

**CRUDE SESAME (*SESAME INDICUM L.*) OIL QUALITY ASSURANCE THROUGH  
HAZARD ANALYSIS CRITICAL CONTROL POINTS AND APPLICATION OF  
NATURAL ANTIOXIDANT IN CRUDE SESAME OIL STABILITY: THE CASE OF THE  
SMALL - SCALE PROCESSING PLANTS IN WESTERN TIGRAY, ETHIOPIA**

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the Doctor of Philosophy Degree in Food Science of Egerton University**

**EGERTON UNIVERSITY**

**MAY, 2024**

## DECLARATION AND RECOMMENDATION

### Declaration

I declare that this thesis is my original work and has not been presented in this university or any other for the award of a degree.


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## **DEDICATION**

This work is dedicated to my late parents (my mom Gerges Hagos and my father Fisseha Gebremeskel), beloved husband Dr Kiros Gebrearegawi, son Merhawi Kiros, brothers and sisters. This is in appreciation of their constant prayers, support, and encouragement while I was conducting this research. May my parents' souls rest in peace in paradise. God bless all of them.

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## ABSTRACT

Sesame (*Sesame indicum*) is a significant crop for the Ethiopian economy, but improper handling causes 22–35% of the seed to be lost along the value chain. The study aimed to reduce post-harvest losses in the sesame value chain in western Tigray, Ethiopia by implementing a Hazard Analysis Critical Control Point (HACCP). To better understand the sesame value chain, a questionnaire survey conducted to 332 actors. Crude sesame oil (CSO) was extracted using a cold press mechanical extraction method. Sesame seeds' proximate composition and CSO physico-chemical quality were determined using the corresponding AOAC standard methods. Microbial quality assessment, chemical (Aflatoxin levels, pesticides, oxidation) and physical hazards analysis was conducted. The moisture content of sesame seed and CSO was found to be 3.36 and 0.37 percent, respectively. Sesame seeds were found to have 50.9% oil, of which 83.15% was made up of unsaturated fatty acids. Three critical control points (CCPs) identified were during sesame roasting, conditioning while pressing, and packaging of CSO. Potential hazards during roasting were fungi and mycotoxins; hazards during extraction were *staphylococcus aureus*, and aerobic plate bacteria; and hazards during packaging were *staphylococcus aureus*, aerobic plate bacteria, and oxidative metabolites during the filling process, to be carefully controlled for safe CSO production. The peroxide value of CSO from raw and roasted sesame seeds was 8.23 and 5.98 meq O<sub>2</sub>/kg oil after 35 days of storage at room temperature. However, after 35 days of storage at 65 °C, the raw and roasted sesame seeds' respective CSO peroxide values were 20.92 and 81.01 meq O<sub>2</sub>/kg. Meanwhile, oxidation was reduced when basil was added. Accordingly, during ambient condition storage, the peroxide values of CSO for raw and roasted seeds were 0.42 - 0.55 meq O<sub>2</sub>/kg, and during accelerated storage, they were 2.62 - 10.13 meq O<sub>2</sub>/kg, respectively. The total aflatoxin level in sesame seeds was found to be 14.98 µm/kg whose B<sub>1</sub> (AFB<sub>1</sub>) component was 0.51 µg/kg. CSO total aflatoxin level was 8.5 µm/kg with the B<sub>1</sub> component being 0.37 µm/kg. Because it lacks a refining procedure and hazards detected, the CSO shelf life was calculated to be no longer than two months based on the available data. The small and medium-scale food processing industries hold significant role in the community and yet lacks attention. As a result, periodic training and restricts regulatory on value addition, hygienic practices, and application of organic preservatives promote safe food production and improve the economic value. Though, spices such as sweet basil have been utilized as flavour enhancer and yet the preservatives effect in semi-packed and packed food is not well utilized.

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## LIST OF ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
GAE	Gallic acid equivalents
TEA	Triethanolamine
p,p-DDT	Dichlorodiphenyltrichloroethane
CSO	Crude Sesame Oil
AFPA	Aspergillus flavus parasiticus Agar
ATA	Ethiopian Agricultural Transformation Agency
CCP	Critical Control Point
CL	Critical Limit
ECX	Ethiopian Commodity Exchange
EU	European Commission
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drug Administration
GMP	Good Manufacturing Practice
SS	Sesame seed
SSC	Sesame seed cake
TDS	Total dissolved solids
GHP	Good Hygienic Practice
HACCP	Hazard Analysis Critical Control Point
SOPs	Standard Operating Procedures
HCC	Hepatocellular carcinoma
LLE	Liquid-liquid extraction
LLE-LTP	Liquid-liquid extraction – low temperature purification
LOD	Limit of detection
LOQ	Limit of Quantification
MRL	Maximum Residue Limit
FSO	Food Safety objectives
OPRPs	Operational prerequisite programs
PRPs	Prerequisite Programs

SFAs	Saturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
QuEChERS	Quick, Easy, Efficient, Rugged and Safe
WHO	World Health Organization
MSFA	Mono Saturated Fatty Acids
THMs	Total trihalomethanes
FID	Flame Ionization Detector
FIFO	First in and first out

## CHAPTER ONE

### INTRODUCTION

#### 1.1. Background Information

Agriculture in Ethiopia is primarily managed by small-holder farmers. The farmers are either livestock keepers or crop producers. The farmers encounter challenges which lead to low productivity. These include use of traditional technologies, financial constraints, climate change and lack of improved varieties (Golla *et al.*, 2020; Mezgebo *et al.*, 2021). The low agricultural productivity leads to persistent poverty in the country (Baye, 2017). The Ethiopian Agricultural Transformation Agency (ATA) was established with the goal of modernizing Ethiopian agriculture (National Planning Commission, 2016; Unique-Kulima, 2017). One of the most important crops produced in Ethiopia is sesame (*Sesame indicum L*) seed either for export or local consumption next to coffee (Teklu *et al.*, 2021; (D. H. Teklu, Shimelis, Tesfaye, & Abady, 2021)Terefe *et al.*, 2012). It is primarily used for edible oil extraction and animal feed (Rodríguez-miranda *et al.*, 2014). The production of sesame oil impacts the social-economic well-being of both the large scale farmers, and small-scale farmers' livelihoods and oil producers (Michler & Josephson, 2017).

Sesame also locally known as “Selit”, is a *Pedaliaceae* family flowering annual oilseed crop (Gebregergis *et al.*, 2018). It is drought tolerant, adaptable to a variety of soil types, harsh environment, and diversified agro-ecological conditions and sowing seasons (Mawcha *et al.*, 2021; Terefe *et al.*, 2012). From the time of plantation to maturity, sesame takes an average of 90 days to harvest (Amare *et al.*, 2019). The maturity indicators for sesame seed readiness for harvest and seed collection includes leaves colour changes to yellow / brown yellow, stem colour darkened, pods bursts. Sesame value chain includes organic cultivation, sesame post-harvest practices, distribution and marketing, and processing (Gebremedhn *et al.*, 2019). Small- and large-scale sesame farmers, traders, brokers, oil millers, regulatory authorities and consumers are involved in the sesame value chain. Sesame farming requires a large labour force since it lacks basic infrastructure and technology for ploughing, weed prevention, and harvesting. Illiterate labour force has a negative impact on the sesame value chain. This contributes to ineffective agriculture, substantial post-harvest losses and hampering the sesame value chain (Gebremedhn *et al.*, 2019). Sesame farming include land preparation, sowing, and fertilizer application, weed control, seed gathering. Field storage, wholesalers and exporters, transporters and value addition, are the basic

sesame post-harvest practices which impacts the safety and economic value. Sesame is sensitive to biological factors (Berhe *et al.*, 2008), and environmental factors such as rainfall, wind, temperature and harvesting time (Mawcha *et al.*, 2021), soil quality and moistness (Barreto-Salazar *et al.*, 2021) which impacts productivity and quality. The use of insecticides (Gebregergis *et al.*, 2018), sesame breed/varieties (Baraki *et al.*, 2020; Baraki & Berhe, 2019), plantation sites, year of plantation, usage of fertilizers and agricultural practices, and agro-climatic conditions (Teklu *et al.*, 2021) affect sesame production yield in western Tigray, whose production range were from 400 to 2044 kg/ha. During harvesting, drying and cleaning, however, between 17-25% of sesame seed is lost due to insect damage, weather conditions and seasonal variation, stacking duration, farm distance and land size, labour educational level and lack of consultation with agricultural experts (Berhe *et al.*, 2008; Gebremichael, 2017; Gebretsadik *et al.*, 2019). Sesame oil recovery and extraction losses ranges from 5 to 10%, having a major negative influence on the economic value and food security of the crop (Teklu *et al.*, 2021).

Globally sesame production is estimated to be at 2.2 million tonnes annually. Ethiopia sesame export valued approximately 262,654 tonnes in 2019, bringing in USD 307 million. This accounted for 8.96% of the global sesame market (Gebregergis *et al.*, 2018; Teklu *et al.*, 2021). Sesame oil is utilized in various industrial applications, including in perfume making, skin conditioning and moisturizers, hair oil, bath oils, hand products and make-up, pharmaceuticals, pesticides, paints and varnishes and as cooking oil (Diamante & Lan, 2014; Warra, 2012) regardless of the extraction technology and physico-chemical variations (Chemat, 2017). Sesame is rich in nutrient considered as superior quality and winning food nutrition deficient and immune compromised consumers (Codex, 2012).

Nonetheless, studies prove that sesame seed and sesame based products are susceptible to chemical, microbiological, and physical contaminants, posing a public health risk and economic losses (Patel & Bahna, 2016). Sesame, according to biological hazards prevalence study conducted elsewhere shows it is sensitive to fungus species. Thus, mycotoxin metabolites have been detected in sesame seeds using HPLC analysis techniques in Sudan, Greece, Syria and India (Kollia *et al.*, 2016). Aflatoxin prevalence in sesame seed reported in Sudan using HPLC analysis were found to be contaminated with the levels ranging from 0.2 to 0.8 µg/kg (Idris *et al.*, 2010). Idris *et al.* (2013) reported that 52-81% of Sudanese sesame oil was contaminated with aflatoxin in the range of 0.5-1.3 µg/kg measured using HPLC, regardless of the seasonal variation. According to Kollia *et al.*

(2016), packed and unpacked sesame products were contaminated with Aflatoxigenic fungi (> 73% of the sampled product cultivated in AFPA media). The aflatoxin B<sub>1</sub> was detected using HPLC method in 77.6% of all samples within the range of 0.1-14.5 µg/kg. This observation confirms how weather, processing and packaging contribute to the prevalence of potential hazards, negatively impacting on food safety.

To prevent biological contaminants, actors in the sesame seed value chain use agro-chemicals from the Agri-value chain. Pesticides are used to prevent pest infestation on the farm and storage facilities, and boost sesame productivity. However, pesticide use and practices were recorded at levels higher than the tolerable limit (Gebregergis, 2018; Mengistie *et al.*, 2017). On the other hand, pesticide residues found in most Ethiopian staple food crops (for example, pepper, teff, maize and coffee beans) using QuEChERS GC method exceed the tolerable limit (Mekonen *et al.*, 2014). Pesticides 2,4-D, Aldrin, Endosulfan, and p,p-DDT were detected in pre-harvest and post-harvest cereal crops cultivated in Ethiopia using GC-MS (Zezelew *et al.*, 2018). Pesticides and their residues risks human health and environment (Geremew *et al.*, 2012; Mekonen *et al.*, 2014). Studies have showed that water, as a process and cleaning medium, can be a source or vehicle for contaminants. Many food processing industries have used water purification. However, purification technology might not be affordable in particular the small-scale food processing industry, where municipal chlorinated water is widely used. Despite this, chlorination techniques and chloride residue exceeding the allowable level endanger human health and causes other food safety risks (Ambelu *et al.*, 2017; Zezelew *et al.*, 2018).

Sesame oil millers are actors in the sesame value chain and food industry groups that supply consumers with crude sesame oil (CSO). However, the quality of CSO is compromised due to pests, agro-climatic factors, development of off-flavour, discoloration and short shelf life (Gebretsadik *et al.*, 2019; Niguse & Alem, 2015). The oxidation reaction, fungi and other microbial and chemical contaminants causes CSO quality decline and storage instability. To the best of our knowledge, Ethiopian sesame and sesame product quality, hazard occurrence and the presence of safety management are scarce. The goal of this research was to look into the sesame value chain and come up with a generic HACCP plan for the CSO extraction line in the small-scale crude sesame extraction plant in western Tigray, Ethiopia to reduce post-harvest loss and contribute to the sustainable developmental goals.

## **1.2.Statement of the Problem**

There is still an issue with the lack of edible oil and the low quality of imported edible oil in the Ethiopian market. Additionally, the food industry and food business owner see as a new window of opportunity due to the availability of oilseed. Sesame is one of the major oilseeds grown in Tigray, Ethiopia where the small-scale food business owners show interest in the oil extraction business. However, the crude sesame oil (CSO) storage instability, unpleasant flavour and discoloration development, particularly turning amber in colour, remains of significant concern for consumer safety. On the other hand, there is a scarcity of traceability information on the reasons of sesame seed post-harvest loss and CSO quality degradation and storage instability. As a result, the study goal was designed to investigate sesame value chain, its actors, and establish a HACCP plan in the CSO extraction plant to improve the quality and safety of crude sesame oil production, enhance the economic valued of sesame seed and food security which contribute to the sustainable development and poverty reduction goal.

## **1.3.Research Objectives**

### **1.3.1. General Objective**

To contribute to the sustainable developmental and poverty reduction goals through reduction of post-harvest losses in the oil seeds and edible oil value chain via development and implementation of Hazard Analysis Critical Control Point protocols in the traditional sesame (*Sesamum indicum*) oil processing in western Tigray, Ethiopia.

### **1.3.2. Specific Objectives**

The specific objectives of the study were:

- i. To conduct a hazard analysis in the cold mechanical pressing line of during crude sesame oil extraction in western Tigray, Ethiopia.
- ii. To determine the critical control points (CCPs) in the cold press crude sesame oil extraction in the crude sesame oil extraction line in the Tigray sesame oil extraction plant.
- iii. To establish the critical limits with the corresponding monitoring and verification procedures for the CCPs identified in the cold press crude sesame oil extraction in the crude sesame oil extraction line in the Tigray sesame oil extraction plant.

- iv. To establish the HACCP plan in the cold press crude sesame oil extraction in the crude sesame oil extraction line in the Tigray sesame oil extraction plant.
- v. To evaluate the oxidative stability of crude sesame oil with and/or without natural antioxidants in western Tigray, Ethiopia.

#### **1.4.Hypotheses**

- i. There is/are no significant hazards presences to jeopardize the CSO safety in the CSO extraction line in western Tigray, Ethiopia.
- ii. There is/are no CCPs identified in the cold mechanical pressing line of crude sesame oil in Western Tigray, Ethiopia.
- iii. The CCPs' implementation in the cold press crude sesame oil extraction line of the Tigray sesame oil extraction plant is not significantly impacted by the CLs and the associated monitoring and verification processes for the CCPs.
- iv. The Tigray sesame oil extraction plant's cold press crude sesame oil extraction method will not be significantly impacted by the HACCP plan in terms of safe CSO production.
- v. The oxidative stability of crude sesame oil (CSO) in Western Tigray, Ethiopia is within the desirable limit with/without the application of natural antioxidant.

#### **1.5.Justification/Significance of the Study**

Sesame is an important oilseed for sesame producer's livelihoods and a raw material in the edible oil industry. Sesame seed is also a significant export commodity in Ethiopia. Strengthening stakeholders in the oilseed value chain improves productivity and quality for greater health and economic advantage and will contribute to the sustainable development and poverty reduction goals. The value chain analysis and use of HACCP in the food industry encourage the establishment of a sustainable food system, the quality and safety of food distribution, and the number of sesame prospective consumers throughout the country and globally. Consumers routinely complained about the availability of edible oil in the market due to their scarcity and poor quality. Furthermore, promoting and encouraging edible oil producers and establishing quality control measures would help with the creation of food business concepts, close the existing traceability gap that currently exist, and promote good practises in the sesame value chain and sesame oil processing safer quality and quality of edible oils. This will be accomplished by investigating the problems in the sesame value chain, with practical solutions to enhance post-

harvest handling reduction, as well as establishing HACCP protocols at the CSO extraction plant can help achieve safe food production and distribution.

For centuries, people have used spices and herbs in their diets in either fresh or dried form as flavouring agent or for its medical value. On the other hand, inorganic preservatives are frequently utilized in packaged and processed foods. However, food researchers do not need to focus on this in order to combat infectious and non-infectious disorders. In the meanwhile, the food industry's use of medicinal plants will function as organic preservatives, preventing chemical and microbiological hazards. Hence, the introduction of basil extract has great promise for preventing CSO oxidation and enhances storage stability. Following the HACCP plan will enhance CSO safety and preserve its quality, ensuring customer health and encouraging food businesses to take food safety procedures.

### **1.6.Scope/Limitations/Assumptions**

Investigating local technologies and creating Hazard Analysis Critical Control Points (HACCP) are the main goals of the current project, which aims to improve food security and safety of typically small-scale processed crude sesame oil. In addition to improving the economic value, food security, and public health of sesame oilseeds, this study will be a trailblazer in post-harvest management in small- to medium-sized setups and value addition, supporting the goals of sustainable development and poverty reduction. However, at the time this study was undertaken, there were challenges due to the COVID-19 pandemic, the continuing civil war, and seasonal changes. In addition to HACCP, other measures needed to be taken into account in the food supply chain to ensure safe food production, distribution, and post-harvest reduction. These measures included the introduction of organic pesticides to open up new business opportunities and provide new opportunities for farmers and other stakeholders in the same value chain, as well as the promotion of green technologies to combat climate change. The use of spices, herbs, and other wild plants is thought to improve the food chain, encourage biodiversity and a greener environment, and help achieve the objectives of sustainable development and poverty alleviation in addition to the value chain and value addition studies.

## 1.7. Definition of Terms

For the purpose of these guidelines, the following definitions apply:

- Food value chain - all the stages through which food is handled from primary production to processing, manufacturing, distribution and marketing to the point of consumption.
- Contamination - incidence of any undesirable chemical, biological and physical agent in the product following the value chain violates the standard or requirement determined by law or does not meet satisfactory food hygiene standards or is unfit for human consumption
- Contaminants – substances, microbial agents, undeliberate or deliberately associated to food at various stages of handling
- Good manufacturing practices- a system to ensure consistent or controlled production according to the specified quality standards to prevent or minimize risks of contaminations
- Hazard Analysis Critical Control Point (HACCP) system - a system that identifies, evaluates and controls hazards that are significant to food safety
- HACCP Plan - a document prepared in accordance with the principles of HACCP, aimed at ensuring the control of hazards that could influence the food safety
- Flow diagram - a systematic illustration of the sequence of steps or operations used in the production or manufacture of a particular food item
- Step - a point, procedure, operation or stage in the food processing from primary production to final consumption
- Hazard - a biological, chemical or physical agent with the potential to cause harm to or affect the safety of the consumer
- Hazard analysis - process of collecting and evaluating information on hazards occurrence to be included in the HACCP system
- Hazard characterization - the qualitative assessment of the nature of any adverse result associated with any biological, chemical or physical agents or a combination of these that might be present in food
- Pest - any animal competent of contaminating or consuming food crops and food directly or indirectly
- Control - all the essential measures or action to ensure and maintain predictability with the criteria of HACCP plan establishment

- Control/preventive measure – any action used to eliminate or reduce a food safety hazard to an acceptable level
- Critical control point - a point, step or procedure in the process at which control must be applied and a food safety maintained or hazard prevented, eliminated or reduced to an acceptable level
- Decision tree - a series of questions that are applied to each step in the process in respect of an identified hazard to identify which steps are CCPs
- Critical limit - a measurable value (s) separating the acceptable from the unacceptable
- Deviation - the failure to meet a critical limit
- Monitor - to perform a planned series of observations or measurements of critical limits to assess whether a CCP is under control
- Corrective action - action taken to eliminate or control the cause (s) of deviation, non-conformity, inadequacy or unwanted situation in order to prevent it from re-occurring
- Validation - obtaining the confirmation that the particular requirements of the HACCP plan are efficient
- Safe - containing no substances likely to cause injury to the end user
- Non-conformity - non-fulfilment of a particular requirement
- Record - a document that provides proof of activities, measures undertaken or results achieved
- Verification - the application of evaluation methods, procedures, and tests to verify compliance with the HACCP Plan
- Aseptic processing – The processes of processing, filling and packaging under sterile condition to prevent viable microbial contamination
- Approved - permitted by the administering authority
- Tolerable Limit- a range of sufficient parameters to meet the objective of safe food production and protecting consumer
- Acceptable - satisfactory to the administering authority or specific governing limits
- Appropriate - acceptable to, or necessary by, the administering authority/standards
- Suitable - considered by the administering authority to be suitable for the purpose
- Adequate - enough to achieve the intended purpose of these guidelines

- Cleaning - removal of dirt, impurities, soil, food residues or other objectionable matter  
container or food container - anything in which or with which food is served
- Establishment or plant - any structure (s) or area (s) in which food is processed, handled  
and the environment under the control of the same management
- Facility - any apparatus, appliance, equipment, implement, storage space, working surface  
or object used in association with the handling of food
- Food handling - any process in the growing, harvesting, preparation, processing,  
packaging, storage, transportation, distribution and sale of food
- Hygiene - all conditions and actions necessary to ensure the safety, soundness and  
wholesomeness of food at all stages, from production until consumption
- Premises - a building, structure, stall or other similar structure including a vehicle, stand or  
place used for or in association with the handling of food
- Food safety - the guarantee that a particular food product will not cause injury to the  
consumer when it is prepared and / or consumed conferring its proposed use
- Suitability - the guarantee that a n establishment is suitable for handling and production
- Disinfection - use of approved chemical agents and physical methods or both to prevent or  
reduce micro-organisms to a level that will not lead to harmful contamination of the food,  
without adversely affecting the food
- Food business operator - dependable person or persons that ensure that requirements of the  
regulations are met within the food company under his or her control and include the  
management as well as the person with the overall power on site or in the exact organization
- Handle - manufacture, process, produce, pack, prepare, keep, offer, store, transport or  
display for sale or for serving
- Hands - includes the forearm or the part of the arm extending from the wrist to the elbow
- Small- scale farmers –are also known as smallholder farmers or family farmers, carry out  
their agricultural operations on small-scale land of conventional technologies and rely on  
labour from family or relatives for household necessities and production
- Small scale food business owners/operators -usually referred as the cottage industries, few  
individuals who manufacture and retain food at small scale level

## CHAPTER TWO

### LITERATURE REVIEW

The Ethiopian topographic diversity and a wide range of agro-climatic condition such as temperature, rainfall, relative humidity supports farmers to cultivate grain crops such as sorghum, barley, maize, millet, cotton, teff, oilseeds and keep livestock. Western Tigray has good climate and excellent soil for the cultivation of various food crops and livestock farming (Mawcha *et al.*, 2021). Sesame, soybean, and Niger seed are the major oilseeds, which contributes to the farmers' livelihood and generated \$430 million in foreign currency in 2018/19 (Bickford, 2020). Sesame seed alone economic worth is estimated to be 307, 512 - 619,033 million per year between 2011 and 2017. The practices and conditions in the sesame value chain significantly affect the economic value and overall sesame yield (Gebremedhn *et al.*, 2019).

Sesame (*Sesamum indicum*) is a flowering annual plant in the family *Pedaliaceae* that takes an average of three months to mature (Amare *et al.*, 2019). The global sesame production is estimated to be at 2.2 million tonnes. According to Ethiopian commodity Exchange (ECX) data, Ethiopia sesame production accounted for approximately 262,654 metric tons (307 million USD) in 2018/19 with an 8.96% share of the global sesame market (Teklu *et al.*, 2021). This increased to 285,000 metric tons in 2019/20 although the cultivated land remained unchanged at 600,000 hectares (Bickford, 2020). Sesame productivity in western Tigray ranged between of 400 and 2044 kg/ha, with productivity variability related to insecticides use (Dong *et al.*, 2021; Gebregergis *et al.*, 2018), sesame breed/cultivar differences (Arslan *et al.*, 2007), plantation location and conditions (Barreto-Salazar *et al.*, 2021; Teklu *et al.*, 2021), fertilizers use (Amare *et al.*, 2019), sesame genotype performance (Baraki *et al.*, 2020; Baraki & Berhe, 2019) and other agro-climatic factors (Mawcha *et al.*, 2021). Sesame is an important oil crop, accounting for over 90% of oil seeds exports (Wijnands *et al.*, 2007).

Sesame is adaptable to a wide range of agro-ecological conditions and is tolerant to extreme environmental conditions (Waha *et al.*, 2018). On the other hand, other researchers have found that sesame susceptibility to different agro- climatic factors including rainfall, temperature, and wind negatively impacts sesame productivity and contributes to poor quality and high post-harvest losses (Baraki & Berhe, 2019). Furthermore, sesame value addition and processing are sensitive

to environmental conditions such as oxygen and light (Gharby *et al.*, 2017), process condition such as roasting (Hama, 2017), and oil extraction method (Ribeiro *et al.*, 2016).

Sesame sensitivity to a number of environmental and biological factors has a negative impact on productivity, economic value, and quality (Barreto-Salazar *et al.*, 2021; Mawcha *et al.*, 2021). Teklu *et al.* (2021) identified unknown sesame seed varieties and high genetic diversity as problems for low sesame yield in western Tigray, as well as limited/ lack of access to improved sesame seed varieties (high price), lack of access to improved varieties and/or high cost of the improved varieties, disease and weeds, low market price and lack of market information (Gebretsadik, 2020). Poor agricultural, handling and agronomic practices, poor post-harvest technology and management, an insufficient regulatory system, a lack of up to date information, the absences of proper seed sector and breed policies, inadequate extension service, climate change and lack or poor infrastructure were the factors that contributed to the challenges (Gebremedhn *et al.*, 2019; Teklu *et al.*, 2021). During harvesting (early and or late harvesting), drying and cleaning, threshing, loading and unloading, transportation and storage, transportation modes and distance, and storage, 17-25% of sesame seed is lost (Gebremichael, 2017; Gebretsadik *et al.*, 2019; Neme, *et al.*, 2021). Untimely rain, insect attack, weather conditions and seasonal variation, stacking duration, farm distance and land size, labour, educational level, lack of consultation and market volatility were decisive factors for sesame loss (Gebretsadik *et al.*, 2019). Oil recovery and extraction losses, which vary from 5 to 10%, have a substantial impact on the economic value and food security.

Due to its pleasant flavour, nutritional benefits, essential supplies for margarine, shortening, salad oil, food preparation at home, restaurant, and food manufacturers (Diamante & Lan, 2014), cosmetics making, pharmaceuticals (vehicle for drug delivery), insecticides and paints and varnishes (Pathak *et al.*, 2014; Warra, 2012), sesame is an important ingredient with an ever increasing demand worldwide for food, cooking oil, health food and sweets. However, sesame maturity index during harvest is crucial factor in ensuring the purity and safety of sesame seed and sesame based products (Dong *et al.*, 2021). Sesame plant colour change from green to yellowish or light brown, leaves begin to fall, and seed pod bursts, indicating sesame is ready to harvest (Ferreira *et al.*, 2017). Sesame seed is available with or without its coat, has a nutty flavour, is crispy, and yields a lot of oil. However, the weak oligopoly market structure, information irregularity, price collusion, and barrier entry impacted the economic value (Gebretsadik, 2020).

## 2.1.Sesame Seed Composition

Sesame is a small seed with an average of 2.74 - 3.16 g per 1000 seed weight, 1190.6-1215.50 kg/m<sup>3</sup> a true density and storable oilseed crop with a low moisture content ( $\leq 6\%$ ) (Zebib *et al.*, 2015). Varieties, soil quality, cultivation practice, agro-ecological condition, genetic and environmental factors, post-harvest handling and processing practices have a significant effect on physico-chemical properties of sesame based products (Baraki & Berhe, 2019; Wei *et al.*, 2015; Zerihun & Berhe, 2020). Because of the fibrous cellulose in the capsules, the size and shape of sesame seed (length, width, thickness, porosity, and capsules angle of repose) increases when moisture content increases. Furthermore, as moisture levels rise, the friction coefficient rises while the rupture force falls, having a substantial impact on oil extraction (Yilmaz *et al.*, 2012). Udoh *et al.* (2017) also reported the coefficient of friction of oil seeds increased with increased moisture content and dropped the deformation forces with optimal moisture content (13.5%). The moisture content also affects the oil recovery, residual oil, pressing rate, oil loss, oil quality and stability (Gawrysiak-witulska *et al.*, 2012).

Sesame seed is composed of approximately 96% dry matter content. Sesame seed is containing high concentration of protein and oils, which is typically used for oil extraction. The sesame seed from western Tigray is composed of crude oil content of 51% (Zebib *et al.*, 2015), white Sudanese sesame oil content accounted 53% (Borchani *et al.*, 2010), Morocco sesame seed oil content 52% extracted using screwless cold press extraction method (Gharby *et al.*, 2017), Sudan seed extracted using petroleum ether for 72 hours at room temperature composed of oil content of 47.4% (El-Khier *et al.*, 2008), Congo-Brazzaville” seeds with Soxhlet method (highest) and liquid method separation of (methanol-water) and organic layer (chloroform), average oil content reported 54% (Nzikou *et al.*, 2009), Nigeria seed extracted using dimethyl ether in a Soxhlet apparatus 36%(Ogbonna & Ukaan, 2013), Turkey seed extracted using hexane at 20 °C for 72 hours 54.3% (Ünal & Yalçın, 2008) and Egypt sesame seed extracted in a continuous overnight agitation in hexane contained 56-60 % oil (Hassan, 2012).

Sesame oil is rich in energy provision 3350 KJ/100 ml oil, which explains its importance in human nutrition (Diamante & Lan, 2014). The fatty acid composition, is a significant quality indicator for the characterization and alteration of oil's nutritional, organoleptic or physico-chemical qualities. Sesame fatty acid composition was found to be 4-16% saturated fatty acids, with palmitic (12.03 - 14.02%) and Stearic acid (4.93 - 5.72%) accounting for the majority of

saturated fatty acids, and 84-85% unsaturated fatty acids, with oleic (45.46 - 47.03%) and linoleic acids (33.79 - 35.01%) at higher proportion. Sesame is a good source of protein 17-40% with significant variation depends on agro-climatic factors, extraction, and analytical procedures/methods. Sesame protein is essential in human nutrition because it provides 18 amino acids, 39% essential amino acids, which supplement daily requirements (FAO & WHO, 2007), the highest of which is glutamic acid, and it can be used to modify the functionality of foods such as digestibility, solubility, foaming making and emulsifications (Fasuan *et al.*, 2018; Salimon *et al.*, 2017). The carbohydrate content of sesame seed varies greatly, ranging from 1-41%, (average ~11%). Sesame seeds are high in dietary fiber, ash and essential minerals (Ca, K, Mg, Mn, Na, Fe, Cu, and Zn) (Elleuch *et al.*, 2007).

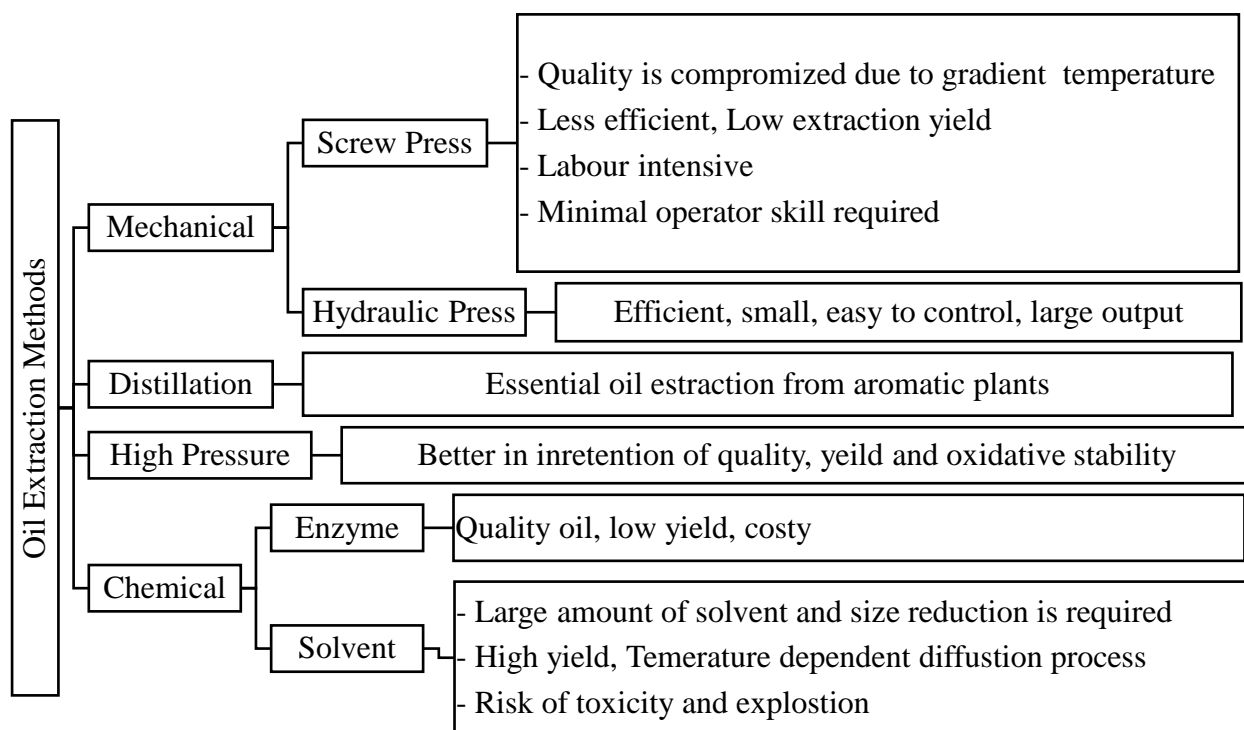
Sesame is also renowned for its medical bioactive compounds, which are important in defence of reactive oxygen species, aging, cancer and oxidative rancidity (Pathak *et al.*, 2014). Lignans (Sesamin, Sesamol, Sesaminol), Phytosterols ( $\beta$ -sitosterol, Campesterol, stigmasterol,  $\Delta^5$  -avenasterol, Sitostanol, Campestanol, and Ergosterol), Tocopherol, phytate, glucosides, carotene, and others are among the beneficial substances (Dar *et al.*, 2019; Gharby *et al.*, 2017). Most of the bioactive compounds are concentrated in the outer cover of the seed where lost during extraction and refining process (Elleuch *et al.*, 2007). Sesame on the other hand, contains anti-nutrients, including phytate (89-467 mg/100g) and Tannin (225-435 mg/100g) which prevents the nutrient availability (Deme *et al.*, 2017). The inconsistency of sesame composition describes how agro-climatic factors and agricultural practices affect the nutritional content, extraction yield and economic value (Wacal *et al.*, 2019).

## **2.2.Oil Extraction Methods**

Oil extraction methods exist, summarized in Figure 2.1, includes water and steam distillation, chemical (solvent, supercritical CO<sub>2</sub>, enzymatic extraction), high pressure extraction method, and mechanical (maceration, hydraulic and screw press) (Trapani *et al.*, 2017). Chemical extraction yields more than the mechanical and green extraction methods (Ribeiro *et al.*, 2016). Chemical extraction method, on the other hand, is expensive because it necessitates the use of solvent, a long process or contact time, smaller particles of oilseed, and the risky of explosion (Paibon *et al.*, 2011; Rodriguez-Miranda *et al.*, 2013; Rodríguez-miranda *et al.*, 2014). Mechanical extraction is easy, safe, and efficient, requiring fewer extraction steps and yielding an average extraction efficiency of 80%. Mechanical extraction methods include rolling press, rotary press, and hydraulic press,

commonly used either in the small-scale or laboratory. Oil extraction involves two parallel steps; 1) washing off of the oil from the surface of the cells or broken cells, which yields the major oil and 2) diffusing the oil stored and released by osmosis in the intact cells (Junpeng *et al.*, 2019). Oil extraction yield is determined by temperature, pressure, moisture and mass of oilseed. Oil extraction efficiency might be improved by changing the extraction temperature, pressure and moisture (12.3%), as well as reducing the mass of the oilseed. However, higher extraction temperature and pressure negatively impacts the biochemical properties of the oil, degrade the anti-oxidant properties and damages seed tissue (Ghiasi *et al.*, 2022; Trapani *et al.*, 2017).

Solid-liquid phase separation techniques is used in the mechanical press method of oil extraction (Kamai-eldin & Appelqvist, 1995). Mechanical extraction is easy, quick, inexpensive cheap and suitable for diverse oilseeds. A batch extraction method requires a minimal amount of ingredients at a time. Feeding rate, restriction dye diameter, temperature, pressure, press rotation speed and mass of the seed are determinant factors to improved extraction efficiency (Elkhaleefa & Shigidi, 2015). Seed properties and pre-treatments such as drying, conditioning, roasting affect oil quality, extraction method and extraction efficiency also affect oil extraction efficiency (Abou-gharbia *et al.*, 1997; Kanavouras *et al.*, 2004; Hama, 2017). Seed shell, moisture content, oil content and type of oilseed / agro-climatic factors are important variable to consider during oil extraction (Abou-Gharbia *et al.*, 2000; Elleuch *et al.*, 2007). Roasting treatment is widely reported as oilseed pre-treatment to improve extraction efficiency though it impacts the anti-oxidants and other essential nutrients (Hou *et al.*, 2019). As a result, roasting temperature over a longer holding time leads to degradation of essential nutrients including the anti-oxidants, unsaturated fatty acids, accumulation of acidity and oxidation products (Jannat *et al.*, 2013). Small-scale extraction plan typically uses the expeller cold press oil extraction method process. Continuous crushing and high rotation speed separate the oil from the husk/fiber, which is then filtered and centrifuged. Cold pressed oil is preferred by the consumers might be due to the intense taste, colour, and aroma that can be consumed with or without refining. To separate the crude oil and oil cake, screw press oil extraction compresses the seed between the screw and traveling cones. However, ineffective cold press results, in a large loss of oil (Benítez Benítez *et al.*, 2016; Bhuiya *et al.*, 2020; Firdaus *et al.*, 2017). In small-scale plants, mechanical press oil extraction is widespread (Benítez Benítez *et al.*, 2016). The yield and extraction efficacy and apparent extractability index is determined by difference before and after extraction (Rodriguez-Miranda *et al.*, 2013; Trapani *et al.*, 2017).



**Figure 2. 1:** Summary of plant-based oil extraction methods commonly used in the oil refinery industry for edible oil production

### 2.3.Oil Oxidation and Implications

Oxidation is defined as a natural process between atomic/molecular oxygen and unsaturated fatty acids in fats, oils and foods caused accumulation of toxic radical metabolites, caramel and other browning reaction induced metabolites. It is defined as the synthesis of undesired metabolites by gaining of oxygen or loosing of hydrogen in unsaturated fatty acids or complex dietary mixtures, leading to unfavourable organoleptic qualities (Loock, 2011; Silverstein, 2011; Zhang *et al.*, 2013). According to Denisov and Afanasév (2005), oxidation is the degradation of quality and the conversion of fatty acids into radicals of primary and secondary oxidative metabolites. Unpaid electrons that are created or present as a result of oxidation during the reaction's commencement and propagation are known as free radicals and are found in oxidized products. Oxidation is catalysed by a number of parameters includes composition, temperature, time, light, package and oxygen availability. Composition the types of fatty and degree of unsaturation, endogenous enzyme and moisture catalyse oil oxidation which determines the storage stability and

edible oil shelf-life. Oxidation is desirable to some extent as it contributes to the organoleptic characteristics of some food products. Nonetheless, exceeding certain limits is carcinogenic to human due to the formation free radicals, peroxide and hydroperoxide and quality degradation (Apak *et al.*, 2013). The primary or secondary oxidative metabolites explain the oxidation status of lipid or complex food mixture.

#### **2.4. Basil Extract and Importance**

Antioxidants widely used to constrain oxidation (Mozuraityte *et al.*, 2016) includes organic and inorganic substance acts as a chain breaker and mitigates oxidation binding metal ions, scavenging and/or deactivating singlet oxygen, disintegration of hydroperoxide to non- radicals products (Carelli *et al.*, 2005). Antioxidants are organic or inorganic chemical substances either synthesized or present in foods or in plant extracts at low concentration or applied to food to prevent, control or delay oxidation in an *in vitro* or *in vivo* assay to preserve quality and extend shelf life (Shahidi & Zhong, 2015; Zeb, 2020). Antioxidants are grouped in to primary, secondary and tertiary antioxidants based on mechanisms of action. Primary antioxidants includes the phenolic compounds synthesized by the shikimic acid pathway in plant foods and microorganisms (Maeda & Dudareva, 2012), neutralize millions of free radicals by donating hydrogen atom or single electron transfer. Secondary anti-oxidants are  $\beta$ -carotenes, neutralize the pro-oxidant catalyst such as metal ions, ethylenediaminetetraacetic acid and citric acid and single free radical. Tertiary antioxidants repair damaged DNA or protein biomolecules and yet limited information on the role in foods. The commonly used anti-oxidants includes flavonoids, carotenoids, citric acid, ascorbic acid, and others (Mozuraityte *et al.*, 2016) obtained from different fruits, vegetable and herb.

The effectiveness of antioxidants however depends on the structural features, concentration, oxidation substrates, and pro-oxidants (Shahidi & Zhong, 2015). Identification, characterization, quantification and utilization, is however, depends on the several factors includes agronomic practices and cultivar/species (Calderón Bravo *et al.*, 2021; Cruz *et al.*, 2020), growth substrate and the fertilizer (Kolega *et al.*, 2020), irrigation and water effect (Ekren *et al.*, 2012), pre-and post-harvest treatments (Loi *et al.*, 2020) includes pretreatments such as drying, roasting, milling (Mohamed *et al.*, 2021), extraction technology (Soquetta *et al.*, 2018), time and temperature (Pedro *et al.*, 2016), solvent use (Bae *et al.*, 2012), and analytical methods (Carbonell-capella *et al.*, 2014; Thouri *et al.*, 2017). Fattahi *et al.* (2019) explored delayed germination of basil seed and minimize

the germinated seed due to the presences of heavy metals in the soil. Enzymatic and solvent extraction gives better yield, increased extraction temperature up to a certain limit and extended holding time increases extraction yield. Though, it is expensive, time, solvent consuming and might not be safe to the environment and human health (Dirar *et al.*, 2019).

Fruits and vegetables, spices and herbs are excellent sources of carotenoids, alkaloids, flavonoids, phenolic compounds, and Vitamin C are among others (Lorenzo *et al.*, 2019). The aromatic plant extract's bioactive compounds exhibit various health benefits, including the prevention of chronic and degenerative diseases, as well as anti-oxidant, anti-microbial, anti-insecticide, medicinal, and food flavour enhancing properties (Chhikara *et al.*, 2021; Majdi *et al.*, 2020; Pandey *et al.*, 2017). The aromatic extracts preservative properties to microorganism reported such as prevention of fungi and fungi metabolites inhibition (Loi *et al.*, 2020; Mmongoyo *et al.*, 2017). According to Medhujith and Sivaakanthan (2019), bioactive compound are phytochemicals that are present in small amounts but are crucial for the proper functioning of food and human health. These compounds include polyphenols, flavonoids,  $\beta$ -carotenoids, alkaloids, vitamins, microorganisms, and other essential oils in fruit and vegetables, spices and herbal plants, cereals and legumes and fermented products (Aswathanarayan & Vittal, 2019; Hu *et al.*, 2017; Pathak *et al.*, 2014; Soquetta *et al.*, 2018).

Basil (*Ocimum basilicum L.*) is a white-purple flowering annual plant used as a flavour enhancer in food, cosmetics and medicine (Castano *et al.*, 2016). Basil contains volatile and non-volatile substance, which enhances aroma and flavour profile of foods. Basil is essential in the functional foods because it enhances food functionality and human functionality, meeting the criteria set forth by Astrini *et al.* (2020). Furthermore, basil is an excellent sources of bioactive compounds that acts as a preservative against *S. aureus*, *C. albicans*, *E. coli* and *B. subtilis* (Adigüzel *et al.*, 2005; Deo *et al.*, 2011), microbial metabolites mitigation (Iram *et al.*, 2016), and anti-oxidant property (flavonoid, alkaloid, phenol and essential oil) (Warsi and Sholichah, 2017) efficient at 10 ppm (Castano *et al.*, 2016). Furthermore, the extraction yield of bioactive volatile and non-volatile basil chemicals varied significantly depending on the extraction solvent, extraction time and temperature (Soran *et al.*, 2009; Tambun *et al.*, 2017). The extraction conditions and techniques also determine the bioactive compound efficiency for the intended objective (Soquetta *et al.*, 2018). In contrast to the use of enzymatic and solvent extraction, (Ribeiro *et al.*, 2016) found that the DPPH radical scavenging capacity of phytosterols and

tocopherols were more effective with pressing extraction (106.18  $\mu\text{mol Trolox g}^{-1}$  of oil), while the L-ORAC was better during enzymatic extraction (371.56  $\mu\text{mol Trolox g}^{-1}$  of oil). However, press extraction, reduces the antioxidants' extraction yield.

The dominant bioactive compounds in basil extract were reported as linalool, methyl chavicol and 1,8-cineole (Chenni *et al.*, 2016; Soran *et al.*, 2009). Anthocyanin is a flavonoid common antioxidant in basil with an optimal extraction yield of 67.4 mg/100 g at 60 °C during 30 minutes (Pedro *et al.*, 2016), have both gram-positive and gram negative bacteria prevention potential (Deo *et al.*, 2011) and fungi inhibition (Sudarno *et al.*, 2017). Organic anti-oxidants serve a dual purpose as they provide nutritional benefits as well as medical value by replacing inorganic anti-oxidant that are thought to constitute a long-term health risks (Anand *et al.*, 2019; Carelli *et al.*, 2005). Basil is one of the most frequent species used as a flavour enhancer in both fresh and dried form. Its practical application in the food processing business as organic preservative is however not fully understood.

## **2.5.HACCP Plan in the Food Processing Plant**

Food safety management is important for consumer health protection through the food safety objectives (FSO). FSOs translated in the food industries through the good manufacturing practice (GMP), good hygienic practice (GHP), Hazard Analysis Critical Control Point (HACCP), international organization for standardization (ISO), total quality management, process/product criteria, acceptance and performance criteria (Kooch *et al.*, 2020; Spiegel *et al.*, 2004). FSOs enables to develop a protocol and or procedure either of to prevent, control and reduce hazards of concern in the food industry. Good hygienic practices are the pre-requisite programs (PRPs and OPRPs) taking it to consideration the layout and building design (Holah & Campdem, 2014), equipment, maintenance and services schedule and procedures, incoming ingredients/ materials handling, processing, cleaning, storage, transport & distribution, personal hygiene, pest prevention and security measures (Bortoletto *et al.*, 2018; Motarjemi *et al.*, 2014; Rossi *et al.*, 2018). However, the inadequacy of good post-harvest practices (Wacoo *et al.*, 2014), unsanitary practices of the storage, food industry and food catering (Garayoa & Nathaly, 2016) and poor personal hygiene (Rossi *et al.*, 2018) impacts food security (Gebremichael, 2017), public health (Khan *et al.*, 2018) and economic value (Agidew & Singh, 2018; Gilbert *et al.*, 2017). Plus, pre-requisite programs are important foundation for HACCP system includes team assembly, defining the product intended use and its description, constructing the flow diagram and validate.

Hazard analysis critical control point (HACCP) is an efficient and effective method for monitoring and prevention of food hazards and a reference method for food safety assurance in the food supply chain. HACCP was first introduced in the USA in the 1960's due to the outbreaks associated with a number of potential hazards and advanced its protocols and implementation in association with National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (Weinroth *et al.*, 2018). HACCP principles was developed by the codex alimentarius with defined objective and procedure tailored to a specific product line (Motarjemi *et al.*, 2014). Organic food safety management (Stanley *et al.*, 2011), mushroom and edible fungi safety (Pardo *et al.*, 2013), traceability of packaging of soybean oil (Miarka *et al.*, 2019) and so many other studies were reported that HACCP is effective for foods safety management system. The seven universal principles of HACCP are: (1) Conducting hazard analysis, (2) Determining Critical Control Points (CCP), (3) Establishing Critical Limits (CL), (4) Establishing monitoring, (5) Establishing corrective actions, (6) Establishing verification procedures and (7) Establishing documentation and records.

#### *2.5.1. HACCP Pre-requisite and Operational Pre-requisite Programs*

Hazard analysis critical control point (HACCP) is a systematic and preventive universal system for safer food production and distribution tailored for a specific product. The HACCP plan development and implementation involve multiple steps, summarized Figure 2.2. The first step of for HACCP plant development and implementation is team assembly (Step 1, Figure 2.2). A multidisciplinary expertise team of 5-8 individuals in processing, unit operation, hazard analysis (biological, chemical, physical), risk analysis articulated to a specific or an equivalent product assembled with the aim of developing a HACCP plan and its implementation to maintain the safety of food following the unit operation and associated activities during processing, production and distribution. Product description (step 2, Figure 2.2) is the foundation for HACCP implementation includes raw material and end product characterization, composition, shelf-life estimation and storage condition specification, packaging, and labelling. The product use (step 3, Figure 2.2) includes details of use to advance in product development as an ingredient, raw material and instructions for preparation and targeted consumers. Process flow diagram contraction (step 4, Figure 2.2) on how the unit operations carried out from raw material reception and handling, storage following all unit operation until the end product. It includes the operational factors such

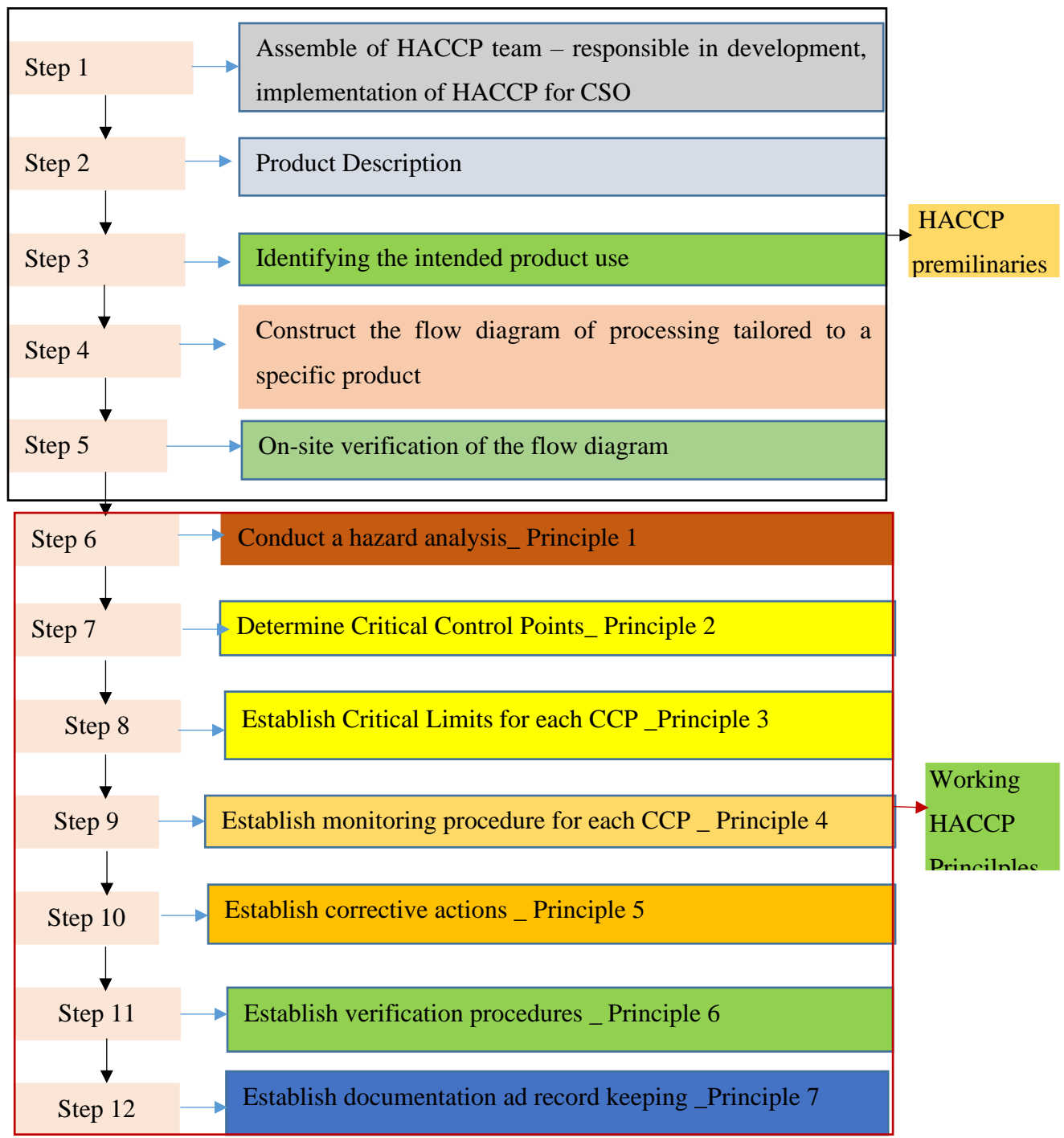
as the time -temperature combination, humidity, moisture content, aging, separation and or mixing, pressurizing, packaging and others depending on the nature of the product. The processing flow diagram validation (step 5, Figure 2.2) is authoritative to verifying on-site on its proper and rightful implementation of the process flow diagram. Step 1- 5, Figure 2.2 also known as the HACCP preliminaries (pre-requisite programs) which are the foundation for food safety management.

### 2.5.2. HACCP Working Principles

Hazard analysis is one of the basic HACCP principle (Principle 6 of Figure 2.2, step 6) in collecting and evaluation (likelihood and severity) of a long list of potential hazards associated with growing, harvesting, raw materials and ingredients, processing/manufacturing, distribution, marketing, preparation and consumption which *compromises* food safety and human health (Kooch *et al.*, 2020). Hazards are defined as any contaminants of biological, chemical and physical agent with the potential to cause adverse health effect, illness or injure or violates food standards (Wallace *et al.*, 2018). During hazard identification a long list of hazards listed taking into consideration of the product characteristics and technology during processing and analytical techniques. Hazard analysis is however evaluation based on frequent occurrence and toxicity/pathogenicity. Critical control point (CCPs) (Principle 7 of Figure 2.2, step 7) is process points, step or condition where control is a must to prevent or minimize identified hazard occurrence or reduce to an acceptable level to ensure food safety. CCPs include use of approved chemicals or additives, controlling product composition (pH, aw, preservatives), sanitary and cleanness conditions, process condition (time, temperature, pressure), packaging and others. Critical limit (CLs) (Principle 8 of Figure 2.2) is the limits or boundaries established to separate process or product safety for the identified CCPs. It is the tolerance that must be met to ensure that a CCPs controlled effectively as the identified biological, chemical and physical hazards eliminated, prevented or reduced to an acceptable level.

Monitoring the CCPs ad CLs (Principle 9 of Figure 2.2) is the procedure on how to check or observe the CCP/CL is under control and proper implementation by either of use of instrumentation, management action to help prevent or minimize deviations. Monitoring frequency and responsible professionals is critical for the accurate implementation of the HACCP. Corrective actions for each CCPs ad CLs (principle 10 of figure 2.2) to be taken if deviations identified during monitoring. Based on the data reflected deviation of the control or process corrective actions taken

either to manage the process, or noncompliance product to fix the causes and recording the actions taken. Depending on the nature of the product and hazard suspect to compromise product safety and public health, possible corrective actions into consideration are (1) terminate the operation/production, (2) hold suspect product, (3) fix the production (4) identify and correct the root causes of deviation or failure, (5) deal with the suspect product (releases, test and divert), (6) corrective actions record and (7) review and improve HACCP plan. Sampling and decision making are critical. Verification procedures (Principle 11 of Figure 2.2) is a confirmation that HACCP is working and food safety is not compromised. HACCP verification can be done by (1) reviewing the HACCP plan, (2) compliance with the established critical control points, (3) confirmation of compliance with the procedures for handling of deviations and records, (4) visual inspection of the operation while a product is in production, and (5) written report. HACCP verification should be based on in-plant laboratory test results, shelf-life studies, consumer complaints. Record keeping (Principle 12 of Figure 2.2) is written evidences documentation for reviewing and tracing the effectiveness of HACCP plan. Record keeping is an integral part of HACCP to trace the history of raw material, the process and end product, record related to CCPs, CLs for each CCPs, monitoring and corrective action of CCPs/CLs should be available for auditing and food regulatory. Monitoring and records data are the proof for HACCP plan implementation and prove of compliance.



**Figure 2. 2:** The hazard Analysis Critical Control Points (HACCP) principles to follow for development and implementation diagram and an overview in the food processing industry tailored to a particular product in a defined and verified process flow diagram(Kooh *et al.*, 2020).

### 2.5.3. Hazard Prevalences in the Food Value Chain

Hazards are contaminants classified as biological, chemical and physical associated to food, feed in the value chain, and environment detected in agri-food value chain. Hazard (s) may exist in different reservoirs such as soil, water, surfaces, warm-blooded animals, and food of different origin along the value chain as summarized in Figure 2.3. Hazards causes either a direct or indirect public health threat to human by itself or its metabolites, resulted nutrient depletion to reduce food quality, increase food wastage, illness, hospitalization and death (Baranyi & Silva, 2017; Bortoletto *et al.*, 2018). Hazards are persistent due to unsuccessful handling and treatment of raw material, food, water and other ingredients including resistant spores from microorganisms categorized as biological, chemical and physical hazards. Thus, According to the codex guidelines, national and food industries legislation, quality assurance recognised as standards to prevent hazards, control process condition and product composition established to regulate public health, promote food market and enhance the economic value quality assurance of foods (FAO & WHO, 2009; Schirone *et al.*, 2017).

Biological hazards refer to microorganism of different bacteria, viruses, protozoa, worms, fungi, prions, and algae determined to cause serious health risks and economic loss. Microbial hazards are different in terms of epidemiology, physiology, host, pathogenicity, virulence, persistence and resistance to antibiotics, remains a dilemma in the food value chain, safe food production and distribution. Microbial hazards have gained attention in the food value chain due to life threatening food-borne or water-borne illness and economic loss. Bacteria, a microbe without nucleus or membrane bound organelle organised in spherical, rod, spiral, comma and corkscrew structure in single cells, pairs and cluster form. Bacteria requires sources of energy for its growth, cellular function besides the variation in specious and strains (Smith *et al.*, 2019). Viruses are infectious microorganism with ultra-small size not retained by bacteria proof filter progressed over longer time, adopted to specific cells. Viruses contains of nucleic acid and cannot reproduce by division without the host, frequently hosted in live animals (Modrow *et al.*, 2013). Studies reported that fungi and fungi metabolites, aerobic count bacteria, *staphylococcus aureus*, yeasts and moulds, salmonella, coliforms and Escherichia coli confirmed in the sesame value chain (Alaouie *et al.*, 2017; Aydın & Başaran, 2018).

Fungi are eukaryotes important in nutrient and carbon cycling, uncultivable with widely diverse taxonomic classification of eco-physiological and biodiversity along environmental

gradient (Tedersoo *et al.*, 2018). Fungi, a potential contaminant which grows at different moisture content and stages of cultivation and post-harvest handling happened to restrain enzymatic and mitotic cell division and protein biosynthesis (Berthiller *et al.*, 2017; Merritt & Gregory, 2016; Podolska *et al.*, 2017). Fungi might and might not be toxic by itself (Thathana *et al.*, 2017) but synthesize toxic metabolites called mycotoxins. The mycotoxins have diverse physicochemical, morphological and physiological properties (Berthiller *et al.*, 2017; Podolska *et al.*, 2017) .

The sources and causes of contamination might be natural, cross contamination due to inappropriate handling and processing, and interaction with other ingredients. For example, several studies revealed, inappropriate handling and treatment of water can be a source of waterborne hazards. Kenyan ground and surface water contaminated with *E.coli*, *Staphylococcus aureus*, *Clostridium pafringens*, *coliforms*, *total coliforms* (Onyango *et al.*, 2018). Similarly, Ethiopian dugouts underground water in the wet season was reported to be contaminated with *E.coli* (Amenu *et al.*, 2014). The municipal water in Jigjiga city, Ethiopia was found to be contaminated with *E.coli*, *Shigella*, *Salmonella*, *Vibrio*, *fecal coliforms* (Asfaw *et al.*, 2016). Reports of Ethiopian drinking water from a storage tank in urban areas contaminated with total coliforms and fecal coliforms has been confirmed (Chalchisa *et al.*, 2017). Other reports indicates that Ethiopian bottled water was contaminated with heterotrophic plate count, mould, yeast, total and thermos-tolerant coliforms, *Escherichia coli* and *Staphylococcus aureus* (Bedada *et al.*, 2018).

Biological and physical hazards are detectable at comparably lower cost than the chemical hazards. Chemical hazards could intentionally added to foods during processing as impurities in some ingredients or unintentionally as contaminants from raw materials. Chemical hazards include (1) residual within or on foods from previous exposure in the food value chain (pesticides, detergents...), (2) applied chemicals (food additives and preservatives), (3) accidental (impurities, cleaning and sanitation chemicals, and packaging chemicals), and (4) background chemicals (naturally occurring, microbial toxins, heavy metals, process induced chemicals...). Biological originated mycotoxin detected in raw, semi-processed and processed food of plant, animal and microbial origin. The most potent chemical toxic metabolites reported in foods in particular durable crops are mycotoxins (Kollia *et al.*, 2016;Thathana *et al.*, 2017;Yoshinari *et al.*, 2007) synthesized from different kinds of fungi species (Somorin *et al.*, 2016; Thathana *et al.*, 2017).

Pesticides are agro-chemicals accidentally or deliberately added in agriculture or foods to improve productivity, protect crops/food against insects, fungi, weeds and other pests and to

protect public health from tropical vector diseases. Pesticides utilized in the sesame Agri-value chain against cotton mealybugs, such as early and late blight, bollworm, spider mites, aphids, trips, powdery mildew, downy mildew, botrytis, nematodes, mealy bugs, and caterpillars (Gebregergis, 2018; Teklu, 2016; Teklu *et al.*, 2021). The overuse or inappropriate use of pesticides causes environment safety problem, soil quality degradation and health complications to human such as gastrointestinal distress, organ damage, and immunological reactions/death and long-term chronic effects, including cancer, hematological alterations, respiratory issues, endocrine dysfunction, neurotoxicity, and infertility (De-Assis *et al.*, 2021). The use and practices of pesticide (s) in Ethiopia are however, beyond the recommended level (Mekonen *et al.*, 2014; Mengistie *et al.*, 2017). Pesticide residue detected in red pepper, teff, maize and coffee beans with and without pulp were above the MRL explains its impact on human health and environmental pollution (Geremew *et al.*, 2012; Mekonen *et al.*, 2014). Although there has been a recent increase use of pesticides and the need for regulation of the use of prohibited pesticides across the supply chain, pesticides are not a major concern in the Ethiopian food system.

Physical hazards are any extraneous object in contact of food and or personnel in the food value chain which may cause illness, injury and overall food safety problem. Physical hazards might be included via raw materials, badly maintained facilities and equipment, improper production procedures and poor employee practices (Wallace & Mortimote, 2005). Physical hazards include (1) impurities with lighter and heavier objects of agricultural by-products, (2) Sharp items may cause injuries such as glass, metals, stones, (3) Metallic and other hard objects that damage extraction machines and affects the overall quality and safety of food products. Thought, Effective cleaning, restrict reception and food quality assurance objectives are the control measures of physical hazards.

## **2.6.The Risks of Hazard Prevalence**

Infectious and poisonous microorganisms, their toxic metabolites, additives and chemical preservatives and foreign matter associated to food, surface and environment are capable of causing acute and chronic health complication and environmental pollution. Some but not all of the hazards are responsible for immediate illness or hospitalization after consumption few minutes to weeks, causes watery/acute diarrhoea, vomiting (Feleke *et al.*, 2018; Rajasingham *et al.*, 2020; Solomon *et al.*, 2020), typhoid, hepatitis and cholera (Derso *et al.*, 2017; Geberselassie *et al.*, 2018; Tariku *et al.*, 2017), gastro-intestinal inflammation, and immunomodulation (Kushiro, 2008). On

the other hand, hazards are potential noiseless threat with reversible and irreversible non-communicable health complications (Allen, 2017; Bhat *et al.*, 2010; Karlovsky *et al.*, 2016; Marchese *et al.*, 2018). For example, fungi metabolites called mycotoxins are chronic (Bryden, 2007; Knutsen *et al.*, 2018), triggered respiratory and neurological dysfunction (Rea *et al.*, 2003), environmental enteric dysfunction (EED) and disturbance of the insulin-like growth factor (Smith *et al.*, 2015), child malnutrition and mortality such as stunting, wasting and growth restriction (Adane *et al.*, 2017; Chen *et al.*, 2018; Mupunga *et al.*, 2017; Tariku *et al.*, 2017; Wirth *et al.*, 2017). Fungi metabolites interfere with protein/micronutrient metabolism leads hepatocellular carcinoma (HCC) and even death (Abebe *et al.*, 2017; Egmond & Jonker, 2003; Marchese *et al.*, 2018) binds nucleic acids and nucleoprotein are essential to cellular viability and build-up of hepatic lipids, causes enlargement of liver, proliferation of bile epithelium and necrosis (Abrar *et al.*, 2013). Aflatoxin (s) are the most toxic, mutagenic and carcinogenic globally (Kumar *et al.*, 2017; Mupunga *et al.*, 2017) affects liver function, exacerbate malnutrition (Knipstein *et al.*, 2015; Verheecke *et al.*, 2015), hepatitis and liver cancer (Abrar *et al.*, 2013; Šarkanj *et al.*, 2018), immune system deficiency, reduces child growth and increase incidences of stillbirth or new-born jaundice (Chen *et al.*, 2018; Verheecke *et al.*, 2015). Minerals, heavy metal, and anionic which is toxic by itself and/or interacts in water, in seeds as pesticides and other chemical preservatives are carcinogenic to human (Brhane, 2019; Gebresilasie *et al.*, 2021), over chlorination treatment (Geremew *et al.*, 2018; Semagn *et al.*, 2020; Vargas *et al.*, 2021).

## **2.7. Causes of Hazard Occurrence**

Hazards exists in a dynamic environment with clinical and non-clinical importance towards public health. Factors influencing the growth and existence of microbial origin hazards and toxin metabolite synthesis include moisture (0.87-0.99), temperature, time (Lahouar *et al.*, 2016; Lappa *et al.*, 2017; Leggieri *et al.*, 2017; Mailoa *et al.*, 2017). Elevated temperature and lengthy incubation/storage time are the favourable growth condition, multiplication and production of toxic metabolites. Depending on the gas composition of the storage houses and package, hazards might be prevalent as microbial hazards may or may not require gas for their prevalence. Indoor environment of hospitals, industries and shopping centres were highly colonized with airborne microorganism due to increased temperature and gases (Nunes *et al.*, 2005), moist wall indoor microbial colonization (Pessi *et al.*, 2002), unsterilized equipment's (Ratajczak *et al.*, 2015). Acidity is also important factors as it alters mutation of the acid tolerant enzymes or amino acids

that support the existence of potential hazards (Brzonkalik *et al.*, 2012; Moreno-pedraza *et al.*, 2015; Qiu *et al.*, 2016; Sandoval-contreras *et al.*, 2017). Composition of the food is a critical factor for the survival and multiplication (Splichalova *et al.*, 2014).

Inadequate hygienic practices and lack of cleanness such as inappropriate water storage, hand washing and personal hygiene/health, safe temperature handling, detergent/sanitizer misuse leads for a potent hazards such as fungi, *staphylococcus aureus*, *fecal coli forms* to grow in food processing industries and food service establishments (Girmay *et al.*, 2020; Kifer *et al.*, 2021; Sogin *et al.*, 2021), street food venders, hospitals (Adane *et al.*, 2018; Abdi *et al.*, 2020; Ferreira *et al.*, 2014). Inappropriate handling and preparation practices due to the inadequate food safety training, supervision, and routine medical check-up (Azanaw *et al.*, 2019), inadequate layout and premises design for cleaning (Djekic *et al.*, 2014), absences of separate latrine for male/ and female or customers inadequate processing and preparation (Sandoval-contreras *et al.*, 2017) are the major causes for health hazard. Lack of awareness about food safety and what good sanitary practices are (Nesbitt *et al.*, 2014), failure to plan, design and implement food safety managements results food/water borne outbreak or sporadic illness (Lee *et al.*, 2021). The human pathogen staphylococcus bacteria recovered from food, air in particular suffocated unhygienic air, surfaces and clinical samples (Sulaiman *et al.*, 2018; Uhlemann *et al.*, 2011).

Workers handling food while sick or signs of sickness, lack of appropriate or insufficient education and awareness (Soares *et al.*, 2012; Verhoef *et al.*, 2013); lack of protective clothing were reported as a sources of contamination. Personal hygiene such as hand washing, premises and equipment hygienic such as presence of foreign matter on the surface of machine or equipment indirect contact of foods (Aarnisalo *et al.*, 2006; Al-Bahry *et al.*, 2014), inadequate washing in the food market (Jaja *et al.*, 2018) were reported the causes of contamination and survival of microbial niche. Presence of animals such as camels, dog, rat and others are carrier for microorganisms such as E.coli are the carrier and transmittion of hazards (Kambire *et al.*, 2017). Hazards survive on glass, wood, vinyl, plastic, fabrics, internal and external premises surfaces and other inanimate objects (Coughenour *et al.*, 2011; Domon *et al.*, 2016), high risk of environmental air contamination in the food production/preparation area due to lack of ventilation (Konieczny *et al.*, 2016). According to Oguntoyinbo (2012) Nigerian fermented legume condiments was contaminated by total aerobic viable count (TAVC), coliform, enterococci, bacilli and staphylococci due to the inappropriate/inadequate hygienic and manufacturing practices. Ready to

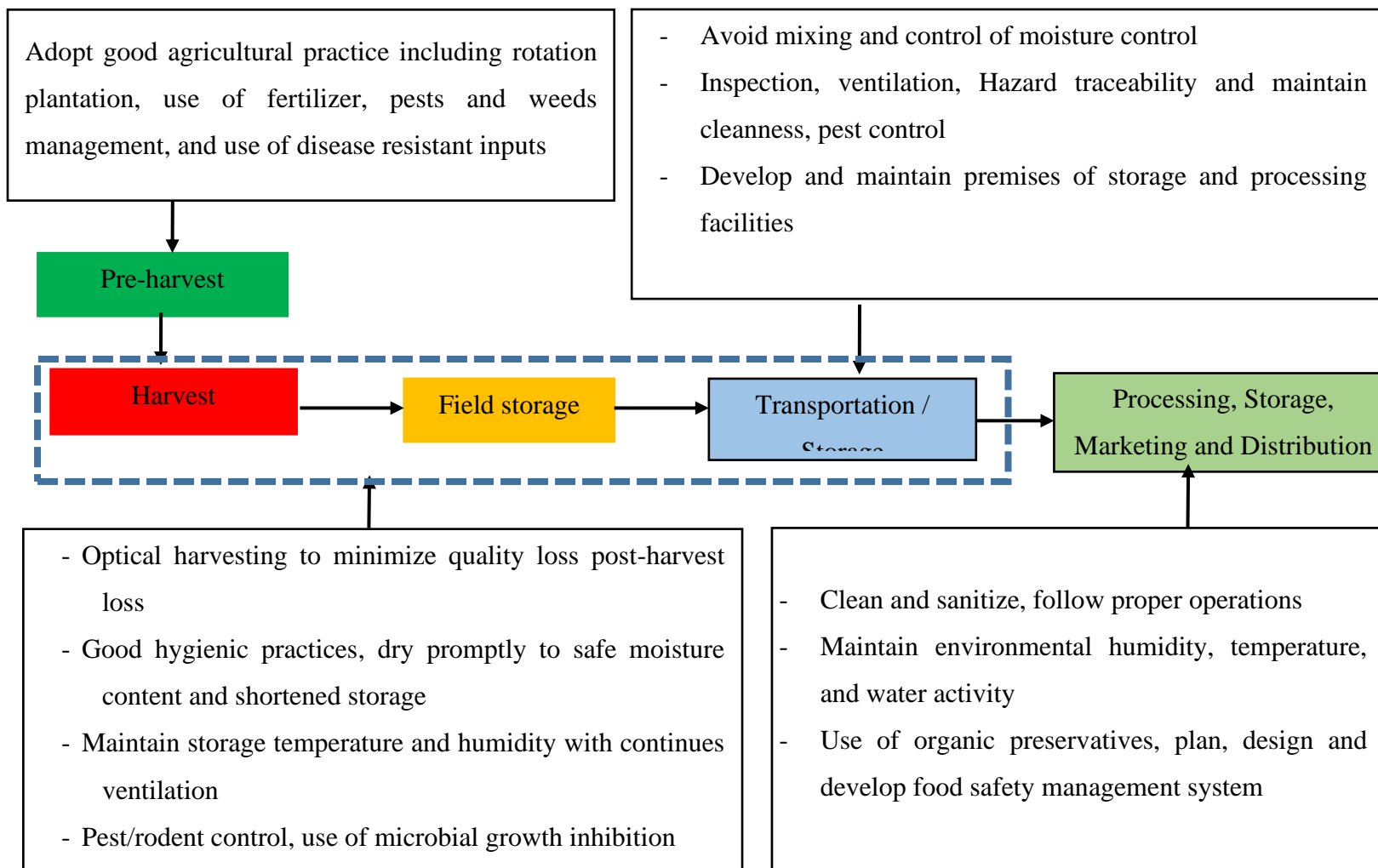
use therapeutic foods of cereals, root crops combined with legume was contaminated with salmonella (Henry *et al.*, 2014), dairy products contaminated with *Staphylococcus aureus*, *Enterococcus faecalis* in water supply, *Escherichia coli*, and aerobic plate counts on the surface of food processing industries or equipment's used for processing and workers hand was detected in Greece ice cream, water and dairy processing industry (Kokkinakis *et al.*, 2011) due to insufficient processing and sanitary practices. Negative consequences of supply chain hazards in the food system include improper resource allocation, high medical costs for both acute and chronic illnesses, and poor life quality that causes growth retardation and mortality. Food safety management and prevention in the food chain today helps to ease the presence of hazardous pollutants, minimize production loss, and improve quality of life because of the significant economic implications of food safety hazards (Focker & Fels-Klerx, 2020).

## **2.8.Prevention of Hazard Occurrence**

Consumer requirement is growing towards food integrity, transparency and reputation as safety and quality remains a challenge due to the association of health hazards (Bosona & Gebresenbet, 2013). In the meantime, prevention of hazard in the food value chain is the best remedy and includes good agricultural practices (GAP), hygienic, storage and good manufacturing practices in the Agri-value chain (Figure 2.2 and Figure 2.3). Good hygienic, storage and processing plant design prevents the occurrence of hazards (Bortoletto *et al.*, 2018; Garayoa & Nathaly, 2016; Holah & Campdem, 2014; Rossi *et al.*, 2018; Wacoo *et al.*, 2014) and personal hygiene (Rossi *et al.*, 2018) which significantly affect food security (Gebremichael, 2017), public health (Khan *et al.*, 2018) and economic value (Agidew & Singh, 2018; Gilbert *et al.*, 2017).

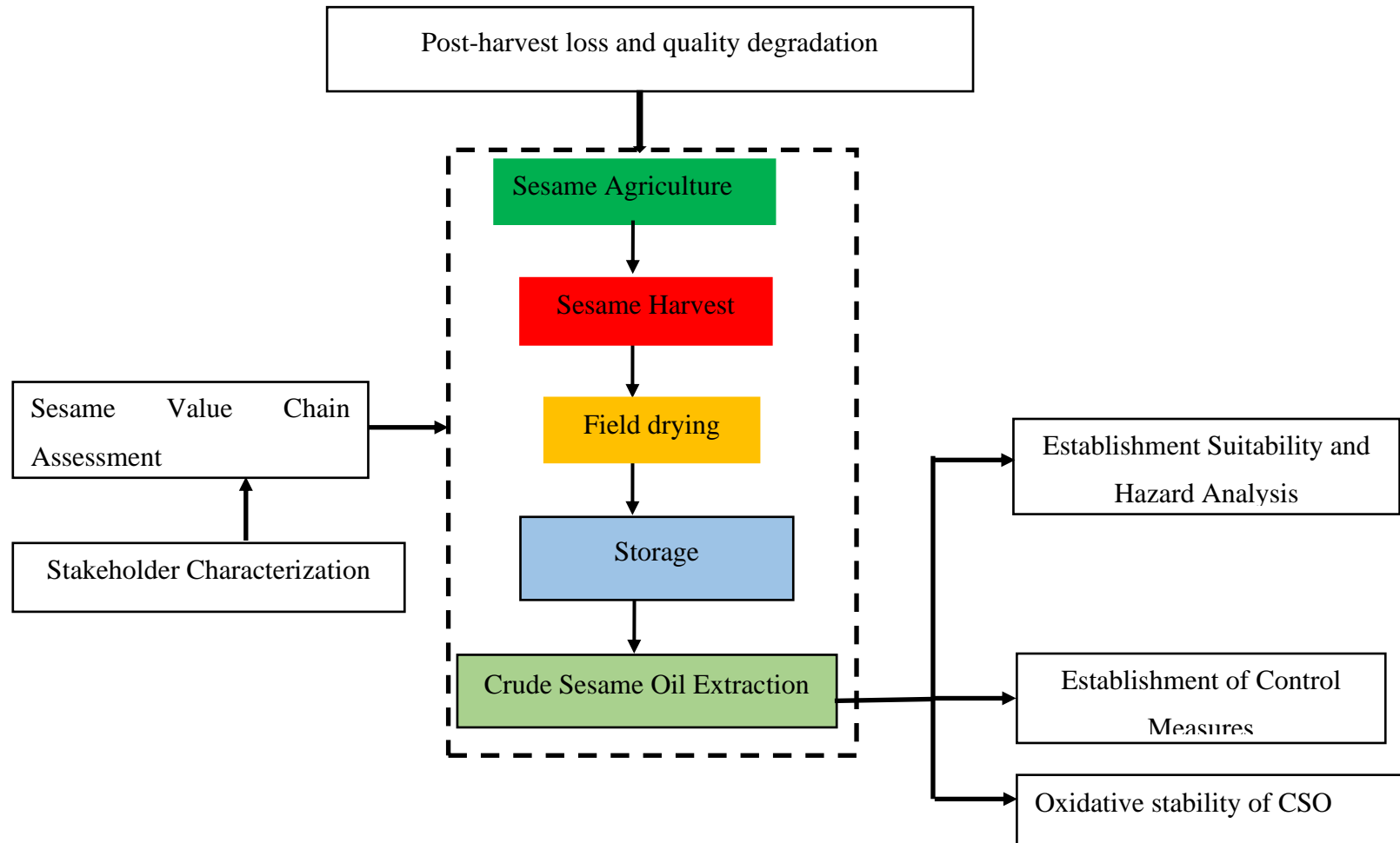
Water chlorination is effective water treatment to prevent waterborne illness and cross contamination (Mengistie *et al.*, 2013; Zelelew *et al.*, 2018). However, over chlorination causes chlorine interacts with other organic matter and accumulated as Total Trihalomethanes (THMs), carcinogenic to consumers with prolonged exposure (FAO & WHO, 2021). Studies reported by Abdullah (2014) and Ambelu *et al.* (2017) shows chloride residue exceeded the commendable limit (< 0.2 mg/L) causes carcinogenic diseases (cancer). Identification of CCPs during food processing tailored to a specific product following defined unit operation are effective in prevention, elimination and or reduction of food hazards. Establishment of CLs, monitoring procedures, preventive and verification procedures for each of the CCPs identified guarantees safe food processing, production and distribution (Jansen *et al.*, 2020; Muncke *et al.*, 2020). Studies

reported, Ethiopian sesame value chain is structured compared other agricultural crops. However, hazard prevalence and its prevention mechanisms are scarce to the best of our knowledge. HACCP was chosen as it is cost effective and have been used for decades in the food system. Thus, the aim of this study was to design protocols to assess the sesame value chain and develop a generic HACCP plan for crude sesame oil. This is summarized in the theoretical frame work (Figure 2.4) according to Gebremeskel *et al.* (2019).



**Figure 2. 3:** Food value chain and recommended practices for the prevention of hazard occurrence, for quality and safe food production and distribution.

## 2.9.Theoretical frame work



**Figure 2. 4:** Theoretical frame work in the sesame value chain and crude sesame oil extraction analysis

## CHAPTER THREE

### HAZARD ANALYSIS IN THE COLD MECHANICAL PRESSING LINE OF SESAME OIL PROCESSING IN WESTERN TIGRAY, ETHIOPIA

#### Abstract

Ethiopian agriculture and its value chains have been using outdated, inadequate agricultural and agronomic practices for thousands of years. Nonetheless, food insecurity and poverty are still widespread issues in the country. Nonetheless, value addition chain startups have lately improved. Sesame is a major crop in both domestic and foreign markets, mostly in the edible oil manufacturing industry. Crude sesame oil (CSO) is the unrefined cooking oil that is produced by mechanically cold pressing sesame seeds. However, it soon loses its flavour and turns discoloured when stored, which reduces the shelf life. The objectives of this research were to characterize the CSO extraction facilities and procedures utilized in the sesame value chain, as well as to conduct a hazard analysis of the extraction of crude sesame oil (CSO) in western Tigray, Ethiopia. The sesame value chain and the viability of CSO extraction setup were the subjects of a focused group discussion and survey. A laboratory experiment for hazard analysis which might compromise CSO safety. A variety of post-harvest procedures (such as storage, sorting, and grading based on colour and impurity, as well as the production of sesame-based food products) are included in the sesame value chain, along with distribution and marketing. The actors in the sesame value chain included farmers, wholesalers and retailers, regulatory bodies, extension agents, and researchers. Stakeholder illiteracy and passive involvement, poor infrastructure, a shortage of improved sesame breeds, and sesame sensitivity to several meteorological and biological conditions all contributed to low sesame yield and substantial post-harvest loss. Sesame seeds (SS) had a moisture content of 3.36% and a CSO of 0.37%. The yield of the sesame seed oil extraction was between 45 to 50.9%, with 83.13% unsaturated fatty acids. Safety was compromised, as evidenced by the ELISA method's detection of total aflatoxin and AFB<sub>1</sub>. Sesame seed (SS) exhibited a total aflatoxin level and aflatoxin B<sub>1</sub> as high as 14.98 and 3.94 µg/kg, exceeding the tolerated limit of 13 µg/kg and 2 µg/kg, respectively. Aflatoxin B<sub>1</sub> and the overall aflatoxin level measured in CSO were found to be 8.54 and 0.37 µg/kg below the acceptable limit, respectively. The safety of CSOs may be compromised by the high total soluble solids in ground water (1046.67 mg/L), which is

significantly higher than the WHO-recommended limit of less than 300 ppm. Because of the unsanitary and improper handling conditions, the majority of microorganisms found in CSO were aerobic bacteria, total Coliforms, fungus, *Aspergillus species*, and *Staphylococcus aureus*. But there were no Shigella, Salmonella, or Escherichia coli found in CSO.

### **3.1.Introduction**

Agriculture is still the backbone of the Ethiopian economy contributes more than 50% of the gross domestic economy, despite being based on old fashioned and inadequate rain-fed system (Davis *et al.*, 2017; Gebre-selassie & Bekele, 2010; Welteji, 2018). Ethiopian agriculture is subjected to natural and man-made disaster, resulting in food insecurity for millions of Ethiopians (Red Cross & Red Crescent, 2022). However, Agricultural Development Led Industrialization policies have been established and approved with the goal of modernizing smallholder agriculture and increasing productivity in order to increase domestic food security and enhance foreign revenues (Welteji, 2018; Zewdie, 2015) The policies were backed up with new technology, improved seed, fertilizers, finance and technical help (Llewellyn *et al.*, 2017). Despite this, poverty persists in the country (Mohamed, 2017). The policies are facing challenges that includes urbanization, limited infrastructure, insufficient market linkage, macro- and micro-economic instability (Dorosh & Rashid, 2013). According to studies, inadequate agricultural extension and outdated agronomic practices are to blame for low crop production and insufficient food value/supply chain (Zewdie *et al.*, 2021). Ethiopian agriculture faces challenges such as policy impediments, low implementation capacity for all crops at all levels for agricultural modernization, a limited industrial sector, a lack of attention for pastoral community development, ineffective and low technology dissemination, a lack of coordination among the respective stakeholders, poor land management, urbanization, and poor planning for an immediate monetary demand and high population growth 2.5-3% annually. Due to inadequate skills, experience and expert consultation, Ethiopian sesame farmers face a higher risk of resource allocation and lack of the best practices (Belete *et al.*, 1993; Edwards *et al.*, 2007). Cooperatives incompetence, household size, insect/pest/disease occurrence, small plot size, market accessibility, poor post-harvest handling, and distance from market significantly impacts on the sesame agro-value chain (Gebregergis, 2018; Gebremichael, 2017; Gebremedhn *et al.*, 2019; Mossie *et al.*, 2020).

Cereals, pulses, oilseeds, coffee, false banana (enset), livestock and flowers are the most important agricultural crops grown (Michler & Josephson, 2017; Taffesse *et al.*, 2012). Sesame

(*Sesamum indicum L.*) is herbaceous oilseed plant of Pedaliaceae family (Sharaby & Butovchenko, 2019) and important crop to Ethiopian economy (Neme *et al.*, 2020). Sesame is composed of more than 95% dry matter, 50-60% that is oil rich in unsaturated fatty acids, fiber, minerals and bioactive compounds (Elleuch *et al.*, 2007). It is an excellent energy source (3350 kJ/100 ml) for humans (Diamante & Lan, 2014; Francčáková *et al.*, 2015). Camel-driven traditional or industrial unit set-up extraction methods are utilized to extract crude sesame oil for human consumption without refining, as shown in Figure 3.1 (Kebede *et al.*, 2017). Camel driver sesame oil extraction, locally known as “Ashera”, is common in the area. A small-scale oil extraction operated by few individuals and was arbitrarily chosen. The factory was found to compromise the hygienic conditions. It was also noted that the suitability of extraction facility and equipment layout was inadequate. The goal of this study was to evaluate sesame value chain activities, as well as the suitability of CSO establishment and hygienic measures.



**Figure 3. 1:** Crude Sesame Extraction in Tigray, Ethiopia A) Camel driven and B) Small scale cold mechanical pressing method of crude sesame oil extraction

Sesame (*Sesamum indicum L.*) is a warm-season annual oil seed plant that is grown in semi-arid lowlands with rain-fed agro-ecological condition. The sesame plant has a variable genotype, which resulted productivity differences (Baraki *et al.*, 2020), nutritional and physical quality, and stability of sesame-based products (Baraki & Berhe, 2019; Kebede *et al.*, 2017; Teklu *et al.*, 2021).

Sesame is an export crop in Ethiopia (Girmay, 2018), a primary ingredient for oil and protein extraction (Doker *et al.*, 2010), confectionery product (Alaouie *et al.*, 2017; Catargiu *et al.*, 2017), and protein supplements in the bakery industry (Fukuda & Nagashima, 2005). Sesame seed cake and straw are used for animal feed and dye production (Aregawi *et al.*, 2013; Feng *et al.*, 2017). Western Tigray sesame is characterized as organic property with a single seed weight 0.003 g and a thousand seed weight of 2.8 g, a nutty, sweet flavour, and a creamy / dull white in colour (Araujo *et al.*, 2018). According to the Ethiopian commodity exchange (EXC) grading standard, impurity and colour are critical quality parameters for sesame grading and pricing. The under graded and grade 4 sesame were largely utilized in the local market for small scale oil extraction. Despite variations in cultivar, cultivation agro-climatic factors and practices, sesame seed has a high oil (36-60% dwb), protein with 18 amino acids, carbohydrate, crude fiber, and ash (Fasuan *et al.*, 2018; Gharby *et al.*, 2017; Onsaard, 2012). It's a good source of minerals especially Ca (1172.71-1225.7 mg/100 g), Zn (4.23-4.45 mg/100 g), and Fe (10.24-10.51 mg/100 g). Sesame seed also contains bioactive compounds such as phenolics (307.61-324.91 mg/100g), ferric iron reducing compounds (23.16-25.69 mg GAE/g) and anti-nutrients phytic acid, (32.31-34.53  $\mu$ . Mol/g) (Alyemeni *et al.*, 2011).

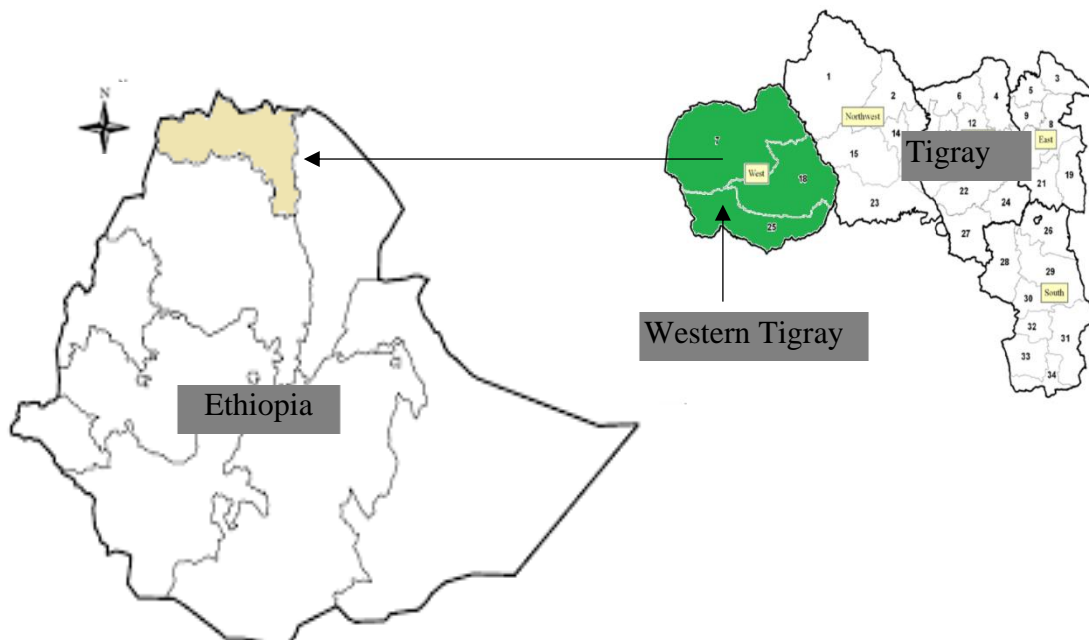
The optimal moisture content of sesame seed for storage is  $\leq 6\%$  and for oil extraction  $< 13\%$ . Moisture is an important factor for oil recovery, reduce residual oil and oil loss, pressing rate and power consumption, oil quality, and stability. Increased level of moisture also impacts to elevate the fire point, acid value, FFA and peroxide value (Udoh *et al.*, 2017). The breakdown of phytosterols increases with increased moisture content, temperature, and storage time (Gawrysiak-witulska *et al.*, 2012). The oil recovery of oil seeds and sediment content reduce as moisture content increases, although residual oil content and pressing rate increases (Singh *et al.*, 2002). The quality of Crude Sesame Oil (CSO) was affected by discoloration and odour development. The goal of this research was to perform hazard prevalence analysis (Figure 2.2, step 6) in CSO.

## **3.2 Materials and Methods**

### *3.2.1. Study Area*

The research was carried out in western (Mi'irabawi) zone of Tigray, Ethiopia, during the periods of 2019-2020 as shown in Figure 3.2, where sesame is largely cultivated, crude sesame oil

is extracted and consumed. Western Tigray is located in Northern part of Ethiopia at latitude  $17^{\circ} 1' 46.7673''$  N and Longitude E  $39^{\circ} 25' 46.9585''$  E. Western Tigray has an average annual rainfall of 500-700 mm and a warm climatic condition with temperature ranging from 18-38 °C. The soil is sandy clay with alkaline water. Western Zone of Tigray is located at 570 and 991 km far from Mekelle and Addis Ababa, respectively. It lies at  $13^{\circ}42'$  to  $14^{\circ}28'$  north latitude and  $36^{\circ}23'$  to  $37^{\circ}31'$  east longitude and share borders with Tahtay Adiyabo Woreda, Sudan, Amhara region and Eritrea in the East, West, South and North, respectively (Gebrekidan *et al.*, 2020).



**Figure 3. 2:** Map of the study area Western Tigray, Ethiopia

### 3.2.2. Survey Based Data Collection

Stakeholder characterization in the value chain and the sesame value chain were evaluated using a semi-structured questionnaire (appendix F and G). Following Bartlett *et al.* (2001) protocol, 320 stakeholders were selected through systematic random selection to complete the questionnaire according to the equation below. These stakeholders included producers of sesame oil, suppliers of sesame seeds, farmers, and regulatory bodies as shown in the equation below. After holding a round table discussion on the study's purpose, content, and clarity, the questionnaire was translated into Tigrigna so that the respondent could comprehend it. A semi-structured questionnaire and check list (appendix G) were created to assess employees' comprehension of the prerequisite programs that the food industry must adhere to. Response possibilities for the questionnaire were critical, minor, random, and not necessarily important. The

criteria that were deemed significant were taken into account when evaluating the suitability of the CSO extraction facility and hygiene requirements. There aren't many workers at the small-scale oil extraction facility. Twelve respondents include the proprietor, employees, a regulatory and health extension specialist completed the questionnaire.

$$n_o = \frac{(t)^2 pq}{d^2}$$

Where  $n_o$  is the sample size,  $t^2$  is the abscissa of the normal curve that cuts off an area  $\alpha$  at the tails = 1.96 at 95 % level of confidence,  $d^2$  is the acceptable margins of error for proportion being estimated = 0.003 desired level of precision,  $p$  is the estimated proportion of an attribute that is present in the population, and  $q$  is  $1-p$ .

### 3.2.3. *Determination of Moisture Content*

In this subsection, the proximate composition analysis procedures of sesame seed and CSO quality assessment methods presented. The moisture content was determined using the oven drying method of the AOAC, 930.15, 2016. This involved drying the samples in an oven until a constant weight was attained. First, the aluminium cup was cleaned, washed and dried in at 104 °C in an oven (Gallenkamp, model OV 880, England) for 30 minutes before being placed in a desiccator to cool. The samples ( $W_1$ ) were then carefully weighed (2 grams) using a digital electronic beam balance (ISTA, 195) and placed into an aluminium cup that had been dried. The samples, along with the aluminium cup with sample ( $W_2$ ), were the placed in the drying oven at 139 °C for 30 minutes before being cooled in a desiccator to room temperature. Finally, the moisture content of the sample was determined by weighing the dried and cooled samples along with the aluminium cup ( $W_3$ ) using digital electronic beam balance. The heating and weighing were conducted three times until a constant weight was obtained. The percentage of moisture content was calculated by expressing the weight loss as a percentage of the initial weight of the sample.

$$\text{Moisture Content (\%)} = \left[ \frac{W_2 - W_3}{W_1} \right] * 100$$

Where:  $W_1$ - Weight of sample (g),  $W_2$  - Weight of aluminium cup and sample,  $W_3$  - Weight of aluminium cup and the dried sample.

#### 3.2.4. Determination of Ash Content

The total ash content was determined using the muffle furnace (Gallenkamp, Model FSL 340-0100, U.K) method of the AOAC 923.03, 2016. Cleaned porcelain crucibles were dried for 30 minutes at 100 °C in an oven. The cleaned, dried crucible was weighted ( $W_1$ ) after cooling in a desiccator for 30 minutes and weighed. About 3.0 g of sample in a desiccator was weighed ( $W_3$ ) burned at hot plate starting from low temperature under a hood at 550 °C for 2 hr. The crucible was removed from the furnace and placed in a desiccator for 45-60 minutes to cool down. Finally, the crucible and burnt sample were weighed ( $W_2$ ). The total ash was calculated as a percentage on dry matter basis as follows:

$$\text{Total Ash (\%)} = \left[ \frac{W_2 - W_1}{W_3} \right] * 100$$

Where:  $W_1$  = Mass of the crucible (g),  $W_2$  = Mass of the crucible and Ash (g) and  $W_3$  = Mass of the crucible and dried sample (g)

#### 3.2.5. Determination of Crude Protein Content

The crude protein content was determined using the Kjeldahl method of the AOAC 981.10, 2016. Grounded sesame flour (0.5 g) was weighed into a digestion flask, and 3 g potassium sulphate and copper sulphate catalyst were added. Twelve millilitre (12 mL) of concentrated  $H_2SO_4$  were added, and the flask was shaken to uniformly mix the contents. The flask was then placed on a digestion burner for 1 hr and at 420 °C allowing digestion until the solution turned green and clear. The samples solution was transferred into a 100mL volumetric flask and filled to the mark with distilled water. Twenty-five (25) mL of 1% boric acid was pipetted into a 250 mL conical flask and 2 drops of mixed indicator (20 mL of bromocresol green and 4 mL of methyl red) solution were added and 15 mL of 40% NaOH solution was added into the decomposition chamber of the distillation apparatus. Ten millilitre (10 mL) of the digested sample solution was

then placed into a Kjeldahl flask. The condenser tip of the distillation apparatus was then dipped into the boric acid contained in the conical flask. The ammonia in the sample solution was then distilled into the boric acid until it completely changed to bluish green. Finally, the distillate was titrated with standardized 0.1N HCl acid to a reddish colour. Total nitrogen and crude protein were calculated using the following equation.

$$\text{Nitrogen (\%)} = \left[ \frac{(V-B) * N * 14.007}{W} \right] * 100 \quad \text{and} \quad \text{Protein (\%)} = \text{nitrogen (\%)} * 6.25$$

Where:

B = Volume of standard HCl solution used in the titration (mL) for the blank test

V = Volume of standard HCl solution used in the titration (mL) for the test material

N = Normality of standard HCl (0.1N) and W = Sample weight on dry matter basis and 14.007 = the molecular weight of nitrogen.

### 3.2.6. Determination of Crude Fiber Content

The crude fiber content was determined using the AOAC 962.09, 2016 procedure. About 1.5 g weighed sesame flour ( $W_1$ ) was transferred into a 600 mL beaker and about 200 mL 1.25% sulfuric acid was added and boiled for 30 minutes. After 30 minutes heating and keeping the level constant with hot distilled water, 20 mL of 28% KOH was added and boiled gently again for another 30 minutes. Subsequently, washing was conducted with 1% sulfuric acid and NaOH solution. After filtration, it was then dried in an electric oven (Mettler 854 Schwabach, West Germany) at 130 °C for 2 hrs. Furthermore, it was cooled at room temperature for 30 minutes in a desiccator and weighed after drying ( $W_2$ ) then transferred the crucibles to muffle furnace (Carbolite Aston Lane, Hope, S20 England.) for 30 minutes ashing at 550 °C. Finally, it was cooled again in a desiccator and reweighed after ashing ( $W_3$ ). The crude fiber content was determined by using the formula;

$$\text{Crude fiber (\%)} = \left[ \frac{W_2 - W_3}{W_1} \right] * 100$$

Where:  $W_1$  = the initial weight of samples,  $W_2$  = weight of crucible and residue after drying and  $W_3$  = weight of crucible and ash after incarnations

### 3.2.7. Determination of Crude Fat Content

The crude fat content was determined based on the Soxhlet extraction method of AOAC 920.39, 2003. Aluminium cup containing boiling chips was dried in an oven (Gallenkamp, model OV 880, England) for 30 minutes at  $102\pm 2$  °C, then allowed to cool at room temperature in a dissector for 30 minutes, after the weight of the cup ( $W_1$ ) was determined. The bottom of cellulose thimble was covered by fat free cotton. About 1-5 g of dried samples were weighed in the thimble ( $W$ ) and covered with fat free cotton at the top of the thimble. The thimble along with the sample was inserted into the Soxhlet extraction unit with the condenser connected and 70-90 mL hexane solvent in each cup was added using the connector on the top of extraction unit and solvent addition kit connected to dispenser. Then the program was started on the control unit by pressing the key. After all the process was finished the extraction cup was removed from the extraction unit. The extraction cup was dried at  $102\pm 2$ °C for 30 minutes in oven and the cup was cooled in dissector. After cooling the cup was weighed ( $W_2$ ).

$$\text{Crude fat (\%)} = \left[ \frac{W_2 - W_1}{W} \right] * 100$$

Where:  $W_1$  = Weight of the extraction cup (g),  $W_2$  = Weight of the extraction flask plus the dried crude fat (g), and  $W$  = Weight of the initial sample (g)

### 3.2.8. Determination of Total Carbohydrates

Total percentage of carbohydrates was determined by using difference method according to Onyeike *et al.* (1995). This method involves adding the total values of crude protein, crude fat, crude fiber, moisture, and ash constituents and subtracting it from 100. The value obtained is the percentage carbohydrate constituent of the sample. Total carbohydrate content of the samples including crude fiber was determined by subtraction of the above tested parameters from 100%. Total carbohydrates including fiber was determined by difference using the following formula: Total CHO (%) =  $100 - [\%MC + \%PC + \%FC + \%Fb + \%AC]$  where CHO is for digestible carbohydrate content, MC is moisture content, PC is protein content, FC is fat content, Fb is fiber content and AC is ash content.

### 3.2.9. Energy Calculation (kcal/100gm)

Energy content was obtained by multiplying the mean values of crude protein, crude fat and carbohydrate by the Atwater factors of 4, 9, 3.75 respectively, expressed kilocalories per 100 g as reported by Onyeike *et al.* (1995). The formula for calculating energy is shown below:

$$\text{Energy (kcal/100 g)} = (4 * \% \text{ Protein}) + (3.75 * \% \text{ CHO}) + (9 * \% \text{ Fat})$$

$$1 \text{ Kcal} = 4.18 \text{ kJ/100 g}$$

### 3.2.10. Fatty Acid Analysis

Fatty acid methyl esters (FAMES) were prepared following the procedure of AOAC 996.01 described by Satchithanandam *et al.* (2001). About 4 ml of 0.5 M sodium hydroxide in methanol was added to 1 ml oil sample, vortex mixed and incubated at room temperature for 20 minutes. About 5 ml of 15% boron trifluoride, freshly prepared in methanol was added to methylate the sample. After 2 min cooling, 5 ml heptane and 2 ml of saturated solution of sodium chloride were added. Then, heptane (15 ml) and saturated sodium chloride (40 ml) solution were added to the extract of FAMES, shaken, left undisturbed for 20 min, the upper phase was separated using a sterile syringe to GC vial together with 1 ml of methyl- undecanoate internal standard. Fatty acids were quantified from the FAMES using a gas chromatograph (Shimadzu Corporation, Tokyo, Japan) with a flame ionization detector (FID). Nitrogen was used as the carrier gas, while a fused silica capillary column HP-88 Agilent Technologies (100 m × 0.25 mm) and thickness of 0.2 μm was used for separation on the FAMES. The capillary column was coated with a stationary phase (88% cyanopropyl, aryl-polysiloxan). The injection volume (1.0 μL) and injector temperature was set at 250 °C while the GC column temperature was programmed at 120 °C for 5 minutes and increased to 240 °C at the rate of 4 °C/minutes for 30 minutes. Identification of the fatty acids was performed by comparing their retention times with the internal standard. Quantitation was by comparison of the peak areas with that of the internal standard expressed in percentages. The calibration curve from the standard solution was used to calculate the response factor and number of fatty acids as follows.

$$R_i = \frac{(A_i)(W_{C13:0})}{(A_{13:0})(W_i)} \quad \text{and} \quad F_i = \frac{(A_i)(W_{C13:0})}{(A_{13:0})(R_i)}$$

Where:

- $R_i$  = response factor for each fatty acid;
- $F_i$  = Amount of individual fatty acid;
- $A_i$  = peak area of the individual fatty acid in the standard;
- $WC_{13:0}$  = weight (mg) of  $C_{13:0}$  in the standard;
- $A_{13:0}$  = peak Area of  $C_{13:0}$  FAME in the standard;
- $W_i$  = weight (mg) of individual FAME in the standard;
- $F_i$  = Amount of individual fatty acid
- $A_{C_{13:0}}$  = peak area of internal standard ( $C_{13:0}$ ) in test sample

$$\text{Total fatty acids calculated as: } Total\ Fat, \% = \left[ \frac{\sum F_i}{W_{sample(g)}} \right] * 100$$

Saturated fat, is the summation of fatty acids with not double bonds in the chain of fatty acids calculated as;

$$Saturated\ Fat, \% = \left[ \frac{\sum \text{staturated fat } F_{iFA}}{W_{sample(g)}} \right] * 100$$

The monounsaturated fat, the summation of fatty acids contains single double bonds in their chain structure;

$$Monounsaturated\ Fat, \% = \left[ \frac{\sum \text{monounstaturated fat } F_{iFA}}{W_{sample(g)}} \right] * 100$$

The polyunsaturated fat, the summation of fatty acids contains more than one double bonds in their chain structure;

$$polyunsaturated\ Fat, \% = \left[ \frac{\sum \text{polyunstaturated fat } F_{iFA}}{W_{sample(g)}} \right] * 100$$

Omega-6 to omega-3 ratio was calculated as

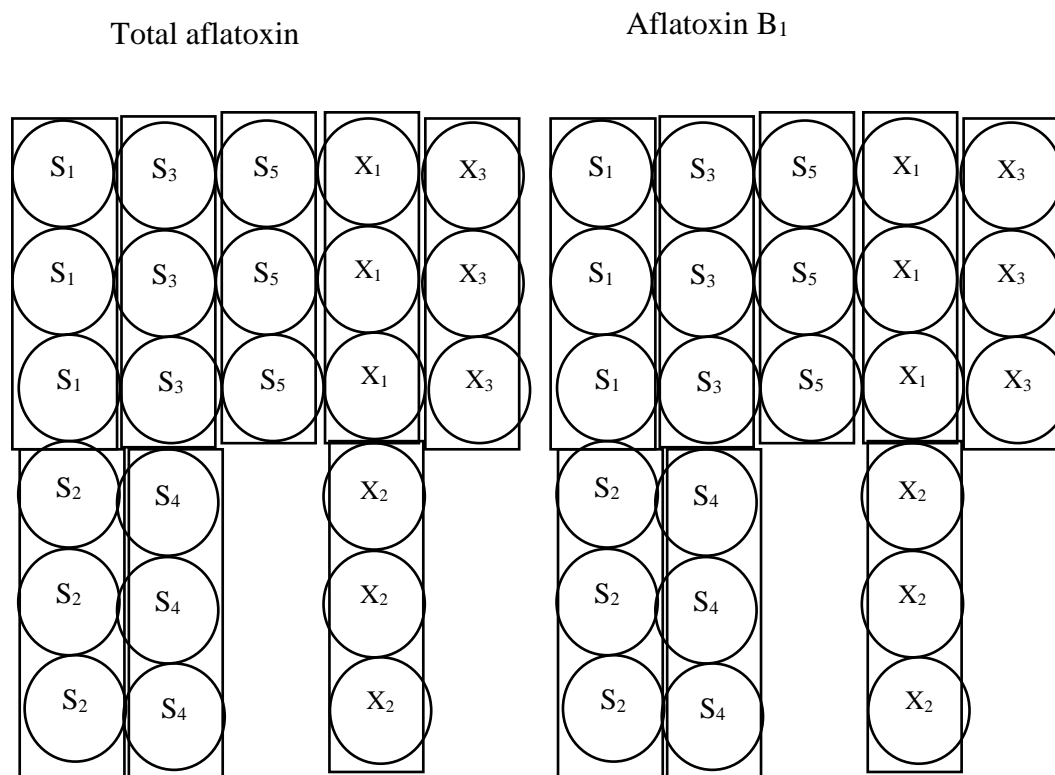
$$\frac{\text{Omega} - 6}{\text{Omega} - 3} = \sum \frac{\text{Omega} - 6 \text{ fatty acids}}{\text{omega} - 3 \text{ fatty acids}}$$

### 3.2.11. Aflatoxin Analysis

The Hazard team conducted a hazard analysis according to Figure 2.2, Principle 1). Hazard analysis is a pillar for HACCP plan implementation, and includes the HACCP team identifying and analysing a long list of hazards following the unit operation during crude

sesame oil extraction. The hazards identified included aflatoxin, pesticides residue, chloride residue and microbial hazards were analysed in the following section.

Sesame seed sample was randomly taken from the crude sesame oil (CSO) plant store in a cotton bag of 50-100 g. The CSO was sampled in a blue-brown glass bottle package after extraction and sesame seed cake in polyethylene bag from the oil extraction plant. Aflatoxin were determined using the enzyme-linked immunosorbent assay (ELISA) (Helica Biosystems Inc., Santa Ana, California, USA) as described by Leszczyńska *et al.* (2001), arranged as shown in Figure 3.3. A 20 g sample of sesame seed and sesame seed cake was grounded so that at least 50% of the grounded sample passed through a 20-mesh screen, and a 20 ml CSO was centrifuged to remove precipitates. The samples were homogenized for 5 minutes in 100 ml of solvent (70% methanol: 30% distilled water). To obtain a clear sample extract for testing, the mixture was filtered through Whatman qualitative filter paper grade 2 (125 mm diameter). One (1ml) of the clear sample extract was transferred to a test-tube, and 3ml phosphate washing buffer (pH = 7.2) were added. The well stripe was labelled as 2, 10, 30, 50, 100 ppb for total aflatoxin (TAF) and aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) analysis, crude sesame oil (CSO), sesame seed (SS) and sesame seed cake (SSC) in triplicates for total aflatoxin and aflatoxin B<sub>1</sub> analysis, as shown in Figure 3.3. Aflatoxin standard solution (50 µl) (Aflastandard for total aflatoxin and aflatoxin B<sub>1</sub> standard for AFB<sub>1</sub> analysis) was added, 50 µl extract sample, and 50 µl aflatoxin-peroxidase conjugate enzyme, and 50 µl of mouse antibody solution against aflatoxin. The mixture was incubated in the dark at room temperature for 30 minutes. The well was washed with phosphate washing buffer (pH = 7.2) five times, then taped on tissue paper to remove residue of the washing buffer. Substrate (Tetramethylbenzidine and urea peroxide) (100 µl) was added to each well which interacts with the conjugated enzyme to produce blue colour, incubated at room temperature in the dark for 5-10 minutes. Reaction was stopped by adding 100 µl stopping solution (sulphuric acid) into each well to change the colour in to yellow, absorbance measured at 450 nm wave length. The total aflatoxin and aflatoxin B<sub>1</sub> were calculated from the standard curve as follows;  $LOD = [b_o + 3S(b_o)]/b_1$  and  $LOQ = [b_o + 10S(b_o)]/b_1$  Where: LOD: level of detection, LOQ: level of qualification, b<sub>o</sub>: response of the blank sample (intercept of the calibration curve), S<sub>b<sub>o</sub></sub>: standard deviation of the blank, b<sub>1</sub>: sensitivity (calibration model slope).



**Figure 3.3:** Standards and sample spiking where  $S_1 = 2$  ppb  $S_2 = 10$  ppb  $S_3 = 30$  ppb  $S_4 = 50$  ppb and  $S_5 = 100$  ppb for total aflatoxin and aflatoxin B<sub>1</sub> and  $X_1 =$  sample 1,  $X_2 =$  sample 2 and  $X_3 =$  sample 3

### 3.2.12. Pesticide Residue Analysis

The pesticide residue Analysis was carried out in accordance with the method described by Wumbei *et al.*(2018). About 5-10 g of the grounded sesame seed sample was poured into 50 ml glassware tube. On top of the sample, 15 ml of 1% glacial acetic acid/acetonitrile (MeCN) was added. The glassware tubes were tightly capped, and shaken for 1 minute to confirm contact between the solvent and the sample matrix. Salt/buffer phase was poured slowly in to the MeCN layer and mixed for 30 seconds until all powder reagents combined with the liquid layer. The mixture was homogenized for 15 minutes on a mechanical shaker and centrifuge (10,000 rpm) for 5 minutes. One millilitre (1 ml) from the top MeCN layer was transferred to a 15 ml clean-up glass ware tube containing appropriate amounts of dispersive solid-phase sorbents and injected into a liquid chromatography – mass spectrophotometer (LC-MS) with electrospray ionization interface.

The column mobile phase of the LC-MS was water, with injection volume (10  $\mu$ L) and an oven temperature (40  $^{\circ}$ C). The first mobile phases was Water + 10 mM ammoniumacetate, while the second mobile phase was acetonitrile + 0.1% formic acid at a flow rate of 0.4 ml/min. Blank samples were spiked with 100  $\mu$ L of mixed standards of the four compounds of Malathion, Fenitrothion, diazinon and Carbaryl in 5 replications. The maximum tolerable level varies depending on the product and types of pesticide. The most prevalent MRL in most commodities and pesticide residue is 0.05 ppm, set by codex Alimentarius (Mekonen *et al.*, 2014).

### 3.2.13. Chlorine Residue Analysis

Water sample was collected from the housing pipelines and ground water in 125 ml brown glass bottles. The bottle was cleaned with phosphate free detergent and tap water, then rinsed and dried with distilled water. According to Meride and Ayenew (2016), the PH and temperature of water were measured using a pH meter and thermometer, respectively, and the total dissolved solids (TDS) were measured using a gravimetric method. Water chloride residue of water was tested using the USEPA method 551.1 described by Baird *et al.* (2017). Before sampling, 1.5mg of ascorbic acid was added to the bottle to eliminate any residual chloride and halt additional chloroform formation. Water was sampled in a bottle with no air bubbles and stored in the dark between 0 and 4  $^{\circ}$ C. Water samples were filtered (45 $\mu$ m millipore), and absorbance was measured using UV/visible spectrophotometry at 254nm with 5mm optical path quartz cells.

### 3.2.14. Microbial Hazard Analysis

To determine the prevalence of microbiological hazards, sesame seed, CSO, and water were collected. The source of those hazards was traced from the environmental air, the surface of the machinery and premises, and protective clothing. Sesame seed (100 g) was taken from the CSO extraction stock with a grain sampler from the bottom, top, and middle of the package, harvested during the 2019/2020 of June-September in western Tigray (latitude 14.032334 and longitude 38.316573, 17.5 – 41.7  $^{\circ}$ C). The seed was ground using a mortar and pestle. About 10 g of grounded sesame seed was mixed with 90 ml peptone water, diluted, aseptically transferred to the appropriate agar containing petri dish. CSO (100 ml) was sampled in a blue-brown glass, and 1 ml of CSO was aseptically transferred for microbial prevalence testing. After 1-2 minutes of flow, pipe Water was sampled, and groundwater was fetched through a hole in a polyethylene plastic

placed in an ice-pack and sampled within 2 hours to determine microbiological purity. The milling and pressing, filtration, and filling machines, sesame store floor, and extraction room wall were all swabbed. Using a moistened cotton swab in buffered peptone water, swabbing was performed 10 by 10 cm in difficult to clean or reach surfaces in two perpendicular directions. A peptone water moistened cotton swap scraped, put, and kept for 5 minutes was used to sample the protective gown from the inner underarms and the inner side of the clove. The swab sample was placed in a sterilized plastic bag, break-off in a test tube according to the method described by Mikelonis *et al.* (2020). The indoor and outdoor air sample was collected using a passive sampling during/after production 1 m above the floor and 1m away from the wall in an agar media containing petri dish (140 mm diameter) left open for 1 hour according to the procedure described by Napoli *et al.* (2012). Incubation for microbial cultivation was performed as shown in Table 3.1.

**Table 3. 1:** Summary of microbial assessment methods applied to assess CSO microbial quality and CSO extraction plant establishment

<b>Microbial cultivation</b>	<b>Agar</b>	<b>Incubation condition</b>	<b>Method</b>
Aerobic plate count	Pour plate methods in a Plate count agar (AOAC 966.23)	37°C / 48 h	Hong <i>et al.</i> (2008)
Total Coliforms	Pour plate method in a Plate count agar (ISO 9308-1:2014)	37 °C/ 48 h	Wohlsen <i>et al.</i> (2006)
Escherichia coli	Pour plate method in a MacConkey agar	44.5 °C / 24 h	Splichalova <i>et al.</i> (2014)
Fungi	Spread plate method in a Rose Bengal Chloramphenicol Agar	22°C / 7 days	AOAC, (2002)
Aspergillus fungi	Sabouraud Dextrose Agar chloramphenicol powder as anti-bacterial growth inhibitor	25°C/5-7 days	Visagie <i>et al.</i> (2014)
Salmonella and Shigella.	Xylose lysine desoxycholate agar with Rappaport-Vassiliadis Soy Peptone Broth	37 °C/ 18 h	Mengist <i>et al.</i> (2018)

<i>Staphylococcus aureus</i>	Mannitol Salt Agar (Oxoid), gram staining, coagulase test	32 °C / 48 h	Reda <i>et al.</i> (2017)
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### 3.2.15. Physical Hazard Analysis

The extraneous materials such as bone chips, metal flakes or fragments, sand/mads, pieces of product packaging, stones, glass, or wood fragments, insects or other filth, personal items, or any other foreign materials was analysed by visual inspection.

### 3.2.16. Hazard Preventive Measures

The preventive measures for each hazard identified in the CSO extraction process following the process flow diagram as presented in Figure 3.8 was established based on the experimental, literature and legislatives for food.

### 3.2.17. Data Analysis

The descriptive data was produced using IBM SPSS Statistics version 22, and it is shown in Table 3.3. In order to assess the adequacy of the CSO extraction plant and sanitary conditions (pre-requisite program), a sample Kolmogorov-Simonov non-parametric test method was used. Chemical hazards (Total aflatoxin, aflatoxin B<sub>1</sub>, four pesticides and free chloride residue) data were organized in an excel sheet. Microbial colonies were enumerated using an illuminated magnifying colony counted, organized in an excel Microsoft transformed into log<sub>10</sub> Colony forming units. The microbial colony was expressed as log<sub>10</sub> CFU/g for sesame seed, log<sub>10</sub> CFU/ml for CSO and water, log<sub>10</sub> CFU/m<sup>2</sup> for swab samples, and log<sub>10</sub> CFU/m<sup>3</sup> for air sample. The APC, total Coliforms, fungi, *Aspergillus species*, *staphylococcus aureus* count colonies were tabulated and converted log<sub>10</sub> colony forming units in an excel sheet. Data analysis was performed using analysis of variance (ANOVA) in a SAS V8 software to separate the means and standard deviations. Tukey was used to test for statistical significance difference at a P-value of 0.05.

## 3.3. Results and Discussion

### 3.3.1. Sesame Value Chain

In contrast to other crops and livestock, the sesame value chain is well structured. Sesame farming, harvesting, storage, value addition, processing, primarily edible oil extraction with

sesame seed cake as a by-product and distribution are presented in Figure 3.7. Land preparation, access to improved seed and fertilizer, cultivation, weed prevention and pesticides use, labour, and technology utilization are aspects of sesame plantation and production. Farmers interviewed stated that rainfall fluctuation, weed, environmental conditions such as unexpected rainfall and wind at the stage of maturity, insufficient labour, or a lack of harvesting machinery were the major obstacles. Sesame harvest readiness indicators are the colour of the capsules or aging of the sesame leaves. Cuts of sesame bundle locally named as “hilla” kept in the field for 15 days to dry and facilitate seed separation from the parent plant. However, this post-harvest treatment caused the seed to be susceptible to insect infestation such as termite, sesame webworm, sesame seed bug, and gall midge. These pests lead to huge post-harvest loss and quality defect of the seed.

Other studies have reported that sesame is sensitive to a variety of factors in the field, storage, marketing and processing including seedling rate and plantation period (Thandar *et al.*, 2010) and growing location (Gharby *et al.*, 2017). It is also sensitive to pests such as webworm *antigastra catalaunstra* (Duponchel) (Gebregergis *et al.*, 2018), Indian Meal Moth *Plodia interpunctella* (Lepidoptera) (Gebremichael, 2017), Cotton Mealybug *Phenacoccus solenopsis* Tinsley (Gebregergis, 2018), sesame seed bug, *Elasmolomus Sordidus* F. (Berhe *et al.*, 2008), toxigenic microorganisms such as fungi and fungi metabolites (Ezekiel *et al.*, 2014; Idris *et al.*, 2013). The insect damages the crop and reduces seed quality, resulting in considerable post-harvest loss (~24%) (Gebretsadik *et al.*, 2019). To reduce loss and quality defect, studies suggested the bundles should be placed in a clean plastic mat and pesticides be used around the bundle to prevent insect attack. However, the mats were prohibitively expensive for the farmers. After 15 days of drying alongside parent plant, the seed is threshed and separated on a plastic mat. However, During harvesting and early post-harvest practices, around 17% of sesame was lost due to inappropriate farming technologies, delayed harvesting time, poor handling of the crop and farm winnowing leading losses (Neme *et al.*, 2020). As shown in Table 3.2 and Figure 3.4, sesame seed is sorted and graded against sesame growing place, impurity and colour for storage and marketing. White sesame seed is most preferred and grade 1- 3 sesame seed is considered export grade. The impurities mainly sand and soil, small and larger sesame plant parts, and weed cleaned for storage. The whitish *Humera* sesame is premium quality considered as grade one with less than or equal to 1 % total impurity and less than or equal to 1 % non-white contrasting sesame seed colour. The reddish sesame seed is also considered as quality seed. However reddish sesame seed requires

dehulling. The under-grade sesame seed is mostly utilized in the local market and relatively cheaper.

**Table 3. 2:** Sesame seed grading parameters in western Tigray based on the Ethiopian commodity exchange

<b>Parameters</b>	<b>G<sub>1</sub></b>	<b>G<sub>2</sub></b>	<b>G<sub>3</sub></b>	<b>G<sub>4</sub></b>	<b>UG</b>
<b>Whitish Humera and reddish sesame seed</b>					
Total Impurity (Foreign Matter and DSW) Max % by weight	1	3	5	7	15
Contrasting Colour, max % by Weight	1	2	4	6	7
<b>Mixed Humera and Mixed Reddish Sesame Seed</b>					
Total Impurity (Foreign Matter and DSW) Max % by weight	1	3	5	7	15
Contrasting colour, max % by Weight	> 7				

DSW: Damaged, Shrivelled, Weevil bored, UG: under grade sesame seed, G<sub>1, 2,3,4</sub>: Sesame grade

Sesame storage is important to the overall sesame quality. Farmers store grain, including sesame seed, in subterranean pits known as “Bahiri Hidmo”, which has been practiced for centuries. Crops filled in a local silo built from animal wastes called as “Kofo” placed inside the subterranean pits believed to balance the storage environment and protect from insect and rodent entry. However, because of the high gradient heat due to lack of ventilation and gas accumulation, the underground subterranean pit storage has an impact on the overall quality such as seed colour, taste and odour change, which has a substantial impact on market value and safety of sesame seed (Kuyu & Bereka, 2020). As a result, home-based underground pit storage has been gradually replaced out in favour of warehouses or other ventilated environments arranged in sack with/without plastic laminated from inside to prevent moisture migration.

Sesame farmers and traders kept sesame in bulk in the warehouses, as indicated in Figure 3.5, depending on the level of productivity and end use of sesame. The warehouses are located in unsanitary environment where the external environment is full of grasses and trees that help to hide the insects and rodents. The humid and warm climatic condition creates conducive environment and encourage the survival/growth of microorganisms and entry of macro-organisms, which increases post-harvest and quality loss. As a result, registered and unregistered pesticides has been used to prevent insect and rodent attack and microbial growth. Meanwhile, the regulatory and agricultural extension system have been challenged as a result of the illegal pesticides from

entering from the bordering market. Furthermore, the application and conditions of pesticide use were random, without understanding the consequence of using them and health risk (Mekonen *et al.*, 2014; Mengistie *et al.*, 2017).

Sesame stored in a stainless-steel storage warehouse packed in polypropylene bags has a significant weight loss rate. According to Neme *et al.* (2021), sesame stored in polypropylene bags lost up to 17% of its weight after 12 months of storage, but sesame stored in metal silos, grain pro bags, and Purdue improved crop storage bag lost 2.4, 3.4, and 1.7%, respectively. Monitoring the storage condition and selection of packaging within the storage warehouse is important to reduce post-harvest loss regardless of the differences in crops (Kumar & Kalita, 2017). The temperature inside the stainless-steel warehouse was quite high in a humid environment, which is ideal for microbial growth including fungi and fungi metabolites. Furthermore, the warehouse's external environment was devoid of grass, shrubs, and trees, which eases to hide out macro-organisms such as mice and rats, birds, and termites and attack sesame during storage. On the other hand, warehouses with concrete walls, smoothly cemented floor and walls were clean with enough space for ventilation. Sesame packed in a polypropylene 100 kg bags arranged in the concrete warehouses with adequate space and ventilation reduce storage losses. Figure 3.4 depicts the unprocessed sesame seed value addition process, which includes cleaning and colour sorting, and as well as a de-hulling process that meets sanitary and phytosanitary requirements. Sesame value addition carried out to meet the grading specification as presented in Table 3. 2, where impurity sorting, colour sorting and dehulling value addition carried as presented in Figure 3.4, which determines the economic value of sesame seed at diverse market segments. Sesame agriculture is regulated by regulatory agencies, and farmers are trained to avoid using unregistered or unpermitted pesticides on the farm, after harvest field drying, and storage. Studies in Myanmar suggested that integrated sesame agricultural and value chain is critical to improving sesame yield and reducing sesame loss (Myint *et al.*, 2020).

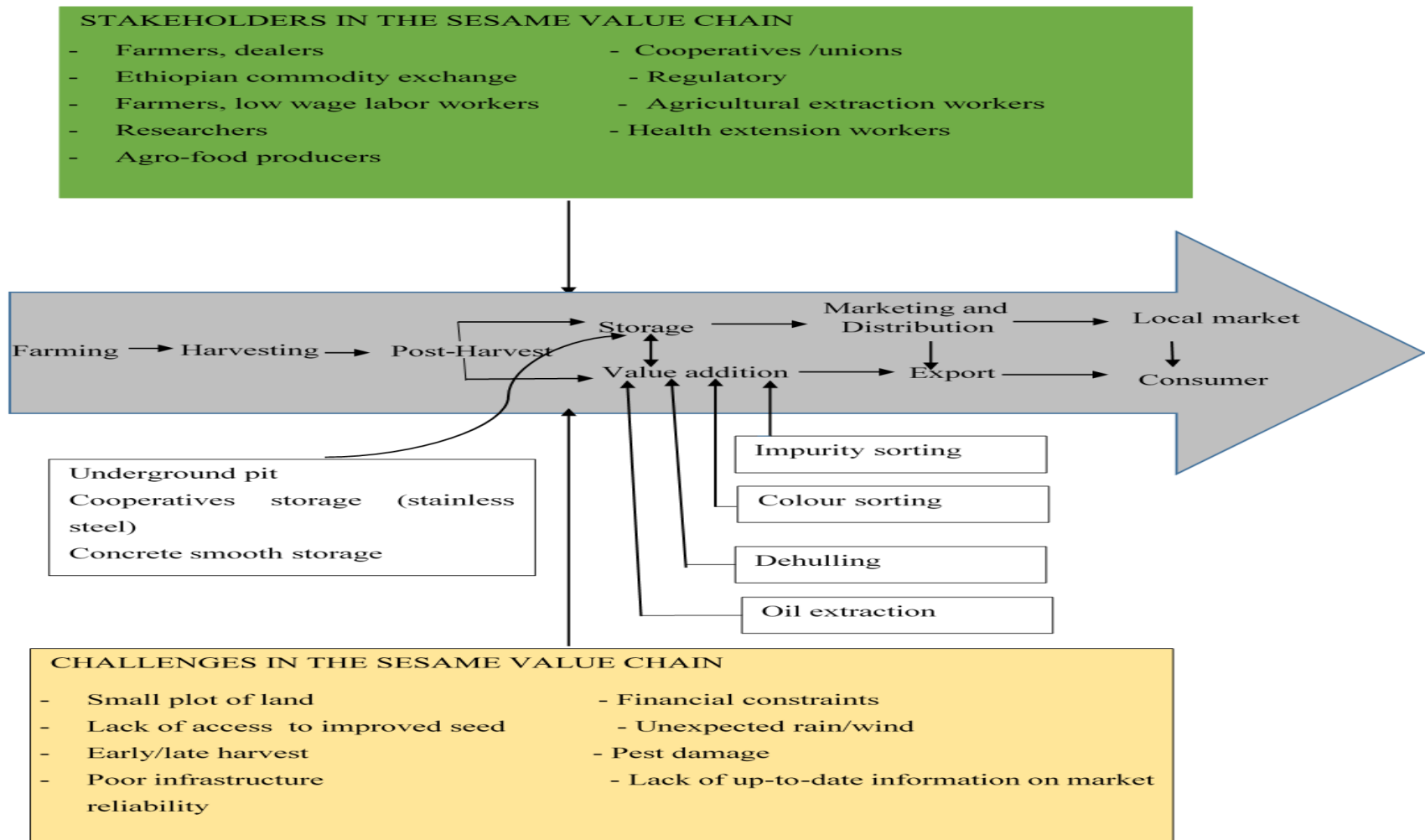


**Figure 3. 4:** Sesame value addition (a) laboratory-based homogenizer to screen and grade sesame based on impurity level, (b) Industrial based sesame sorting based on colour and (c) industrial based sesame de-hulling

Ethiopian commodity exchange (EXC) certifies sesame by inspecting the quality and grading based on impurity levels and contrasting colour, presented in Table 3.2, and identifying the appropriate market channel. Sesame marketing continues to grow, on the other hand, sesame quality and market value defects are noticeable due to inadequate value addition, illegal broker interference, lack of storage and transportation, poor infrastructure, disparity between stakeholders, limited access to market, and low price. Besides export earnings from the unprocessed seed, sesame is locally used for food and non-food application. It is a primary ingredient in the extraction of refined and unrefined edible oil. Sesame seed cake is a by-product of edible oil extraction, which is used to make animal feed and soap. Sesame straw utilized as animal feed and as a source of biomass energy sources in the cement industry. The sesame value chain is summarized in Figure 3.6.



**Figure 3. 5:** Sesame seed storage warehouses in western Tigray, Ethiopia



**Figure 3. 6:** Schematic description of Sesame Value Chain in Western Tigray, Ethiopia

### 3.3.2. Stakeholders' Characterization in the Sesame Value Chain

The demographic factors of stakeholders include gender, age, educational background, experience and their role in the sesame value chain are presented in Table 3.3. Farmers, researchers, regulatory authorities, technical experts, and traders with active and passive participation in the sesame value chain are the stakeholders. Studies reported that, the composition, educational status, and experience of stakeholders was substantial for improved farming, value addition, and resource utilization in the sesame value chain (Mezgebo *et al.*, 2021). Stakeholders were gender-inclusive (37.2% women, 62.8% men), with 26.7% of the stakeholders being farmers of different scales of farming. More than 74% of the stakeholders were above 30 years of age, which contributes to an effective sesame value chain. Experience of stakeholders was reported for an effective resource utilization and manage the value chain (Amare *et al.*, 2019). Small-scale farmers and low-wage employees made up the majority of those with no formal education estimated to be 25.6%. Farmers and low-wage workers use outdated technology and conventional farming methods, according to studies, are at a disadvantage (Gebremedhn *et al.*, 2019; Gebretsadik *et al.*, 2019) Farmers' farming practices were laborious and time-consuming, especially for small farmers lacked or could not afford to rent a sesame driller/ploughing machine (Gebre, 2020). Aside from the economic benefit, safety may be jeopardized due to the emergence of toxigenic microorganisms and associated metabolites, chemical either used to improve productivity, prevent venter diseases or insect attach, consistent with other studies (Luo *et al.*, 2018; Scaramuzzi *et al.*, 2020; Velmourougane *et al.*, 2014). Stakeholders in the sesame value chain operate their routine practices with inadequate support and lack of training (18.9%) on pesticide use, getting access to improved sesame seed variety, pest, disease and weed prevention, sesame harvesting and handling, value addition, and market linkage consistent with the reports of Gebregergis *et al.* (2018).

Agricultural extension, soil and water conservation, pest control, post-harvest management, community health, and post-harvest technologies were among the technical experts surveyed (27.2 percent). Regulatory (8.9%) came from health extension, agricultural specialists to manage pesticide usage, post-harvest technologists, and food scientists to regulate agro-processing, including sesame-based product processing. A multi-disciplined researcher (8.2%) with the goal of increasing sesame productivity, introducing new and diseases resistance sesame breeds (Golla

*et al.*, 2020), post-harvest handling, and loss reduction, and value addition. Studies revealed that the causes and extent of post-harvest loss, as well as the introduction of best practices with the aim of improving the sesame value chain and food security, were carried out in the sesame value (Gebretsadik *et al.*, 2019; Neme *et al.*, 2020; Neme *et al.*, 2021).

Sesame seed market dealers make up the 22.2% of the stakeholders participated, collect and supply sesame to the worldwide market via the Ethiopian commodity exchange office (EXC, market intermediary, 6.7%) and the local market. Sesame market dealers though face challenges on getting timely sesame market information included obtaining reliable and timely market information, financial constraints, deviation price setting, and identifying marketing channels (Gebretsadik, 2020; Goshme *et al.*, 2018). Sesame traders collect the seeds and store in a thin metal sheet-built storage warehouse or well-cemented block warehouses. The metal (stainless steel) warehouses were not clean of grasses and bush around. While, sesame storage in a warehouse builds of blocks, cemented floor, inlet/exit door, and windows with sufficient space and a recommended size dimensions did not have grasses and bush. However, in addition to the field loss, extended storage, loading and unloading, transportation, and packaging register significant economic losses.

**Table 3. 3:** Characteristics of stakeholders involved in the sesame value chain in western Tigray, Ethiopia (n=320)

<b>Variables</b>	<b>Frequency</b>	<b>Percent</b>	<b>Variables</b>	<b>Frequency</b>	<b>Percent</b>
<b>Gender</b>			<b>Role of stakeholders</b>		
Women	119	37.2	Market intermediary	21	6.7
Men	201	62.8	Traders	71	22.2
<b>Age (years)</b>			Farmer	86	26.7
18-25	7	2.3	Regulatory authorities	29	8.9
26-30	75	23.3	Technical expert	87	27.2
> 30	238	74.4	Researcher	26	8.2
<b>Experience (in years)</b>			<b>Communication</b>		
< 1	15	4.7	Telephone	160	50
1-2	60	18.6	Letter	40	12.5
2-5	119	37.2	Telephone, Letter and		
> 5	126	39.5	Internet	120	37.5
<b>Educational Level</b>			<b>Management Support</b>		
Upton 7 <sup>th</sup> grade	82	25.6	No	60	18.9
8 <sup>th</sup> -12 <sup>th</sup> grade	90	27.9	Yes	260	81.1
Certificate and above	150	46.7			

### 3.3.3. HACCP Plan Development in the Crude Sesame Oil Plant

At this section the prerequisite (PRPS) and operational prerequisite programs (OPRPs) during Crude sesame oil extraction are presented.

### 3.3.4. HACCP (Operational) Pre-requisite Programs

According to the WHO, pre-requisite programmes are defined as the recommended practises for safer and quality food production and handling in a food system (Mortimore & Warren, 2014) and a foundation for HACCP implementation (Everstine, 2017; Lelieveld *et al.*, 2014) to prevent biological, physical and chemical contaminants (Rossi *et al.*, 2018). In chapter 3 the pre-requisite programs in the crude sesame oil plant, which compromise supplier quality assurance, equipment installation, raw materials and supply specification are summarized. The oil extraction plant is closer to infrastructure, water and power supplies, as well as a road, but it is hampered by urbanization and inadequate space for sesame seed and CSO storage. In addition to that, the premises was not designed for food processing. Meantime, dusty non-food processing enterprises such as wood and metal work, near the CSO extraction plant, have a negative impact on the sanitary conditions and jeopardise the safety conditions. The external environment of the oil extraction plant was dry, windy, dusty, with weeds, brush, and litter was gathered, creates a convenient environment for macro-organism to grow, multiply and hide, thereby disrupting the overall sanitary condition and creates conducive conditions for microorganism growth. The muddy external premises allowed water stand the drainage system was not as per the recommendation.

As a result, fumigation carried out in the indoor and outdoor premises and track to prevent the pests and other macro-organism, in accordance with the manufacturer's instructions. Pests in the area identified were ant, cockroach, rats, dog and cats. However, methyl bromide and other fumigants causes health problems in employee's nervures system, pollute the environmental, and pose a safety risk as a residue in the food crops has been utilized (Park *et al.*, 2020). According to Hashimoto *et al.* (2020), a microencapsulated allyl isothiocyanate fumigant with no or little health risks and the ability to kill/prevent pest. The CSO extraction plant's interior was well-cemented, with an acidic or alkaline resistant floor and adequate windows to allow a controlled light passage and ventilated air sealed to prevent the entry of insects and larger macro-organisms. The raw material entry door and final product distribution, on the other hand, were the same. Worker's

deficiency in understanding of external and internal premises and cleanness standards, pest control, and process quality continue to be a barrier in maintaining food safety. Wanniarachchi *et al.* (2016) and Okpala and Korzeniowska (2021) addressed how the ethics, knowledge and skills of designers and workers are being challenges to meet food safety standards in the design, layout and hygienic practices of food processing facilities.

Personal hygiene includes control of belonging (jewellery, phone and personal and protective clothing) and inadequate practices were the most current concerns of safety problems. Gender, educational background, and other demographic characteristics, on the other hand, influenced personal hygiene (Adikari *et al.*, 2016; Barjaktarovic-Labovic *et al.*, 2018). According to studies, personal belonging such as mobile phone and jewellery are prohibited in the production area because they interfere with workers cleanliness and productivity (Ismail *et al.*, 2016). Unequipped hand washing infrastructure with inadequate supplies of soap and paper towel, and money transaction are also challenges. Inappropriate hand washing between food preparations, during money transactions, lavatory breaks, uncovered air, and inadequate access to water and power increases the risk of food safety (Cortese *et al.*, 2016). Signs of safety preconditions were introduced for workers and visitors not to touch oil and apparatus in direct contact with the oil with bare hands, and to wash up before and after production and lavatory visit. Visitors who were coughing or sneezing were not permitted to enter production facility. During sesame reception and processing, no specification was adopted, although the first-in and first out rule was maintained to avoid cross-contamination. The build-up of oily dirt on the surface of machines and the interior premises explains the inadequacy of cleaning and cleanliness, which encourages the growth of microorganisms and biofilm formation, affecting the quality and safety of CSO. Food safety awareness provides a basis for safer and healthier food distribution, both with and without formal education (Barjaktarovic-Labovic *et al.*, 2018) is a foundation for safer and healthier food distribution (Angelos *et al.*, 2016; Angelos *et al.*, 2017). The PRPs/OPRPs assessment and employee awareness on what PRPs/OPRPs in the food business were conducted according to the code of practice general principles of food hygiene (CAC/PCP, 2003; CAC/GL, 2013) and questionnaires which includes the premises and structure, employee facilities, overall hygienic practices and GMPs. PRPs criticized as critical, minor and randomly considered during designing and practices. The major area of consideration for the PRPs and suitability for the CSO extraction plant were presented.

### 3.3.5. *HACCP Team Assembly*

A team of five individuals was assembled consisting of business owner, mechanical engineer, food scientist, operator and regulatory expert. The purpose of the study was presented, commitment and regulatory assistance was requested, and the oil extraction plant/manager owner consent was signed. The team was trained and consulted to develop and test HACCP plan for CSO following crude sesame oil extraction flow diagram.

### 3.3.6. *Suitability of Establishment for CSO Extraction and Hygienic Condition*

Conformance criteria for food extraction and processing facility suitability include site selection and plant construction, process performance and product quality, hygienic practises, and pest control, as shown in Table 3.4 to Table 3.7 and Figure 3.7. These criteria are important for assessing the suitability of the establishment and activities within it. Because to a lack of awareness, insufficient space, poor technology, and poor infrastructure, even the best designed prerequisite programmes did not meet the recommended level. Poor hygienic conditions and practices, a lack of / inconsistent inspection, and insufficient process control, recall procedure, and pest management are all disadvantages that have a substantial impact on overall good manufacturing practises. Workers' understanding of precondition practises and the function of PRPs in ensuring safer food production and distribution is lacking, as shown in Figure 3.7. Employees' lack of awareness is primarily caused by poor management, unprofessional employment, a lack of training, sloppy regulations, and inadequate infrastructure. Figure 3.7 depicts the awareness of workers regarding the PRPs and OPRPS to ensure safe oil production. Regardless of the qualification workers have poor knowledge on premises standards for food production, raw and fished product handling and process, hygienic practices and pest control.

### 3.3.7. *Crude Sesame Oil Extraction Plant Site Selection*

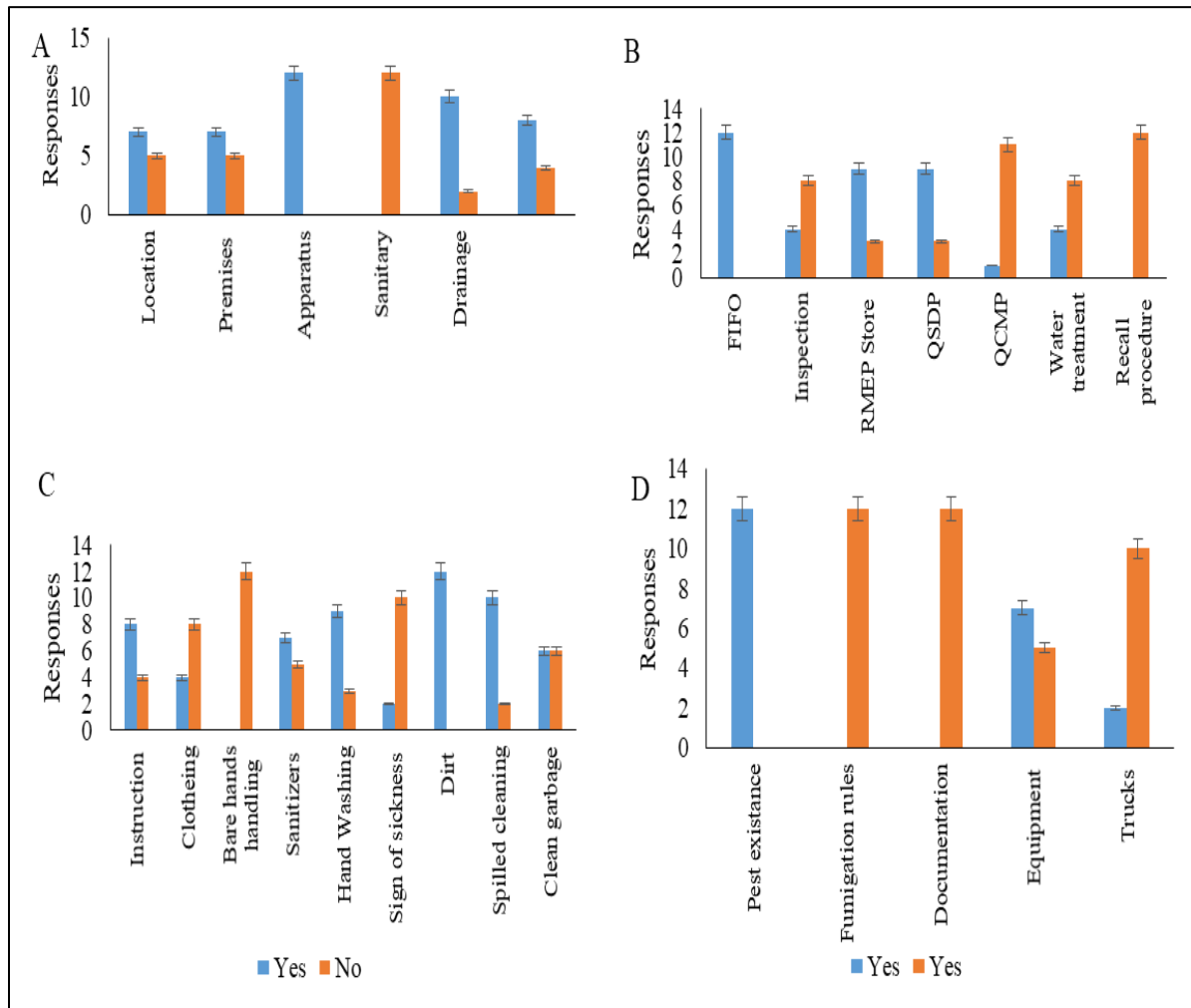
Figure 3.6 elucidates that knowledge of workers and across directly or indirectly involved in the Agro- processing industries. The worker knowledge is limited or twisted on how and where the oil extraction plant should be located. The site where the oil extraction plant selected partially meet the standard requirement where and how to build it. The standards include nonduty environment and no chemical industries nearby due to the fact that oily matter has easily absorb

dirt and notorious chemical smell. The extraction plant was located proximity to raw material, infrastructure (power, water supply, and road) and market. However, the drainage system to avoid standing water was not hygiene friendly in particular the external premises. Plus, the extraction plant was affected urbanization and continued infrastructure which creates dusty environment and limit future expansion. The oil extraction plant was constructed by concrete cement where the internal premises were not hygiene friendly which violates the recommended internal premises for food processing plant. The recommended internal premises of food processing should be with non-corrugated materials, with loading docks 1 m high from the ground, concrete wall, monolithic concrete chemical resistant and moisture proof floor and windows and doors designed to permitted lightening and prevent insect and rodent entry. The foundation of processing facilities should be moister proof, hygiene friendly and opening (windows and doors) in a way to prevent insect entry. Trees and grasses should not be planted near the plant at least 10 m and 1m, respectively to prevent rodent and other macro-organisms. However, the owners and employee's awareness were not as per the standards for safe production and distribution presented in Figure 3.6.

#### *3.3.8. Equipment Layout in the CSO Extraction Plant*

Equipment layout and hygienic condition includes the equipment appropriateness for processing of specific product which permits the processing flow diagram layout (raw material reception, storage, processing, packaging, end product storage and dispatch). The extraction plant appropriateness for oil extraction was established, and the preconditions and criteria required to meet in the CSO extraction premises are listed (Table 3.4), process control practices (Table 3.5), hygienic practice (Table 3.6) and pest control practices (Table 3. 7). The workers responses on the prerequisite programs are presented in Table 3.6. The oil extraction plant, which was located in a government-built lodging designed for small and medium-sized manufacturing businesses, was close to the sesame farm and infrastructure (energy, water supply and transportation). The plant was at a located distance from chemical manufacturing plant or sewage accumulation/treatment facilities. The plant was built using cinder concrete blocks, the wall and floor were smoothly cemented and a stainless-steel covered of the roof with appropriate drainage system and sanitation friendly. The exterior environment, however, was not free of grass, shrubs, and trees, and it was dusty due to the dry and windy conditions, as well as a randomly mounted light pole for security purposes. Studies suggested that lightening ensures quality preservation, sanitary purpose,

security, and prevents insect attraction and contamination (D’Souza *et al.*, 2015). The windows and doors were controlled to prevent insects and rodent entrances, however, the only door for raw material and end product dispatch was old and folded somehow, encourages insect entry. The extraction equipment’s (milling, pressing, and filtration machine) was appropriate. Despite this, the sanitation and handling practices were poor due to limited access to sanitary inputs, such as insufficient water supply, detergent, and other cleaning inputs, poor sanitation infrastructure, including operation and maintenance, and poor hygiene habits jeopardize food safety. Sesame seed procured either from farmers, cooperatives and other retailers without quality specification, which influence the quality and safety issues. Because of the warm climate and unsanitary conditions, the sesame store in the extraction facility lacks ventilation and temperature control system, creates a favourable condition for the revival of contaminants.



**Figure 3. 7:** Respondents suitability response on the prerequisite programs of the CSO extraction plant. a) Premises quality, b) Raw material, process and end product quality, c) Hygienic practices and d) pest control

**Table 3. 4:** Survey based assessment of the crude sesame oil extraction plant premises compatibility (n=12)

<b>Premises</b>	<b>Checklist</b>	<b>Questionnaire</b>	<b>Description</b>
Is the plant located at the right location?	Compliant	Compliant	<ul style="list-style-type: none"> <li>- The plant located in a government build shades proximity to the raw material, infrastructure and market. Thought, experience power and water supply cut-outs, and affected by urbanization</li> <li>- Assumed the plant is randomly located as it was mixed with non-food dusty small scale manufacturing industries.</li> </ul>
Is the premises primarily designed for sesame oil extraction with separate separated store for raw materials, end product and other supplies?	Noncompliant	Compliant	<ul style="list-style-type: none"> <li>- Designed for any kind of small and medium scale enterprises and noncompliance with food processing facility standards with limited space for storage, offices and rest room. The possibilities of fungi growth are high due to lack of space.</li> <li>- Oil extraction is based on the demand of customers.</li> </ul>
Is the extraction apparatus designed for sesame oil extraction?	Compliant	Compliant	<ul style="list-style-type: none"> <li>- Thought, the same crushing machine use separate spare parts for different product line (peanut, and other oilseeds)</li> </ul>

Is the facility clear of litter, weeds, grass and brush and dust free environment?	Noncompliant	Noncompliant	- Dry, windy and dusty external environment with no signs of litter bins or signs to prevent litter disposal
Is the drainage system (slope) well designed to prevent standing water in the interior or exterior area of the plant?	Noncompliant	Compliant	- The interior part of the facility is smooth, well cemented, chemical resistant floor with no cracks/holes and well drained nevertheless the exterior is rough, flat, and not clean of grasses and allow water to stand. - The dusty and standing water creates a conducive environment from macro/microorganisms and easily pass through the holes and cracks.
Is the in and out doors and windows have fine mesh screens or tightly sealed for insect entry control?	Noncompliant	Compliant	- The opening is sealed with metal without mesh which affect natural ventilation

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**Table 3. 5:** Compatibility assessment of the crude sesame oil extraction plant process control practices (n=12)

<b>Process Condition</b>	<b>Checklist</b>	<b>Questionnaire</b>	<b>Description</b>
Is the sesame seed stored on the basis of first in and first out to avoid spoilage?	Compliant	Compliant	
Are the incoming sesame seed inspected against specification?	Noncompliant	Noncompliant	- Purchase of raw material based without specification except moisture, impurity and no signs of fungi infestation (manual) in compliance with ECX quality specification used as primary deal with the suppliers. However, farmers also supply sesame with low quality.
Is the oil and non-food items stored separate?	Compliant	Compliant	- End product (CSO) dispatched within few hours of production based on customer orders.
Is the water used for processing and cleaning treated?	Noncompliant	Noncompliant	- Municipal tap water is chlorinated while ground water is used during water supply shortage which is untreated and hard water.
Is there a recall procedure?	Noncompliant	Noncompliant	- No recall procedure and workers no aware of what the recall procedure is?

**Table 3. 6:** Assessment of the crude sesame oil extraction plant hygienic conditions and practices (n=12)

<b>Hygienic conditions and instructions</b>	<b>Checklist</b>	<b>Questionnaire</b>	<b>Description</b>
Is instruction available on avoiding jewellery, dressing and use of mobile phones on the production area?	Compliant	Compliant	- Prohibited to use or wear during the working hours
Is the workers wear protective and hygienic clothing? Gloves, clothing, hair and foot cover?	Noncompliant	Noncompliant	- Insufficient clothing
Do operators and actors handle oil with bare hands?	Complaints	Compliant	
Are the hand wash facilities equipped with soap, sanitizers and paper towel?	Complaint	Compliant	
Do workers and visitors wash up their hands before and during production or toilet visitation?	Noncompliant	Compliant	- It is inconsistent and random with random soaps placed
Do workers blow/cough their nose while handling or preparation?	Compliant	Compliant	- Visitors/workers are not allowed to enter the facilities
Is there a build-up of seepage such as oily dirt, cleaning solvents, lubricants on the surface of extraction machine which can contaminate the oil?	Noncompliant	Noncompliant	- The sticky nature of oily soils attracts dusts and others dirt on the surface of the wall and roof explains the infrequent cleaning of the premises
Is spilled oil or solvents cleaned up quickly to avoid cross contamination?	Compliant	Compliant	

Is garbage quickly cleaned, covered and removed?

Noncompliant Noncompliant - Dust beans placed from  
outside of the plant is not  
covered exposed to animals

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**Table 3. 7:** Assessment of the crude sesame oil extraction plant pest control practices (n=12)

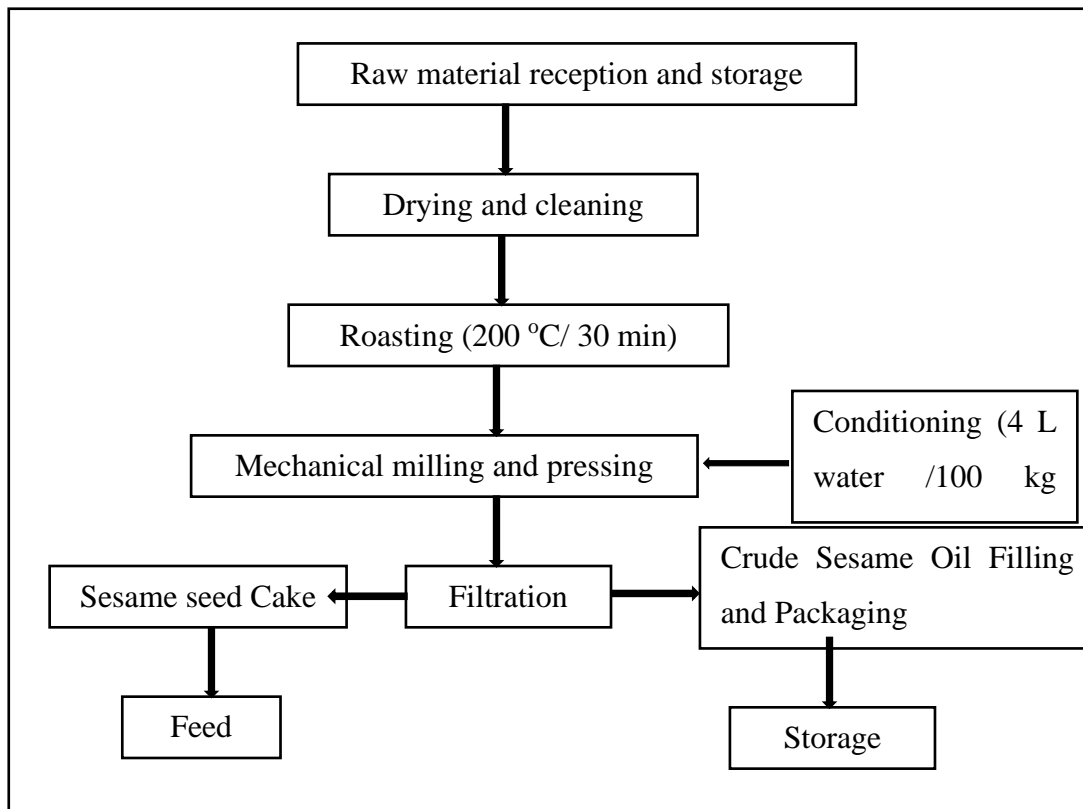
Premises	Checklist	Questionnaire	Description
<b>Pest Control</b>			
Are insects and macro-organisms including domestic animals exist in the indoor and outdoor environment?	Noncompliant	Noncompliant	- Cockroach, rats, rodent, dog and cats exist hide in the external premises due to the presences of grass, small tree and poor hygienic with no specific independent pest control practices. - However, random pests' control with the application either of dry or spray pesticides were administered and performed based on the manufacturer instructions and logo was introduced and assisted by the agricultural and health extension worker
Are safety rules observed during fumigation?	Noncompliant	Noncompliant	- Required to be introduced for worker protection and avoid in contact of the commodities
Are the pest control logs and documentation readily available?	Noncompliant	Noncompliant	- Needs to be in place
Are pesticides or applicable equipment readily available?	Compliant	Compliant	- Pesticides, spraying equipment and instruction of use supplied from the agricultural extension offices
Are trucks inspected and cleaned?	Noncompliant	Noncompliant	- End product delivering tracks wash up frequently, however the cleanness is not inspected.

### 3.3.9. *Crude Sesame Oil Extraction Process Flow Diagram and Validation*

The Tigray sesame oil extraction facility employs a cold press, mechanical extraction technology. The incoming inputs include sesame seed, water, and various sizes of polyethylene packaging bags. As shown in Figure 3.8, the processes and unit operations involved in crude sesame oil extraction include raw material reception and storage, cleaning, drying, roasting, pressing, filtration, filling and packaging. Sesame seed is packaged in 100 kg polypropylene bags supplied by farmers, unions, cooperatives and EXC. The mean value of sesame seed moisture and oil content was 3.37 % and 50.9 %, respectively. Municipal and borehole water sample was collected in a blue brown bottle. [1] Receiving and storing raw material; Sesame seed is the raw material for CSO extraction was purchased by the oil extraction facility from wholesalers and farmers. Sesame seed quality is in compliance with the EXC quality parameters and specification, suitable for the local and domestic market. Sesame quality parameters for sesame market includes foreign matter and colour, moisture content, absences of extraneous matter, discoloration, odour, fungi and other insect damage. Machine lubricants and cleaning detergents were acquired from authorised local vendors. The oil extraction facility is small scale not equipped with cold and ventilated storage, and the storage temperature is higher than the ambient temperature in a dry environment. [2] Drying: the mean moisture content of sesame seed is 3.37%, however, in case of sesame seed purchase during the early harvest, seed sun dried to reduce moisture content to lower than 7% to improve storage stability and prevention of fungi and other microbial hazard occurrence/growth. [3] Cleaning: removal of impurities such as foreign matter, damaged or unsound seed crops, discoloured, metals, hulls, and other foreign impurities.

[4] Roasting: Roasting sesame seeds for 30 minutes at 200°C on a hot plate improves extraction yield, reduce the extraction time and energy expenditure, and inactivate the endogenous enzymes responsible for biochemical reactions. Yet, the functional and nutrient recoveries organoleptic quality and storability could be negatively impacted. [5] Mechanical milling and Pressing: The screw press machine was manually loaded with sesame seed. The screw press has a vertical feeder and a horizontal screw rotate at high gradient pressure to crush the seed and separate CSO from sesame seed cake. In one batch extraction: 50 kg of seed loaded continuously conditioning with approximately 2 L potable water to enable oil release and prevent burning due to the gradient temperature while milling. Approximately 2 L water (at least three times) is applied

per batch (50 kg of sesame), while continuous mechanical milling and pressing till a strong foggy oil appears and dry sesame seed cake is produced. The oil was separated by pressing from the sesame seed cake. [6] Filtration: To remove the sesame seed cake and other solid particles, a plate filtration machine with a filter bed, an oil pump, and filter was assembled. The filtered oil was collected in a non-corrosive paint-coated metal tank. [7] Filling and Packaging: manual filling in a ½, 1, 3, 5, 20 liter polyethylene terephthalate plastic (PET) packaging in transparent white and yellow colour. PET is amorphous, semi-crystalline, has a high boiling temperature, is mechanically strong, thermostable, has a low molecular weight, and has excellent barrier property to moisture and gas. Because of its low cost and reduced weight, PET packaging plastic bottles are often used to carry liquid foods. PET plastic bottle is polar in nature with crystallite level of 22-25%, 300 µm thickness, 1-36 g/cm<sup>3</sup> density and glass transition temperature of (+78°C). PET, on the other hand, may interact with food and the environment. The interaction may be responsible for the production of hazardous metabolites such as Benzoic acid, 4-ethoxy-, ethyl ester and flavour compounds migrates to foods and vis versa that jeopardise the consumer's health (Heindler *et al.*, 2017; Lahimer *et al.*, 2017; Willige *et al.*, 2000). The packaging material replacement to coloured bottles or righted light non-transparent plastic should be considered [8] Storage: crude oil stored at ambient condition protected from sunlight in an opaque packaging PET. The HACCP team validated the cold press extraction method of CSO with actual operation.



**Figure 3. 8:** Crude Sesame Oil extraction using a mechanical milling and cold pressing method

### 3.3.10. Hygienic Practices and Preventive Measures During CSO Extraction Process

Preventive measures taken in to account to prevent hazards occurrence and recontamination during CSO extraction and associated facilities, surfaces and environment are summarized. The overall personal hygiene procedure could be followed as presented in Table 3.8. Hand washing Figure 3.9, hygienic and good handling practices to follow during raw material and other supply reception and storage simmered in Table 3.9, water handling and treatment summarised in Table 3.10, CSO extraction process practices summarized in Table 3.11 and crude sesame oil packaging material selection summarized in Table 3.12. The cleaning and disinfection procedure of the equipment, inner and outer premises of the CSO extraction facilities should be followed as in the Figure 3. 10. following the preventive control measure will assure the safety of CSO.

### 3.3.11. *Personal Hygiene*

When visiting the CSO extraction plant, using the restrooms, and entering and exiting the building, staff members and guests should adhere to the personal hygiene procedures listed in Table 3.8.

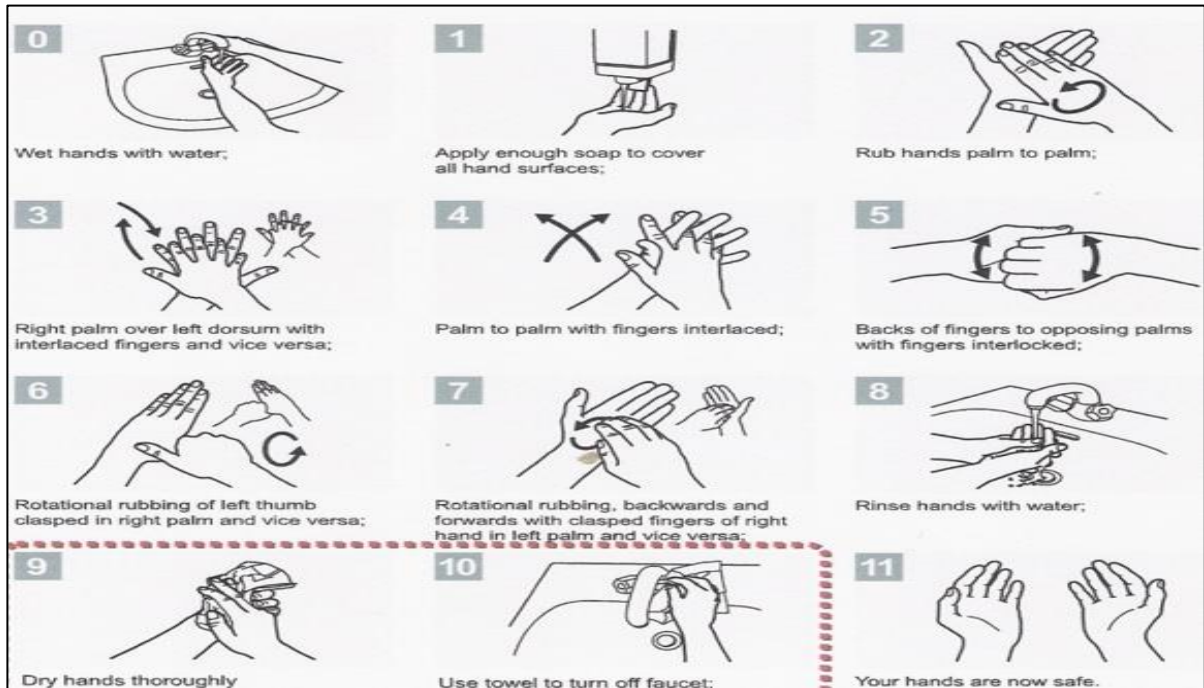
**Table 3. 8:** Personal hygiene procedures posted at the frequency visited area with in the oil extraction plant

Personal hygiene comprises the following:

- Employee with the signs of illness should be prohibited from entering the plant or making any contact with any surface or equipment within the plant
- Wearing jewellery and carrying personal belongings in the plant should be prohibited
- Personal protective clothing including hair cover, shoes, gloves and gown /overcoat / lab coat /apron should be mandatory
- Install sanitary facility with the appropriate supply (detergents, sanitizer of mild alkaline solution and drying towel)
- Follow the standard hand washing procedure as shown in Figure 3.9
- Before and after visiting restrooms and coming into contact of equipment's or surfaces,
- Stickers include washing and sanitary instruction should be posted on the appropriate site within the plant.

### 3.3.12. Hand Washing Procedure

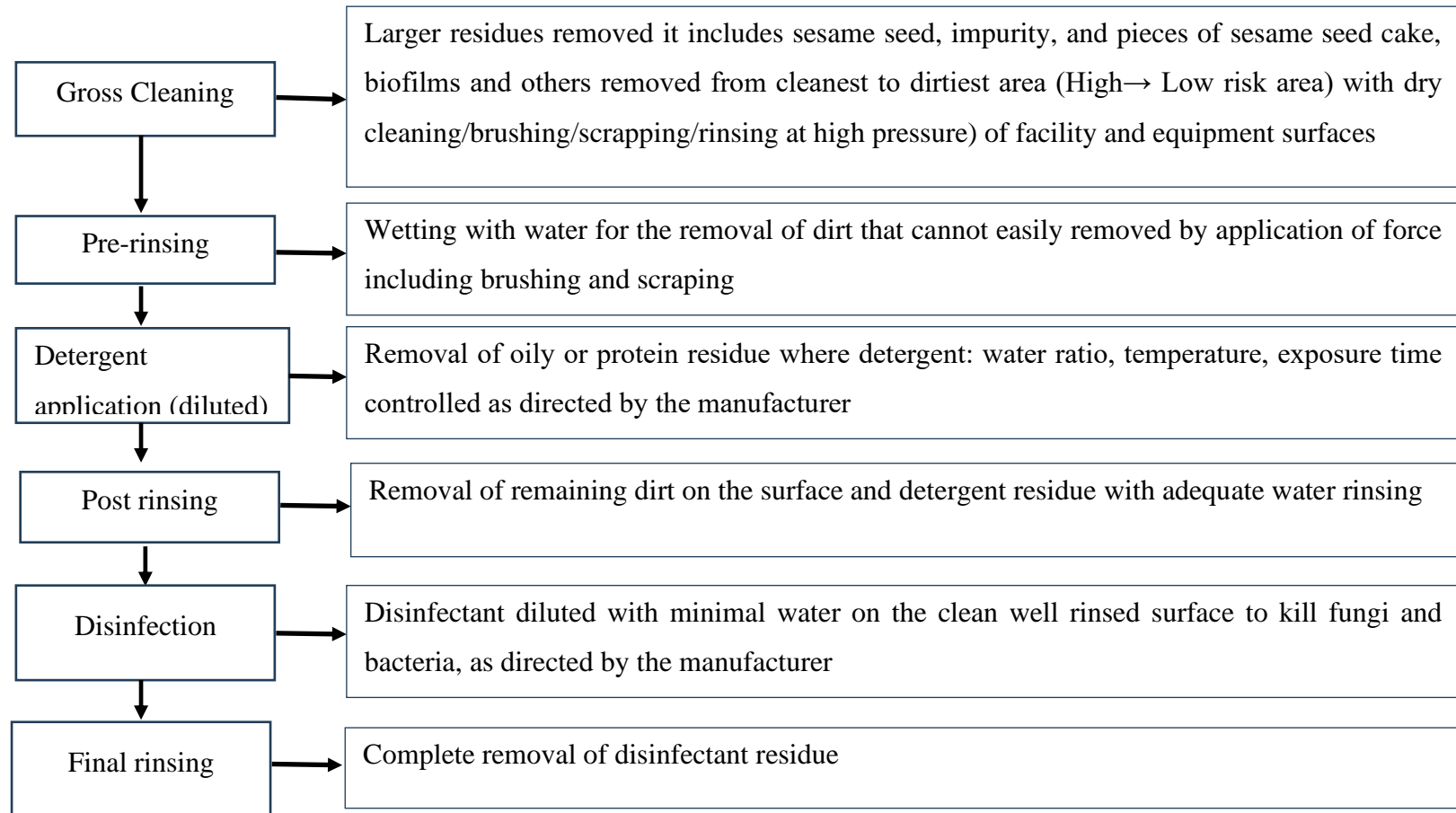
The WHO suggested technique for hand washing when visiting the processing plant and using the restrooms is depicted in Figure 3.9, were introduced and encouraged to follow.



**Figure 3. 9:** Standard hand washing procedure adopted from WHO poster posted in the entry of rest room while visiting rest rooms with detergents and drying tissues

### 3.3.13. Cleaning and Sanitation Procedure

Figure 3.10 shows the cleaning and sanitation procedures used in the CSO processing plant which includes the equipment's and internal premises of the CSO extraction plant.



**Figure 3. 10:** Effective cleaning and disinfection procedure in the oil extraction surfaces in contact of CSO and facility premises

**Table 3. 9:** Hazard preventive control measures during sesame seed reception and storage

Hazards	Sources and reason of hazard occurrence	Preventive measures
Microbial and Macro organisms' infection (B)	Longer sun drying in an open environment	Drying at $\leq 40$ °C to lower than 10 % moisture content
	High storage temperature in a humid environment without ventilation and limited space	Design storage house with controlled lightening, ventilation, humidity (< 70%) at ambient condition
	Inadequate hygienic of the storage facility	Maintain the external and internal premises cleanness clear from trash, trees and dryness to discourage growth, survival and entry of macro-organisms Follow the rule of first in and first out (FIFO) during storage and subsequent process Selection of packaging bags with moisture barrier properties such as Purdue Improved Crop Storage bags, metal silos, and GrainPro bags (Neme <i>et al.</i> , 2021)
- Pesticide residue (C)	Pesticides applied to prevent insect attack in the field and storage	Use of bio-pesticides useful repellents, antifeedants, insecticides, fungicides, weedicides, nematicides, molluscicides (Carpinella <i>et al.</i> , 2003; Dimetry, 2014; Maia & Moore, 2011).
	Detected below the MRL	Maintain the external and internal premises cleanness clear from trash, tress and dryness to avoid pest entry If no, use registered pesticides following the manufactures instruction, maintain the warrant as manufactories/suppliers' assurance
Aflatoxin	Due to the continued fungi growth in the field and storage premises and	Eco-friendly fungi and fungi metabolites mitigation technology (Dimetry, 2014). Control of the storage temperature and humidity and raw material moisture content and clean of impurity

convenient environment for metabolites synthesis Follow the first in and first out (FIFO) rule during storage and oil extraction

Foreign and trace elements (P) Early harvesting, post-harvest handling stress and insufficient sorting of impurities Effective cleaning to confiscate any kind of debris, mechanical damage, under matured seed, debris and the foreign materials should be measured and cleaned

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**Table 3. 10:** Hazard preventive measures during water handling and storage to prevent hazards in water and recontamination

Item	Sources and reason of hazard		
	Hazards	occurrence	Preventive measures
Water	Microbial (M)	Inadequate water treatment and hygienic practice Longer residence time in the water tank due to the irregular limit water supply	Water supply chain hygienic/cleaness practices and conditions of control Reduce residence time and adequate renewal of the washing water Water treatment such as chlorination and others possible
	Chemical residues (C)	Use of untreated water contains solid content for cleaning or seed conditioning purpose	Water treatment include removal of minerals Integrity of storage tank

**Table 3. 11:** Hazard preventive measures during crude sesame oil extraction process

Process step	Sources and reason of		
	Hazards	hazard occurrence	Preventive measures
<b>Cleaning and drying</b>	Microbial (M)	Inadequate hygienic condition	Effective cleaning and disinfection program
		Longer drying time in an open environment	Drying to < 10% MC in a controlled drying condition at ≤ 40°C
	Impurities (P)	Early harvesting, post-harvest handling stress and insufficient sorting of impurities	Effective cleaning with no sign of discoloration/ off odours, mould growth
<b>Roasting</b>	Browning (C)	The roasting time temperature is higher	Optimized roasting time temperature combination with continuous mixing for homogeneous roasting and avoid burning
	Burnt seed and lighter husk (P)	High roasting condition	Screening and cleaning
<b>Conditioning and pressing</b>	Microbial (M)	Inadequate hygienic condition and sporadic cleaning	Conditioning and pressing machine cleanness before and after extraction with clean water and appropriate detergents
		Microbial growth initiated due to conditioning	Scheduled disinfection of the conditioning and pressing machine
			Replace the pressing machine with controlled and automated machine recommended
			Conditioning water treatment

	Oxidation (C)	Uncontrolled extraction condition where light, environmental air is in contact	Protect the conditioning and pressing machine from direct/indirect environmental exposure while extraction Reduce the extraction holding time and temperature protected from environmental exposure
	Detergent residue (C)		Adequate rinsing with clean water
	Foaming		Application of emulsifier, stabilizers and anti-foaming agents Protein fractionation
<b>Filtration</b>	Microbial infection (C) Turbidity (C)	Inadequate hygienic condition and sporadic cleaning	Machine cleanness before and after filtration Improve Filtration efficiency to remove solids impurities or sediments
<b>Filling</b>	Microbial (M)	Inadequate hygienic condition and sporadic cleaning Lack of aseptic filling	Good hygienic practices with appropriate clothing to avoid contact of the product with bare hands Automation of filling and packaging process recommended
	Deterrent residual		Adequate rinsing with clean water

**Table 3. 12:** Hazard preventive measures during crude sesame oil packaging and storage

Process Item/ step	Sources and reason of hazard occurrence			Preventive measures
<b>Packaging</b>	Microbial cross-contamination (M)  Detergent residual (P) Migration of chemicals (C) Oxidation (C)	Inadequate hygiene leads to microbial growth  Lack of aseptic packaging  Inappropriate use of packaging/packing	Leak test, Moisture test, temperature and humidity control  Foil pack test  Good personnel hygiene practices  Packaging conformance recommended for edible oil  Control of washing and water flushing  Good hygienic and manufacturing practices  Recommended use of less reactive packaging material with proper barrier properties  Removal of residual oxygen and maintain the recommended headspace in less transparent packaging material	
<b>End product storage (CSO)</b>	Microbial (M)  Rancidity (C)	Cross contamination due to unhygienic practices  Uncontrolled storage  Lacks refining where enzymatic induced hydrolysis/oxidation might be induced	Good storage practice to prevent microbial cross contamination due to leakage and unhygienic practices  Good storage practices far away from light and high gradient temperature with control in contact of environmental air  Recommended to use organic preservatives (See Chapter 4, as an example)	

Aflatoxin (C)	Continued fungal growth on the surface of the facilities/premises due to sporadic cleaning and convenient environment for fungi metabolites synthesis	Effective processing to the acceptable end product moisture content Apply organic and eco-friendly preservatives Storage in dry cold storage protected from direct sunlight exposure
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### 3.3.14. Crude Sesame Oil Product Description

Crude sesame oil (CSO) description is summarized in Table 3.15. Crude sesame oil (CSO) is an unrefined oil that can be used for cooking and salad dressing for consumers without specification. CSO has a moisture content of 0.37%, and 83.15 % unsaturated fatty acids made up with 95.5 omega-6 to omega-3 ratio. Unrefined sesame oil also used in the oil refinery industry as a blending ingredient.

### 3.3.15. Sesame Seed Proximate Composition

The proximate composition of sesame seed data Table 3.13 and the physico-chemical properties data of crude sesame oil is presented in Table 3.14. Proximate composition analysis is the first insight for identification of the general composition. Sesame seed is an excellent source of oil, protein, fiber, and minerals. It also has a higher energy content than other food grains. Sesame seed moisture content ( $3.36 \pm 0.17\%$ ) is an important quality parameter for its storage stability, was within the recommended limit ( $< 10\%$ ) for oilseeds and other durable crops. Other studies found that sesame seed moisture content ranged from 1.19 to 3.96%, despite substantial variability due to geo-agricultural and climatic factors (Zebib *et al.*, 2015), maturity stage during harvest (Araujo *et al.*, 2018), storage conditions and containers (Neme *et al.*, 2021). This explains why, when the environment's temperature reduces the moisture as a result of dehydration, matured sesame seed composed of more solid contents and less moisture, and the barrier properties qualities of packing and storage containers prevents migration.

Ash, which is the leftovers after combustion of organic matter and an estimator for total mineral content, is one of the most important proximate quality estimation of foods (Miller & Fennema, 2008). Though, the ash content estimation is significantly variable with sample size, ashing temperature and duration (Liu, 2019). The crude ash content of sesame seed was  $5.47 \pm 0.06\%$ , indicating that sesame is an excellent sources of minerals, superior than some broad beans (Kmieciak *et al.*, 2000; Sharaby & Butovchenko, 2019). The volatility and availability of minerals to processing are explained by the ash content changing throughout processing and storage. Ash is a determinant factor for the physico-functional properties of foods, including the colour, viscosity and density, in addition to the nutritional composition (Dongmo *et al.*, 2020; Yegrem *et al.*, 2021).

Sesame, in addition to oil, is an excellent source of protein, which is important in the biological system for nutritional, structural, catalytic and other functions such as foaming, flavour binding, water binding and viscosity. The protein content and quality, on the other hand, highly variable on the food type. The protein content of sesame seed grow in Humera, Tigray was found to be  $22.11 \pm 0.92\%$ , which is consistent with the values reported by Zebib *et al.*(2015). Nonetheless, the protein content of sesame varies depending on varieties, plantation condition and agro-ecological factors (Bahkali *et al.*, 1998; Mawcha *et al.*, 2021; Zerihun & Berhe, 2020). The majority of the sesame protein was globulin, which is water soluble (Idowu *et al.*, 2021). Protein is important for emulsification, foaming and oil stability, and its function is influenced by acidity and salt content. According to studies, sesame protein includes 18 amino acids but is deficient in lysine. It is digestible by the pepsin–pancreatin enzyme systems (Onsaard, 2012) and meets the FAO/WHO recommendations for daily consumption (Fasuan *et al.*, 2018).

Fiber is one of the important nutritional components in human diet. It is carbohydrate polymers that are resistant to enzymatic digestion in the intestine and provides various health benefits such as of gastrointestinal track functioning, lowering blood cholesterol by sweeping salt and preventing salt reabsorption, and prevents heart diseases. For a healthier diet, nutritionist recommend 25-50 g of dietary fiber on daily basis. Sesame seed composed of  $6.09 \pm 0.16\%$  dietary fiber lower than the findings of Zebib *et al.* (2015) and higher than the Pakistan cultivars (Saeed *et al.*, 2015). Sesame seed composed of  $11.95 \pm 1.51\%$  carbohydrates, 592 kcal/100g designated as it provides essential amino and fatty acids (Bahkali *et al.*, 1998). The dry matter content explains the solute fraction in sesame seed which account to 96.6 %, durable under optimal storage condition. Studies have found the differences in the proximate composition of sesame cultivars, which explains why extraction method and cultivar resulted in different chemical composition and extraction yield.

**Table 3. 13:** Sesame Seed proximate composition expressed in percentage

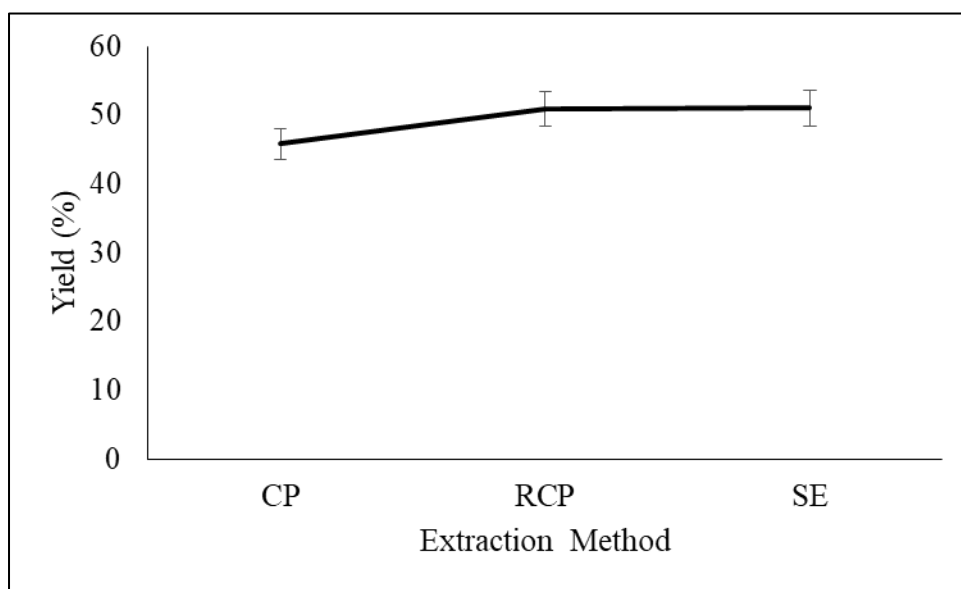
Quality parameter	Mean Values
Moisture (%)	3.36 ± 0.17
Ash	5.47±0.06
Protein	22.11±0.92
Oil	50.9±0.89
Dietary Fiber	6.09±0.16
Carbohydrate	11.95±1.51
Dry Matter	96.64±0.17
Energy (Kcal/100g)	592.54±5.95

### 3.3.16. Crude Sesame Oil Extraction Yield

The oil extraction yield of white sesame seed was obtained 50.9% and 45.8% with and without roasting treatment, respectively, presented in Figure 3.11. The raw sesame oil extraction yield without roasting treatment using a laboratory based Soxhlet extraction method was however 51.03 % equivalent with the cold press mechanical commercial extraction method was sesame seed roasting treatment explains roasting facilitates oil extraction process. Sesame oil extraction yield is however increased with increased extraction temperature, pressure, holding time (Elkhaleefa & Shigidi, 2015) and decreased smaller particle size (Dokeret *et al.*, 2010) and extraction method (Junpeng *et al.*, 2019), operating conditions and solvent type/solvent: seed ratio (Osman *et al.*, 2019). Sunflower seeds oil expression efficiency was improved with increased thermal treatment, explains seed mild thermal treatment improve oil the extraction efficiency (Guradil *et al.*, 2020).

This explains selection of the seed treatment and extraction method significantly affects oil extraction efficiency, seed economic value and overall quality. Sesame oil extraction yield was in agreement of with other studies reported by different researchers. According to Zerihun & Berhe (2020), the oil yield of different sesame varieties in Tigray was reported within the range of 44.4 - 50.84% and sesame varieties in Ethiopian were within the range of 44- 56% (Kebede *et al.*, 2017). Meanwhile, studies reported that the oil yield and oil quality of sesame seed is highly variable regardless of the genetic variation (Kurt, 2018; Wei *et al.*, 2015) cultivation practices and agro-climatic conditions (Gharby *et al.*, 2017), seed physico-chemical composition (Chakraborty *et al.*,

2017), extraction method (Benítez Benítez *et al.*, 2016; Ribeiro *et al.*, 2016) whereby extraction yield using solvent was higher 59.9%, cold press mechanical extraction yield (42%) and enzymatic green (36.65%), treatment method such as thermal treatment (Ghosh *et al.*, 2014; Kiralan & Ramadan, 2016), roasting (Hama, 2017), and storage time (Gulla and Waghray, 2011).



**Figure 3. 11:** Crude sesame oil extraction yield of sesame cultivars from western Tigray, Ethiopia Where CP is cold press mechanical extraction, RCP is cold press mechanical extraction with seed roasting treatment and SE is hexane based Soxhlet extraction

### 3.3.17. Physico-Chemical Properties of Crude Sesame Oil

The physico-chemical characterization of CSO data is presented Table 3.14. The physico-chemical properties of CSO includes colour, acid value, iodine, primary and secondary and overall oxidation indicators, used to determine the quality and storage stability of oils and fats throughout processing and storage. Colour is one of the physical quality parameters of edible oils. The CSO was found to be yellow, though develops light amber colour as a result of roasting treatment and oxidation reaction. The colour change could be attributed due to Maillard reaction, which also produces a deep pleasant flavour. Acid value (AV) is a chemical property that is used to estimate the carboxylic acids presents in the fatty acid chains, as well as the effect of processing when it converts oxidized metabolites and esters produced.

Acid value expressed as the number of KOH (in milligrams) required to neutralize the organic acids in 1 g of fat, important for quick characterization of oil quality, exceeding the tolerable limit (0.6 mg KOH/ g oil) is considered deteriorated, unfit for consumption (Kardash & Tur, 2005). The AV of the CSO was  $1.59\pm 0.03$  mg KOH/g of oil within less than 24 hours of production, exceeded the limit for all kinds of edible oil (0.6 mg KOH/g oil) according to the FAO/WHO recommendations and exceeding 3 mg KOH/g oil is not fit for consumption unless further refining process accomplished (Zhang *et al.*, 2015). The higher the AV of CSO, the more reaction occurs since the oil is unrefined, resulting in a prolonged extraction condition in an open environment. Increased heating time reduced the acid, oxidation of sesame oil (Valantina *et al.*, 2016). On the other hand, heating reduces quality degradation owing to due to the enzyme inactivation and polymerization. Other research, however, indicated that the AV of edible oil increased during storage due to high storage temperature and other environmental factors (Kuselman *et al.*, 1998).

**Table 3. 14:** Crude Sesame oil physic-chemical properties with roasting treatment of sesame seed

Quality parameter	Mean Values
Colour	Yellow
Moisture (%)	$0.37 \pm 0.03$
Acid value (mg KOH/g oil)	$1.59 \pm 0.03$
Peroxide value (meq O <sub>2</sub> /kg oil)	$0.49 \pm 0.01$
p-Anisidine value (meq O <sub>2</sub> /kg oil)	$1.10 \pm 0.03$
TOTOX value (meq O <sub>2</sub> /kg oil)	$2.09 \pm 0.05$
Iodine value (g of I <sub>2</sub> /100 g)	$112.30 \pm 0.88$

Iodine value (IV) is the measure of degree of unsaturation of fats and oils expressed as the amount of iodine absorbed. The IV was calculated according the procedure described by Kyriakidis and Katsiloulis (2000) after the fatty acid analysis conducted using the fatty acid methyl esters (FAMES) following the procedure of AOAC 996.01 described by Satchithanandam *et al.* (2001) using gas chromatograph. The Iodine value represents the halogens required to bind 100 g of oil in the C=C double bond of the unsaturated fatty acids. The IV are significantly variable depending on the type of fatty acids and amount of the fatty acid in the test sample. The reference

values for IV calculation were IV = 89.9 for oleic, IV = 181 for linoleic and IV = 273 for linolenic acids. The iodine value of crude sesame oil was measured 112.3 g of I<sub>2</sub>/ 100 g of oil). It is used as a process guide in the edible oil industry. The more unsaturated fatty acid with multiple double bonds in the long chains of fatty acid explains the higher the IV detected. The IV is an indicator for the physical and chemical correlation and oxidative stability of oils and melting point (Knothe, 2002). Calculation of IV from the fatty acids profile of CSO generated by GC according to Kyriakidis and Katsiloulis (2000) was as follows;

$$IV = xC_1 + yC_2 + zC_3$$

Where: C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub> refers the sum of unsaturated fatty acids with one, two and three double bonds, Table 4.4 and x, y, z refers to the coefficient of oil. The coefficients of the sum of mono, di and tri unsaturated fatty acids for the evaluation of iodine value was calculated 1.1, 1.57, and 2.60, respectively. The Peroxide value, p-anisidine and Totox Value of CSO was measured 0.49, 1.10, 2.09 meq O<sub>2</sub>/kg oil within less than 24 hours of analysis after extraction explains the CSO was not protected from an open environment. According to Rounizi *et al.* (2021) reports crude sesame oil extracted using a cold press extraction method was detected high oxidation value than refined sesame oil explain refining halts oxidation. Then again, roasting, storage temperature and times hastens oxidation (Valantina *et al.*, 2016; Olaleye *et al.*, 2019; Mohamed *et al.*, 2021).

**Table 3. 15:** Summary of crude sesame oil description, composition, use, and shelf-life

Product name	Crude sesame oil with light amber colour/deep yellow colour
Composition	Fat 83.15% unsaturated fatty acids with omega-3/ omega-6 ratio moisture content (0.37%) exceeded the commendable limit (0.2 %)
Product Intended use	Cooking oil, salad dressing, blending component in the oil refining industry, cosmetics and pharmaceutical
Processing stages	Sesame seed reception and storage, cleaning and drying, roasting, conditioning during cold mechanical pressing, filtration, filling and packaging
Packaging type	½, 1, 3, 5, 20 litter in an amorphous, semi-crystalline polyethylene terephthalate plastic (PET) and HDPE package with transparent and yellow colour.

Storage and shelf-life	CSO should be stored at ambient condition protected from sunlight for no more than 2 months
Labelling specification	Company name, product type, product size, net weight, area code, and production and expiration date was included

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### 3.3.18. Crude Sesame Oil Fatty Acid Composition

The fatty acid profile of CSO from roasted sesame seed presented in Table 3.16, were palmitic, stearic, oleic, linoleic, linolenic, and Arachidic and Eicosanoid acids. The dominant fatty acids of CSO were omega-6 Linoleic acid followed by oleic acid. CSO composed of 83.15% unsaturated fatty acids. It is rich in omega-6 fatty acids with omega-6 to omega-3 ratio of 95.3 which is nearly 100:1 higher than the recommended ratio (3:1-4:1) (Simopoulos, 2010), increases oxidation sensitivity. The higher the level of the unsaturated fatty acids is more sensitive to oxidation and storage stability (Syed *et al.*, 2016). Omega-6 fatty acids are fatty acids obtained from diet which body cannot synthesis it. While, omega-3 fatty acids could be synthesised in the body. The omega-6 to omega-3 ratio essential fatty acids are important in defence of up to 70% health risks of cardiovascular diseases, defence of infection and metabolic disorders (Hashempour-baltork *et al.*, 2017; Hashempour-baltork *et al.*, 2018; Nehdi *et al.*, 2019, Van Den Elsen *et al.*, 2015), promote a healthy development (Candela *et al.*, 2011), lessened the saturated fatty acid intake. The omega-3 fatty acid in crude sesame oil (0.45 %) equivalent to some selected fish (Miller & Fennema, 2008), and yet oxidative deterioration reduces the omega-3 fatty acids. The lower level of linolenic acid (omega-3 fatty acid) as the plant-based oils are rich in omega-6 fatty acids and omega-3 fatty acid might be oxidized. According to Yun and Surh (2012) investigation fatty acid composition of edible oils are the best predictors of oxidation and oxidative stress, besides the impact of seed pre-treatments and processing (Abou-gharbia *et al.*, 1997; Abou-Gharbia *et al.*, 2000). Moisture content of oil is also a critical parameter for the storage stability. CSO moisture content was measured at 0.37% exceeded the recommended limit for storage and overall oil quality (0.2%). Choe and Min, (2009), Choe and Oh (2013) and Gumus and Decker (2021) investigated increased in moisture content of oils and oily foods accelerates oxidation.

However, the total fatty acid content, individual fatty acid level, saturated and unsaturated fatty acids are significantly affected by the soil composition and climate condition, season and year of

harvest (Wacal *et al.*, 2019) and processing (Dar *et al.*, 2019; Hama, 2017). The higher the level of the unsaturated fatty acids are the more sensitive to oxidation and storage stability (Syed *et al.*, 2016). Imbalanced intake of omega-6 to omega-3 fatty acids leads metabolic disorder where the omega-3 fatty acid acids have valuable effect to prevent cardiovascular diseases and stable with insignificant difference during cooking and storage time at not higher than 20 °C (Douny *et al.*, 2015). Packaging and barrier properties of packaging to light and environmental air plays a significant role. According Gargouri *et al.*(2015) to investigation of tin containers and dark/blue brown glass bottles were better to prevent oxidation, physico-chemical and sensorial properties of vegetable oils and reduce anti-oxidant degradation than the polyethylene plastics and clear bottle.

**Table 3. 16:** Fatty Acid Composition of crude sesame oil with roasting pre-treatment of sesame seed at 200 °C for 30 min

<b>Name of FFAs</b>	<b>Fatty Acid level (%)</b>
Total Oil content	50.9 ±1.38
Saturated Fatty Acids (SFAs)	15.01±1.71
Palmitic (C16:0)	9.7±0.93
Stearic (C18:0)	4.82±0.47
Arachidic (C20:0)	0.49±0.14
Monounsaturated fatty acids (MUFAs)	39.8±1.19
Oleic (C18:1nω9)	39.4±1.91
Palmitoleic (C16:1ω7)	0.18±0.04
Eicosanoid (C20:1ω9)	0.22±0.16
Polyunsaturated fatty acids (PUFAs)	43.35±1.15
Linoleic(C18:2ω6)	42.9±1.70
Linolenic (C18:3ω3)	0.45±0.12
PUFA: SFAs	2.9
Omega-6/Omega-3	~ 95.3

### 3.3.19. Aflatoxin Contamination

Hazard analysis conducted as the HACCP principles presented in Figure 2.2, principle 1. The hazard analysis conducted in this chapter are presented below. The prevalence of aflatoxin and

AFB<sub>1</sub>, pesticide residue and water quality data in sesame seed, sesame seed cake, CSO, municipal and ground water is presented in Table 3.17 and Table 3. 18. The total aflatoxin level in sesame seed and sesame seed cake was as high as 14.98, 16.87 µg/kg, respectively, exceeding the tolerable limit (10 µg/kg) immediately after extraction amended by the European Union. CSO's total aflatoxin level was 8.54±1.44 µg/kg lower than the tolerable limit. However, because fungi growth was abundant, aflatoxin synthesis could have been continued during storage of seed or the oil. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) was present in sesame seed at the level of 0.51 µg/kg and also in CSO at the level of 0.37 µg/kg which did not exceed the tolerable limit recommended by European Union (2 µg/kg). Meanwhile, the AFB<sub>1</sub> for sesame seed cake was 3.94 µg/kg exceeded the limit, explains the sensitivity of sesame and sesame-based products to fungi and its metabolites. Studies have found aflatoxins to be synthesized by fungi of different species, in particular the *Aspergillus flavus* and *Aspergillus parasiticus*. The warm environmental factors in the sesame value chain, inadequate hygienic practices, and increased CSO moisture contents contributed to fungi development with a variety of physical features and aflatoxin synthesise.

The spore forming fungi grow everywhere in the indoor and outdoor environment and synthesis aflatoxin where humidity, temperature, hygienic condition and product composition is conducive (Kumar *et al.*, 2017) might or might not be toxic by itself (Amaike & Keller, 2011). As a result, the toxicity of fungus was confirmed as the *Aspergillus* species were able to grow in Sabouraud Dextrose Agar chloramphenicol powder as anti-bacterial growth inhibitor at 25 °C for 7 days. Fungi and their metabolites have been found at high dose levels in soils, walls and house utensils (Diba *et al.*, 2007), house dust and walls (Visagie *et al.*, 2014), house utensils and damaged buildings (Gray *et al.*, 2003). Fungi have been found in agricultural commodities (Fountain *et al.*, 2015; Lai *et al.*, 2015; Liu *et al.*, 2016; Thathana *et al.*, 2017), fresh and stored sorghum (Taye *et al.*, 2016), sesame seed and millet (Ezekiel *et al.*, 2014), rice grains (Lai *et al.*, 2015), livestock (EFSA, 2013) and food stuffs like sun dried potato chips (Amri and Lenoi, 2016), various vegetable oils (Mariod & Idris, 2015; Waqas *et al.*, 2021; Yang *et al.*, 2011), sesame oil (Idris *et al.*, 2010; Idris *et al.*, 2013; Kollia *et al.*, 2016), sunflower oil (Mmongoyo *et al.*, 2017); groundnut, sesame and cottonseed oils (Idris *et al.*, 2010), groundnut and sunflower oils (Mariod & Idris, 2015), dried vine fruits (Kollia *et al.*, 2014), wheat grain (Sifuentes *et al.*, 2013) and wheat flour (Li *et al.*, 2016) and melon seeds consumed raw (Somorin *et al.*, 2016).

Due to decay and synthesis of metabolites at a wide range of moisture contentment and temperature (5-35 °C) (Hassane *et al.*, 2017; Leggieri *et al.*, 2017; Mannaa & Kim, 2017), gas, storage and incubation time and composition (Lahouar *et al.*, 2016), and inadequate hygiene, storage and processing practises spore forming fungi are potent treat for human health threat and economic loss (Gacem & Hadj-khelil, 2016; Gray *et al.*, 2003; Hawkins & Windham, 2005; Rea *et al.*, 2003). Fungi growth impacts food safety by restraining the enzymatic activity, disrupting of mitotic divisions of meristematic cells and restrained protein biosynthesis, as well as producing secondary metabolites (Podolska *et al.*, 2017) that could cause acute and chronic health problems (Darwish *et al.*, 2014; Krska *et al.*, 2008; Krska *et al.*, 2016; Tola & Kebede, 2016) such as immunosuppression, potent cancer and even death (Zain, 2011).

Preventing the growth of spore forming and toxigenic fungi, as well as the synthesis of their metabolites, has been explored in studies. Understanding of the position or distribution mechanism of fungus in the surfaces and food raw materials, as well as metabolite concentration, biosynthesis mechanisms, is important for their prevention approaches. Yu *et al.* (2004), for example, elaborated that *Aspergillus parasiticus* driven aflatoxin synthesis involves more than twenty-five enzymes in 14-15 pathways. However, calcium signalling channel blockers such as LaCl<sub>3</sub> suppress aflatoxin production (Li *et al.*, 2019; Rentel & Knight, 2004). Extracellular hydrogen bonds serve as communication links between exogenous fungi and crop/food (Gacem & Hadj-khelil, 2016; Fountain *et al.*, 2015). Controlling stress inducing factors such as humidity, hotness and mechanical stress, quorum sensing, protein signalling pathways (Gilbert *et al.*, 2016) aid in the inhibition of fungus growth and toxic metabolites synthesis (Gacem & Hadj-khelil, 2016).

**Table 3. 17:** The summary of chemical hazards detected in sesame seed, CSO, and Sesame seed cake

Sample	Hazard	Mean value	Recoveries	LOD	LOQ	Tolerable limit	P-value, R <sup>2</sup>
SS	TAT (µg/kg)	14.98 <sup>a</sup> ±0.16					
	B <sub>1</sub> (µg/kg)	0.51 <sup>b</sup> ±0.37					
CSO	TAT (µg/kg)	8.54 <sup>b</sup> ±1.44	90.5	0.038	0.072	10	
	B <sub>1</sub> (µg/kg)	0.37 <sup>b</sup> ±0.09	85.125	0.095	0.21	2	< .0001, 0.95
SSC	TAT (µg/kg)	16.87 <sup>a</sup> ±0.55					
	B <sub>1</sub> (µg/kg)	3.94 <sup>a</sup> ±0.58					
SS	Diazinon	0.062 <sup>a</sup> ±0.02	97	0.0005	0.012	0.1	< .0001
	Fenitrothion	0.048 <sup>ab</sup> ±0.02	109	0.0019	0.009	0.05	0.44
	Malathion	0.031 <sup>bc</sup> ±0.013	106	0.0004	0.001	8	
	Carbaryl	0.016 <sup>c</sup> ±0.023	95			1	

NB: Means ± Stdev, ND: not detected; Where SS = sesame seed, SSC = sesame seed cake, CSO = crude sesame oil, TAT = total aflatoxin, and B<sub>1</sub> =Aflatoxin B<sub>1</sub>

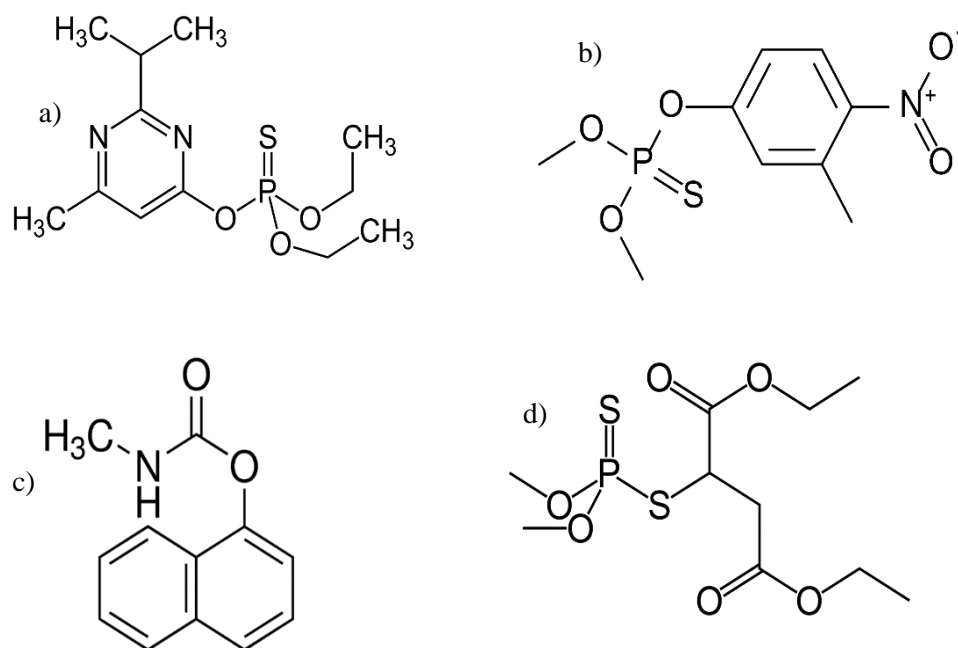
### 3.3.20. Pesticide Residue Contamination

Pesticides are agrochemicals that are deliberately or unintentionally added to agricultural crops and the human indoor and outdoor environment to prevent pest damage, increase productivity, and disease prevention. Pesticide use, on the other hand, is having an emerging poisoning effect on soil/agricultural biodiversity/sustainability, and food safety by reducing nutrient availability or by increasing disease incidence (Meena *et al.*, 2020). Farmers in developing countries who lack formal education, lack the basic skills and knowledge to follow the precautions for pesticide use and application, risks towards human and environment safety because they use pesticides without due diligence on side effects of not following what they have been guided to practice (Apeh, 2018). Ethiopian farmers and growers use more than 212 types of registered pesticides that includes insecticides, herbicides, fungicides and pesticides. The utilization of these pesticides increases from time to time. Small scale farmers and stakeholders with no formal education, on the other hand, have experienced challenges due to lack of information on pesticides adaptation with pests, soil and weeds, use of conditions include the frequency, timing, doses, toxicity to humans and environmental issues. The motives for the use of pesticides were due to the immediate yield increase and disease prevention. Diazinon, Fenitrothion, Malathion and Carbaryl, Figure 3.12 and Table 3.17, are registered agrochemicals that have been used to protect sesame from caterpillar, aphids, moths, butterflies, worms, locusts, grasshoppers, lice, blowflies, ked, ticks and other insects in the farm, and sesame storage warehouses. Those pesticides are volatile in the environment persistence exceeding the residual limit poses a health risk and pollutes the environmental. The residues of Diazinon, Fenitrothion, Malathion and Carbaryl found in sesame seed that do not exceed the FAO/WHO guideline limit. MRL established are however crop and pesticide type specific to limit the health impact (Duijn, 2008).

Diazinon is an organophosphate insecticide used in sesame farm and storage warehouses to control aphids, beetles, scales and pill bugs and its residue detected was as large as 0.062 ppm in sesame seed not exceeding MRL (0.1 ppm) (EC, 2013). Fenitrothion (O, O-dimethyl-O-[3-methyl-4-nitrophenyl]-phosphorothioate) is an insecticide extensively used to suppress chewing and sucking insects, flies, mosquitos, and cockroaches and the residue discovered was 0.048 ppm, which was not exceeded the MRL of 0.05 ppm. Carbaryl is spectrum of Naphthalen-1-yl N-methyl carbamate insecticide applied to control pests in sesame agriculture in the field and storage found

to be 0.016 ppm residual in sesame seed, not exceeded the limit of tolerance (1 ppm). Malathion (MAL) S-(1,2-dicarbethoxyethyl) O, O-dimethyl dithiophosphate, is an Organophosphorus insecticides applied before and after harvest to control a wide range of pests and eradicate disease vectors organisms during sesame cultivation, field drying, and post-harvest storage. The residue of Malathion detected in sesame seed was 0.031 ppm. Malathion, on the other hand, should not be found in crops or food stuffs due to serious health problems. Though, malathion is used in the farm and storage due to insect infestation of sesame seed. Farmers are advised not to use malathion by regulatory and extension workers since it affects the market value as it causes health problem and environmental risk (Aktar *et al.*, 2009).

Pesticides that have been outlawed in the industrialized world due to their harmful effects on human health and environmental have been found to be used in developing countries, according to studies. According to Al-Attar *et al.* (2018), the risk of diazine pesticide includes reduced body weight gain, increased serum albumin, glucose, low density lipoprotein cholesterol, and cardiac health risk while high density lipoprotein cholesterol, glutathione and superoxide dismutase were decreased to advance acute and chronic reproductive problems, organ failure, neurotoxicant, respiratory tract, interference with the nervous system, weakening neuro and immune system, skin irritant and environmental pollution (Al-Attar *et al.*, 2018; Badr, 2020; Koshlukova & Reed, 2014; Vásquez-Castro *et al.*, 2008; Vásquez-Castro *et al.*, 2012). Despite the fact that the pesticide residue detected were below the MRL, with frequent use, poor handling and storage practices, inappropriate use and dosage have a negative impact on the environmental and human health. The usage of unnamed/unknown pesticides concerned agricultural extension workers. According to studies, noncompliance with suggested procedures and pesticides policy has an impact on consumer health and environmental safety (Mengistie *et al.*, 2017). Lack of training, poor pesticide handling and storage system and lack of strict pesticide policy are the disadvantages of using banned/unnamed pesticides (Mekonen *et al.*, 2019; Mekonnen & Agonafir, 2002; Mengistie *et al.*, 2016; Mequanint *et al.*, 2019). Pesticide could be decreased or destroyed during processing, according to Bajwa & Sandhu(2014).



**Figure 3. 12:** Chemical Structure of (a) Diazinone ( $C_{12}H_{21}N_2O_3PS$ , MW: 304.35 g/mol) (b) Fenitrothion ( $C_9H_{12}NO_5PS$ , MW: 277.23 g/mol) (c) Carbaryl ( $C_{12}H_{11}NO_2$  and Molecular Weight: 201.22 g / mol) and (d) Malathion ( $C_{10}H_{19}O_6PS_2$ , MW: 330.36 g/mol) (Naughton & Terry, 2018)

### 3.3.21. Chloride Residue in Water

Water is important because it is utilized during processing as cleaning medium, as an ingredient in the food industry or for direct consumption. To qualify as a process and cleaning medium as well as direct consumption, water must be portable, clean, and colourless and fulfil the microbial and chemical limits. Water, on the other hand, can be a source of contamination and cause of water borne diseases when used directly or indirectly. Table 3.18 and Table 3. 19 shows both ground and municipal water was found to be contaminated with potential microorganisms includes yeasts and moulds, potential pathogenic microbes and solutes which impacts its quality for cleaning, processing and or direct consumption. The sources of water contamination were primarily due to poor hygienic condition, leakage along the pipelines, longer residence time and inefficient treatment. Studies revealed that water is a vehicle of microorganisms in regards of the sources and lack of treatments (Asfaw *et al.*, 2016; Onyango *et al.*, 2018), hygienic and handling

practices (Chalchisa *et al.*, 2017) and processing (Bedada *et al.*, 2018), and wet and dry seasons for microbial survival (Amenu *et al.*, 2014).

In Ethiopia, water contamination has resulted in serious sporadic and outbreak illness occurrence (Adane *et al.*, 2017). As a result, chlorinating water has been used as an effective mitigation (Mengistie *et al.*, 2013; Zelelew *et al.*, 2018). Chloride, on the other hand, interact with other organic materials in water, accumulating as unsafe metabolites to human health (Zelelew *et al.*, 2018). The lower level of total dissolved solids (TDS) is suitable for quality water used both for direct consumption or a process and cleaning medium and as part of the ingredient. The Higher the TDS level in ground water (1046.67 ±148.94 mg/L) explains the presences of minerals, metals, ions and other organic solutes. Meanwhile, Municipal distributed pipe water TDS level was 219±2.0 mg/L far below the limit of WHO recommendation for drinking water (600 mg/L) (WHO, 2006). However, the TDS level of municipal water for processing and cleaning purpose might not fit, the remaining solutes interact with food and other solids affects the overall quality and safety (Fawell *et al.*, 2003).

**Table 3. 18:** Water quality assessment mean value expressed in terms of the total solids and chlorine residue

Sample	Hazard	Mean value	Tolerable limit	P-value, R <sup>2</sup>
MW	Free chloride (mg/L)	0.21 <sup>a</sup> ±0.01	0.2-0.5	<0.0001, 0.99
	Temperature (°C)	17.5 <sup>a</sup> ±0.75		0.06, 0.63
	pH	7.56 <sup>b</sup> ±0.24		0.0324, 0.73
	TDS (mg/L)	219 <sup>b</sup> ±2.0	<1000	<0.0001, 0.96
GW	Free chloride (mg/L)	Nd		
	Temperature (°C)	15.98 <sup>a</sup> ±0.67		
	pH	6.97 <sup>a</sup> ±0.21		
	TDS (mg/L)	1046.67 <sup>a</sup> ±14		

NB: Means ± STDEV, ND: not detected; Where MW = municipal water, GW = ground water, and TDS for total soluble solids

Municipal pipe water temperature and pH values were found to be higher, on average 17.5 °C with alkaline properties (pH = 7.56). While, ground water temperature and pH was lower 15.98 °C, neutral (pH = 6.97), it might affect the sensorial quality and microbial quality. Zhu *et al.* (2016) found out that water electrolytes significantly affect the organoleptic quality of wines. Municipal water free chloride residue was found to be 0.21±0.01 mg/L within the WHO standard which is (0.2 - 0.3 mg/L) at delivery point for drinking water. Free chloride residue was not detected in groundwater. Nevertheless, microbial contaminants were detected in both ground and pipe water, presented in Table 3. 19, even though chlorination treatment was practiced explains the inefficiency of water chlorination treatment process (4 mg/L should be added) according to the centre for disease control and prevention. This explains the conditions and frequency of chlorination might be not as per the recommended practices. The free chloride residue detected in municipal pipe water used for cleaning and sesame condition while oil extraction might be responsible for quality reduction such as discoloration, unpleasant flavour development and toxic metabolites accumulation due to side interactions.

### 3.3.22. Microbial Hazards

Microbial contaminants of aerobic bacteria, total Coliforms, fungi, *Aspergillus species*, and *Staphylococcus aureus* were predominantly identified in sesame seed, CSO, water, employee clothing, equipment, indoor and outdoor air and premises are presented in Table 3.19 and Table 3.20. *Staphylococcus aureus* and *Aspergillus species* were the potential pathogens identified. However, *Escherichia coli*, salmonella, and *Shigella* were not detected. The source of microbial contaminants was found to be the extraction establishment after examining the surface of equipment and premises, protective clothing, indoor, and outdoor air and water. Aerobic plate count bacteria (APC) are microbial population grow and survive in an aerobic and mesophilic environment. APC are important microbial indicators to assess product quality such as organoleptic quality and shelf life, manufacturing and hygienic practices, and cleanness of food establishment and product safety to a lesser extent (Jaja *et al.*, 2018; Maharjan *et al.*, 2019) and process efficiency for safer produce (Ratajczak *et al.*, 2015). The APC for CSO was 2.44 log<sub>10</sub> CFU/ml oil lower than the acceptance limit of < 6 log<sub>10</sub> CFU/ml which applies to raw foods (Mailoa *et al.*, 2017). The sources of APC contamination were the surface of equipment and premises (4.68 log<sub>10</sub> CFU/m<sup>2</sup>), water (2.16 log<sub>10</sub> CFU/ml), Indoor and outdoor air (2.08 log<sub>10</sub>

CFU/m<sup>3</sup>), and personal protective clothes (2.08 log<sub>10</sub> CFU/m<sup>2</sup>) with significant variability. Groundwater was more contaminated than pipe water and indoor air during production recorded significantly higher counts. The prevalence of APC explains unhygienic handling, inefficient and sporadic cleaning as well as environmental factors such as warm climatic conditions, dry and dusty environment.

Coliforms are non-spore-forming and *Enterobacteriaceae* group of gram-negative rod-shaped microbial flora capable of lactose fermentation and can survive in soil, water, human and animal intestine at aerobic and facultative anaerobic conditions at nearly body temperature. Coliforms indicate pathogenic microorganisms originating from the gastrointestinal tract with a potential to cause food and waterborne illness as a result of poor environmental sanitation of food establishment, food handlers, and improper food processing and handling conditions (Blanch *et al.*, 2007; Nkere *et al.*, 2011). Total coliforms identified in CSO were as large as 2.39 log<sub>10</sub> CFU/ml oil lower than the acceptance limit of < 4 log<sub>10</sub> CFU/ml applied to processed foods. However, coliforms detected in animal manure (5.81 log<sub>10</sub> CFU/ml), the surface of equipment, and establishment premise (4.36 log<sub>10</sub> CFU/m<sup>2</sup>) were above the limit explains the inefficiency of hygienic practices in the premises and equipment. Coliforms in water (2.43 log<sub>10</sub> CFU/ml), protective clothing (2.0 log<sub>10</sub> CFU/m<sup>2</sup>) and environmental air (1.6 log CFU/m<sup>3</sup>) were within the limit. The prevalence of coliforms in the product, protective clothing, surface, and environment confirms that poor hygienic practices compromise CSO safety and quality. Nevertheless, *E.coli* was not detected in CSO.

Fungi, a microbial flora grow and survive in a wide range of environmental conditions such as humidity, water activity, temperature, time, acidity, and air (Lahouar *et al.*, 2016; Leggieri *et al.*, 2017) and in wider agricultural produce, food, food processing and storage establishments (Houbraken *et al.*, 2014; Visagie *et al.*, 2014). Fungi are categorized as beneficial and also as a hazardous microbial flora with diverse physical features (Hutkins, 2006). However, hazardous fungi to humans and animals are gaining attention due to their diverse pathogenic character and toxic metabolites (Amaike & Keller, 2011; Fri'as-De-Leon *et al.*, 2018), resistances during handling, processing, and cooking (Fountain *et al.*, 2015). Fungi were detected in sesame seed, sesame oil, and associated surfaces, environment and protective clothing. Surfaces of extraction equipment and premises were found convenient environment for fungi growth and survival (4.15 log<sub>10</sub> CFU/m<sup>2</sup>) whereas the filling machine was detected lower fungi colony count. The average

fungi identified in the indoor and outdoor environmental air during and after production was also as high as  $1.82 \log_{10} \text{CFU/m}^3$  with insignificant variability. Fungi detected were variable in size, shape, texture, and colour explains diversified fungi species were prevalent due to convenient environmental temperature (22-27 °C), humid environment, and poor hygienic practices. Fungi colonies with powdery and fluffy white, fluffy black, deep brown, green-black, mountain-like rough yellow colonies of different sizes and structures were detected. In the meantime, Fungi pathogenicity was confirmed as the *Aspergillus* species were detected in a Sabouraud Dextrose Agar with chloramphenicol powder added as a bacterial growth inhibitor at 25 °C during 5-7 days of incubation. The prevalence of *Aspergillus* species was confirmed ( $3.45 \log_{10} \text{CFU/m}^2$ ) on the selected surface of the storage and extraction facilities and  $1.25 \log_{10} \text{CFU/ml}$  in sesame seed and CSO with insignificant variability.

**Table 3. 19:** The Microbial count in log<sub>10</sub> colony forming unit of the sesame seed, CSO, Water and animal manure

<b>Sample</b>	<b>Aerobic bacteria</b>	<b>Total coliforms</b>	<b>Fungi</b>	<b>Aspergillus species</b>	<b>Staphylococcus aureus</b>
Sesame seed (log <sub>10</sub> CFU/g)	-	-	2.31 <sup>cd</sup> ±0.13	1.17 <sup>b</sup> ±0.13	-
Crude sesame oil (log <sub>10</sub> CFU/ml)	2.44 <sup>cd</sup> ±0.07	2.39 <sup>d</sup> ±0.14	2.31 <sup>cd</sup> ±0.08	1.33 <sup>b</sup> ±0.01	2.09 <sup>dc</sup> ±0.12
Municipal water (log <sub>10</sub> CFU/ml)	1.58 <sup>f</sup> ±0.44	1.46 <sup>f</sup> ±0.28	-	-	0.67 <sup>g</sup> ±0.49
Ground Water (log <sub>10</sub> CFU/ml)	2.76 <sup>c</sup> ±0.23	3.41 <sup>c</sup> ±0.14	-	-	0.83 <sup>gf</sup> ±0.31
Animal manure (log <sub>10</sub> CFU/ml)	-	5.81 <sup>a</sup> ±0.48	-	-	1.24 <sup>gef</sup> ±0.28

<sup>1</sup>All values indicate log<sub>10</sub> colony forming unit ± standard deviation. Values with different superscript letters in each column are significantly different (P-value < 0.05)

*Staphylococcus aureus* is a small (0.5-1.5 µm) spherical gram-positive facultative anaerobic multi-resistant bacteria of Micrococcaceae family staphylococcus genera. *Staphylococcus aureus* is catalase-positive, and thermostable (Seo & Bohach, 2013) animal and human pathogen associated with poor hygienic human, animal, and environmental conditions. *Staphylococcal* poisoning is known worldwide as it produces *Staphylococcal* enterotoxin when ingested the strain/ its toxins in food or in contact with surfaces contaminated with *Staphylococcus aureus*. *Staphylococcus aureus* a potential infectious pathogenic bacteria were detected in CSO as large numbers as 2.09 log<sub>10</sub> CFU/ml a few hours after production which is below the acceptable limit (< 4 log<sub>10</sub> CFU/ml). However, *Staphylococcus aureus* growth might be continued during CSO storage. It was also detected at the surface of the equipment and different sites in the CSO establishment, protective clothing. This was due to unhygienic and inappropriate handling practices. Studies have reported *Staphylococcus aureus* to survive in a wide range of surfaces including plastics and fabrics (Mcnitt *et al.*, 2021), household and marketing equipment, glass, pipes, and metallic containers (Coughenour *et al.*, 2011; Kusumaningrum *et al.*, 2003), bottled and unblotted water of different sources (Levin-Edens *et al.*, 2011), animal and animal by-products (Dabele *et al.*, 2021; Dweba *et al.*, 2019) and food (Tesfaye *et al.*, 2021), bio-aerosol (Moon *et al.*, 2014), public areas and hospitals (Weldeselassie *et al.*, 2020). It survives in a wide range of conditions such as relative humidity, high gradient temperature, salinity, and diverse environment and deceases with time and light exposure (Levin-Edens *et al.*, 2011; Mcnitt *et al.*, 2021) due to unhygienic practices and inappropriate food-handling (Ho *et al.*, 2015). Water might be directly or indirectly utilized in the plant and is a vehicle of potential microbial (Adane *et al.*, 2017; Chalchisa *et al.*, 2017) and chemical contaminants (Abdullah, 2014; Ambelu *et al.*, 2017; Mengistie *et al.*, 2013; Semagn *et al.*, 2020). Packaging materials are important to conserve and protect food, convenient for consumers, ease transportation (Muncke *et al.*, 2020). However, packaging material selection based on the nature of the product is important.

**Table 3. 20:** The microbial count in log<sub>10</sub> colony forming unit of the swap samples of equipment, premises, clothing, and indoor and outdoor environmental air by passive air sampling during and after production in the crude sesame oil extraction facilities

<b>Sample</b>	<b>Aerobic bacteria</b>	<b>Total coliforms</b>	<b>Fungi</b>	<b>Aspergillus species</b>	<b>Staphylococcus aureus</b>
<b>Surface ((log CFU/m<sup>2</sup>))</b>					
Milling and pressing machine	4.55 <sup>ab</sup> ±0.12	4.54 <sup>b</sup> ±0.12	-	-	2.87 <sup>b</sup> ±0.19
Filtration machine	5.06 <sup>a</sup> ±0.09	4.52 <sup>b</sup> ±0.13	-	-	2.56 <sup>bc</sup> ±0.31
Filling machine	4.34 <sup>b</sup> ±0.15	3.99 <sup>bc</sup> ±0.05	3.73 <sup>b</sup> ±0.09	-	-
Floor of sesame store	4.83 <sup>ab</sup> ±0.13	4.47 <sup>b</sup> ±0.20	4.47 <sup>a</sup> ±0.07	3.50 <sup>a</sup> ±0.09	3.22 <sup>ab</sup> ±0.05
Wall of the extraction plant	4.62 <sup>ab</sup> ±0.18	4.28 <sup>b</sup> ±0.11	4.27 <sup>ab</sup> ±0.20	3.37 <sup>a</sup> ±0.16	-
<b>Protective clothing</b>					
Glove (log CFU/glove)	2.11 <sup>def</sup> ±0.20	1.78 <sup>def</sup> ±0.22	1.18 <sup>e</sup> ±0.29	-	3.77 <sup>a</sup> ±0.36
Gown (log CFU/gown)	2.05 <sup>def</sup> ±0.10	2.23 <sup>cd</sup> ±0.20	3.01 <sup>c</sup> ±0.33	-	3.26 <sup>ab</sup> ±0.14
<b>Air (log CFU/hr)</b>					
Indoor air during production	2.55 <sup>cd</sup> ±0.19	1.63 <sup>ef</sup> ±0.28	2.06 <sup>d</sup> ±0.11	-	1.82 <sup>de</sup> ±0.04
Indoor air after production	2.17 <sup>de</sup> ±0.18	1.36 <sup>f</sup> ±0.39	1.73 <sup>de</sup> ±0.23	-	1.54 <sup>def</sup> ±0.09
Outdoor air during production	1.18 <sup>ef</sup> ±0.10	1.70 <sup>ef</sup> ±0.16	1.70 <sup>de</sup> ±0.57	-	1.35 <sup>def</sup> ±0.18
Outdoor air after production	1.79 <sup>ef</sup> ±0.08	1.73 <sup>def</sup> ±0.12	1.79 <sup>de</sup> ±0.08	-	0.74 <sup>g</sup> ±0.20

<sup>2</sup>All values indicate log<sub>10</sub> colony forming unit ± standard deviation. Values with different superscript letters in each column are significantly different (P-value < 0.05)

### 3.3.23. Physical Hazards

Physical hazards were important quality parameters considered for sesame grade as indicated in Table 3.2. The physical hazards identified in sesame seed and sesame value chain were sesame straw, sesame leaves, grasses, non-sesame seed such as weeds, immature underweight sesame seeds, clay mud from the field, fragments of insects, pieces of product packaging, and wood fragments, and burnt seed after roasting treatment. Impurity and seed colour was the prime factor in sesame grading and price setting and most visual quality specification. The sources of physical hazards were included in the raw materials, badly maintained facilities and equipment, improper production procedures and poor employee practices which require thorough cleaning and proper handling.

### 3.4. Conclusion

Organic farming, post-harvest practices, value addition, marketing and CSO extraction are the dominant sesame value chain. Farmers, researchers, regulatory authorities, technical experts, and market dealers with an active and passive ways of participation were the actors play a significant role in the sesame value chain. Variability of agro-climatic factors, illiteracy, inadequate infrastructure and technology, absences or unaffordable price of improved breed, sesame sensitivity to external factors, volatility or low market price, and lack of market information are some of the challenges that contribute to low sesame productivity and post-harvest and economic losses. The internal premises of the CSO extraction plant was partially met of the sanitary requirement with no written sanitation program. While, the external premises, violate the sanitary requirement of food processing plants. Standardizing operational and sanitary capabilities requires extensive training and low enforcement. The inadequate agricultural practices, insufficient food hygiene and handling practices were found to be hampering the sesame value chain. Potential hazards included pathogenic microorganisms, chemical and physical hazards that were detected in the sesame value chain. Moulds and *Aspergillus* species fungi with different physical features were detected. Following the growth of fungi in sesame, CSO, sesame seed cake, prevalence for aflatoxin were detected exceeding the tolerable limit. Pesticides were also used in the sesame value chain in particular in the field for pest prevention, field drying and sesame storage warehouses, were detected in levels not exceeding the tolerable limit. Thought, the skills and

knowledge of labour for pesticide application were lacking with the conditions, frequency, time and dose of applications. Water was found to be a vehicle of contaminants associated in as an ingredient during sesame conditioning or used as cleaning medium. Microbial hazard, chloride residue and TDS in water was persistent. Furthermore, potential microbial hazards include total aerobic counts, coliforms, *staphylococcus aureus* detected in sesame seed and sesame-based products, surfaces, and personal clothing and indoor and outdoor environmental. In conclusion, training on good agricultural practices, hazard prevention, hygienic practices and strict food policy, regulation is critical to improve the food value chain and food safety policy.

## CHAPTER FOUR

### DETERMINATION OF THE CRITICAL CONTROL POINTS IN THE COLD MECHANICAL PRESSING LINE OF CRUDE SESAME OIL EXTRACTION LINE IN THE TIGRAY SESAME OIL EXTRACTION PLANT.

#### Abstract

Sesame is economically and nutritionally important oil seed crop used as a cooking, dressing and frying oil in human diet. For safer and quality oil extraction, handling and quality control are critical. The objective of this research was to determine the critical control points (CCPs) during CSO extraction following the unit operations. Three CCPs were identified were sesame seed roasting, pressing while conditioning, and filling (packaging). Hazards critically controlled were included fungi with different physical features and fungi metabolites as large as 14.98  $\mu\text{g}/\text{kg}$  during roasting, pathogenic bacteria including *staphylococcus aureus* (2.87 log CFU/g), aerobic plate count bacteria (4.55 log CFU/g) and chlorine residue (0,21 mg/L) during extraction while conditioning and *aerobic plate count bacteria* (4.34 log CFU/g) and oxidative metabolites as large as the PV, p-AV and TOTOX of 81, 23.5 and 174.6  $\mu\text{g}/\text{kg}$ ), respectively, during filling operations. Due to a lack of awareness, and economic barriers, the prerequisite programs, which included the extraction facility placement, and the design of the premises were inadequate. As a result, cross contamination and unhygienic condition were the causes for the prevalence of the potential hazards and identified to be critically controlled.

#### 4.1 Introduction

Food safety has enormous challenges along the value chain, particularly in the small-scale food processing sectors that include value addition and food service provision establishments in densely populated low -income societies. Food safety and handling culture are inextricably linked though in most cases severely compromised as a result of the complex and dynamic nature of food, consumer demographic characteristics, processing and handling conditions and other factors. Food traceability tools used in hazard analysis critical control point (HACCP), standard operating procedure (SoPs) and other have been utilize and proven to be useful in maintaining food safety suited to a specific food product in a well-defined unit operations and process flow diagram

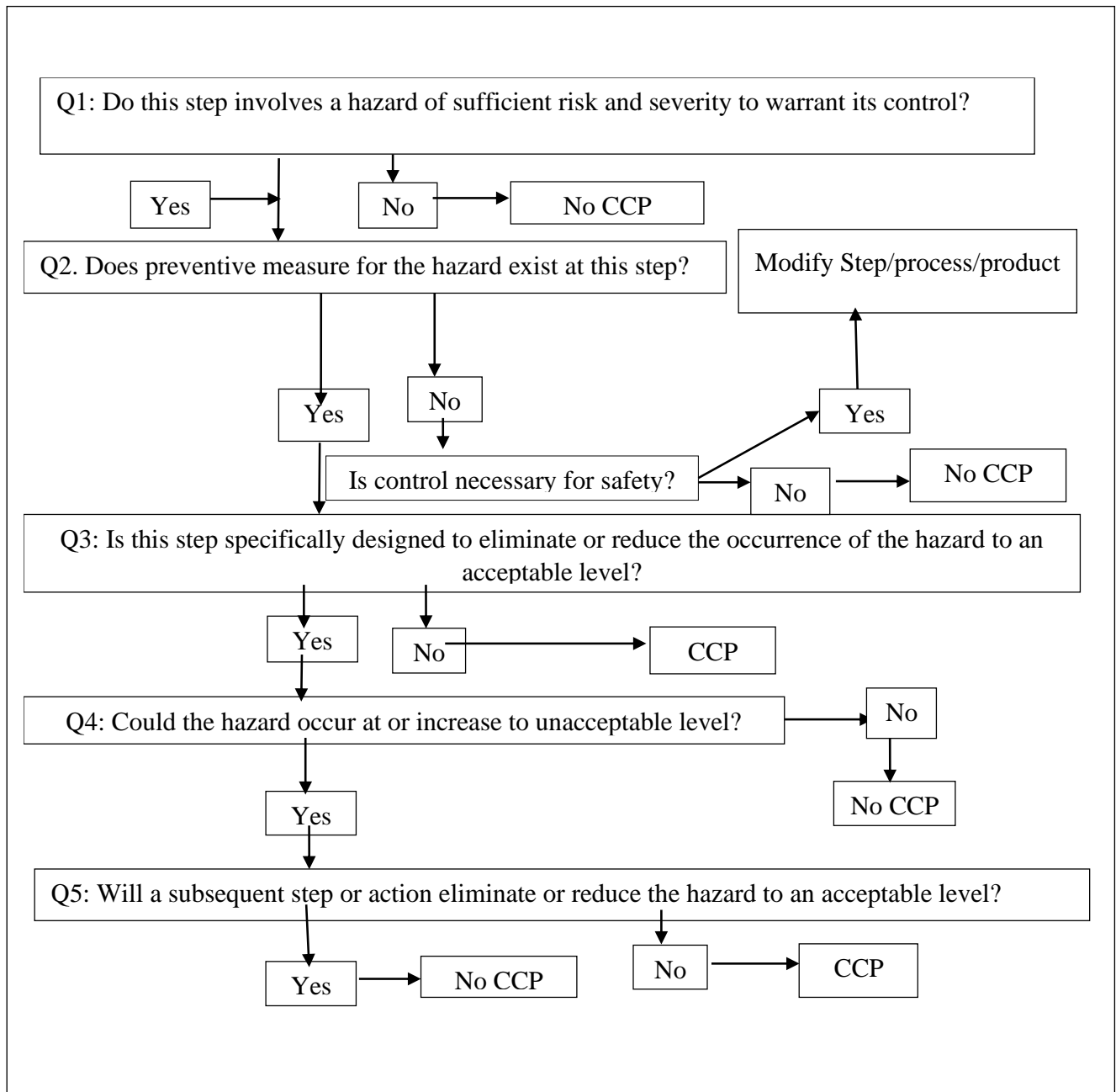
(Miarka *et al.*, 2019). Hazard control/preventive measures are actions, activities, process/procedures in the CSO extraction plant based on the evidences provided to prevent, eliminate or minimize food safety hazard (s) to an acceptable level (Ceylan *et al.*, 2021). The control measures include raw material and supplier inspection, good extraction practices, premises hygienic condition and infrastructural supply. Control/preventive measures include the location selection and design of the premises, internal and external premises cleanliness, smoothness of the internal premises, zoning within the production area to include wet and dry area separation, high risk and low risk area, equipment layout, and restricted passage and others. Control measures are designed with the nature of the food or hazard(s) in mind, as well as the interaction with the process, and the critical limit for each important control point determined (Amit *et al.*, 2017).

Critical Control Points (CCPs) are identified along the process, where control is used to prevent, eliminate, or reduce to an acceptable level of any hazards identified, with the help of a CCP decision tree (Figure 2.2, task 7). Microbial contaminants were detected in numerous foods, causing major health problems, according to studies, and HACCP was found to be effective method of preventing food hazards. Four CCPs were identified along the process to assure mushroom safety and edibility, with unauthorized phytosanitary, pathogens and heavy metals as a potential hazards that needed to be controlled (Pardo *et al.*, 2013). Cooking, fermentation, drying and sieving have been identified as CCPs for a safer Turkish traditional food Tarhana processing prevent survival pathogens and other microorganisms and foreign material (Dalgıç & Belibağlı, 2008). Mohamed *et al.* (2015) reported the use of four CCPs during olive oil extraction to protect olive fruit reception from microbial growth, insect infection, pesticide residue and enzymatic induced reactions. Cleaning water, malaxation and centrifugal extraction were recognized as CCPs to prevent the occurrence of potential hazards during olive oil production. Hung *et al.* (2015) reported two CCPs throughout the pasteurization and freezing process processes of peanut butter. Ozturkoglu-Budak (2017) found five CCPs critically important in dried red chili pepper production to inhibit fungal growth and mycotoxin synthesis prevention during washing and sorting, drying in oven, grinding, packaging and bulk storage. However, too many CCPs are not

recommended in the process from the perspectives of operational management and cost implication.

#### **4.2 Materials and Methods**

Hazards analysis was carried out following Figure 2.2, Step 7, as described in Chapter 5, and CCPs were determined in the crude sesame oil extraction line. The CCPs were determined in the crude sesame oil extraction process assisted by the CCP decision tree, according to Codex, (2009), presented in Figure 4.1 and supplementary data presented in appendix I. The hazards required to critically controlled during crude sesame oil extraction were fungi and fungi metabolites, *staphylococcus aures*, aerobic plate bacterial, oxidative metabolites and chlorine residue. Fungi and fungi metabolites were potential hazards where the handling effectiveness could be measured. CSO oxidation level is a storage stability indicator where CSO shelf life were determined. The oxidation value exceeds the tolerable limit the oil is considered unfit for consumption. Aerobic plate count bacteria, good indicator for the operation and cleanness of food processing facilities and easiest microbe where food business operators used for verification. *Staphylococcus aureus* was a potential pathogen were detected in CSO, water and associated surfaces. Those hazards were selected to critically controlled based on their frequent of occurrence and associated risk to incurred in consumer health, simplicity of analysis and associated cost implications to control during the actual operation and analytical techniques (Sperber, 2001).



**Figure 4. 1:** The critical control point decision tree with the a series of queries in identifying CCPs in the crude sesame oil extraction process (Codex, 2009)

### 4.3 Results and Discussion

Tables from 3.8 – 3-12 and Figure 3.9 and Figure 3.9 in chapter 3 summarises the PRPs and OPRPs which are important for the set-up plant for HACCP plan in the crude sesame oil extraction plant. The preventive measures and critical control points (CCPs), critical limits (CLs) for every CCPs identified and the monitoring, corrective action and verification procedures for each CCPs implemented summarized. CCPs identified were raw material reception and storage, conditioning and pressing, and packaging (filling) during CSO extraction. The hazards detected during raw material reception and storage includes presence of fungus, *Aspergillus* species, fungi toxic metabolites, pesticide residue in sesame storage and chloride residue in water and microorganisms. Moisture content, regulatory maximum residue levels (MRL) for pesticide and chloride residue concentration were established as critical limits. Moisture content, microbiological, pesticide and chloride residue analysis and records have been developed to monitor and verify the implementation of CCPs. Sesame seed reception and storage, conditioning while press extraction and filling processes were recognized as CCPs for safe CSO production. The following section will discuss the critical control points identified and verification procedures during crude sesame oil extraction in the processing plants during the study. This section includes sesame reception and storage (CCP<sub>1</sub>), Conditioning and packaging (CCP<sub>2</sub>) and filling (packaging) stage (CCP<sub>3</sub>), presented in Table 4.1.

#### 4.3.1. Sesame Seed Roasting (CCP<sub>1</sub>)

The presence of fungus infection and fungi metabolites, were the potential hazards in sesame seed which compromises safety and contributes to quality loss. Fungi were cultured appeared with various physical features includes in light yellow, blue, green and oranges coloured colonies with different size and texture. The texture of the fungi was mountain like rough, sponge –like, and tree like structures of the colonies. The fungus toxicity and ability to produce mycotoxin were confirmed in Sabouraud Dextrose Agar chloramphenicol powder added as anti-bacterial growth inhibitor, *Aspergillus* species were discovered, total aflatoxin, aflatoxin B<sub>1</sub> and pesticides residues levels were potential hazards, as shown in Table 3.17, Table 3.18 and Table 3.19. CCP established during sesame seed roasting (Table 4.1) to prevent fungi growth and associated metabolites (mycotoxin). Seed moisture (< 10%), seed roasting temperature and roasting time (200 °C for 20-

30 min) were settled as critical limits. The CCP implementation could be verified with mycotoxin analysis and should be within the tolerable limit.

#### 4.3.2. *Sesame Seed Conditioning and Pressing Process (CCP<sub>2</sub>)*

CCP was considered during crushing/pressing sesame seed while continued conditioning whereby the pathogenic and hygiene indicator bacteria were the potential hazards with no subsequent process to prevent. Conditioning while pressing was one of the operations to expel the oil from the seed with the aim of easing the crushing process, minimize the energy expenditure and reduce holding time. Though, no other chemical treatment were added. The hazards that needed to be controlled were *Staphylococcus aureus*, aerobic plate count bacteria, and chlorine residue. The critical limit was determined to be the water chlorine residue and the cleanliness of the plant's internal premises and machinery. Additionally, a microbiological challenge test was employed to verify the implementation of the CCP. The pre-HACCP aerobic plate count bacteria (4.55 log CFU/m<sup>2</sup>), total Coliforms (4.54 log CFU/m<sup>2</sup>), *staphylococcus aureus* (2.87 log CFU/m<sup>2</sup>) and oxidative metabolites were detected in CSO syrup of swab samples of the milling machine while pressing, presented in Table 3.19. The reasons for those microbial contaminants were due to poor hygienic practice. The post HACCP aerobic bacteria count shows a significant reduction during pressing, and filtration as well as the raw material store and wall of the extraction plant, as shown in Figure 3.8. The CLs established were absence of pathogens or below the acceptable limit, other hygiene indicator microorganisms below the tolerable limit and water chloride residue below 0.2 mg/L, summarized in Table 3.18, Table 4.12 and Table 4.13.

#### 4.3.3. *Packaging (CCP<sub>3</sub>)*

Packaging (filling) (CCP<sub>3</sub>) were identified to be critically controlled to avoid cross contamination, as there is no subsequent process/mechanism to prevent CSO cross contamination. *Staphylococcus aureus*, total aerobic bacteria count and oxidative metabolites detected in CSO after HACCP implementation were below the tolerable limit. *Staphylococcus aureus* was not detected in the filling machine with post-HACCP practices explains the efficiency of cleanness. Although, CSO safety cannot be granted as those microorganisms might be continued to revive and jeopardize CSO safety. Aseptic sterilization and filling, as well as CSO moisture content ( $\leq$  0.2%), and coloured PET and non-transparent glass packaging material were established as critical

limits in addition to the cleanness and use of protective equipment/clothing was considered as the monitoring procedure/techniques during filling and packaging. CSO storage at lower temperature (< 15 °C) in a tightly packed package and use of organic preservatives to prevent the revival of those microorganisms and prevention of oxidation reaction shows a promising potential in CSO shelf-life extension and quality retention.

Crude sesame oil expected shelf life with roasting treatment at ambient condition storage was established based on the Rancimat method assuming the first order TOTOX accumulation. The rate of reaction was calculated as 0.13 per day and CSO quality index of 7.7 daily. The reaction rate was calculated as  $\frac{d(TOTOX)}{dt} = -K(TOTOX)^n$ , Where TOTOX is overall oxidation value, dt is change in storage time (days), K is rate of reaction and n is the order of reaction. The CSO quality index (QI) is the inverse of rate of reaction  $K = \frac{1}{QI}$ . Arrhenius' Equation was used to estimate the CSO expected shelf life, as follows  $K = C(TOTOX)e^{\frac{-E_a}{RT}}$ . Where Ea is activation energy (kJ/mol) to trigger reaction and accumulation oxidative metabolites, R is the gas universal constant (8.318 kJ/mol), and T is the absolute temperature (°K). Accordingly, the CSO expected shelf life was no more than 2 months. The shorter CSO storage life explains the lacking refining process and inappropriate handling condition.

**Table 4. 1:** Summary of the HACCP plan and corresponding hazard, critical control points, critical limit, monitoring procedure, corrective action, verification procedure and records during CSO extraction

CCPs	Process step	Hazards
CCP <sub>1</sub>	Roasting	Fungi (M)
		Mycotoxins (C)
CCP <sub>2</sub>	Conditioning while pressing	<i>Staphylococcus aureus</i> (M)
		Aerobic plate count (M)
		Chlorine residue (C)
CCP <sub>3</sub>	Filling (Packaging)	APC bacteria) (M)
		Oxidative metabolites (C)

#### **4.4. Conclusion**

Three critical controls points identified to prevent fungi and fungi metabolites, aerobic plate, *staphylococcus aureus* bacterium, and an oxidation reaction were found throughout the sesame seed roasting, conditioning while pressing, and filling processes. Understanding and implantation of the CCPs significantly reduces the occurrences of those potential hazards. Yet, the moisture content of CSO was measure above the acceptable limit for edible oil. This moisture will affect the storage stability of CSO during storage by initiating undesirable biochemical reaction such as discoloration and unpleasant deep flavour and oxidation reaction. The moisture content mitigation without refining process requires further investigation.

## CHAPTER FIVE

### ESTABLISH THE CRITICAL LIMITS OF CCPS WITH THE CORRESPONDING MONITORING AND VERIFICATION PROCEDURES IN THE COLD PRESS CRUDE SESAME OIL EXTRACTION IN THE CRUDE SESAME OIL EXTRACTION LINE IN THE TIGRAY SESAME OIL EXTRACTION PLANT.

#### Abstract

Critical limit and corresponding monitoring and corrective action are the basics for the implements of CCPs. Falling to understand and implement the CLs will lead to unsafe CSO production and shorten the shelf life. The CLs established were roasting temperature and time (200 °C/20-30 min), absences of *staphylococcus aureus* or below the tolerable limit ( $\leq 25$  CFU/ml) (11-88% reduction), absences of APC bacteria or below the tolerable limit (3 log CFU/g), residual chloride  $\leq 0.2$  mg/L during CSO extraction while conditioning, and APC bacteria absence or  $< 3$  log CFU/ml (31-79% APC reduction), oxidative metabolites levels, Primary ( $< 10$ ), secondary (30), TOTOX ( $< 50$  meq O<sub>2</sub>/kg oil) during packaging. The monitoring and corrective procedures were process condition, cleanness with alkaline detergent followed by sanitization, introduction of organic anti-oxidant (16-90 % oxidation prevented) and care full selection of packaging material with excellent barrier properties to light and environmental air under controlled storage condition. Implementation of the established CLs and corresponding monitoring and corrective action guarantee safe production and protect public health. Yet, training and encouragement on the CLs and corresponding monitoring/corrective action is crucial to develop confidence and ownership.

#### 5.1.Introduction

Critical Limit (s) (CLs) are measurable, monitored, and confirmed standards of a single or multiple parameters that are specified for each CCP found to ensure an absolute tolerance of hazards and food safety management (Figure 2.2, task 8). The CLs comprise the upper and lower boundaries in order to prevent, eliminate and or reduce potential hazards while maintaining product quality. The CLs are also used to categorize product as safe or unsafe, if CL is violated, product is deemed unsafe or lost its quality and acceptance. The CLs are of criteria used to describe the hazard(s) behaviour and factors that influence its activation or inhibition, based on literature,

experts, experimental data and predictive computer models (Mannaa & Kim, 2017; Parra & Magan, 2004). According to Pardo *et al.* (2013), CLs determined were based on the type of hazard of pathogenic bacteria and heavy metals, as well as, the application of authorized phytosanitary with the optimal doses and duration. Dalgiç and Belibağlı (2008) establish CLs for Tarhana as a combination of time and temperature, as well as and impurity level. Hung *et al.* (2015) identified optimal pasteurization (80-85 °C/15 minutes) and cleanness of the freezing process for safe peanut butter processing. Controlling the water activity (0.492-0.59) was critical to ensuring the safe honey of distribution (SerİN *et al.*, 2017). Grain water activity, storage temperature and controlled ventilation prevents the growth of fungus and mycotoxin synthesis in maize grain (Medina *et al.*, 2014).

Monitoring is HACCP established during the development and implementation of HACCP plan (Figure 2.2, task 9) based on the activities and actions conducted in the process through visualization, measurement and traceability record keeping, and action taken when deviation is observed. CCPs are monitored based on the who, what, when, and how inquiries concerning the control measures (Ozturkoglu-Budak, 2017). Microbiological examination of mushroom casting materials was used as a monitoring parameter during raw material reception, casting materials replacement being the corrective measure, and analytical records as a verification parameter during mushroom production (Pardo *et al.*, 2013). Corrective actions are activities and procedures taken when the CLs established for each CCPs are violated and the product safety is jeopardized (Figure 2.2, task 10). The Changing of the casting material was a necessary corrective procedure (Pardo *et al.*, 2013).

HACCP verification is a HACCP principle (Figure 2.2, task 11) that is defined as the validation of a HACCP plan after a single or multiple parameter analysis, tracing record, and document keeping procedures other than activities monitoring procedures to confirm HACCP system operation in accordance with the designed plan. The international life science institute for risk analysis develops a verification and validation procedure for HACCP based on microbiology analysis to support scientific and technical evidences, measure the efficiency of producing safe and sound food by adopting and implementing a HACCP plan (ILSI, 2001). Microbial data provide useful for validating HACCP plan in the food processing industry (Swanson & Anderson, 2000). According to Hong *et al.* (2008) presences of aerobic bacteria was considered effective to validate the HACCP in pork processing plant. Presences of hygiene indicator microorganisms at certain

levels were considered as cleanness verification in poultry slaughterhouses (Hutchison *et al.*, 2006).

The final HACCP principle is documentation and record keeping (Figure 2.2, task 12). Besides the nature of the raw material and nature of food product, documentation and record keeping includes material receipt data with some measured parameters, storages conditions, analytical record, consumer complaint records, product record and product dispatch records. During mushroom production, records of microbiological analysis, casting usage and replacement were used as a verification parameter (Pardo *et al.*, 2013). However, the majority of food processing industries, particularly small-scale facilities, fail to establish control system and documentation on routine activities.

## **5.2. Material and Methods**

Critical limits and corresponding monitoring and corrective actions for each CCPs determined were established as presented in Table 4.1. According to Zwietering *et al.* (2010) and Ackah *et al.* (2018), HACCP plan practice verification was evaluated by microbiological challenge testing against a specific microbe. Aerobic plate count bacteria (Hong *et al.*, 2008) and *staphylococcus aureus* (Reda *et al.*, 2017) were used to validate the HACCP plan effectiveness according to the standard microbiological testing method.

## **5.3. Results and discussion**

The critical limits for each of the identified CCPs which are discussed in chapter 4 as well as the associated monitoring and corrective action protocols are compiled in the table below Table 5.1.

### *5.3.1. Critical limits for the CCP Identified*

The critical limits established for each CCPs identified during CSO extraction are summarized, in Table 5.1. The roasting time and temperature at which fungus and their metabolites (mycotoxin) were prevented to the lowest possible level were the crucial parameters for CCP<sub>1</sub> during the roasting of sesame seeds. The high heat treatment (200 °C/20–30 min) hindered the growth of the fungus, as table 3.19 shows. The critical limit of residual chlorine in water, which is  $\leq 0.2$  mg/L, was established as the critical limit during CSO extraction while conditioning to prevent chlorine presences in the CSO or prevent side undesirable reaction. This limit was also set for the absence of *staphylococcus aureus* and or ( $\leq 25$  CFU/ml), total aerobic bacteria absences or be  $\leq 3$  log

CFU/g. These limits are outlined in Table 3.18. The critical limit established during CSO filling (packaging) were absolute absence of *staphylococcus aureus*, oxidative metabolites include peroxide value < 10 meq O<sub>2</sub>/kg oil, p-anisidine value < 30 meq O<sub>2</sub>/kg oil, TOTOX value < 50 meq O<sub>2</sub>/kg oil) and aerobic count bacteria absences or below the tolerable limit (3 log CFU/g).

### 5.3.2. *Monitoring and Corrective Actions for the CCPs*

The corresponding monitoring and corrective measures for the detected CCPs during CSO extraction are discussed and summarized in Table 5.1. In order to prevent cross-contamination, the monitoring and corrective action during the roasting of sesame seeds (CCP<sub>1</sub>) involves measuring the roasting temperature and roasting duration of each batch using a thermometer and an automatic timer while roasting process follows the FIFO. Corrective measures, such as visual examination of over- or under-roasted sesame seeds, the culture method of fungal cultivation, and mycotoxin analysis by random sample when visual quality defect identified, are implemented when the roasting condition deviates. Refer to Figures 4.2a and Figure 4.4b for the monitoring and corrective procedure during CSO extraction while conditioning (CCP<sub>2</sub>). Additionally, the cleaning and conditioning water must meet the water specification and be free of microorganisms, level of TDS, and chlorine residue. The machinery and internal CSO extraction premises must be cleaned with 30% alkaline detergent (caustic soda) in warm water (50–55 °C) and followed by sanitization (0.2–0.5% NaOCl) effective after extraction. Use of water should be stopped if a deviation was detected and no chemical or thermal water treatment was done. Aseptic sterilization while filling with 0.5 ppm H<sub>2</sub>O<sub>2</sub> at 80 °C for 15 seconds, filling condition and storage adjustment with controlled humidity (67%) and storage and humidity (67%) at a temperature of < 20 °C, use of protective clothing, standard hand washing procedure, refer to Figures 4.3 and 4.4, and introduction of organic preservatives were the monitoring and corrective action during CSO filling (packaging) (CCP<sub>3</sub>). When there is a recorded deviation from the CLs, the CSO should be recalled unless they undergo an additional heating process of > 121 °C for 28–30 minutes, excuse employees and visitors who exhibit symptoms of illness or who are not wearing protective gear, and use packaging materials that have barrier qualities against light, air, and corrosion when the CSO comes into contact with the package.

**Table 5. 1:** Summary of the critical limit and corresponding monitoring and corrective measures, verification procedure and records during CSO extraction

CCPs	Critical limits	Monitoring procedures	Corrective actions
CCP <sub>1</sub>	Roasting temperature and time	Roasting temperature and holding time using thermometer and timer of every batch, refer Table 3.19	Visual inspection / culture method of analysis of signs of fungi infection Follow FIFO Reject, reprocess at a temperature (> 100 °C)
CCP <sub>2</sub>	Absence of pathogens or below the tolerable limit ( $\leq 25$ CFU/ml)	Cleanness with alkaline detergent (30% caustic soda) with warm water (50-55°C) followed by sanitization (0.2 -	Avoid use of poor water quality for conditioning or cleaning
	Absence of APC or below the tolerable limit (3 log CFU/g)	0.5 % NaOCl) after extraction, refer Figure 4.2 and Figure 4.4a.	
	Residual Chloride $\leq 0.2$ mg/L in water, Table 3.18	Meeting water specification Avoid use of ground water, unless treated	Avoid use of water with chlorine residue and high total soluble solids exceeding the acceptable limit
CCP <sub>3</sub>	Aseptic sterilization and filling	0.5 ppm H <sub>2</sub> O <sub>2</sub> at 80 °C for 15 seconds	Reject, unless further heating process at above 121 °C for 28-30 min
	APC bacteria absence or < 3 log CFU/ml	Storage and humidity (67%)	Reject, unless there is heating process

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	temperature (< 20 °C), Use of protective clothing, follow standard hand washing procedure, refer Figure 4.3 and Figure 4.4.	Excuse sign of illness of employees /unprotective clothing
Oxidative metabolites levels, Primary (< 10), secondary (30), TOTOX (< 50 meq O <sub>2</sub> /kg oil)	Measuring the oxidative metabolites Signs of discoloration and off flavour development Introduction of antioxidants (organic), refer Figure 5.5	Packaging with good barrier to light and environmental air

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### 5.3.3. HACCP Verification

Preventive measures for food safety management, such as prerequisites and HACCP implementation, are cost effective and help to increase the food market value and competitiveness. Plus to the monitoring procedures, verification is described as activities to assess the validity of HACCP plan and its effectiveness of implementation in accordance with the plan. HACCP may be carefully planned, but due to engineering matters, lack of knowledge, untrusted food safety legislations/regulations, financial and skilled human resource limitation, lack of competitiveness, successful food safety tool implementation remains a challenge (Kafetzopoulos *et al.*, 2013). HACCP verification activities / tools vary depending on the scale of production, nature of the product, the technology and general infrastructure of the processing facility. Hence, there is no approved technique of HACCP verification. Spiegel *et al.* (2004) presented the possible models for HACCP and others food safety management tools in food business validation based on 1) system/process approach, 2) customers accustomed horizontal approach, 3) Goal/Question/Process indicators (metric approach), 4) horizontal approach, 5) performance approach, 6) critical success factors, 7) balanced scorecard (Financial, customer, internal business, and learning and growth perspectives) and 8) performance of indicators. All the HACCP validation techniques are based on scientific publications, experimental trails and operational data, experience, regulatory documents, and survey (Brackett *et al.*, 2011; Scott, 2005 ). When it comes to HACCP, verification is are vital, and every employee should be trained through theoretical, practical audio/video visual training, technical support and improving the physical state of the food processing facilities (Sharma *et al.*, 2019; Wallace *et al.*, 2005).

HACCP verification measures during CSO extraction are vital for oil safety and quality distribution. Microbial contamination and oxidation reactions were observed and determined in this study, presented Table 5.2. This was as a result of improper handling, sporadic and insufficient hygienic practices, and convenient ambient state. Cleaning was deemed the best indicator for post-HACCP verification. A microbiological challenge test was used to confirm the cleanness. APC and *staphylococcus aureus* were chosen as indicators of HACCP efficacy and sanitary practice because APC is easier to analyse and *staphylococcus aureus* was the potential pathogen existing at different surfaces including air happen with inadequate processing and poor hygienic practice. Studies revealed that, *Staphylococcus aureus* was found in public areas, equipment surfaces, air,

animals and animal feces as a result of poor hygienic practices (Kozajda *et al.*, 2019). This was confirmed with positive detection of *staphylococcus aureus* in the indoor and outdoor air, surfaces of machines and inside premises (wall and floor), personal clothing, water, animal manure and CSO. A post-HACCP microbial challenge test was performed, which confirmed a 9-88% drop in aerobic plate bacteria count and a 31-79% drop in *staphylococcus aureus*. CSO's pre- and post-HACCP aerobic bacterial counts were 2.44 log CFU/ml and 1.68 log CFU/ml, which were within the tolerated limits 3 log CFU/ml for final product, dropped by 31%, while dropped by 60.5% in ground (borehole) water. CSO's pre- HACCP *staphylococcus aureus* counts was log 2.09 CFU/ml exceeded the tolerated limits log 1 CFU/ml for final product and post-HACCP *staphylococcus aureus* count of CSO was log 0.61 CFU/ml according to the Codex standards, dropped by 70.1%, while dropped by 56.1 and 79.4% in ground (borehole) and municipal water, respectively. The rate of bacterial count reduction was estimated as the difference between pre- and post HACCP count divided by pre- HACCP count multiplied by 100 shows high variation explains the process efficiency variation, higher initial microbial count and handling inadequacy. According to Hong *et al.* (2008), aerobic bacteria were shown to be sufficient to validate HACCP in the pork processing industry.

**Table 5. 2:** Summary of the verification procedure and records during CSO extraction

CCPs	Verification	Records
CCP <sub>1</sub>	Absences of fungi	Records of fungi analysis
	Mycotoxin absences or below the tolerable limit, Table 3.17	Records of mycotoxin analysis
CCP <sub>2</sub>	Absence of or below the acceptable limit of <i>Staphylococcus aureus</i>	Microbial analysis
	Absence or below the tolerable limit of APC bacteria	Microbial analysis
	Chlorine residue below the tolerable limit	Chlorine residue analysis
CCP <sub>3</sub>	Microbial challenge test	Record of microbial analysis
	APC bacteria absence or below 3 log CFU/ml	APC analysis records
	Oxidative stability records	Oxidative stability or off flavour/discoloration reports

As a result of washing and soaking with 3% sodium hypochlorite (NaOCl) sanitizer for 5-10 minutes at ambient condition, the post-HACCP APC in gown and glove detected at levels of 0.38 log CFU/gown (81.3% reduction) and 0.8 log CFU/glove (61.6% reduction), respectively. Karale *et al.* (2011) reported that NaCl is effective in prevention of potential microbial hazards stimulated public health problem. Meanwhile, post-HACCP *staphylococcus aureus* counts in the gown and glove detected were 0.55 log CFU/gown and 2.05 log CFU/glove, reduced by 41.6% and 45.7%, respectively. This explains the insufficient disinfection. The reduction rates of APC and *staphylococcus aureus* on the equipment surface and the inner part of the premises were 52-78% and 31.2-50%, respectively. To summarize, HACCP was designed to assure the safety of CSO and the microbiological challenges tests were sufficient to validate the HACCP system. Although, due to dynamic proliferation, the post-HACCP bacteria count was worrying for post CSO treatment. Absolute microbial prevention might not be possible although optimal concentration and exposure time of cleaning agent is vital for efficient cleaning.

Other studies reporting microbiological criteria has been used as an effective tool to verify and test the effectiveness of HACCP plan and its implementation. Aerobic and total coliforms

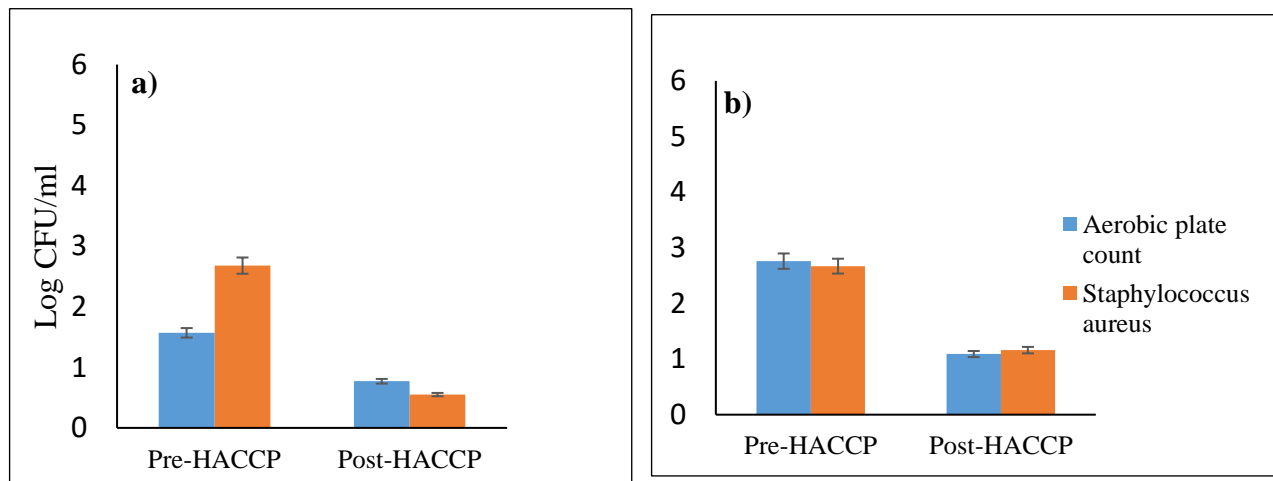
microorganisms have been commonly used in dairy and pork processing plants and packaging equipment's (Ackah *et al.*, 2018; Domenech *et al.*, 2013; Hong *et al.*, 2008). In this study, cleaning and disinfection of processing equipment's effectively decreased the occurrence of pathogens. According to Ackah *et al.* (2018) HACCP was implemented and microbial challenge test conducted on shito, a spicy smooth dark brown sauce hermetically packed product with a self-life of 12 months primary developed for vegetarians was witnessed a significant reduction of potential pathogens. Plant and equipment design and structure, unfavourable for hygienic and cleaning purposes, lack of traceability, transportation and lack of supply were cited as non-conformities with the HACCP system in the small scale ice-cream sector (Domenech *et al.*, 2013).

#### 5.3.4. Microbial Challenge Test Verification Procedure

The small-scale crude sesame oil extraction plant's adherence to the HACCP plan is tested in this section using a microbiological challenge test on the water used, personal hygiene, surfaces, and products addressed.

Water is used as an element in the oil extraction plant as a conditioning and cleaning medium. According to the Pre-HACCP microbial analysis, water sample from the municipal water distribution pipe lines and from the ground (borehole) had high numbers of aerobic bacteria, total Coliforms and *staphylococcus aureus* (see, Figure 5.1), which explains water could be a vehicle for contamination. Water treatment, on the other hand, exhibits a large reduction of the microbial contaminant. Plant based water treatment is the most effective in the food processing industry. A study by Desye *et al.* (2021) reported that plant based water treatment is effective and could remove up to 91% of the total Coliforms, and 99% faecal Coliforms. Water purification, according to Ethiopian studies, helps to prevent waterborne illness. Boiling, bleaching, straining, filtration, Solar Water Disinfection (SODIS) and letting it stand were among the water treatment methods practiced at household level and small and medium scale food processing business (Geremew *et al.*, 2018; Geremew & Damtew, 2020; Soboksa *et al.*, 2019; Tafesse *et al.*, 2021). However, issues arose as a result of insufficient water supply design, poor hygienic habits, urbanization, and the high cost of heating water. As a result, providing safe water remains a challenge in the food processing industry, for direct human consumption and other environmental aspects (Wang *et al.*, 2014). However, it is expensive to invest in, particularly in small scale food processing business.

Considering the nature of contaminants in water and dirt at the surfaces of the equipment and premises in the small-scale food businesses use boiling (heat) and chlorination method of water treatment methods are commonly used for effective cleaning. As a result, community-based water chlorination treatment technologies with affordable cost and easy to operate, shows in a significant increase in water safety. Warm water with medium thermal treatment (50-60 °C) is also used during sesame conditioning and cleaning to facilitate the oil extraction. On the other hand, warm water application may cause oxidation, hasten microbial growth and other undesirable side effects. The pre-and post-treatment microbial count differences divided by pre-HACCP count multiplied by 100 was used to calculate the percentage of microbial reduction in water samples as shown in Figure 4.2. The pre-and Post HACCP aerobic plate count bacteria result shows a reduction of 60.5% and 51.39% for ground (borehole) and municipal water, respectively. The pre-and post HACCP *staphylococcus aureus* result shows a reduction of 56.5 and 79.36% for ground (borehole) and municipal water, respectively. The lower reduction in ground water could be explained by a higher initial microbial dose, and presences of substrates in water promote continued growth, conducive condition for their revival and inefficient water treatment.

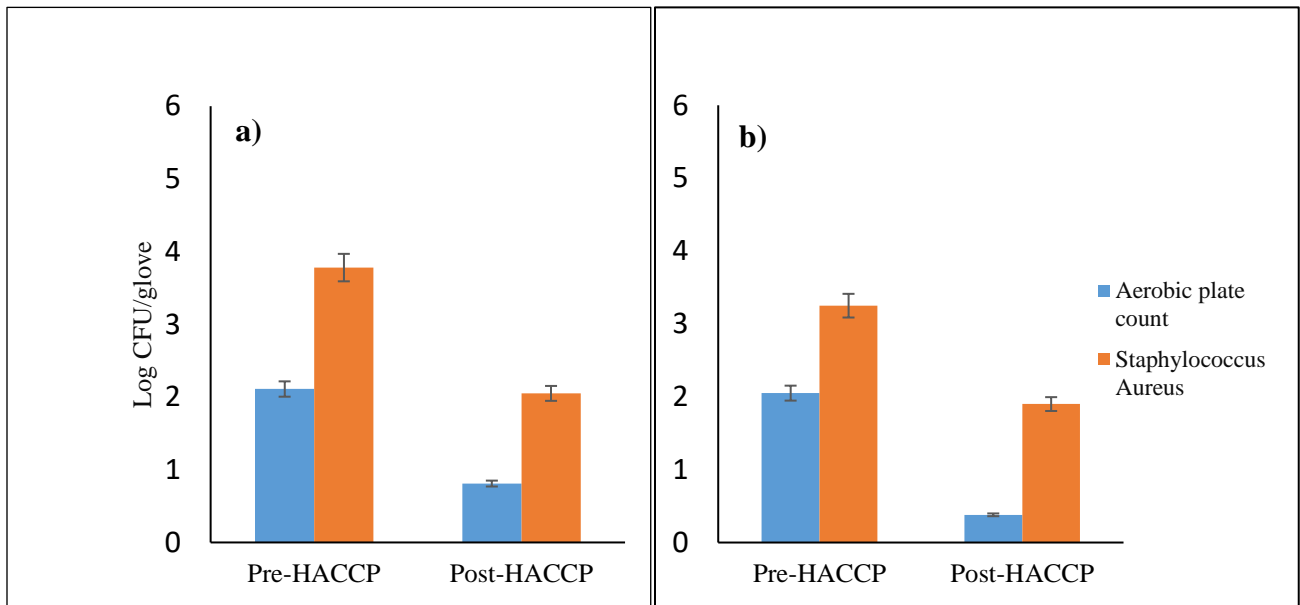


**Figure 5. 1:** The microbial quality of pre- and post- HACCP implementation in log CFU/ml of water (a) Municipal water and b) Ground water, that could be utilized as a conditioning medium or cleaning purpose

Cross contamination might occur when food is handled with bare hands, sneezing, being in contact with food with clothing kept unhygienic. As the hands, noses and other part of the body

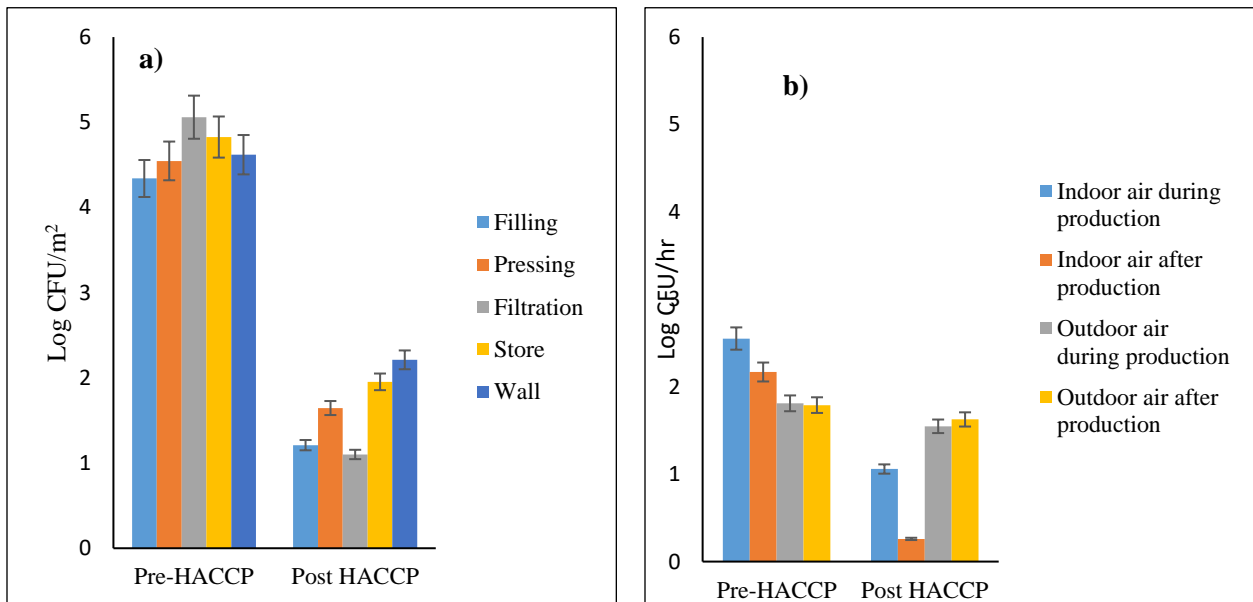
might be either the hosts of microflora inherently or transient due to poor hygienic practices. To ensure personal hygiene, while working and visiting the food business establishment, protocols to follow are presented in chapter 3, Table 3.8 and Figure 3.9 including washing with the recommended detergents and sanitizers and wearing protective clothing, is crucial in maintaining food safety in the food industry and food service facilities (Ghosh *et al.*, 2020; Marriott & Gravani, 2006; Moerman, 2017). The employee should be trained on the necessity of personal cleanliness and the implications of not practicing hygienic practices.

The protective clothing cleanliness was verified by the chemical-thermal cleaning method. Washing water (< 60 °C), detergents and sanitizers added (< 5%), and the gown and glove soaked, washed, rinsed with clean water and dried. The microbial challenge tests for gown and gloves, as shown in Figure 5.2, of the pre- and post-HACCP practices reveals a significant reduction in aerobic plate count bacteria by 81.3 % and 61.6%, respectively, and *staphylococcus aureus* bacteria reduction by 41.56% and 45.7%, respectively. The rate of microbial reduction, however, depends on the initial contamination level, composition of the soils, types of contaminant and efficiency of cleaning (time, temperature, chemicals and the area of sampling) (Bockmühl *et al.*, 2019).



**Figure 5. 2:** Employee clothing hygienic indicators a) gown and b) glove expressed in log CFU/gown or glove

Aerobic plate count (APC) bacteria swab sample analysis conducted on the surface of the pressing, filtration, filling machine, and store floor and extraction premises wall before and after thorough cleaning shows a significant reduction within the range of 52-72%, as shown in Figure 5.3a. With through cleanness the APC bacteria detected below the tolerable limit ( $5 \times 10^5$  CFU/ml) for most foods. The indoor and outdoor passive air samples aerobic plate count bacterial analysis shows a significant reduction as indicated in Figure 5.3b. The APC bacteria reduction in the Indoor air during and after extraction accounted to 55 and 88%, respectively. Yet, the APC bacterial level of reduction during and after extraction from the outdoor environment was smaller compared to the indoor air. The APC bacteria level of reduction before and after though cleaning during and after CSO extraction accounted 14.5 and 9%, respectively. This explains sanitation is effective means to avoid hazard association in the surrounding extraction establishment. Meanwhile, thought a significant reduction was observed trace of microorganism might be easily revived.



**Figure 5. 3:** The Pre- and Post HACCP Aerobic plate count bacteria microbial population of selected instruments, inside premises of the CSO extraction plant, indoor and outdoor air of the extraction plant (a) swab samples of selected surfaces and (b) indoor and outdoor air of the crude sesame oil extraction plant

Cereals, legumes and oilseeds are durable grain crops that might be stored for longer periods of time at low moisture content and controlled storage condition. However, as a results of the infestation, microbial growth and operational losses in the durable crop value chain have an impact on both health and economic value (Abass *et al.*, 2014). Sesame oil crop in western Tigray is lost up to 35% of its production during harvesting, drying, un-threshed, piles transportation, storage and packaging, and cleaning (Gebretsadik *et al.*, 2019). Storage is the major contributor and packaging during storage with moisture barrier properties reduce loss and contains the quality. According to Tola *et al.* (2021) investigation, the post-harvest loss of sesame was shown a significant reduction during 12 months of storage with different packaging materials. The packaging materials with increasing level of sesame loss rate were purdue Improved Crop Storage bags, metal silos, GrainPro bags and polypropylene bags. Besides to the reduction of post-harvest loss reduction, packaging and storage condition selection contributes to preserves the nutritional and functional properties (Fotouo-M *et al.*, 2020). When it comes to edible oil packaging and handling, the choice of packaging material and storage conditions is critical. CSO packaging in yellow PET away from direct sunlight exposure were deemed as the preventive measures for the final product CSO, summarized in chapter 3.

PET is popular because of its light weight, stiffness, high strength, creep characteristics, low interaction with oil and chemical resistance property, strong moisture, light and gas barrier properties at affordable cost (Demirel *et al.*, 2011; Panowicz *et al.*, 2021). As a result, aseptic sterilization with 30% hydrogen per oxide ( $H_2O_2$ ) at 80 °C for 15 seconds holding time was found to be the most effective for preventing microbiological contamination without causing damaging to the plastic packaging material (Ansari & Datta, 2003). A study by Ramezani (2004) which investigated the oxidative stability of sunflower oil after storing in the dark for one year concluded that using yellow PET and stainless metal packaging material was better than the clear PET and HDPE packages. Packaging is also necessary to preserve the benefits of the product after the process is completed, allowing it to travel over long distance while maintaining the safety and originality. However, while choosing packaging, it is necessary to balance between cost, barrier features, and environmental impact, as well as ease of use (Marsh & Bugusu, 2007).

Records and its documentation procedures is important for the assessment of HACCP plan adequacy, compliance and recall traceability and auditing. Based on the literature information, the records at the desk of the food safety management should contain specifications of raw materials and other minor/ functional ingredient, policy and regulatory guidelines, certificate of assurance of all supplies, analysis and operational records, compliance and recall records (Arvanitoyannis & Traikou, 2005; Mauropoulos & Arvanitoyannis, 1999; Sanddrou & Arvanitoyannis, 2000). Violating the proper documentation and record keeping however results failure of the HACCP or leads to lack of transparency on its implementation (Arvanitoyannis & Savelides, 2007). The records and documentation required in the CSO extraction plant are summarized in Table 5.2 and Table 5.3.

**Table 5. 3:** List of records documented in CSO plant for traceability and HACCP plan implementation control

<p>In the CSO extraction plants records documented were;</p> <ul style="list-style-type: none"> <li>– For every delivery of sesame seed records were taken, this included the capacity, delivery date, delivery mechanism, price, area of harvest, seed colour, seed moisture content and impurity level</li> <li>– Crude sesame oil extraction yield, distribution, by-product, Dispatch and marketing records</li> <li>– Delivery of cleaning detergents and sanitizers</li> <li>– Storage condition records (humidity, temperature, duration)</li> <li>– Operational and analysis records</li> <li>– HACCP plan, CCPs, CL, monitoring, corrective and verification records</li> <li>– Hygiene guidelines and records</li> <li>– Visitors (regulatory, customers, researcher) records</li> </ul>
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#### 5.4. Conclusion

Measurable critical limits that align with mentoring and corrective action protocols are useful for implementing CCPs and HACCP. The microbial challenge test was used to confirm the efficacy of HACCP demonstrates a notable decrease in the possible risks that were found to require strict management in order to produce and distribute CSOs more safely. The results of the

microbial challenge test show an improvement in the hygienic situation with a microbiological decline within the allowed range. Inadequate sanitary conditions were the source of those hazards, and maintaining cleanliness required cleaning as a necessary course of action. But, if a post-filling operation is not carried out, there remains a possibility that the microbial hazard will become dangerous. Nonetheless, food safety, on-site hygiene standards, regulatory enforcement and industrial infrastructure enhancement are crucial.

## CHAPTER SIX

### ESTABLISH THE HACCP PLAN IN THE COLD PRESS CRUDE SESAME OIL EXTRACTION IN THE CRUDE SESAME OIL EXTRACTION LINE IN THE TIGRAY SESAME OIL EXTRACTION PLANT.

#### Abstract

HACCP plan is a complete product tailored tool for safer food production and distribution. HACCP Plan for CSO during production shows a significant importance in improving CSO safety where the potential hazards were prevented and or reduced to the limit of acceptance. However, commitment and enforcement are important as the food business owners and employee understanding on HACCP and other food safety tools is poor requires attention.

#### 6.1.Introduction

In the food industry, food safety management is critical, could not be undermined as it possesses to protect public health, bust confidence of the food business owners and develops consumer trust. Total quality management, food safety objectives and hygienic practice and HACCP are the commonly tools used in the food industry to build safety practices (Arvanitoyannis & Varzakas, 2009; Lee *et al.*, 2021). HACCP is codex Alimentarius guideline for its implementation. HACCP follow the three steps grouped as the pre- requisite (PR), operational pre-requisite program (OPRPs) and critical control points (CCPs) following the HACCP working protocols summarized in Figure 2.2. It includes the hazard identification and analysis on the potency to causes public health problem, how frequent occurred and response to the processing condition. Upon implementation of HACCP plan, it compromises the product, unit operation, human nature, over all plant condition and technology (Maupoulos & Arvanitoyannis, 1999). HACCP plan is the whole set of HACCP principles, tailorized to a specific product with primary objective of safe food production (Arvanitoyannis & Savelides, 2007; Dalgiç & Belibağli, 2008; Kourtis & Arvanitoyannis, 2001; Sanddrou & Arvanitoyannis, 2000; Tzouros & Arvanitoyannis, 2000). However, due to lack of understand and transparency, HAACP plan failure have been a challenge in the food industry which jeopardize food safety and negatively compromise public health

(Arvanitoyannis & Varzakas, 2007). Thought, HACCP plan works to significantly prevent the potential hazards, it requires continuous modification and strict follow up.

## 6.2. Material and Method

The data from chapter 3, chapter 4 and chapter 5 makes up the HACCP plan in the CSO extraction plant in western Tigray, Ethiopia.

## 6.3. Results and discussion

HACCP plan for safe CSO production and distribution is summarized in Table 6.1 (product description), Figure 6.1 (CSO production diagram with corresponding CCPs identified), Table 6.2 (HACCP plan) and Table 6.3 (CCP check list). During HACCP plan implementation everyone involved in the CSO are responsible however, management commitment is crucial. Hence, for the HACCP plan implementation and continued follow-up the quality control department, if available as the CSO extraction is small-scale or the regulatory are responsible for its implementation, as presented in Table 6.2.

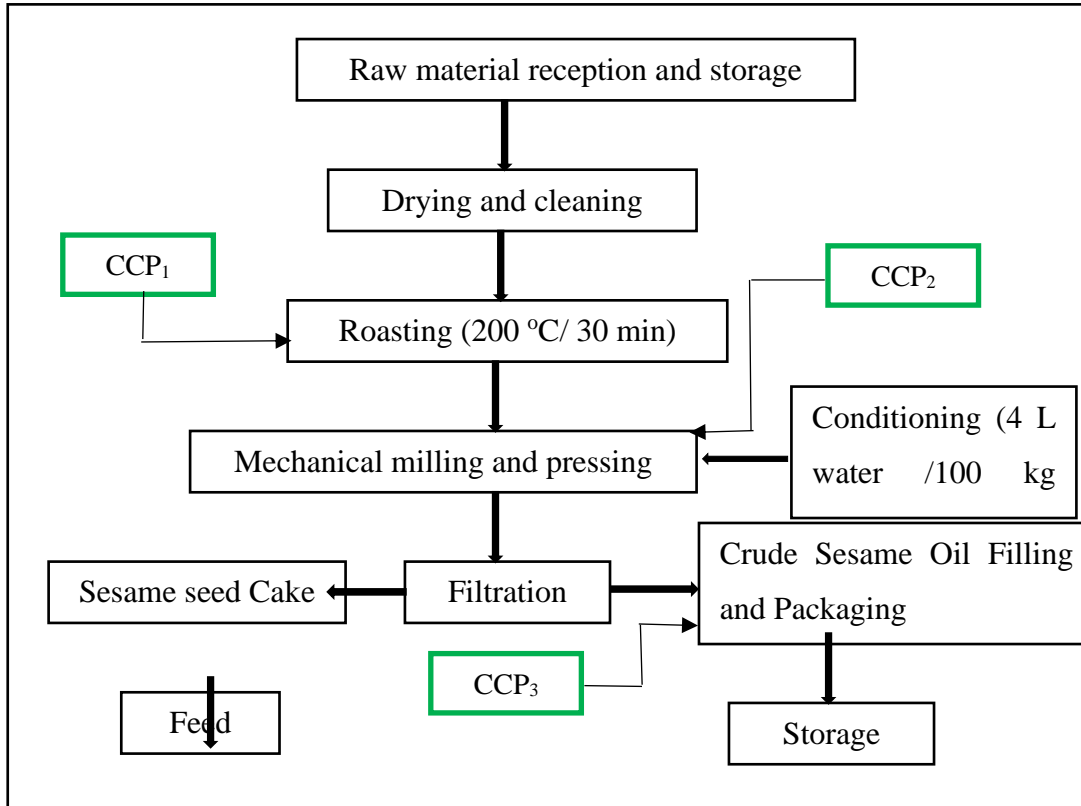
### 6.3.1. Crude Sesame Oil Product Description

**Table 6. 1:**Crude Sesame Oil Product description

Product name	Crude Sesame oil (CSO) cooking oil
Raw material	<ul style="list-style-type: none"> <li>• Sesame seed</li> <li>• Water</li> </ul>
CSO Composition	<ul style="list-style-type: none"> <li>• Moisture content (0.37%), requires further investigation</li> <li>• Unsaturated to saturated fatty acid</li> </ul>
Packaging	<ul style="list-style-type: none"> <li>• Coloured Polyethylene terephthalate</li> <li>• High Density Polyethylene</li> <li>• Coloured glass bottles</li> </ul>
Expected shelf life	<ul style="list-style-type: none"> <li>• No more than 2 months</li> </ul>
Use	<ul style="list-style-type: none"> <li>• Cooking, if possible, exclude using for salad making due to the lack of refining process</li> </ul>

### 6.3.2. Crude Sesame Extraction Flow Diagram and Associated CCPs

Figure 6.1 shows the CSO extraction flow diagram in the western Tigray, Ethiopia. It is a small-scale oil extraction plant where strict follow up and low enforcement to adopt to the HACCP system. This will encourage to the other small and medium scale food processing facilities.



**Figure 6, 1:** Crude Sesame Oil extraction flow diagram using a mechanical milling and cold pressing method and the critical control points

### 6.3.3. HACCP Plan During CSO Extraction

Table 6.2 summarize the HACCP plan during CSO extraction. the three CCPs, critical limits for each CCPs and corresponding monitoring and corrective measures and verification procedure. Though, HACCP is every one's responsibility the HACCP team and management commitment is critical for its implementation.

**Table 6. 2:** HACCP plan during crude sesame oil extraction

Process step	Hazards	Critical limits of CCPs	Monitoring procedures	Corrective actions	Verification	Records	Responsible
Roasting (CCP <sub>1</sub> )	Fungi growth (M)	Roasting temperature and time	Roasting temperature and holding time using thermometer and timer of every batch, refer Table 3.19	Visual inspection Follow FIFO	Absences of fungi Follow FIFO	Records of fungi analysis	Quality assurance department/regulatory
	Mycotoxins synthesis from fungi (C)			Reject, reprocess at a temperature (> 100 °C)	Mycotoxin absences or below the tolerable limit, Table 3.17	Records of mycotoxin analysis	Quality assurance department/regulatory
Conditioning while pressing (CCP <sub>2</sub> )	<i>Staphylococcus aureus</i> (M)	Absence of pathogens below the tolerable limit ( $\leq 25$ CFU/ml)	Cleanness with alkaline detergent (30% caustic soda) with warm water (50-55°C) followed by sanitization (0.2	Detergent and sanitizer concentration Water temperature and contact time	Absence of Microbial or below the acceptable limit of <i>Staphylococcus aureus</i>	Microbial analysis	Quality assurance department/regulatory

Aerobic plate count (M)	Absence of APC or below the tolerable limit (3 log CFU/g)	- 0.5 % NaOCl) after extraction, refer Figure 4.2 and Figure 4.4a.		Absence or below the acceptable limit of APC bacteria	Microbial analysis	Quality assurance department/regulatory
Chlorine residue (C)	Residual Chloride $\leq$ 0.2 mg/L in water, Table 3.18	Meeting water specification Avoid use of ground water, unless treated	Avoid use of water with chlorine residue and high total soluble solids exceeding the acceptable limit	Chlorine residue below the tolerable limit	Chlorine residue analysis	Quality assurance department/regulatory
Filling (Packaging) (CCP <sub>3</sub> )	APC bacteria (M)	Aseptic sterilization and filling APC bacteria absence or < 3 log CFU/ml	0.5 ppm H <sub>2</sub> O <sub>2</sub> at 80 °C for 15 seconds Storage and humidity (67%) temperature (< 20 °C), Use of protective	H <sub>2</sub> O <sub>2</sub> concentration And aseptic condition at above 121 °C	Microbial challenge test APC bacteria absence or below 3 log CFU/ml	Quality assurance of department/regulatory Record of microbial analysis APC analysis records

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clothing, follow  
 standard hand  
 washing  
 procedure, refer  
 Figure 4.3 and  
 Figure 4.4.

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Oxidative metabolites (C)	Oxidative metabolites levels, Primary ( $< 10$ ), secondary (30), TOTOX ( $< 50$ meq O <sub>2</sub> /kg oil)	Measuring the oxidative metabolites Signs of discoloration and off flavour development Introduction of antioxidants (organic), refer Figure 5.5	Packaging with good barrier to light and environment al air	Oxidative stability records	Oxidative stability or flavour/discolora tion reports	Quality assurance department/regulato ry
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6.3.4. CCPs Checklist During the Implementation of HACCP Plan

**Table 6. 3:** CCPs check list during CSO extraction

<b>Sesame seed Roasting</b>				
	Reject	Reprocess	Accept	Remark
Fungi contamination	_____	_____	_____	_____
Mycotoxin contamination	_____	_____	_____	_____
<b>CSO extraction while conditioning</b>				
Prevalence of <i>Staphylococcus aureus</i>	_____	_____	_____	_____
Prevalence of APC	_____	_____	_____	_____
Residual chlorine in water	_____	_____	_____	_____
<b>CSO Filling (packaging)</b>				
Prevalence of APC	_____	_____	_____	_____
CSO Oxidation PV	_____	_____	_____	_____
p-AV	_____	_____	_____	_____
TOTOX	_____	_____	_____	_____

#### **6.4.Conclusion**

Understanding the CSO production lines and HACCP implementation greatly lowers the likelihood of a hazard occurrence to the safest level. This study could be regarded as a pioneer in the field, especially for small- and medium-sized food processing businesses, food catering, and other related fields where upholding public health is a primary objective. However, further work from both internal and external bodies might be needed to monitor the HACCP strategy. In order to preserve public health and foster customer trust, it is crucial that regulations adhere to this and support food service companies in developing their own HACCP plans.

## CHAPTER SEVEN

### EFFECT OF ROASTING, STORAGE TEMPERATURE AND ETHANOIC BASIL (*OCIMUM BASILICUM L.*) EXTRACT ON THE OXIDATIVE STABILITY OF CRUDE SESAME SEED (*SESAMUM INDICUM L.*) OIL

#### Abstract

Crude Sesame oil (CSO) is unrefined edible oil, under uncontrolled process condition and with the absences of preservatives, thus, could be impacted the storage stability and CSO safety. The purpose of this study intends to determine the oxidative stability of CSO. In a complete randomized (2\*2\*2) factorial design, clean sesame seeds (3.92 % moisture content) was roasted at 200 °C. The roasted sesame seed oil extraction yield was higher (50.9%) composed of 83.15 % unsaturated fatty acids with an omega-6 to omega-3 ratio of 95.3 and CSO moisture content (0.37%), exceeded the tolerable limit. Roasting and accelerated storage accelerate oxidation exceeded the tolerable limit after 25 days of storage while, the ethanoic basil extract was found to inhibit oxidation in the range of 16.38-90% (P-value <0.05). The PV, p-AV and TOTOX value of CSO with 50 ppm basil extract was detected within the range of 0.29 - 3.92, 0.75 – 2.59 and 1.57 – 8.6 meq O<sub>2</sub>/kg oil, respectively, below the tolerable limit. Nevertheless, basil extract anti-oxidant property was declined during prolonged storage in particular at elevated temperature storage. The use of organic extracts of locally available sweet basil herb is capable to mitigate oxidation and substitute inorganic anti-oxidant for a healthier diet.

#### 7.1.Introduction

Oils are important structural and functional components of foods (Akoh, 2017). The global oil consumption is rising to approximately 600 million metric tons in 2019/2020, plant-based edible oil accounted to 203.83 million metric tons by 2018/19 (Statista, 2019). The use of plant-based edible oils raising interest in the consumer market due to its nutritional and energy provision on daily diet, organoleptic profile enhancing property, pharmaceutical and cosmetic, health benefits, and non-food applications (El-hamidi & Zaher, 2018; Kumar *et al.*, 2016; Mensah *et al.*, 2018). Sesame seed is a rich source of protein and essential fatty acids, minerals, and anti-oxidants (Deme *et al.*, 2017). It has medical importance due to the presences of sesamin and sesamol to lower cholesterol accumulation, prevent high blood pressure, triglyceride linoleate inhibit

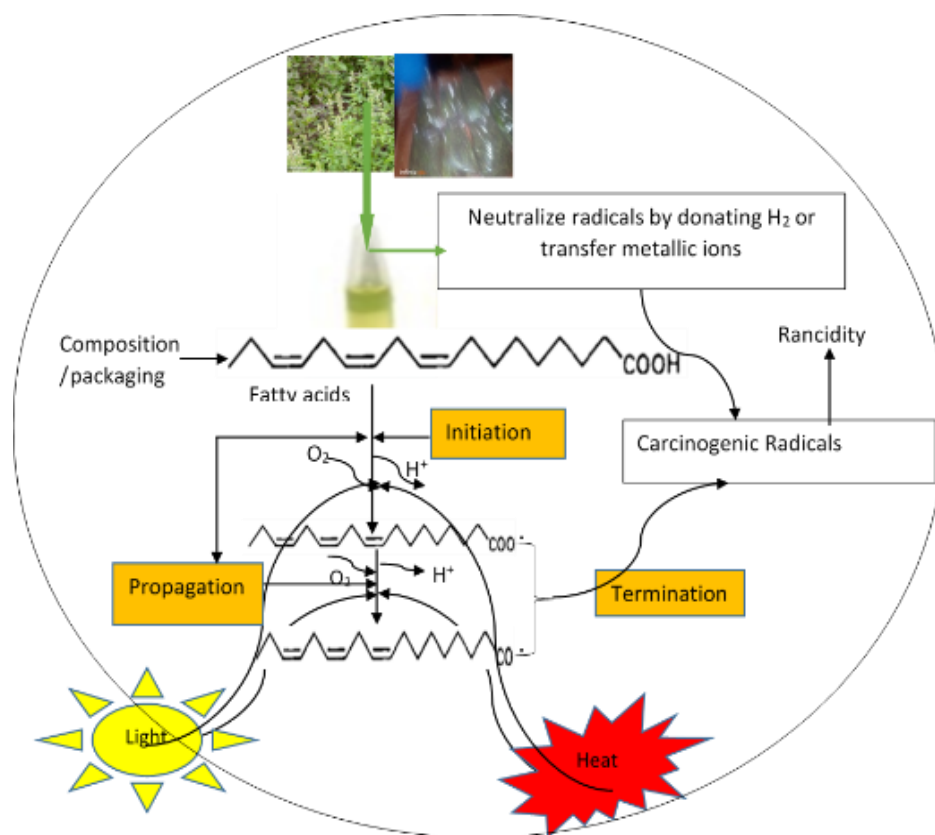
malignant melanoma growth and demulcent, used as cosmetics such as mildly laxative, emollient, poultice, a preservative as antimicrobial activity and antioxidant (lignans) to prevent oxidation (Anilakumar *et al.*, 2010; Gharby *et al.*, 2017). Flavour compounds in sesame based products include hydrocarbons, aldehydes, ketones, alcohols, acids, esters, furans, sesamol, and pyrazine which significantly changed sesame oil quality and stability regardless of seed composition and physical quality (Elkhaleefa & Shigidi, 2015) and differences of extraction methods (Hou *et al.*, 2019; Ribeiro *et al.*, 2016).

Edible oil in the Ethiopian markets are of imported, costly and shortage of demand. As a result, the small and medium scale entrepreneurs inspired with the idea of supplying extract edible extraction of locally available oil crops. Sesame is the dominant oilseed and crude sesame oil extraction and consumption is pre-dominant in the community. However, discoloration, rancid odour, unpleasant flavour and quality degradation due to storage instability and oxidation metabolite formation and use of inorganic antioxidants impacts the safety of oil and public health. Oxidation metabolites are the results of quality degradation such as fatty acids interactions, cleavage of short-chain and physico-chemical structure transformation of fatty acids (Madhujith & Sivakanthan, 2019; Vaskova & Buckova, 2015), undesirable organoleptic and toxic metabolites formation (Adibhatla & Hatcher, 2010; Ramana *et al.*, 2017). Studies reported that use of oils and oily food with oxidative metabolites composed of reactive oxygen species (ROS) damage various organs in the body by producing cytotoxic and genotoxic compounds leads chronic diseases (Jung *et al.*, 2016), disturbance of the redox state of the body, weakening the antioxidant network associated with a higher risk for atherosclerosis, diabetes and obesity (Sies *et al.*, 2005), cancer (Rockenbach *et al.*, 2011; Zhong & Yin, 2015) increases pathogenesis of myeloma and lymphoma by damaging DNA (Tandon *et al.*, 2013), Chemotherapy in Non-Hodgkin's Lymphoma (Elmezayen *et al.*, 2015), aging (Russo *et al.*, 2018), increase plasma total cholesterol level, larger atherosclerotic lesions (Hao & Friedman, 2014) and atherogenesis affects artery wall leads to heart diseases (Münzel *et al.*, 2017) and cell death (Gaschler & Stockwell, 2017; Stockwell *et al.*, 2017; Wenzel *et al.*, 2017).

The rate of oxidation is however can be controlled or reduced. Hence, understanding of the type, concentration, and valency of the metal ion and fatty acids in oil (Thanonkaew *et al.*, 2006), processing and handling condition and mechanisms of oxidation. The mechanisms of oxidation involve abstracting hydrogen from the double bonds of fatty acids or convert the triple oxygen to

a single oxygen catalysed by numerous factors, summarized in Figure 7.1. Factors to facilitate or constrain oxidation include; oilseed pre-treatments (Kombe & Temu, 2017), direct light exposure (Dalsgaard *et al.*, 2010), presences of enzyme ferrous atoms interacting with the *cis-cis* configured fatty acid during extraction in the presence of oxygen at elevated temperature (Madhujith & Sivakanthan, 2019; Silvagni *et al.*, 2012; Vaskova & Buckova, 2015), storage period (Liu *et al.*, 2019) and presences of anti-oxidants (Taghvaei & Jafari, 2015). Oxidation involves three steps: (I) initiation (II) propagation and (III) termination as shown in Figure 7.1.

Sweet basil (*Ocimum basilicum L.*) is a culinary herb belongs to the Lamiaceae family, widely produced and used as a fresh vegetable or dried form in food as a preservative and flavour enhancer. The sweet basil contains a wild range of phenolic compounds, flavonoids, and anthocyanins such as rosmarinic acid (an ester of caffeic acid of 3, 4-dihydroxyphenyl lactic acid), linalool (monoterpenoid), Estragole (phenylpropene), Terpenoids (Stereoisomer of 3,7-dimethylocta-2,6-Dane-1-ol) exhibit as antioxidant property (Anand *et al.*, 2019; Grabmann, 2005). The extraction yield is, however, significantly affected by plantation techniques and use of growth regulators, agro-climatic condition, and extraction solvent type. The extraction yield of basil essential oils was reported 27.9 kg/ha and a significant increment was reported with the application of growth regulators (Mirzajani *et al.*, 2015), methanol (6.12%), ethanol (3.42%), acetone (3.32%), hexane (3.14%) and ethyl acetate (3.12%) (Avetisyan *et al.*, 2017; Majdi *et al.*, 2020). Basil extract radical scavenging ability increases with increased concentration (Warsi & Sholichah, 2017). However, 50 µg/ml basil extract was found nearly optimal to inhibit oxidation (Jayasinghe *et al.*, 2003). The aim of this study was designed to determine CSO oxidation with roasting treatment and application of ethanoic basil extract during 35 days of storage at ambient condition and 65 °C.



**Figure 7. 1:** Summary of oil oxidation mechanisms involves initiation, propagation and termination reaction to produce stable and unstable radicals and other derived compounds responsible for oil rancidity and application of natural anti-oxidants donating a hydrogen atom or transforms metallic ions to prevent oxidation and neutralize radical formation

## 7.2. Materials and Methods

### 7.2.1. Sample Preparation

Sesame seeds were collected from Western Tigray, Ethiopia (latitude 14.032334 and longitude 38.316573, 17.5 – 41.7 °C) and transported to the oil extraction plant located in Axum, Tigray (14.1340° N, 38.7473° E). The whole fresh sweet basil (*Ocimum basilicum*) plant was collected from an irrigation area of Feresmay, Hahaile Province (1800-2100 m altitude above sea level). The old stem of sweet basil was removed, gently washed to remove dirties such as mud and non-basil plants. The clean basil plant materials were dried in an oven at 50 °C till constant moisture content (11.52 %) obtained. The dry basil was powdered (mortar and pestle grinder), sieved (250 µg) and packed in a polyethylene plastic bag wrapped with aluminium foil. Sesame

seed was roasted at 200 °C for 30 minutes in a temperature adjustable oven. Crude sesame oil (CSO) was extracted as shown in Figure 3.8 from both raw and roasted sesame seed. The PV and p-AV of CSO stored in a blue-brown glass bottles for 35 days were analysed as presented in Table 7.1. The CSO sample were drawn during 0, 3, 7, 12, 17, 21, 25, 30 and 35 days for PV and p-AV analysis in triplicates. CSO from roasted sesame seed was analysed for fatty acid composition.

**Table 7. 1:** Experimental design for the determination of crude sesame oil oxidation during 35 days of storage in a blue brown glass bottle

<b>Treatment</b>	<b>Storage Temperature</b>	<b>Basil extract</b>
Raw	Ambient	No
		50 ppm
	65°C	No
		50 ppm
Roasted (200°C/30min)	Ambient	No
		50 ppm
	65°C	No
		50 pm

#### 7.2.2. Crude Basil Extract Preparation

The ethanoic basil extract was extracted according to Warsi and Sholichah (2017) procedure. Basil powder (gram) and petroleum ether (millilitres) were mixed in a ratio of 1:4 in an extraction flask and kept with periodic shaking for 24 hours. The mixture was filtered over Whatman No.1 paper using Büchner funnel. The residue was dried in the fume hood until dry and remove the smell of petroleum ether. The dried residue was soaked with 70% ethanol (1:4), stirred for 30 minutes, and left for 24 hours with periodic shaking. The extract was filtered over Whatman No.1 paper using Büchner funnel. The residue was soaked again two times and the filtrate added to the original filtrate. The viscous ethanol extract was dried in a Büchi rotary evaporator at 60°C to evaporate ethanol. The viscous ethanol extract was packed in a blue-brown bottle and stored below 4 °C.

### 7.2.3. Determination of Moisture Content

The moisture content was determined using the oven drying method of the AOAC, 930.15, 2016. This involved drying the samples in an oven until a constant weight was attained. First, the aluminium cup was cleaned, washed and dried in at 104 °C in an oven (Gallenkamp, model OV 880, England) for 30 minutes before being placed in a desiccator to cool. The samples ( $W_1$ ) were then carefully weighed (2 grams) using a digital electronic beam balance (ISTA, 195) and placed into an aluminium cup that had been dried. The samples, along with the aluminium cup with sample ( $W_2$ ), were the placed in the drying oven at 139 °C for 30 minutes before being cooled in a desiccator to room temperature. Finally, the moisture content of the sample was determined by weighing the dried and cooled samples along with the aluminium cup ( $W_3$ ) using digital electronic beam balance. The heating and weighting were performed three times until a constant weight was obtained. The percentage of moisture content was calculated by expressing the weight loss as a percentage of the initial weight of the sample.

$$\text{Moisture Content (\%)} = \left[ \frac{W_2 - W_3}{W_1} \right] * 100$$

Where:  $W_1$ - Weight of sample (g),  $W_2$  - Weight of aluminium cup and sample,  $W_3$  - Weight of aluminium cup and the dried sample.

### 7.2.4. Determination of Acid Value

The acid value (AV) of CSO was determined using the pH-meter method according to the procedure described by Kuselman *et al.* (1998). About  $3 \pm 0.1$  g of CSO was weighted, placed into a pH meter cell. About 50 ml of TEA-KNO<sub>3</sub> (0.2M TEA and 0.01M KNO<sub>3</sub> in a solution of 50% water and 50% isopropyl alcohol) reagent into pH meter cell contains the oil sample. The electrodes were inserted into the cell and stirrer were on to mix the reagent with the oil sample and extract the free fatty acids into the TEA-KNO<sub>3</sub> reagent for about 4 minutes and stable pH<sub>1</sub> was recorded. Then 0.3 ml of 0.5M HCl was added, stirred for 1 minute and pH<sub>2</sub> value recorded. Change in pH (pH<sub>1</sub>-pH<sub>2</sub>= 0.19 - 0.2) was calculated. Then the AV was calculated as follows;

$$\text{AV, mg} \frac{\text{KOH}}{\text{g of oil}} = \frac{56.11 * N_{\text{HCl}} * V_{\text{HCl}}}{m} * (10^{\Delta\text{pH}} - 1)$$

Where:  $N_{HCl}$  is normality of HCl = 0.5 M,  $V_{HCl}$  is volume of HCl and  $m$  is sample weight, 56.11 is molecular weight of KOH.

#### 7.2.5. Determination of the Peroxide Value

The peroxide value (PV) of the CSO was determined according to the International Dairy Federation (AOAC 920.160) as described by Shantha and Decker (1994). All the reagents used (barium chloride dehydrate, iron (II) sulfate solution (0.5 g  $FeSO_4 \cdot 7H_2O$ ), hydrochloride acid, ammonium thiocyanate and chloroform-methanol) were analytical grade. Iron (II) chloride solution and Ammonium thiocyanate solution were prepared in the laboratory. About 0.2 g of sesame oil was put into a conical flask (250 ml), vortexed with 9.8 ml chloroform-methanol (7:3V/V) for 5 second. A 50  $\mu$ l Ammonium thiocyanate solution was added and vortexed for 4 seconds, followed by the addition of 50  $\mu$ l iron (II) solution. The mixture was vortexed for 2-4 seconds, absorbance reading at 500 nm performed immediately but no longer than 10 minutes. The absorbance of the blank cell contained all reagents except the oil sample was measured at a wavelength 500 nm. The peroxide value was computed as;

$$PV \text{ (meqKg}^{-1}\text{)} = \frac{(A_s - A_b) * S}{55.84 * m_o * 2}$$

Where:  $A_s$  is absorbance of sample solution,  $A_b$  is Absorbance of blank,  $S$  is slope,  $m_o$  is sample weight, 55.84 is atomic weight of Iron.

#### 7.2.6. Determination of the Para -Anisidine Value

The p-Anisidine value (p-AV) was determined according to AOCS cd 18-90 (1995) method as described by Abdelazim *et al.* (2013). Analytical grade chemicals Isooctane and p-anisidine in glacial acetic acid (1 mL 0.25%, w/v) were used for analysis. Oil samples (0.5 L) in a flask dissolved with Isooctane (25 mL) to measure the absorbance at 350 nm using a UV-Vis spectrophotometer. Five millilitres of the above mixture were mixed with 1 mL 0.25% p-anisidine in glacial acetic acid (w/v), stand for 10 min, absorbance was taken at 350 nm using a UV-VIS spectrophotometer against the blank. TOTOX value was as the summation of the para anisidine value and twice of the Peroxide value. The p-anisidine value (AV) and TOTOX was computed as follows,

$$AV = \frac{25 * (1.2A_s - A_b)}{m} \quad T_{otox} = AV + 2PV$$

Where:  $A_s$  is the absorbance of the sample solution after reaction with the p-anisidine reagent;  $A_b$  is the absorbance of the fat solution without the p-anisidine reagent and  $m$  is the mass of oil sample (g) and 25 ml is the volume of isooctane.

### 7.3.Data Analysis

A complete randomized design with a factorial arrangement was used. The means  $\pm$ Stdev was generated by a JMP ®Pro 13.0.0 statistical software at 95% confidence interval, Tukey for statistical significance test was used whereby the predictor variables were roasting temperature, storage temperature, basil extract, and storage time.

### 7.4.Results and Discussion

#### 7.4.1. Crude Sesame Oil Oxidation

The residual are the differences with the observed and predicted value of oxidation expressed in a scatter plot presented in Figure 7.2, explains the residual oxidative data were inclined to one side in particular the peroxide and TOTOX residual with nearly normally distributed with nonlinear association. The para anisidine residual data is, however scattered and normally distributed which explains the oxidative data were defined to predict the oxidative quality of CSO during storage. The mean value of CSO oxidative quality is presented in Table 7.2, pre-treatment, storage and basil extract effect are discussed below (Appendix L).

**Table 7. 2:** The mean value of CSO oxidative stability in meq O<sub>2</sub>/kg oil during 35 days of storage

Treatment		Storage Condition	Peroxide Value	Para-anisidine Value	
Raw	control	Ambient	3.54 <sup>ab</sup> ±1.8	12.58 <sup>b</sup> ±0.26	
		65°C	9.39 <sup>b</sup> ±0.09	4.15 <sup>ab</sup> ±0.07	
	Basil Extract	Ambient	0.29 <sup>a</sup> ±0.11	0.99 <sup>a</sup> ±0.04	
		65°C	1.19 <sup>a</sup> ±0.08	0.57 <sup>a</sup> ±0.02	
Roasted	control	Ambient	11.8 <sup>b</sup> ±0.02	13.07 <sup>b</sup> ±0.08	
		65°C	31.05 <sup>b</sup> ±.64	5.62 <sup>ab</sup> ±0.07	
	Basil Extract	Ambient	0.83 <sup>a</sup> ±0.05	2.59 <sup>a</sup> ±0.04	
		65°C		3.39 <sup>ab</sup> ±0.42	0.76 <sup>a</sup> ±0.04

Data are expressed as the mean ± standard deviation. Mean values with different superscripts in the same column are significantly different at  $p < 0.05$ .

#### 7.4.2. Effect of Sesame Seed Roasting on the Oxidative Stability of Crude Sesame Oil

The effect of roasting treatment on CSO oxidation is presented in Table 7.3. Roasting improves oil extraction yield, minimize extraction holding time and energy expenditure as a result of improving oil expression efficiency by reducing the seed hardness. Crude sesame oil extraction yield with roasting treatment was obtained 50.9% greater than oil yield without roasting treatment (45.8%). This clearly illuminates roasting significantly enhances oil extraction yield. Earlier studies reported that roasting improves extraction yield, physico-chemical properties of the oil, organoleptic quality and nutritional value (Durmaz & Gökmen, 2010; Suri *et al.*, 2019). CSO from roasted sesame seed was amber in colour, deep flavoured and thicker in viscosity might be due to the roasting induced maillard reaction, oxidation, and degradation of the major constituents includes fats, proteins, carbohydrates, and cleavage of short chained fatty acids during roasting. During sesame oil extraction, water was added at the ratio of 100 kg sesame seed to 4 L water to ease seed breakage and increase oil release. Udoh *et al.* (2017) and Junpeng *et al.* (2019) described moisture affect yield, oil quality, and energy expenditure of sesame seeds. Elkhaleefa and Shigidi (2015) described the seed quality, operating conditions, and processing techniques such as increased holding time (increase yield), roasting temperature (150-250 °C), pressing and stirring

speed ( $\geq 350$  rpm), extraction temperature ( $\geq 40$  °C), moisture content considerably affects sesame oil extraction yield and quality. According to Dymińska *et al.* (2017) reports oil stability, however influenced by the process condition and composition. Pumpkin and brown sesame seeds oil yield, saturated and monounsaturated fatty acids were increased while the moisture content and polyunsaturated fatty acids were decreased with roasted seed (Ali *et al.*, 2017; Hama, 2017). Nevertheless, the higher moisture content of the seed results increased moisture content of the unrefined oil (0.37%) might be responsible to initiate enzyme induced oxidation and microbial growth (Choe & Oh, 2013). Xu-yan *et al.* (2012) investigated the flavour compounds of different sesame seed varieties were increased with increased roasting temperature. The amber colour was developed due to temperature induced oxidation, Maillard reaction, hydrolysis, and nutrient side reaction. Ribeiro *et al.* (2016) and Yan *et al.* (2016) reported that roasting impact physical quality, flavour compounds, anti-oxidants, essential fatty and amino acids. Unsaturated fatty acids were largely transformed as a result of heat-induced oxidation and fatty acid modification (Hama, 2017; Hou *et al.*, 2019).

The PV of CSO measured immediately after extraction was as high as 0.13 and 0.49 meq O<sub>2</sub>/kg oil and p-AV was 0.02 and 1.10 meq O<sub>2</sub>/kg oil with and without roasting treatment, respectively. This explains environmental exposure, longer holding time and reaction induced oxidation was prominent. The higher PV and p-AV of CSO without roasting treatment after extraction explains enzymes were lively to induce oxidation and other side reaction while roasting represses enzymes due to the roasting treatment. According Fu *et al.* (2009) and Silvagni *et al.* (2010) reports endogenous enzymes enhances biochemical reactions including oxidation and rancid development. The exposure of extraction machine to environmental air with longer holding time during milling and pressing significantly affects the oxidative value. Silvagni *et al.* (2012) illustrates the use of extraction machine and machine type responds differently to oil oxidation. In addition, other studies reported that agro-ecological condition, environmental factors (water stress, or salinity and coldness) and botanical families, extraction method and types of fatty acids contributes to oxidation differently (Gharby *et al.*, 2017). Oilseeds composition includes moisture content, types of fatty acids and antioxidants (Carvajal *et al.*, 2009; Silvagni *et al.*, 2010; Silvagni *et al.*, 2012), seed maturity and quality, storage and handling condition (light, temperature, air) (Sanaeifar & Jafari, 2019; Silvagni *et al.*, 2010) affects the oxidative stability of edible oils.

The rate of PV formation was continuous, faster (0.437 days<sup>-1</sup>) with roasting treatment than without roasting treatment (0.071 days<sup>-1</sup>). PV formation increases during the first 12 days of storage and declines with a second polynomial association ( $Y = -1.064 X^2 + 11.49 X - 11.96$ ,  $R^2 = 0.70$ ) in particular with roasting treatment, while the PV formation without roasting treatment was linearly associated ( $Y = 0.68 X + 0.142$ ,  $R^2 = 0.98$ ). The increase and decrease PV pattern might be due to the conversion of primary metabolites to secondary oxidation metabolites. The mean peroxide value of the CSO from raw sesame seed was found to be 5.96 meq O<sub>2</sub>/kg of oil and 7.82 meq O<sub>2</sub>/kg from roasted sesame seed considering roasting treatment during 35 days storage below the tolerable limit. The p-AV of CSO was 23.5 meq O<sub>2</sub>/kg of oil without roasting treatment and 21.05 meq O<sub>2</sub>/kg of oil after roasting treatment during 35 days, below the tolerable limit, formed at a higher rate ( $Y = 2.76X - 1.22$ ,  $R^2 = 0.99$ , 0.087 days<sup>-1</sup>) than PV formation. The higher the p-AV at increased thermal treatment might be due to the short-chained aldehydes and alcohols absorb a wider spectrum wavelength (Abdelazim *et al.*, 2013; Dobarganes & Velasco, 2002).

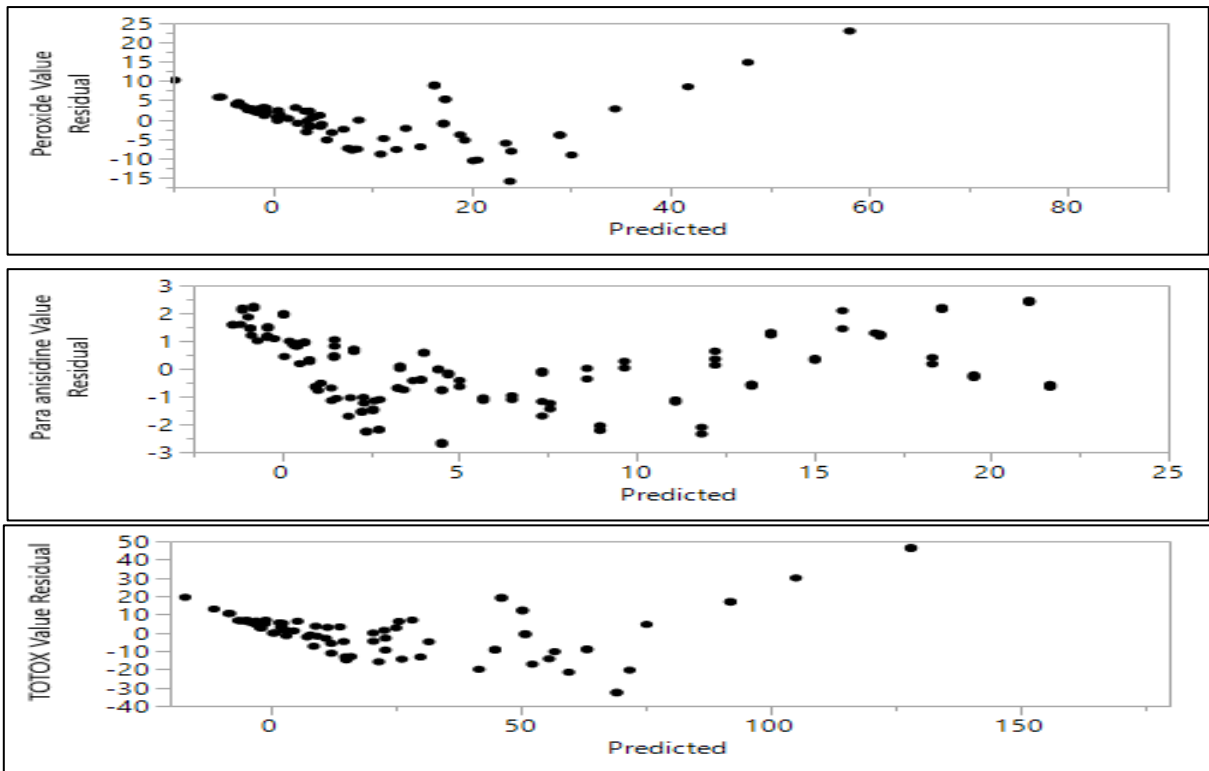
**Table 7. 3:** The oxidative value of crude sesame oil (meq O<sub>2</sub>/kg oil) (n=6) during 35 days of storage at ambient condition in a blue-brown glass before and after roasting treatment

Storage (days)	Peroxide Value		Para anisidine value		TOTOX Value	
	Raw	Roasted	Raw	Roasted	Raw	Roasted
0	0.49 <sup>a</sup> ±0.01	0.13 <sup>a</sup> ±0.02	1.10 <sup>a</sup> ±0.03	0.12 <sup>a</sup> ±0.02	2.09 <sup>ab</sup> ±0.05	0.39 <sup>a</sup> ±0.05
3	1.43 <sup>a</sup> ±0.01	5.43 <sup>cd</sup> ±0.02	4.51 <sup>a</sup> ±0.04	1.83 <sup>a</sup> ±0.04	7.38 <sup>b</sup> ±0.05	12.70 <sup>bc</sup> ±0.07
7	2.29 <sup>b</sup> ±0.01	5.43 <sup>cd</sup> ±0.02	7.23 <sup>abc</sup> ±0.04	6.25 <sup>abc</sup> ±0.11	11.81 <sup>bc</sup> ±0.06	17.12 <sup>cd</sup> ±0.14
12	3.06 <sup>ab</sup> ±0.01	25.11 <sup>h</sup> ±0.17	9.95 <sup>bcd</sup> ±0.04	15.07 <sup>efg</sup> ±0.05	16.07 <sup>cd</sup> ±0.05	65.29 <sup>hi</sup> ±0.40
17	3.76 <sup>ab</sup> ±0.00	22.63 <sup>gh</sup> ±0.10	12.66 <sup>cde</sup> ±0.04	17.46 <sup>def</sup> ±0.38	20.18 <sup>de</sup> ±0.04	62.73 <sup>h</sup> ±0.59
21	4.40 <sup>bc</sup> ±0.01	16.20 <sup>g</sup> ±0.16	15.38 <sup>def</sup> ±0.04	18.0 <sup>def</sup> ±0.01	24.19 <sup>def</sup> ±0.05	50.39 <sup>ghi</sup> ±0.32
25	4.94 <sup>c</sup> ±0.00	13.98 <sup>f</sup> ±0.04	18.08 <sup>def</sup> ±0.04	18.59 <sup>efg</sup> ±0.14	27.96 <sup>ef</sup> ±0.04	46.56 <sup>gh</sup> ±0.22
30	5.56 <sup>cd</sup> ±0.09	9.48 <sup>ef</sup> ±0.06	20.79 <sup>efg</sup> ±0.04	19.25 <sup>ef</sup> ±0.05	31.91 <sup>fg</sup> ±0.21	38.21 <sup>ef</sup> ±0.17
35	5.94 <sup>d</sup> ±0.01	7.82 <sup>e</sup> ±0.09	23.50 <sup>g</sup> ±0.04	21.05 <sup>g</sup> ±0.05	35.39 <sup>fgh</sup> ±0.05	36.70 <sup>def</sup> ±0.22

Data are expressed as the mean ± standard deviation. Mean values with different superscripts in the same column are significantly different at  $p < 0.05$ .

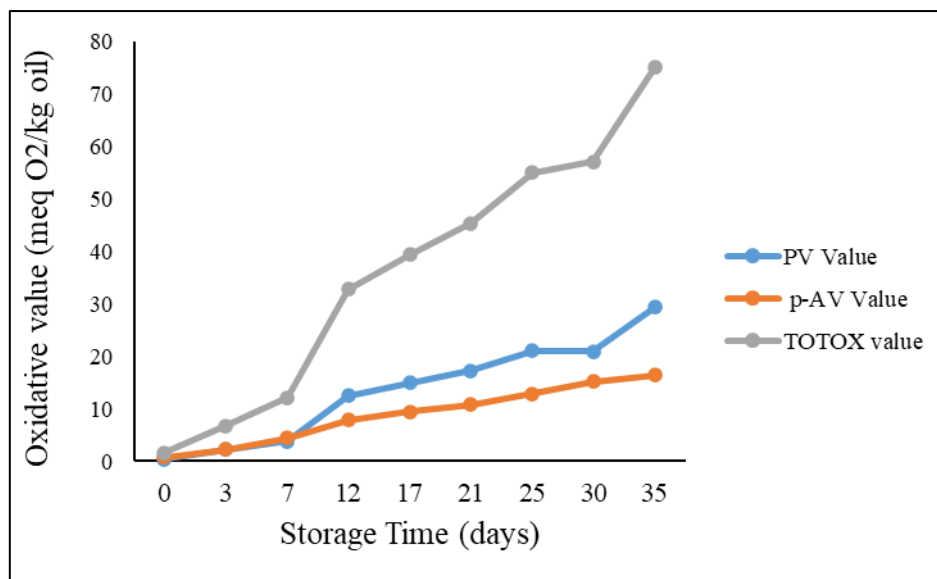
### 7.4.3. Effect of Storage Time on the Oxidative Stability of Crude Sesame Oil

CSO oxidation was linearly associated and continued to build-up during storage as shown below in Figure 7.3. The overall oxidation of CSO exceeded the tolerable limit according to the Codex recommendation for edible vegetable oil after 25 days of storage at ambient condition explains the shorter CSO shelf life might be inadequate processing condition includes the endogenous enzymes initiated biochemical reaction, CSO moisture content, exposure to oxygen and light during extraction, filling and storage condition. CSO is unrefined oil, where enzyme induced reaction boost up chemical reaction and quality degradation, including oxidation shortened shelf life and affects the suitability for consumption. Vidrih *et al.*(2010) reported unrefined oils are vulnerable to oxidation than refined oil. In the meantime, pre-treatment, processing, packaging, storage condition, and preservatives affects the oil quality, safety and stability (Ali *et al.*, 2017; Manzocco *et al.*, 2020). Almeida *et al.* ( 2019) explains oxidation is continuous and yet can be catalysed or hindered and Li *et al.* (2020) explains the role of environmental air, humidity, lightness and temperature to control oil oxidation.



**Figure 7. 2:** The Residual Vs actual scatter plot for data normality of the peroxide, Para anisidine and TOTOX value of crude sesame oil

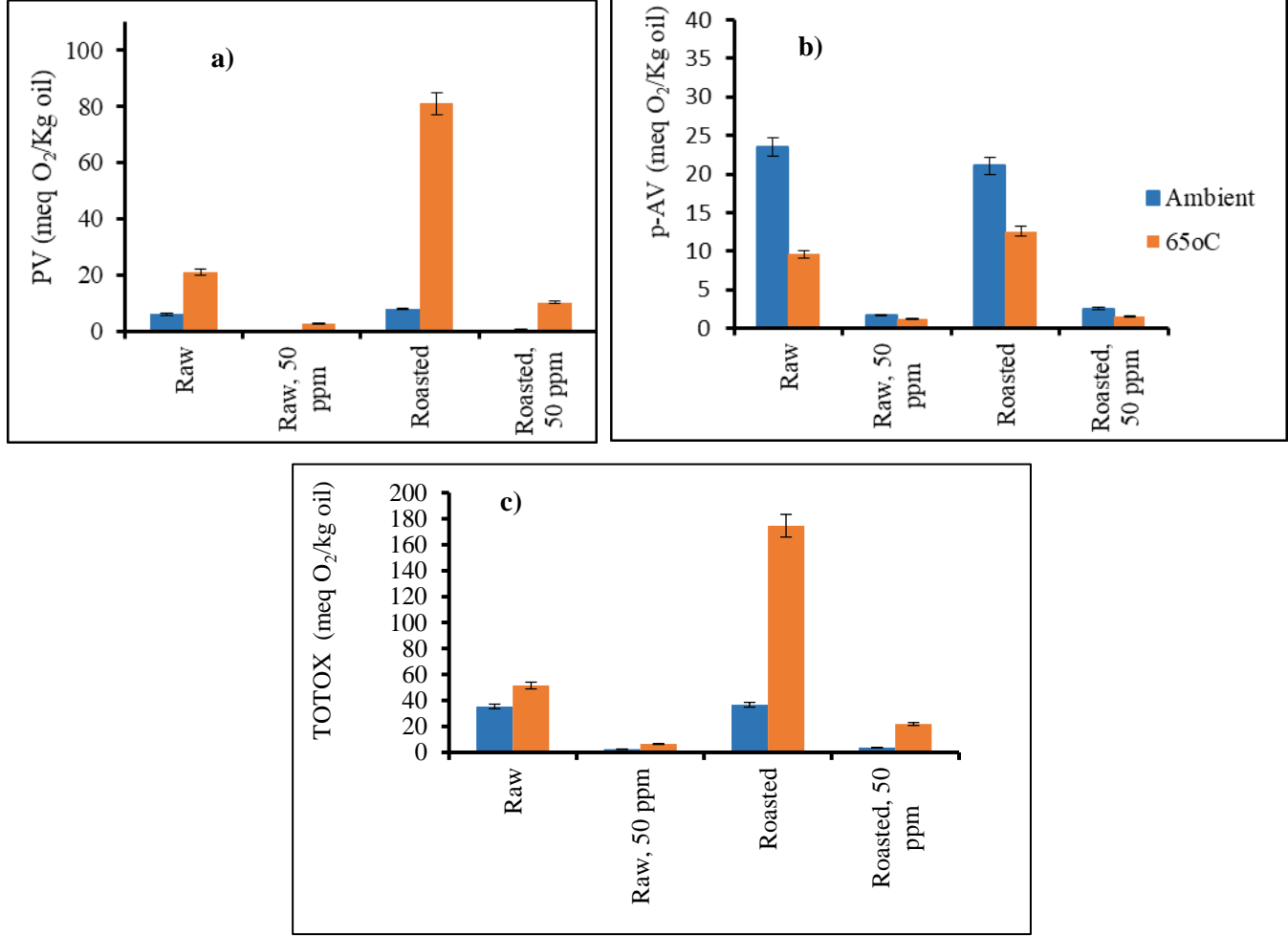
Peroxide value (PV), the primary oxidative quality indicator to assess CSO oxidation estimated 28.92 meq O<sub>2</sub>/kg oil, exceeded the tolerable limit (10 meq O<sub>2</sub>/kg of oil) at ambient temperature during 35 days of storage. The rate of PV formation also shows faster (0.116 days<sup>-1</sup>) with a linear positive association ( $Y= 3.596X - 4.03, R^2 = 0.97$ ). Manzocco *et al.* (2020) reported that the shelf life of edible oil or fried products was significantly influenced as the level of peroxide was increased during storage. Para-anisidine value (p-AV) is a secondary oxidation metabolite indicator, an important parameter for oil quality assessment (Abdelazim *et al.*, 2013). The p-AV of CSO was estimated 16.7 meq O<sub>2</sub>/kg oil not exceeding the tolerable limit (30 meq O<sub>2</sub>/kg of oil) at the rate of 0.082 days<sup>-1</sup> with linear positive association ( $Y= 1.97 X - 0.98, R^2 = 0.99$ ). The rate of PV formation was prompt compared to the rate of p-AV formation. TOTOX is the sum of twice of PV and p-AV, important parameter for quality regulation and shelf-life estimation. The TOTOX value of CSO estimated to be 74.92 meq O<sub>2</sub>/kg oil exceeded the tolerable limit (50 meq O<sub>2</sub>/kg of oil) established by the Codex alimentarius for edible vegetable oil. However, the TOTOX value oils varies with oil composition (SFAs: unsaturated fatty acids) (Xu *et al.*, 2015) and external factors (Maszewska *et al.*, 2018).



**Figure 7. 3 :** Crude sesame oil oxidation (meq O<sub>2</sub> / kg oil) pattern during 35 days of storage at ambient condition in a blue-brown glass bottle

#### 7.4.4. Effect of Storage Temperature on the Oxidative Stability of Crude Sesame Oil

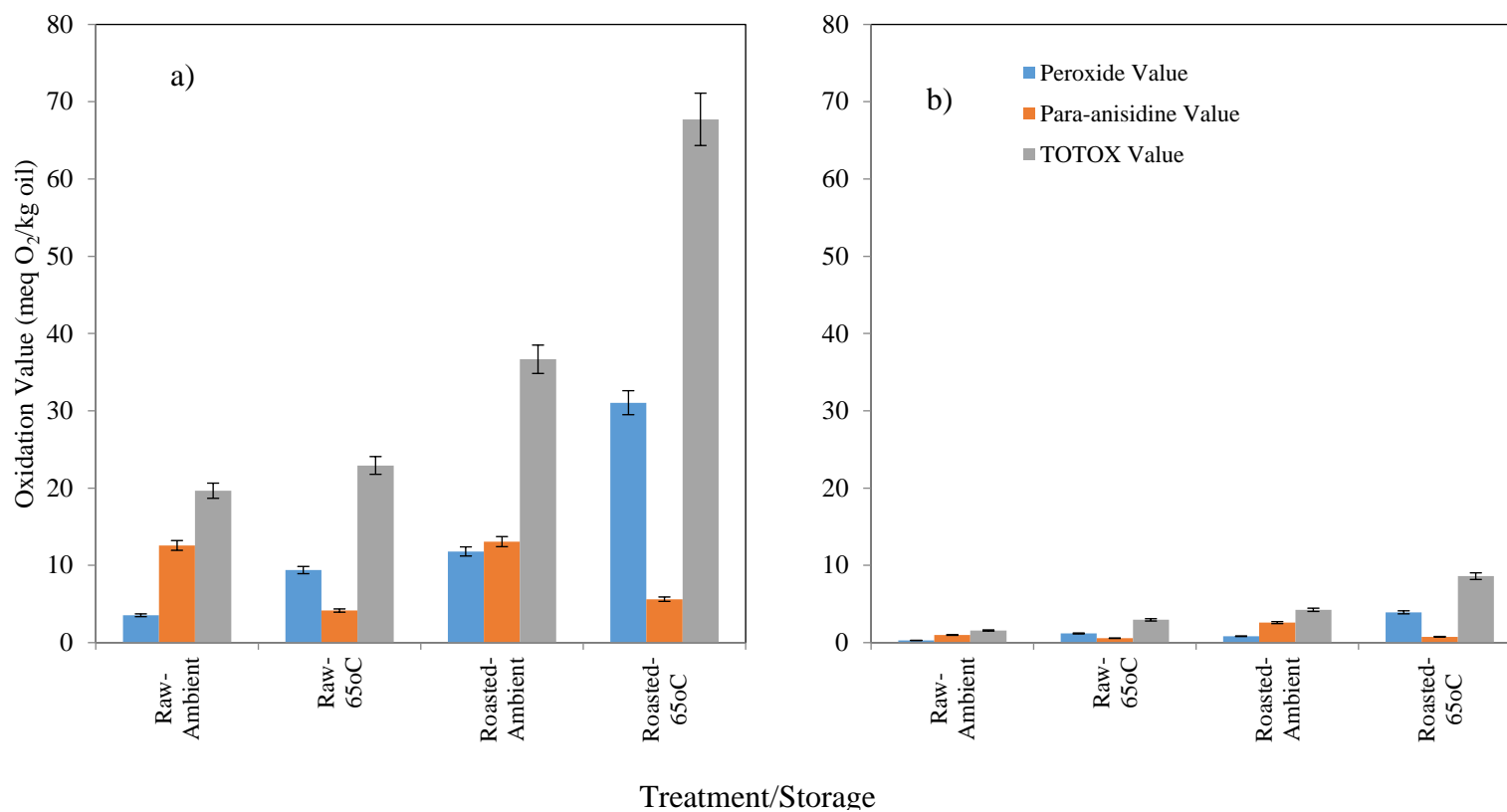
Effects of storage temperature on the PV OF CSO during 35 days of storage at 65 °C temperature and ambient condition presented in Figure 7.4 were linearly correlated ( $Y = 2.601 X - 3.61$ ,  $R^2 = 0.98$ ) and ( $Y = 0.68 X + 0.14$ ,  $R^2 = 0.99$ ), respectively. The rate of PV formation during accelerated storage was prompt ( $0.107 \text{ days}^{-1}$ ) than ambient storage condition ( $0.071 \text{ days}^{-1}$ ). The higher the rate of PV formation explains disruption of fatty acid bonds catalysed by either of high gradient temperature, insertion of single oxygen or interaction of fatty acids with fatty and non-fat nutrients results decomposition and formation of radicals. Unlike PV, the rate of p-AV formation was relaxed during accelerated temperature storage ( $Y = 0.84e^{0.27X}$ ,  $R^2 = 0.99$ ,  $0.062 \text{ days}^{-1}$ ) and ambient condition storage was linear ( $Y = 2.76 X - 1.2215$ ,  $R^2 = 0.99$ ,  $0.087 \text{ days}^{-1}$ ). The faster p-AV formation during ambient condition compared at 65 °C might be due to continuous oxidation reaction while increasing storage temperature facilitates polymerization. Besides oxidation, studies reported that increased thermal storage of edible oil speed up polymerization reaction and colloid formation due to the fatty acid degradation and digestion (Khor *et al.*, 2019; Siepmann *et al.*, 2019). The TOTOX value of the CSO was 35.39 meq O<sub>2</sub>, 51.5, and 174.6 meq O<sub>2</sub>/kg oil at conditions of ambient, accelerated storage temperature and combined roasting pre-treatment with accelerated storage condition, respectively, exceeded the tolerable limit. Ali *et al.* (2017) and Khor *et al.* (2019) reported that high thermal treatment and handling accelerates the p-AV and TOTOX of edible oils as the total polar compound, triacylglycerol oligomers and saturated fatty acids increases. However, free fatty acids, unsaturated fatty acids and peroxides were decreased with the heating temperature and time.



**Figure 7. 4:** The Oxidation value of crude sesame oil (meq O<sub>2</sub>/kg oil) during 35 days of storage in a blue-brown glass bottle at ambient and accelerated temperature storage with/without ethanoic basil extract (50 ppm) a) peroxide value, b) p-anisidine value and c) TOTOX value

#### 7.4.5. Effect of Basil Extract on the Oxidative Stability of Crude Sesame Oil

Oxidation can be not be avoided yet it can be minimized as the rate of oxidation is determined considering processing and handling conditions. The use of basil extract shown a prominent potential to prevent oxidation detected below the tolerable limit. The application of ethanoic basil extract (50 ppm) illustrated in Figure 7.5 (a) without basil extract and Figure 7.5 (b) with basil extract shows a promising oxidation inhibition by 16.38-90% during storage. The PV, p-AV and TOTOX value of CSO preserved with 50 ppm basil extract was detected within the range of 0.29 - 3.92 meq O<sub>2</sub>/kg oil, 0.75 – 2.59 meq O<sub>2</sub>/kg oil and 1.57 – 8.6 meq O<sub>2</sub>/kg oil, respectively. Although, CSO without basil extract was detected more than threefold increase in the range of 3.54 - 31.05 meq O<sub>2</sub>/kg oil, 4.15 - 13.07 meq O<sub>2</sub>/kg oil and 22.93 - 67.72 meq O<sub>2</sub>/kg oil, respectively. Nevertheless, during prolonged storage the rate of oxidation increased explains the anti-oxidant properties of basil extract began to degrade. Studies reported that use of basil extract has anti-microbial and anti-oxidant property (Sudarno *et al.*, 2017; Warsi & Sholichah, 2017), nutritional and health benefits, potential to substitute the inorganic anti-oxidants with a radical scavenging activity (1.29 mg/ml) (Ahmed *et al.*, 2019). The oxidation inhibition potential of basil extract during ambient and 65 °C storage was with a second-order polynomial pattern in the rate of 0.02-0.08 days<sup>-1</sup>, respectively.



**Figure 7.5:** Crude Sesame oil oxidation value (meq O<sub>2</sub>/kg oil) during 35 days of storage in a blue-brown glass with roasting treatment and during ambient and 65 °C storage condition a) in the absences of ethanoic basil extract and b) With the application of 50 ppm ethanoic basil extract

## 7.5. Conclusion

Vegetable oils are a staple of human daily nutrition. The problems are carcinogenic metabolite buildup and oxidation-induced quality degradation. CSO is an unprocessed cooking oil that is high in nutrients. Regardless of the composition and conditions of processing and handling, CSO oxidation occurs continuously. Roasting speeds up oxidation, colouring from the maillard reaction, flavourful oil viscosity from the cleavage of proteins and carbs, and thick oil viscosity regardless of the CSO extraction yield increment. The rate of oxidation is intensified when oil, oil seeds, and oil storage are treated at elevated thermally treated. Meanwhile, quality deterioration and polymerization could be initiated due to the thermal treatment. Because oxidation metabolites absorb hydrogen and create unstable radical molecules, they are hazardous to humans and should be avoided. On the other hand, oxidation could be prevented even though it cannot be totally avoided and is somewhat desired within the tolerable limit. Oxidation could be prevented by either process improvement such as controlled extraction and storage condition, and light-protected packing. Introduction of organic preservative (basil extract) shows a significant reduction of CSO oxidation. During the storage period, the PV, p-AV, and TOTOX values of CSO with basil extract were measured within the ranges of 0.29 – 3.92 meq O<sub>2</sub>/kg oil, 0.75 – 2.59 meq O<sub>2</sub>/kg oil, and 1.57 – 8.6 meq O<sub>2</sub>/kg oil, respectively. On the other hand, the oxidation value of CSO in the absence of basil extract were detected with more than three times. However, research into the bioactive components of sweet basil is necessary before it can be commercialized for use in food processing as a possible preservative and as a functional food for a healthy diet.

## CHAPTER EIGHT

### GENERAL DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

#### 8.1. Major Findings

- i. The substantial post-harvest loss of sesame seeds in the sesame value chain could have been avoided. Since stakeholders' inactive involvement and misunderstandings of what constitutes appropriate agriculture, handling, and manufacturing procedures were the main causes of the difficulties.
- ii. Inappropriate/inadequate farming methods, post-harvest handling, and hygiene practices in the sesame value chain and crude sesame oil extraction facility were potential challenges for the occurrence of potential hazards to jeopardize the CSO safety. Consequently, three CCPs are used in the sesame seed roasting, and conditioning of raw materials during pressing and packaging (filling). In order to confirm the HACCP plan, appropriate critical limits, monitoring, and corrective actions for the CCPs were established. A microbiological challenge test was also carried out to verify HACCP plan.
- iii. The shelf life of crude sesame oil should not exceed two months unless additional refining is done and organic preservatives are used. When followed exactly, HACCP is beneficial for small-scale food processing businesses and food service providers. It is thought that the underutilized wild plants and spices have potential uses in medicine and as preservatives in food. It is proven to have a great potential for preventing CSO oxidation and enhancing storage stability with ethanolic basil extract.
- iv. Comprehend to adhere to food safety management protocols, reduce post-harvest losses, and foster trust will enhance the commercial value of sesame seeds, improving the standard of living for farmers, food entrepreneurs, and other value chain participants while also advancing sustainable development objectives and the fight against poverty.

#### 8.2. Conclusions

- i. Ethiopia's primary source of income remains agriculture, even after decades of experience. However, the nation is facing challenges due to labour-intensive, time-consuming, fast population growth, and outdated, ineffective agricultural practices that are unable to satisfy consumer and market needs. Small and medium-sized agro-processing businesses are

encouraged to start with structured funding and quick training. The organic farming, harvesting, post-harvest handling (impurity and colour sorting, dehulling, and oil extraction), and marketing to various market sectors are important activities linked to the sesame value chain. However, 22 - 35 percent of the seeds are lost within the value chain, which significantly affects the economic value and the livelihood of the sesame farmers. Incompetent and illiterate actors, inattentive involvement, negligent rules, and inadequate infrastructure were the root causes of the high post-harvest rate. CSO is acceptable in the consumer market and yet, strong Flavors and discoloration lower overall quality and shorten shelf life. The employee at the extraction plants lacks a fundamental understanding of hygienic practices, process control, pest control, and general requirements for food processing facilities for safe production and distribution.

- ii. Potential hazards in the sesame value chain detected were included fungi, aerobic bacteria, total Coliforms, aspergillus fungal species, staphylococcus aureus, mycotoxin, pesticides and chloride residue, oxidative compounds detected in sesame seed, CSO, sesame seed cake, and associated surfaces, among others. Nevertheless, CSO did not contain any Shigella, Salmonella, or E. Coli. The primary factor contributing to the hazard (s) prevalence was the unhygienic handling and environmental factors. Actors, in the value chain lack to know enough about what defines acceptable practices, which include things like how the place appears and the things that need to be there to meet that standard, the equipment's suitability and arrangement, process control, hygienic conditions and regulations, and pest control. Therefore, further effort is required to guarantee via training on safe production and handling procedures. To protect consumer health and foster consumer confidence, small and medium-sized food businesses might be further effort on PRPs and OPRPs.
- iii. Three CCPs identified in the CSO extraction line were sesame seed roasting, extraction while conditioning, and filling (packaging). The potential hazards identified to critically controlled for safe oil production were fungi and fungi metabolites, *Staphylococcus aureus* and *aerobic plate count*, chlorine residue, and oxidative metabolites. The causes for the prevalence of those hazards were environment and compositional factors, cross contamination due to the unhygienic practices. With thoughtful management while CCPs

implementation the hazards were significantly prevented or reduces to tolerable limit where safety of CSO was improved.

- iv. Critical limits for each CCPs were roasting condition, cleanliness, aseptic sterilization and filling, introduction of natural antioxidant and careful selection of packaging the implementation of CCPs as planned and prevention potential hazards to possess CSO safety and public health.
- v. HACCP plan implementation significantly reduces the occurrence of potential hazard and improve CSO safe production. Yet, strict management and continued training is crucial.
- vi. Humera sesame seeds have 96.64% dry matter where 45–51.03% oil, and 22.11% protein. On the other hand, inadequate handling, pretreatments, and operational losses negatively impacted the output and quality of sesame oil. Higher extraction yields are obtained from roasted sesame seeds when combined with Soxhlet than mechanical cold press extraction techniques, as opposed to the latter, which is usually used in small and medium-sized oil extraction operations. The safety and quality of the oil were significantly compromised by oxidation reaction resulting from incomplete or poorly maintained extraction conditions, prolonged storage at high gradient temperatures, and uncontrolled or inadequately maintained extraction conditions. This is because the oxidative stress exceeded the acceptable limit, causing short storage times, polymerization, quality degradation, and unfavourable side reactions. Meanwhile, the use of organic preservatives significantly reduces the risk of oxidation. Thus, introduction of organic preservatives of endogenic spices, herbs, and other underutilized plants may be advantageous along the entire edible oil value chain and biodiversity.

### **8.3. Recommendations**

- i. It is believed that the implementation of integrated agriculture will boost output, lower post-harvest loss, and support the objectives of sustainable development and poverty alleviation. Periodic training on farming, harvesting, post-harvest handling, and value addition was necessary for post-harvest management, and there were stringent rules about the use of pesticides.
- ii. To safeguard consumer health and foster trust, hygienic and sanitary standards ought to be applied to the food processing sectors, regardless of their level of production and the nature of the product.

- iii. Adherence to CCPs and preventive measures in the CSO extraction facility would enhance the overall quality and safety of CSO as well as storage stability. This will incentivize the food service industry and small- to medium-sized food processing businesses to adopt safety protocols, comprehend food standards, and take responsibility for safeguarding the public's health.
- iv. Regardless of the capability of the food processing industries HACCP failure cost and negatively compromises food safety as it lacks understanding what food safety and HACCP is, which might be require further training.
- v. In addition to its flavour-enhancing and anti-oxidant qualities, basil extract's anti-microbial qualities in CSO and stability over extended storage needed more research and use.

#### **8.4. Area for Further Research**

- i. Further investigation is needed on the traceability studies in the Ethiopian food value chain to strengthen the food policies and regulation and to comply with the sustainable developmental goals and poverty reduction.
- ii. The anti-microbial properties of the basil extract in CSO requires further studies
- iii. The preservative potential of other spices and herbs locally grown requires further investigation and utilization to replace the inorganic preservatives
- iv. Serious HACCP plan and other food safety management methods / tools need to be considered in the food system in particular the small and medium scale food processing industries are not given attention.
- v. Training and strict regulatory is required on hyenine and sanitary practices, post-harvest handling and value addition and sanitary and phytosanitary requirement in the food value chain.

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## APPENDICES

### Appendix A: Prevalence and controlling mechanisms of mycotoxins

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#### FOOD SCIENCE & TECHNOLOGY | REVIEW ARTICLE

## Prevalence and controlling mechanisms of mycotoxin

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Additional information is available at the end of the article


**Abstract:** Fungi grow everywhere in agricultural produce, food and surface of indoor and outdoor environment. The aim of this paper is to expand the growth and synthesis contributing factors and prevention mechanisms of fungi and its metabolites. Fungi, grouped as hydrophilic, mesophilic and xerophilic, grow under a wider range of water activity, temperature, pH, gases and substrate. Besides the beneficial properties, the harmful fungi species are gaining attention due to their toxicity effect on consumer and economic losses. Taking into consideration their prevalence, food group, daily intake, sampling, analytical techniques and consumer type's regulatory limit have been established and promising prevention mechanisms discovered. Prevention of growth and production of toxic metabolites includes good practices, use of plant extracts/probiotics, oxygen-reactive scavenging substances and molecular silencing technology for a wide range of commodities. Nevertheless, application and commercialization of those techniques are limited.

**Subjects:** Food Chemistry; Food Analysis; Food Microbiology; Nutrition; Food Engineering; Food Packaging; Preservation; Processing; Product Development; Food Laws & Regulations

**Keywords:** Food value chain; fungi; mycotoxin; health; tolerable limit; detoxification

Appendix B: The Sesame (*Sesamum indicum L*) Value chain and Microbiological Quality of crude sesame oil, a case study in western Tigray, Ethiopia

## The Sesame (*Sesamum indicum L.*) Value Chain and Microbiological Quality of Crude Sesame Oil, a Case Study in Western Tigray, Ethiopia

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### Abstract

Crude sesame oil (CSO) is the widely used unrefined edible oil. Storage instability, off-flavour, and discoloration are, however, challenges in the industry. The purpose of this study was to map the sesame value chain, assess the suitability of CSO extraction plant, and analyze the microbial quality of CSO and premises environment. A structured Questionnaire and checklist were used to assess the sesame value chain and evaluate the suitability of the CSO extraction plant. Microbiological quality assessment was conducted using standard analytical methods. Stakeholders in the sesame value chain were inclusive of farmers, market mediators, traders, regulatory, extension workers and researchers. Though, illiteracy, inadequate technology, and infrastructure were the drawbacks. The CSO extraction plant was suitable apart from inadequate ingredients and CSO handling and unhygienic practices. Total aerobic bacteria ( $4.34 - 5.06 \log_{10}$  CFU/m<sup>2</sup> on swap surfaces,  $2.44 \log_{10}$  CFU/g in CSO), total Coliforms ( $5.81 \log_{10}$  CFU/g of animal manure and  $1.36 \log_{10}$  CFU of indoor air after extraction), yeasts and moulds ( $2.31 \log_{10}$  CFU/g of sesame seed and CSO and  $4.47 \log_{10}$  CFU/m<sup>2</sup> of swap sample), *Aspergillus* species ( $1.17 - 1.33 \log_{10}$  CFU/g of sesame seed/CSO,  $3.37 - 3.50 \log_{10}$  CFU/m<sup>2</sup> of swap samples), and *Staphylococcus aureus* ( $2.09 \log_{10}$  CFU/g of CSO,  $2.56 - 3.22 \log_{10}$  CFU/m<sup>2</sup> of surface swaps,  $3.26 - 3.77 \log_{10}$  CFU/protective clothing,  $0.74 - 1.82 \log_{10}$  CFU of the indoor and outdoor air) were detected. *Escherichia coli*, Salmonella and Shigella were not detected. In conclusion, potential microbial pathogens were detected to impose food safety problems and economic loss. To improve the sesame value chain and CSO quality workers training on good handling and hygienic practices and thoughtful regulatory implementation are significant.

# Appendix C: The effect of roasting, storage temperature, and ethanoic basil (*Ocimum basilicum L.*) extract on the oxidativity stability of crude sesame (*Sesamum indicum L.*) Oil

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ORIGINAL RESEARCH

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## The effect of roasting, storage temperature, and ethanoic basil (*Ocimum basilicum L.*) extract on the oxidative stability of crude sesame (*Sesamum indicum L.*) oil

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### Abstract

Sesame (*Sesamum indicum L.*) is an annual oilseed crop that is widely used for crude sesame oil (CSO) extraction. CSO is unrefined, thus, may impact the storage stability due to the uncontrolled processing and handling conditions, and absence of preservatives. The purpose of this study intends to analyze the fatty acid profile and oxidative stability of CSO. In a complete randomized (2\*2) factorial design, clean sesame seeds (3.92% moisture content) were used for oil extraction with/without roasting treatment (200°C). The fatty acid profile and oxidative value of the CSO were analyzed. The roasted sesame seed oil extraction yield was higher (50.9%) and composed of 83.15% unsaturated fatty acids with an omega-6 to omega-3 ratio of 95.3. The ratio of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs) was 2.9, while the moisture content of the CSO was 0.37%, exceeding the tolerable limit. The predominant fatty acids of CSO include palmitic, stearic, oleic, and linoleic acids. Elevated thermal condition during processing and handling speeding up oxidation exceeded the tolerable limit after 25 days of storage, while the ethanoic basil extract was found to inhibit oxidation in the range of 16.38%–90% ( $p$ -value < .05). The peroxide value (PV), para-anisidine value (p-AV), and total oxidation (TOTOX) value of CSO with 50 ppm (parts per million) basil extract were detected within the range of 0.29–3.92, 0.75–2.59, and 1.57–8.6 milliequivalents (meq) O<sub>2</sub>/kg oil, respectively, below the tolerable limit. Nevertheless, basil extract's antioxidant property was declined during prolonged storage, in particular, at elevated temperature. The use of organic extracts of locally available sweet basil herb is capable of mitigating oxidation and substituting inorganic antioxidant for a healthier diet.

### KEYWORDS

basil extract, crude sesame oil, oxidation, roasting, sesame, storage

# Appendix D: Poster Presentation 1

## Effect of Roasting, Storage Temperature and Ethanoic Basil (*Ocimum basilicum* L.) Extract on the oxidative stability of crude sesame (*Sesamum indicum*) oil

Abraham F. Gebremeskel, Peninah N. Ngodo, Elizabeth W. Komau-Mutitu, Symon W. Wachungu

Poster Category: Applied science/results for development

### ABSTRACT

Sesame (*Sesamum indicum* L.) is an annual oil seed crop. Crude Sesame oil (CSO) is unrefined, thus, may impact on the storage stability due to the uncontrolled processing and handling condition, and absence of preservatives. The purpose of this study was to analyze the fatty acid profile and oxidative stability of CSO, in a complete randomized (2<sup>3</sup>) factorial design. Fatty acid composition, peroxide value (PV), and p-anisidine value (p-AV) of CSO stored in blue brown glass bottle at ambient (22-27 °C) and accelerated temperature (42 °C) storage for 25 days with/without ethanoic basil extract (20 ppm) was determined. The roasted sesame seed oil extraction yield was 20.9% composed of 92.12 % unsaturated fatty acids with omega-3 to omega-6 ratio of 17.3. Roasting and accelerated storage accelerate oxidation. However, the ethanoic basil extract was found to lower by 87-93%. Nevertheless, basil antioxidant property declined during prolonged storage. The use of organic extract of locally available sweet basil is capable to mitigate oxidation and substitute inorganic antioxidant for a healthier diet.

### INTRODUCTION

- Lipids are important structural and functional components of foods (Adey, 2017).
- Oxidation is however a challenge in the edible oil value chain resulted quality degradation. Contributing factors and mechanisms of lipid oxidation are:

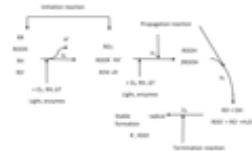


Figure 1: Lipid Oxidation Phases and chronic radical formation catalyzed by environmental oxygen, in addition with other fatty acids, gradient temperature.

### OBJECTIVE

- The purpose of this study was to analyze the impact of processing, handling and organic extracts on the CSO oxidative stability and fatty acid profile of CSO

### MATERIALS AND METHODS

- Sesame seed and Sweet basil (*Ocimum basilicum*) was collected
- CSO was extracted
- Ethanoic basil extract (Shelshah, 2017), Moisture content (AOAC, 2000, P25.04), Fatty acid methyl esters and GC analysis (AOAC 996.01) (Satchithanandam et al., [2001], Peroxide value (AOAC P20.160) (Sene and Navarro et al. 2014), p-Anisidine value [AOCS method 1995] (Abdelstam et al. 2013).

### RESULTS

\* CSO Fatty Acid Composition of CSO extracted from roasted sesame seeds

Name of FF	Fatty Acid level (%)
Molauic content	0.27 ± 0.03
Total Fatty Acids (TFA) (seed)	20.9 ± 0.08
Saturated Fatty Acids (SFA)	10.04 ± 0.71
Palmitic (C16:0)	6.7 ± 0.66
Stearic (C18:0)	4.82 ± 0.47
Arachidic (C20:0)	0.69 ± 0.16
Unsaturated fatty acids (MUFA)	20.9 ± 0.19
Oleic (C18:1n-7)	28.6 ± 1.51
Palmitoleic (C16:1n-7)	0.19 ± 0.04
Elaeosteric (C18:1n-3)	0.25 ± 0.19
Polysaturated fatty acids (PUFA)	42.55 ± 1.15
Linoleic (C18:2n-6)	42.6 ± 1.73
Linolenic (C18:3n-3)	0.69 ± 0.13

### Effect of Roasting

- Roasting improves improve of extraction yield, minimize holding time and energy expenditure
- However, Accelerated Oxidation and unpleasant Organoleptic quality development

Storage (Days)	Peroxide Value		p-anisidine value		TOTOX Value	
	Raw	Roasted	Raw	Roasted	Raw	Roasted
0	0.07 ± 0.01	0.18 ± 0.02	1.10 ± 0.02	0.19 ± 0.02	0.29 ± 0.02	0.29 ± 0.02
5	1.28 ± 0.02	2.49 ± 0.02	4.27 ± 0.01	1.89 ± 0.02	7.28 ± 0.02	10.25 ± 0.07
10	2.28 ± 0.02	3.49 ± 0.02	7.28 ± 0.02	4.27 ± 0.01	11.87 ± 0.04	17.10 ± 0.14
15	3.27 ± 0.02	4.49 ± 0.02	10.25 ± 0.02	7.28 ± 0.02	16.27 ± 0.02	24.29 ± 0.02
20	4.27 ± 0.02	5.49 ± 0.02	13.22 ± 0.01	10.25 ± 0.02	21.19 ± 0.02	32.79 ± 0.07
25	5.27 ± 0.02	6.49 ± 0.02	16.19 ± 0.01	13.22 ± 0.01	27.19 ± 0.02	42.29 ± 0.02
30	6.27 ± 0.02	7.49 ± 0.02	19.16 ± 0.01	16.19 ± 0.01	33.19 ± 0.02	52.29 ± 0.02
35	7.27 ± 0.02	8.49 ± 0.02	22.13 ± 0.01	19.16 ± 0.01	39.19 ± 0.02	62.29 ± 0.02

### CONCLUSION

- Roasting improves oil extraction yield, impacts organoleptic quality with an amber color and rancid flavor, accelerate oxidation which promotes carcinogenic metabolites formation, continuous with time
- Storage significantly affects the oxidative stability of oils and fatty acid composition
- Organic extracts such basil extract have the potential to mitigate oxidation, can be a substitute the inorganic antioxidant
- Further intervention and scaling up of the local available herbs and spices are important for human healthier diet

### Storage time Vs Oxidation

- Oxidation is a continuous process
- Exceeded the tolerable limit within 25 days of storage at ambient temperature (22-27°C)
- Primary oxidation (PV = 0.116 day<sup>-1</sup>) is promote than the secondary oxidation (p-AV = 0.082 day<sup>-1</sup>)

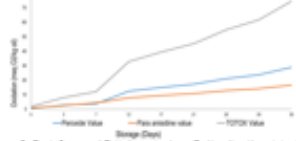


Figure 2: Crude Sesame oil Oxidation value (mg O<sub>2</sub>/kg oil per hour) during 25 days of storage at ambient condition in a blue brown glass bottle to prevent light interaction

### Effect of Storage Temperature

- Accelerated storage accelerate oxidation
- PV is promote than p-AV

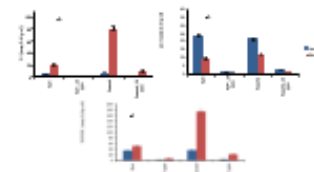


Figure 3: The Oxidation value of Crude Sesame Oil (mg O<sub>2</sub>/kg oil per hour) during 25 days of storage in a blue brown glass at ambient and accelerated temperature storage with/without ethanoic basil extract (20 ppm) at peroxide value and p-anisidine value and TOTOX value

### Effect of basil extract

- Ethanoic basil extract was found to have a potential to mitigate oxidation (14.24-90%)
- Thought, with extended storage of increased temperature starts to degrade its antioxidant properties

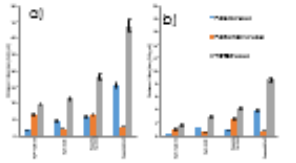


Figure 4: Crude Sesame oil oxidation value during 25 days of storage in a blue brown glass with roasting treatment and storage condition a) in the absence of ethanoic basil extract and b) with the application of 20 ppm ethanoic basil extract

### ACKNOWLEDGEMENT

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# Appendix E: Poster Presentation 2

## Fungi and Fungi Metabolites Mitigation Mechanisms for Better Food and Nutrition Security

Abrehet F. Gebremeskel, Peninah N. Ngoda, Elizabeth W. Kamau -Mbuthia, Symon M. Mahungu

Theme: Management of Mycotoxigenic Fungi and Their Metabolites

### ABSTRACT

Fungi and its toxic metabolites are carcinogenic, exist almost everywhere at a wide range of conditions causes economic loss and public health problem. Review was conducted on the prevention mechanisms of fungi and its metabolites for better food system. Environment friendly and cost effective prevention mechanisms have been reported includes good handling and processing practices, use of organic preservatives (plants extract, probiotics), oxygen reactive scavenging substances and molecular technology. However, those methods remain a the research facilities. Organic preservatives are unstable.

### INTRODUCIN

- Fungi are microbial toxins and synthesis chemical toxin metabolites "Mycotoxins" which impact the economic value and public health
- Almost everywhere where humidity and temperature is conducive and hygienic condition is compromised

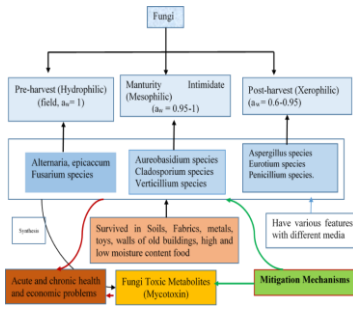


Figure 1: Fungi classification and contributing factors for their survival

### OBJECTIVE

The objective of this review was to address the fungi and fungi metabolites mitigation mechanism for better Food and Nutrition security

### Fungi and Fungi Metabolites Mitigation

- Good agricultural/ handling/processing practices
- Mycotoxin reduction within the ranges of 25-68% with strict receiving and inspection criteria, appropriate storage conditions, and proper processing practices

### Probiotics

- Probiotics are microorganism with beneficial property to enhance the function, sensorial and nutritional value of foods and considered as organic preservatives
- Functions as single or mixed strains, mixed strains are more effective Via mutual interaction, biotransformation or absorption of toxins

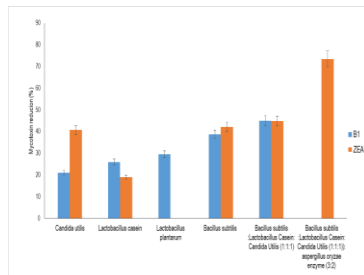


Figure 2: Lactic Acid bacteria spp. as a potential probiotic in prevention of mycotoxins

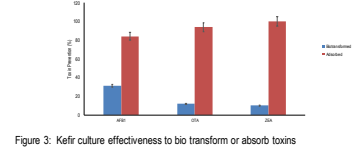


Figure 3: Kefir culture effectiveness to bio transform or absorb toxins

### Plant Extract

- Plant extracts are organic preservatives, underutilized in the food sectors
- Trachyspermum ammi oils (essential oils) are found to have strong antifungal, spore germination and anti-aflatoxin (1 µl/ml - 2 µl/ml of oil)
- Pomegranate Peels (5%) and Clove Powders (2%) anti-aflatoxin and OTA.

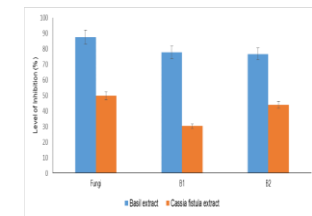


Figure 4: Organic preservatives Effectiveness of Ozonation in prevention of toxins in bulk storage of durable crops

### Use of Oxygen reactive substance inhibitors

- Ozonation:
  - DON inhibition at ≤ 75mg/L Ozone, with no effect on the nutritional quality thought the higher tenacity and whiteness, as well as lower extensibility and yellowness
  - Aflatoxin inhibition: AFG<sub>1</sub> > AFB<sub>1</sub> > AFG<sub>2</sub> > AFB<sub>2</sub> (2 ppb - 10 ppb ozone during 3-7minutes) and complete destruction of AFG<sub>2</sub> and AFB<sub>2</sub>

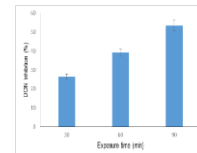


Figure 5: Effectiveness of Ozonation in prevention of toxins in bulk storage of durable crops  
 - H<sub>2</sub>O<sub>2</sub> induced by the fungicide Prothioconazole: H<sub>2</sub>O<sub>2</sub> induced by sub lethal doses of the Strobilurins and triazoles fungicide triggers DON biosynthesis from fusarium  
 - Superoxide dismutase in the cytoplasm and mitochondria detoxify or decreases accumulation of patulin toxins

### Molecular Technology

- mRNA silencing Technology prevents the growth of aspergillus flavus, aspergillus parasiticus and AFB<sub>1</sub> reduced by 98, 97, and 97% respectively
- Nano technology (Fe<sub>3</sub>O<sub>4</sub> particles, 10- 40µg, 9.99 nm via magnet attachment inhibit;
  - Aspergillus parasiticus (24.2%) and
  - A. ochraceous (28.2%) at the optimal concentration of 40µg, highest inhibitory effect.
  - Aflatoxin, 12-95%
- The level of reduction is concentration dependent (±3 mg)

### CONCLUSION

- The use of inorganic preservatives in the food sector is common which is cost ineffective and has side effect to health and environment
- Medical plants including spices and herbs are underutilized in the food processing industry and community
- The different studies reported potential mitigation mechanisms of fungi and mycotoxin lacks stability studies and scale-up to the pilot plant or community based use
- Current study focused on the qualitative prevalence rather than mitigation

Where: B<sub>1</sub>, B<sub>2</sub>G<sub>1</sub>, G<sub>2</sub>, OTA, DON : Aflatoxin B<sub>1</sub> and B<sub>2</sub>G<sub>1</sub>, G<sub>2</sub> Ocratoxin, deoxynivalenol, respectively

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## Appendix E: Questionnaires to be filled by respondents to assess sesame value chain

### Section I: Introduction

Dear respondents,

My name is Abrehet Fisseha Gebremeskel, a Ph.D student at Egerton University, Kenya. My PhD research title is “sesame (*sesame indicum*) oil quality assurance through hazard analysis critical control point implementation in the traditional processing in western Tigray, Ethiopia”. I will be collecting the practices in the sesame seed and oil extraction to improve quality and safety. A questionnaires’ will be distributed among you and thank you for taking time to fill the questionnaires. This survey will help us to understand the current scenario, identify the gaps and good practices. This survey will take 5-10 minutes to complete. I would like to assure you that all information you provided will be kept in strict confidence. Give your answers in the space provided

### Section II: Personal and company information of the respondent

1. Name of Institution: \_\_\_\_\_ Age of respondent: \_\_\_\_\_ Sex \_\_\_\_\_
2. Responsibility in the organization: \_\_\_\_\_
3. Years of work experience \_\_\_\_\_
4. Under line Level of education: BSc/BA/MSc/PhD, Specialization \_\_\_\_\_
5. Qualification \_\_\_\_\_
6. What are the major activities of your organization?  
\_\_\_\_\_.
7. Means of communication
  - i. Internet
  - ii. Telephone
  - iii. Letter
  - iv. Others
8. Do you have management/legislation support? \_\_\_\_\_
9. How do you measure consumer satisfaction? Consumer feedback
10. Do you have competitors? Yes\_\_\_\_, No\_\_\_\_
11. If you have competitors; what is the uniqueness and similarities of your product with the competitor’s product? \_\_\_\_\_.

Respondents Questionnaires of the Pre-requisite programs

S/No	Pre-requisite program checklist for auditing the processing practice for safer oil production in the Ethiopian conventional and commercial oil processing facilities	Control measures			Compliance	Comments
		Critical	Major	Minor	Yes/No/N/A	
1	Establishment design and facilities					
	Is the company located nearby raw material sources and market access?					
	Was the company established primarily for sesame oil processing?					
	Does the company owe appropriate equipment for sesame oil extraction?					
	What quality parameters and specification for raw materials and other imputes for oil extraction do you have?					
	Do you have appropriate equipment for monitoring the process and incoming raw material, containers for waste and inedible substances?					
	How frequent the equipment's are calibrated for the accuracy of measuring and monitoring of the quality and avoid contamination					
	Premises and Rooms					
	Is the design and layout of the company as per the recommendation of food plant?					
	Is the Internal structure and fittings of smooth and easier for cleaning?					
	Temporary/Mobile Premises and vending machines					
	Transportation conveyance and bulk, design, use and maintenance					
	Equipment					
	Does the company have quality control and monitoring equipment?					

Does the company have containers for waste and Inedible Substances handling and treatment?

Are the calibration procedures written and standardized?

Facilities

Does the company have continues water supply?

Does the company have drainage and waste disposal system?

Cleaning, hygiene facilities and toilets

Does the company have Temperature, ventilation and lightening control system?

Does the company have well designed storage for raw material, ingredients and end product (Oil)?

---

**2 Establishment maintenance and sanitation**

---

Do you have written cleaning program?

Personnel hygiene

How frequent the oil processing facilities is cleaned?

Which part of the oil processing area is requires to be cleaned frequently

Before processing

During processing

After processing

Is the cleaning procedures, methods for personnel cleanness, cleanness behavior and health status specified and displayed proper in the areas of the company?

How the environmental, production, handling, storage and transport hygienic activity is managed?

What detergent and sanitizers and how it is applied?

Does the company have pest control systems?

Do you apply pesticides for pest control? Which type of pesticide? →

Algicides, Avicides, Bactericides, Fungicides, Herbicides, Insecticides, Miticides, Molluscicides, Nematicides, Rodenticides, Virucides

For how long the raw materials including oil seed is stored in the factory stock?

Does the company have waste management system?

Does the company have monitored the effectiveness of overall operation?

---

### **3 Control of operations**

---

What possible hazards are expected in the oil processing industry?

Incoming Material

Packaging

Hygiene Control Systems and hygiene codes

→Time and Temperature Control

→Microbiological Cross-Contamination

→Physical and Chemical Contamination

Water as an Ingredient, Ice and Steam or cleaning medium

Management and Supervision

Documentation and Records

Recall Procedures

---

**4 Training and responsibility of employee**

---

Training Programmes

Instruction and Supervision

Refresher Training

---

**5 Product information and consumer awareness**

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→Lot Identification

→Product Information

→Labeling

→Consumer Education

→ Vender procedures

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General

Comments:

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## Appendix G: Checklist/questionnaire to assess the suitability of the extraction plant

Item	Check list		
	Yes	No	Remark
<b>Premises design</b>			
Is the plant located at the right location?			
Is the premises are primarily designed for sesame oil extraction with separated store for raw materials, end product and other supplies?			
Is the oil extraction plant located in dust free environment?			
Is the extraction apparatus designed for sesame oil extraction?			
Is the drainage system (slope) well designed to prevent standing water in the interior or exterior area of the plant?			
Is the facility clear of litter, weeds, grass and brush?			
Is the floor acidic or alkaline chemicals resistant?			
Is the in and out doors and windows have fine mesh screens or tightly sealed for insect entry control?			
Is the roof free of leaks to allow entry of rain, direct light?			
Is the plant have holes or cracks for the entry or facilitate growth of insects?			
<b>Process</b>			
Is the sesame seed stored on the basis of first in and first out to avoid spoilage?			
Are the incoming sesame seed inspected against specification?			
Is the oil and non-food items stored separate?			
Is water used for processing and cleaning treated?			
Do they have recall procedure set up?			

### **Hygienic conditions and instructions**

Is instruction available on avoiding jewellery, dressing and use of mobile phones on the production area?

Is the hand wash facilities equipped with soap, sanitizers and paper towel?

Is the workers wear protective and hygienic clothing? Gloves, clothing, hair and foot cover?

Do operators and actors handle oil with bare hands?

Do workers and visitors wash up their hands before and during production or toilet visitation?

Do workers blow their nose while handling or preparation?

Is there a build-up of seepage such as oily dirt, cleaning solvents, lubricants on the surface of extraction machine which can contaminate the oil?

Is spilled oil or solvents cleaned up quickly to avoid cross contamination?

Is garbage quickly cleaned, covered and removed?

### **Pest control**

Are insects and macro-organisms including domestic animals exist in the indoor and outdoor environment?

Are safety rules observed during fumigation?

Are the pest control logs and documentation readily available?

Are pesticides or applicable equipment readily available?

Are trucks inspected and cleaned?

**AppendixI: CCP decision tree assisted CCP determination and significance of hazard analysis for CSO safety**

Process Item/ step	Hazards	Hazard analysis					Significant Hazard	CCP	
		Q <sub>1</sub>	Q <sub>2</sub>	Q <sub>3</sub>	Q <sub>4</sub>	Q <sub>5</sub>			
<b>Raw material reception and storage</b>	Microbial	Y	Y	N	-	-	N		
	Pesticide residue (C)	N					N		
	Aflatoxin	Y	Y	N	-	-	Y		
	Foreign matter	Y	N	-	-	-	N		
	<b>Water</b>	Microbial (M)	Y	Y	Y	N	-	N	
		Chemical residues (C)	N	N	-	-	-	N	
<b>Cleaning and drying</b>	Microbial (M)	Y	Y	Y	Y	N	N		
	Impurities (P)	N	N	-	-	-	N		
<b>Roasting</b>	Browning (C)	N	N	-	-	-	-	CCP <sub>1</sub>	
	Burnt seed and lighter husk (P)	N	N		-	-	-		
	Fungi and fungi metabolites								
<b>Conditioning and pressing</b>	Microbial (M)	Y		Y	N	-	-	Y	CCP <sub>2</sub>
	Oxidation (C)	N	N		-	-	-	N	
	Detergent residue (C)	N	N		-	-	-	N	
	Foaming							N	
<b>Filtration</b>	Microbial infection (C)	Y	Y	N		-	-	N	

	Turbidity (C)							N	
<b>Filling (manual)</b>	Microbial (M)	Y	Y	N	-	-	Y		CCP <sub>3</sub>
	Deterrent residual (P)	N	N		-	-	N		
<b>Packaging</b>	Microbial	N	N		-	-	N		
	Detergent residual (P)	N	N		-	-	N		
	Oxidation (C)	N	N		-	-	N		
<b>End product storage (CSO)</b>	Microbial (M)	Y	Y	N	-	-	N		
	Rancidity (C)	N	-	-	-	-	-		
	Aflatoxin (C)	N	N		-	-	N		

**Appendix J: Sample data output**

**Microbial challenge test for HACCP validation**

The ANOVA Procedure

Class Level Information

Class	Levels	Values
Sample	28	CSO-A CSO-B Filling1 FillingS Filtre-A Filtre-B G.Water1 G.Water2 Gown-A Gown-B InDoorP1 InDoorP2 InDoorR1 InDoorR2 M.Water1 M.Water2 Miller-A Miller-B OutDoorP OutDoorR OutP1 OutR1 clove-A clove-B store-A store-B wall-A wall-B

Number of observations 84

The ANOVA Procedure

Dependent Variable: log10cfu

Source	DF	Sum of		F Value	Pr > F
		Squares	Mean Square		
Model	27	147.9920692	5.4811877	121.36	<.0001
Error	56	2.5292916	0.0451659		
Corrected Total	83	150.5213608			

R-Square 0.983196  
 Coeff Var 9.921136  
 Root MSE 0.212523  
 log10cfu Mean 2.142121

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Sample	27	147.9920692	5.4811877	121.36	<.0001

Tukey's Studentized Range (HSD) Test for log10cfu

NOTE: This test controls the Type I experiment wise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	56
Error Mean Square	0.045166
Critical Value of Studentized Range	5.53060
Minimum Significant Difference	0.6786

**Oxidation**

**Data** Peroxide value;

Input Roasting \$ ST \$ Basil \$ Storagetime \$ PV;

Datalines;

Raw	Amb	No/Ex	0	0.5
Raw	Amb	No/Ex	0	0.49
Raw	Amb	No/Ex	0	0.49
Raw	Amb	No/Ex	3	1.44
....	.....	.....	.....	.....

;

**ProcANOVA;**

Class Roasting ST Basil Storage time;

model PV = Roasting ST Basil Storage time Roasting\*ST Roasting\*Basil Roasting\*Storage time

```
ST*Basil ST*Storage time Basil*Storage time Roasting*ST*Basil ST*Basil*Storage time
Roasting*ST*Basil*Storage time;
means Roasting ST Basil Storage time Roasting*ST Roasting*Basil Roasting*Storage time
ST*Basil ST*Storage time Basil*Storage time Roasting*ST*Basil ST*Basil*Storage time
Roasting*ST*Basil*Storage time /TUKEY;
RUN;
```

## Appendix L: Sample Result

