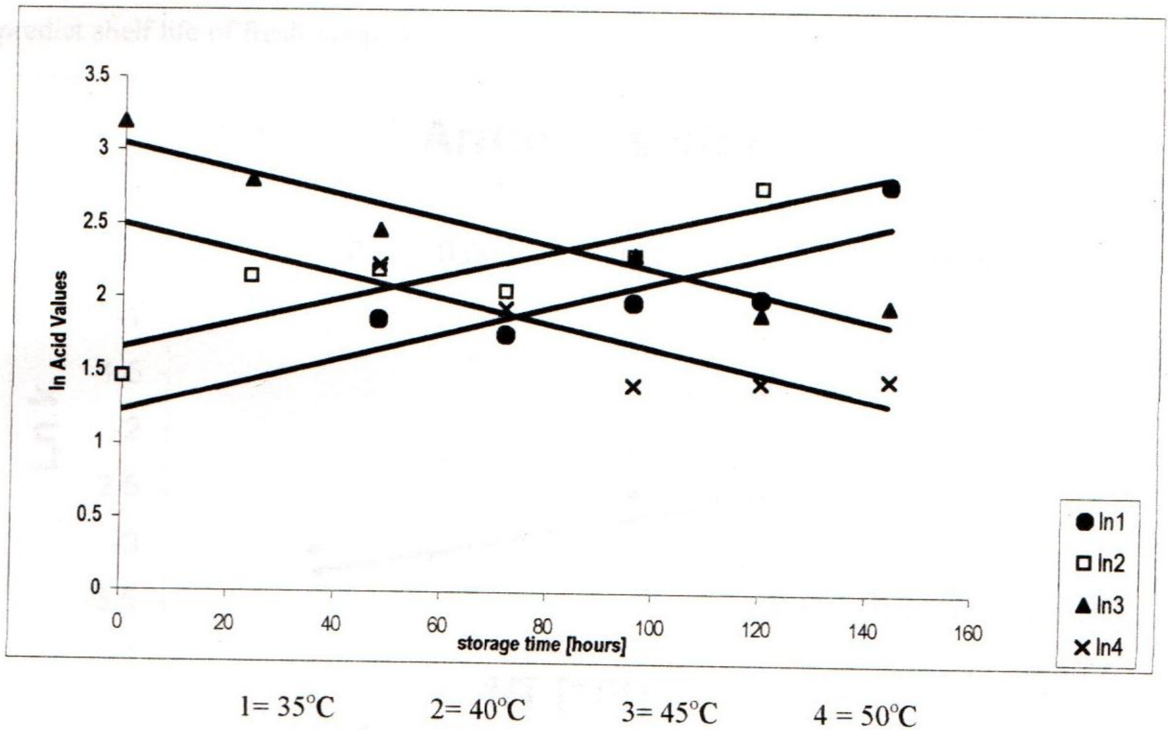
Table 8: Trends of aldehyde content with time and their rate constants [*k*]

Temperature [°C]	Equation of line	Rate constant [dy/dx = *k*]	
35	y = 0.0391x + 10.075	0.0391	k_1
40	y = 0.0794x + 9.4035	0.0794	k_2
45	y = 0.0347x + 8.2696	0.0347	k_3
50	y = 0.0484x + 4.0058	0.0484	k_4

The rate of milk fat oxidation in ready-to-fry chapati was obtained by differentiating the line equations obtained upon plotting the aldehyde levels against storage time. The differences in the rate constants obtained indicated the effect of temperature on rates of lipid oxidation and hydrolysis of milk fat in ready-to-fry chapati [**Table 8**]. The rate constants k_1,

k_2, k_3 and k_4 in mol $s^{-1}L^{-1}$ obtained is given in **Table 8** suggest that the oxidation of milk fat in ready-to-fry chapati with reference to aldehyde concentration follows Pseudo zero-order kinetics. Thus, the rate constant k in mol s^{-1} L^{-1} is independent of concentration of reactants although, k varies with temperature and pressure. It is generally considered to be constant that is, independent of concentration. These findings are explained by the fact that small quantities of off-flavour compounds in this case aldehydes determined are slowly being formed from a large reservoir of parent component [Walstra, 2003] in this case milk fat.

An interesting observation was made when plotting the free fatty acid concentrations against time to estimate the apparent order of the reactions. **Figure 7** shows a shift of the slope observed on the semi log plot where, 35°C and 40°C gave a positive slope while 45°C and 50°C gave a negative slope.



1= 35°C 2= 40°C 3= 45°C 4 = 50°C

Figure 7: Log [acid value] against storage time

This observation was probably due to a change in the reactions apparent order as influenced by temperature of storage among other factors. Ester hydrolysis is catalysed by H^+ as well as OH^- ions hence the reaction rate greatly depends on pH [Walstra, 2003]. As the storage temperature rose, the water content could have been reduced by evaporation and lowering the concentration of H^+ and OH^- ions, thereby affecting the reaction order. Other

factors that could have caused this observation include the complete melting of milk fat at temperatures above 40°C and denaturation of some microbial lipases that may have been catalysing the hydrolysis.

4.3.4. Arrhenius and Shelf Life Plots

Using *k* values obtained **[Table 8]** at the four temperatures tested. An Arrhenius plot was prepared **[Figure 8]** and good fit obtained showing that the kinetics of milk fat oxidation in ready-to-fry chapati fits the Arrhenius model. The steepness of the slope is indicative of the temperature dependence of the reaction [Fu and Labuza, 1997]. Pseudo-zero order reactions have fairly constant reaction rates regardless of initial concentration of substrate. Therefore, despite the high initial concentrations of aldehydes in the samples analysed, the reaction rates obtained can still be utilised to formulate a shelf life plot that could be used to predict shelf life of fresh samples.

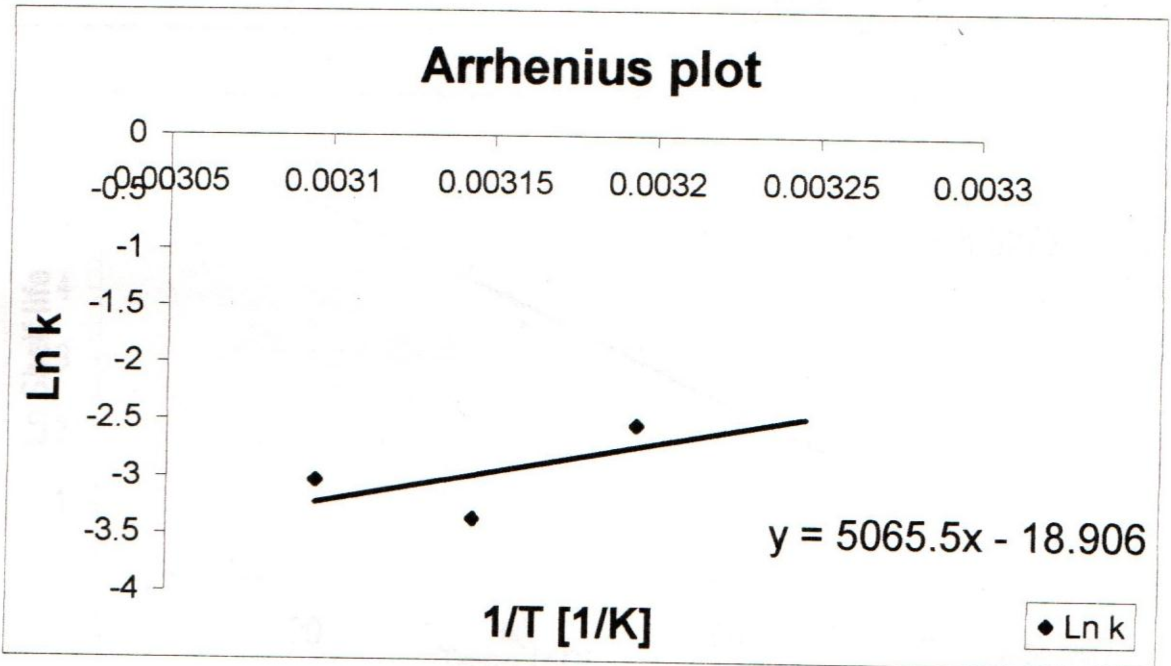


Figure 8: Effect of temperature on lipid oxidation rate constants in ready-to-fry chapati

Shelf life is a vague concept and usually means the time for a product to become unacceptable to a consumer. However, 'unacceptability' is a subjective decision since the factors triggering unacceptability to consumers vary from product to product and storage regime [Reid *et al.*, 2003]. However, shelf life may also be taken to be the time it takes for undesirable product in a food to reach a certain preset value. It has frequently been observed

that most food products will become unacceptable after a 20-30% change from its original form is recorded [Labuza, 1996]. In this experiment 30% change was used to estimate shelf life based on aldehyde concentration. The plot obtained concurs with findings of Labuza, [1996] who he reported that shelf life frequently exhibits approximately logarithmic dependence upon temperature. A good fit was obtained $R^2 = 0.85$ [**Figure 9**]. The values of shelf life calculated [**Table 9**] were used to prepare the shelf life plot [**Figure 9**] for the quick determination of the shelf life of ready-to-fry chapati.

Table 9: Calculated shelf life values at different storage temperatures

Temperature [°C]	Initial p-AV	θ[hrs]	Lnθ
35	9.40	77.30	4.35
40	10.08	35.53	3.57
45	8.27	71.50	4.27
50	4.01	24.83	3.21

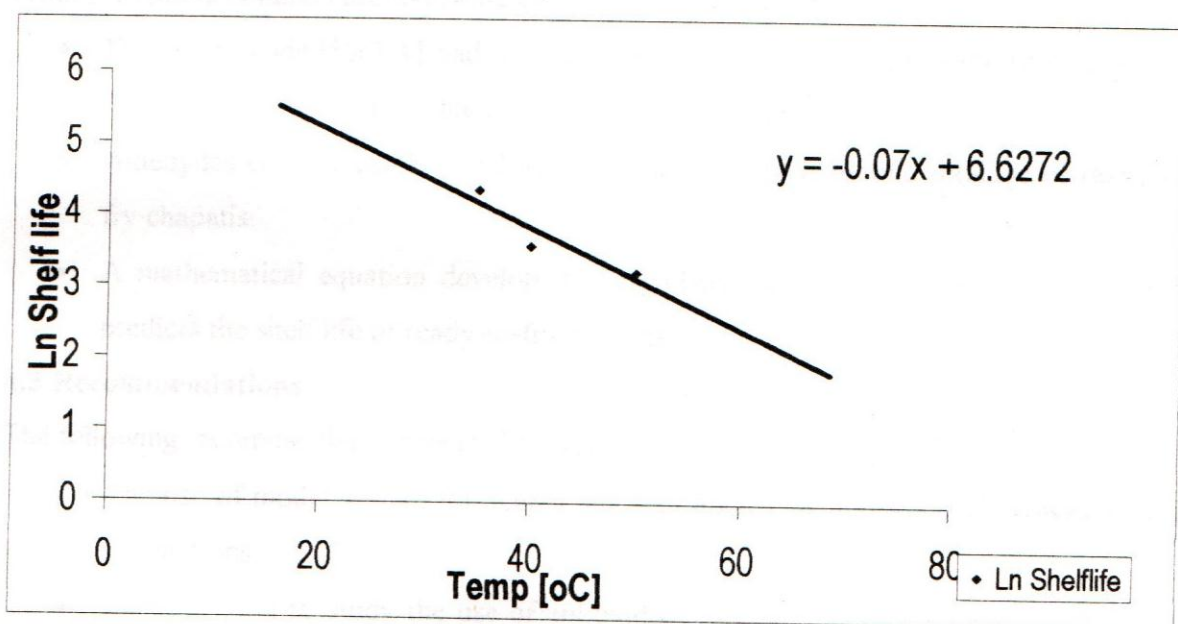


Figure 9: Shelf life plot for ready-to-fry chapati

From the shelf life plot for ready-to-fry chapati, the Q_{10} values for 35 °C and 40 °C were calculated as $Q_{10} = \frac{\text{Shelf life at T}}{\text{Shelf life T + 10°C}}$ and found to be 2.02 and 2.01 respectively.

These findings were found to concur with the Q_{10} values reported by Walstra, 2003. Using the shelf life plot obtained [**Figure 9**], the shelf life equation was found to be:

ln θ = -bT + c OR **θ = exp [-bT+ c]**. The equation may also be rewritten as $\theta = e^{(-bT+c)}$

Where:

θ is shelf life in hours,

b is the slope of the shelf life plot,

T is the storage temperature,

c is the intercept.

For example, using the shelf life equation generated in this study [$\theta = \exp(-0.07T + 6.6272)$], when **T** = 25°C then **θ** = 131.2626 hours. Therefore, the shelf life of ready-to-fry chapati prepared for this study, based on milk fat oxidation as a quality index was 131.26 hours [~5.5 Days] at 25°C. Similarly, using the shelf life plot **ln θ** = 4.8, hence **θ** = **exp** 4.8 = 121.5104 hours [~5.1 Days] at 25°C. This shows that both the equation and the shelf life plot may be used for quick determination of ready-to-fry chapati.

4.4 Conclusions

From the results obtained the following conclusions were drawn:

- Free fatty acids [%FFA] and aldehydes are produced during storage of ready-to-fry chapatis and the reaction rate is temperature dependent.
- Aldehydes content can be used as a reliable index of oxidative spoilage of ready-to-fry chapatis.
- A mathematical equation developed using observed lipid oxidation data objectively predicts the shelf life of ready-to-fry chapatis.

4.5 Recommendations

The following recommendations were then made:

- The use of model system for deeper study of lipid oxidation since it involves a chain of reactions.
- There is need to study the use of antioxidants and antimicrobial agents in ready-to-fry chapati to prolong their shelf life.

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