Addis Ababa University

College of Natural and Computational Sciences

Center for Food Science and Nutrition

ASSESSMENT OF QUALITY AND SAFETY OF SUPER CEREAL-CORN SOYA BLEND (SC-CSB) PROCESSED AT FACTORY LEVEL IN ETHIOPIA

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A THESIS SUBMITTED TO THE CENTER FOR FOOD SCIENCE AND NUTRITION, COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES, ADDIS ABABA UNIVERSITY

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Declaration

I the under-designed, declare that this thesis is original work and has never been presented in any other universities as well as research centers and all source of materials used for this thesis has been fully acknowledged.

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Contents

List of Abbreviations and Acronyms

%	Percent
±	Plus or minus
<	Less than
>	Greater than
≥	Greater than or equal
°C	Degree Celsius
µg	Microgram
µl	Microliter
µm	Micrometer
µS	Microsimens
AAS	Atomic Absorption Spectrometry
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
ANOVA	Analysis Of Variance
AOAC	Association of Official Analytical Chemists
a_w	Water Activity
Ca	Calcium
CCV	Calibration Curve Verification
cfu	Colony form units
cm	Centimeter
CS	Control Sample
ECSA	Ethiopia's Central Statistics Agency
EFMHACA	Ethiopian Food, Medicine and Healthcare Administration and Control Authority
ESA	Ethiopian Standards Authority
FAO	Food and Agricultural Organization of the United Nations
FBF	Fortified Blended Food
Fe	Iron
g	Gram
GMP	Good Manufacturing Practice
GOE	Government of Ethiopia

HACCP	Hazard Analysis and Critical Control Points
HPLC	High Performance Liquid Chromatography
hr	Hour
ICV	Initial Calibration Verification
ISO	International Organization for Standardization
IU	International Units
K	Potassium
L	Liter
LC-MS-MS	Liquid Chromatography-Mass Spectrometry- Mass Spectrometry
LSD	Least Significant Differences
MAM	Moderate Acute Malnutrition
MB	Method Blank
mg	Milligram
Min	Minutes
ml	Milliliter
mm	Millimeter
MRM	Multiple Reaction Monitoring
NGO	Non-governmental organization
NOPA	National Oilseed Processor Association
NSI	Nitrogen Solubility Index
NSI	Nitrogen Solubility Index
PDI	Protein Dispersibility Index
ppb	parts per billion
ppm	parts per million
QPM	Quality Protein Maize
rpm	Revolutions per minute
SAM	Severe Acute Malnutrition,
SC-CSB	Super Cereal-Corn Soy Blend
SOP	Standard Operating Producers
STD	Standard Deviation
UNICEF	United Nations Children's Fund
VAD	Vitamin A Deficiency
WB	World Bank
WFP	World Food Program
WHO	World Health Organization

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Abstract

Super-cereal-Corn Soy Blend (CSB) is a cooked blend of milled, heat-treated corn and soybean and fortified with a vitamin and mineral premix. The ingredients are partially cooked through either extrusion or roasting. CSB is the most widely used foods in supplementary feeding programs as it is naturally wholesome blended food, nutritious and precooked for ease in use and handling. During pre and post-harvesting of the raw materials and processing of the CSB in the industries, hygiene and sanitation procedure should be followed in order to prevent different contaminations. Moreover the super cereal- corn-soya blend should be free from any hazardous contamination like aflatoxins and heavy metals since infants do not have the capacity to resist such kind hazards. Thus, this study was conducted to examine the quality and safety of CSB flour collected from four Ethiopian SC-CSB manufacturing factories based on their cooking processing methods (two extrusion and two roasting). Proximate compositions, minerals (Fe, K, and Ca), Vitamin A, physicochemical and microbiological composition were analyzed by official methods of AOAC, ICP-OES, High Performance Liquid Chromatography with DAD detector (HPLC/DAD) methods used, respectively. Aflatoxin B1, B2, G1, and G2 were determined by immuno-affinity column cleanup and reversed-phase liquid chromatography with fluorescence detection. identification, limit of detection (LOD), limit of quantitation (LOQ), linearity, precession, accuracy and recovery tests were done. Out of four SC-CSB factories, proximate composition (crude fiber) for each cooking methods were in the range of 2.34 g/100g to 3.75 g/100g. Mineral contents (Iron) of SC-CSB for all cooking method were in the range of 10.2 mg/100g to 13.4 mg/100g. Vitamin A contents for each factory were above the expected range between 21.8 mg/100g to 40.9 mg/100g. Physicochemical properties (peroxide value and urease index) for all cooking methods were not detected. Three of them were positive with a range of aflatoxin B1 between 2.49 and 4.66µg/l and one factory sample was less than Limit of Detection. Total aflatoxin level for each sample was detected with a range of 6.47 to 14.13µg/l. Total Aerobic Plate count/gm, yeast and molds and total Coliforms of sample for roasting cooking processing have positive (1.6×10^4 cfu/gm and 4.0×10^3 cfu/gm), 1.77×10^3 cfu/gm and 1.54×10^3 cfu/gm and (4.3×10^1 confirmed coliforms/gm and 3.9×10^1 confirmed coliforms/gm) respectively. It can be concluded that, the vitamin A result showed that all factories have used a higher concentration of Vitamin A fortificant during the processing of SC-CSB. Samples taken from factories using roasting cooking method were highly contaminated with total aflatoxin and aflatoxin B1 which is out of the range of maximum allowable limit according to CES, EU, and WFP standard. The super cereal corn soya blend manufacturing companies should have a system of checking the risk of containment of aflatoxin and other contaminant factors starting from the raw material selection and also via out the process. The concerned stakeholders need to make sure that the products are free of aflatoxin before the products delivered to consumers.

Key words: *Aflatoxin, Corn Soy Blend flour, Fortification, Immuno-affinity Cleanup, Vitamin*

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Complementary food is the food given to infants alongside breast milk with the bid to supply the necessary nutrients and calories that are hitherto inadequate in the breast milk (Adelekan, 2003). Complementary foods are tightly regulated and specifically formulated to meet the precise needs and requirements of infants and young children in good health. Complementary foods should be of the right consistency, energy dense and the variety to provide all nutrient demands of a growing child. When breast milk is no longer enough to meet the nutritional needs of the infant, complementary foods should be added to the diet of the child (WHO, 2012).

Legumes and cereals are the main sources of nutrients for traditional complementary foods in developing countries. The main cereals such as soybean, sorghum, rice, wheat and maize constitute about 85% of total global cereals production amounting to about 200 million tons of harvest annually at an average of 10% protein content, out of which a sizeable proportion goes into human consumption (Tufa, *et al*., 2016).

Super Cereal- Corn- Soya Blend is a product from maize and soya bean and vitamins and minerals. It has been discovered that maize is nutritionally superior to other cereals in many ways, except in protein value. The term Super Cereal used as a market name of Corn- Soya Blend products. Maize is low in two essential amino acids, lysine and tryptophan. The addition of soya bean which is rich in lysine and tryptophan was meant to enhance the nutritional capacity of the composite flour. Soya bean is an important legume. It has been found that soya bean is a very rich source of protein and fat, photochemical and minerals. Corn soy blends (CSB) are fortified blended foods which used as complementary foods throughout the world. CSB is a good source of protein and a good source of energy, carbohydrates, protein, fat, and micronutrients for target groups. It is fortified with a variety of vitamins and minerals. Various formulations of corn soy-based fortified blended foods have been used in food aid for almost 50 years, evolving with the advances in scientific evidence of their nutritional value and impact (Wimalawansa, 2013).

In USAID programming, CSB should be programmed with Vitamin A and D fortified vegetable oil to increase nutrient values and caloric density. CSB is provided as a fortified supplement to traditional complementary foods for children, pregnant and lactating women in Maternal and Child Health Programs to prevent nutritional deficiencies, address wasting, and promote child

growth (prevent stunting) during the first 1,000 days and for treatment of children 6-59 months who are moderately malnourished. Currently, CSB is used as an emergency food distribution as an alternative to a ready-to-use supplementary food for adults and children (Sopov, *et al*., 2015).

Fortified blended flours, such as corn-soya blend (CSB), prepared as porridge, are the most widely used foods in supplementary feeding programs (Karakochuk, *et al*., 2012). Children with moderate acute malnutrition (MAM) are often treated with fortified blended flours, most commonly a corn-soy blend (CSB). It is a product preferred for young children aged above six months. The product is to be used as a complement to breastfeeding but it is not a breast-milk replacer. This blended food prepared from heat treated corn and soybeans, vitamins and minerals. CSB can often be made from locally available, low-cost ingredients and are culturally and organoleptically acceptable in many settings. Super cereal- corn-soya blend is a product used for adult and children over six months. CSB is a blend of milled, cooked, heat-treated corn and soybeans and fortified with a vitamin and mineral premix. The ingredients are partially cooked through wet or dry extrusion or roasting. CSB is processed under conditions that permit improvements in the digestibility of starches and protein and in particular the de-activation of trypsin inhibitors in soy (Lagrone, *et al.*, 2012).

Nutritionally, soybean protein resembles animal protein more closely than other vegetable proteins form oilseeds and legumes. Soybean protein constitutes about 40% of the total solids and plays a very important role in the enrichment of cereal-based food products. It is also a rich source of vitamin, minerals and is relatively low in crude fiber (Okoye, *et al*., 2008). This leguminous plant provides a wide range of opportunities for improving household food and nutrition security. The majority of the population in Ethiopia does not have access to an expensive animal protein such as eggs, milk, and meat, while child and maternal malnutrition are among the highest in the world. The Government of Ethiopia and major relief food aid agencies are mobilizing resources to procure corn-soya blend as part of the drought relief effort (Sopov, *et al*., 2015). The principal buyers of CSB were the World Food Program and other aid organizations for drought and famine-affected areas in Ethiopia and neighboring countries.

According to Belayneh, 2011 in Food Security in Sub-Saharan Africa of nutritional strategies; malnutrition has the most significant impact on rapidly growing infants and young children and can seriously harm their physical and mental development. The nutritional needs of infants and young children are significantly different from those of the rest of the population.

Even though CSB is the most usable food in supplementary feeding programs it is considered to be ineffective in addressing MAM, because of inadequate composition (micronutrients, energy density, lipids, fibers, and anti-nutrients) and occurrence of Aflatoxins and toxic

microorganisms, therefore this thesis will study about assessment of quality and safety on Super cereal- corn soya blend processed at in factory level in Ethiopia.

1.2 Statement of Problem

Micronutrient deficiencies represent a most invisible but often devastating form of malnutrition (Allen, *et al.,* 2006). In Ethiopia, Micronutrient deficiency is the major challenge and to fight against this micronutrient deficiencies food fortification is a proven and preferred strategy in the prevention and management of micronutrient deficiencies. It is also the most cost effective and has the potential to achieve high coverage. On food fortification sectors there are obstacles to produce a high quality fortified product to the target groups from these problems one of the major adverse effect of processing and inappropriate storage conditions regarding to retention of fortified Vitamins and minerals from finished foods (Berry Ottaway, 2010). It is obvious that blended foods like CSB is highly needed to fight against micronutrient deficiencies but, as much as its production amount this product is not able to address this problem because of the nutritional composition is below the given standard. Moreover the existence of different contaminations basically aflatoxins and the other challenge is a quality problem such as under limitation of fortificants, physicochemical, microbiological problems due to inappropriate harvesting and post harvesting conditions, processing condition and even from the raw material itself.

Super cereal- corn-soya blend is a product used for adult and children over 6 months. CSB is a cooked blend of milled, heat-treated corn and soybeans and fortified with a vitamin and mineral premix. The ingredients are partially cooked through wet or dry extrusion or roasting. So that the super cereal- corn-soya blend should be free from any hazardous contamination like aflatoxins and heavy metals. Because, the infants have limited capacity to resist such kind hazards, in addition to that, mostly the product supplied to drought affected infants and adults. Due to this problem, the study will design to investigate the quality and safety of super cereal-corn soya blend processed at the factory level.

According to Ethiopian National Compulsory Standards for super cereal –corn soya blend should meet the requirements for each quality and safety parameters. The requirements includes moisture (10 max, % by mass), total ash (4.1 max, % by mass), Protein (14 min, % by mass), Fat (6 min, % by mass), fiber (10 max, % by mass), peroxide value (10 max, meq/Kg fat), Iron (9.4-14.1,mg/100gm), Calcium (340-510,mg/100gm), Potassium (580 – 870, mg/100gm), Vitamin-A (2770 – 4160, IU/100g), Coliforms (< 10, cfu/g), Yeasts and moulds (< 1000, cfu/g), Mesophyllic aerobic Bacteria (<10,000, cfu/g) and Aflatoxin-B1 (2.0 max, μg/kg). This study

also aims to compare and contrast the research finding with the above stated minimum requirements (CES 139: 2015). The Ethiopian National Compulsory standards for Super cereal – Corn soya blend are indicated in Annex A.

According to Ethiopian Food, Beverage and Pharmaceutical Development Institute (EFBPDI), from the past assessment stated, from 32,000 mt of CSB that were processed by the seven CSB processing companies around 28,000 mt of the CSB was not able to fulfill the Aflatoxins contamination level as per Ethiopian compulsory CSB standard (CES 139) (Source: Personal contacts). Based on this fact, this thesis studied thoroughly to examine quality and safety tests by taking samples of CSB from the purposely selected factories. Furthermore, information regarding the composition of corn soya blend, which was not reported previously in Ethiopia, will be explored.

1.3 The significance of the study

- It highly aids to identify safety parameters and changes they go via inappropriate conditions or processing methods.
- To initiate and increase the level of awareness of concerned bodies and partners to extend their effort in this area so as to achieve the common goals of improving child nutrition and safety.
- It also highly aids to identify each quality and safety parameters and changes due to inappropriate operations.
- Researchers, students and academicians can use the findings of the study as a reference material.
- The study may serve as a baseline for further studies that can be done on assessment of quality and safety of foods in factory level.
- Maintaining the well-being of Ethiopian economy by preventing loss of products from damage.
- For government bodies like EFMHACA (Ethiopian Food, Medicine and Healthcare Administration and Control Authority) and Ethiopian Standards Authority (ESA) to set the limit for regulation.

1.4 Objectives

1.4.1 General Objective

To assess the quality and safety of corn soya blend flour produced at factory level.

1.4.2 Specific Objective

Specific objectives of this study were to:-

- Evaluate the proximate composition of CSB (Moisture, Ash, Fat, Protein and crude fiber) based on processing methods (roasting and extrusion)
- Evaluate the mineral (Fe, K and Ca) content of CSB processed based on extrusion and roasting.
- Determine and compare the vitamin A level in CSB processed with roasting and extrusion.
- Evaluate Physico-chemical characteristics (Peroxide value, particle size and urease index) and other determinant factors of CSB quality.
- Determine the safety of the CSB processed with roasting and extrusion from Aflatoxin.
- Evaluate the microbiological parameters (Total Aerobic plate count, Coliforms, and Yeast and mold) quality of CSB processed with roasting and extrusion parameters.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Complementary Foods

Exclusive breast feeding for the first 6 months is the recommended method of feeding full-term infants with healthy and well-nourished mothers (Kikafunda *et al*., 2003, WHO, 2000). However, breast milk cannot sustain the nutrient and calories requirements of the infant after the age of 6 months; this gives room for the introduction of complementary food that can meet the nutritional requirements of the growing child ((Adelekan, 2003) (Ikujenlola and Fashakin, 2005)).

Complementary foods are defined as any solid or liquid foods with a nutritional value other than breast milk, offered to breast-fed infants and for other groups which needs nutritional rich foods. These foods should be rich in energy, proteins and micronutrients, free from contamination, easily digestible and in adequate amount. Complementary feeding is needed to provide energy and essential nutrients required for continued growth and development (Fashakin, *et al*., 1986).

In Nigeria, like many other developing countries, there are various complementary foods that have been developed and served to infants in various localities ((Obayanju & Ikujenlola, 2002) (Ikujenlola and Fashakin, 2005)). The complementary foods developed based on cereal and starchy root have been associated with the incidence of protein-energy malnutrition among the young infants (Inyang & Idoko, 2006). Complementary foods produced from cereals are known to be deficient in certain essential amino acids which are required for the adequate growth and healthy living of infant.

The essential amino acids; lysine and tryptophan are in short supply in normal maize, however, a new hybrid called Quality Protein Maize (QPM) contains reasonable quantity of these essential amino acids (Abiose, *et al*., 2015). Apart from the problem of inadequate nutrients plaguing the complementary food produced from cereal, high dietary bulk and high viscosity are factors which affect the quantity of food a child could consume per meal; this invariably affects the quality of the nutrients available to the children. The traditional complementary foods are associated with a high viscosity which causes choking and suffocation of infant during feeding. The complimentary food that will support growth and maintain good healthy living must contain adequate nutrients and be of low viscosity. Soybean is generally known to be of good nutritional quality in terms of its protein quantity and quality (Iwe, 2003).

2.2 Super Cereal-Corn Soya Blend/SC-CSB/

Corn Soy Blend (CSB) is a fortified blended food (FBF) used in food aid programs throughout the world for almost 50 years. CSB is made from heat treated maize and soy beans. Vitamins and minerals are added after the heat treatment. CSB is consumed by adults and children over 6 months of age. It is prepared by mixing CSB with clean water (e.g. 40 g CSB with 250 g water). The mixture is brought to a boil and then simmered for 5-10 minutes **(**WFP Technical Specifications for CSB, 2010**)**.

Corn Soy Blend (CSB) is a cooked blend of milled, heat-treated corn and soybeans and fortified with a vitamin and mineral premix. The ingredients are partially cooked through wet or dry extrusion or roasting. CSB is a naturally wholesome blended food, highly nutritious and precooked for ease in use and handling. CSB contains 69.5% cornmeal; 21.8% soy flour; a premix of 3.0% minerals and vitamin antioxidant and 5.5% soy oil (Figure 2.2). CSB is processed under conditions that permit improvements in the digestibility of starches and protein and in particular the de-activation of trypsin inhibitors in soy.

Soybean (Glycine max L.) is an important legume among vegetable foods because it is a source of high-quality digestible proteins, dietary fiber, minerals, and essential fatty acids (Carvalho, *et al.,* 2013). According to Ethiopia's Central Statistics Agency (CSA), soybean production during the 2014/15 was about 72,000 metric tons, up from the previous year's volume of 61,025 metric tons and of this amount, roughly 40,000 metric tons, or more than half, was consumed on farm, while 27,000 metric tons were exported, leaving approximately 5,000 metric tons for other local needs, such as the production of corn-soya blend (CSB), edible oil, and animal feed (Ethiopia's Demand, 2016).

Fortified blended flours, such as corn-soya blend, prepared as porridge, are the most widely used foods in supplementary feeding programs. However, concerns about the nutritional adequacy of these blended foods in combination with issues around preparation at home (making the porridge too thin or inadequate boiling of water) and the documented success of new treatment foods for SAM have led to the development of alternative foods for the treatment of MAM (Karakochuk, *et al.,* 2012).

Fortified blended flours, specifically CSB, are the most commonly used supplementary foods for MAM. CSB can often be made from locally available, low-cost ingredients and are culturally and organoleptically acceptable in many settings (Karakochuk, *et al.,* 2012).

Figure: 2.1 Corn soya based porridge

Source:- (WFP,Food Fortification in Malawi, 2008)

Corn Soy Blend (CSB) is a cooked blend of milled, heat-treated corn and soybeans and fortified with a vitamin and mineral premix (Ashagrie and Paulos,2017). It should be prepared by mixing an appropriate proportion of flour and clean water. Corn Soya Blend has traditionally been an attractive investment in Ethiopia and the major food processing companies such as FAFFA, Hilina, GUTS Agro, Health Care, and Tigers Group have CSB in their product assortment.

2.2.1 Corn Soya Bend Ingredient Formula

In Ethiopia, CSB has currently been used as a major relief food for drought affected areas of the country. About 200,000 tonnes of CSB is required to address moderate acute malnutrition needs during the first half of 2016 and of this amount; Ethiopia has already nearly 90,000 tonnes form local production and import. The remaining 110,000 tonnes could be produced in the country, if locally-grown soybeans and the pre-mix of vitamins and minerals are available (Ashagrie and Paulos, 2017).

Table: 2.1 CSB Ingredient composition **(**USDA commodity requirements, 2014**)**

S/N	Ingredient	Formula CSB % by weight	Formula CSB with sugar % by weight
1	Corn (Maize)	78.3	64.24
2	Soyabeans	20.0	24.0
3	Sugar	0.00	10.0
4	Vitamin mineral premix (FBF-V-13)	0.20	0.20
5	Dicalcium phosphate Anhydrous	1.23	0.80
6	Potassium Chloride	0.27	0.76
	Total	100.0	100.0

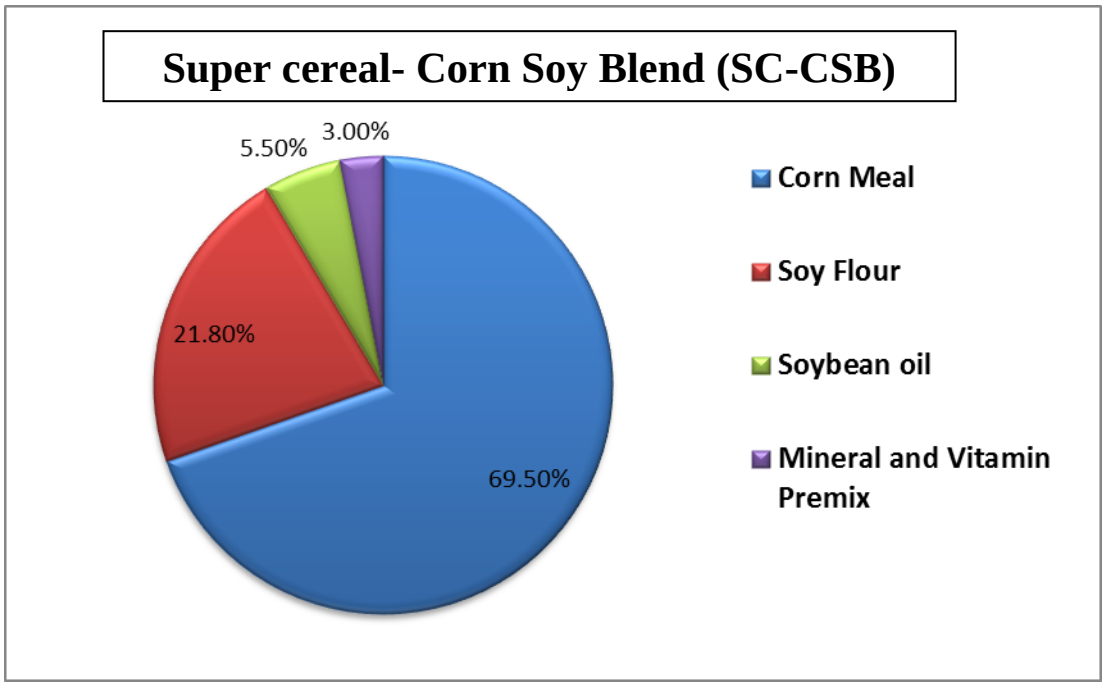


Figure: 2.2- CSB ingredient composition **(**USDA Commodity requirements, 2014**)**

To respond to these needs, Ethiopia has contr acted with seven local food processors (Table 2.2) to produce 39,000 tonnes of CSB for delivery (approximately 8,000 tonnes per month) to the drought hotspot areas over the next several months. Ethiopia is reported to have purchased and is supplying these operations with the necessary pre-mix of minerals and vitamins for this locally-contracted amount.

Table 2.2: Ethiopian food processors making CSB.

S/N	Company Name
1	Abbay International PLC
2	FAFA Food Share Company
3	Guts Agro Industry PLC
4	Health Care Food Manufactures PLC
5	Kidan International PLC
6	Norish Business Group PLC
7	Picana PLC

Corn soya blend shall be manufactured from fresh maize grain and soya bean of good quality, free from foreign materials, substances hazardous to health excessive moistures, insect damage and fungal contamination and shall comply with all relevant national food laws and standards. Maize and soya bean must be stored under dry, ventilated and hygienic conditions. Only safe insecticides may be used for fumigation control. Corn is one of the most suitable cereals for development of mycotoxins like aflatoxins.

Currently, CSB used as an emergency food distribution as an alternative to a ready-to-use supplementary food for adults, children and if the CSB is in poor quality due to contamination with microorganisms and aflatoxins and other factors, these children's will be in worst condition regarding their health, even they can be die.

2.2.2 Corn Soya Blend Cooking Process

Super-cereal- corn soya blend shall be processed as partial pre-cooked food under condition which permit improvement in digestibility of starch and proteins and in particular the de-activation of trypsin inhibitors in soya bean. Macronutrients (vitamins and minerals) are used at the rate of 1.6 kg of vitamins, 2 kg of potassium chloride and 9 kg of di-calcium phosphate per metric tons of finished products (CES 139:2015).

If CSB is consumed as porridge, it should be prepared by mixing an appropriate proportion of flour and clean water that mean 40 gram of corn-soya blend with 250 gram of water followed by boiling time at simmering point from five to ten minute. A recent review recommended that 30 g of oil be used per 100 g of CSB to increase energy density, micronutrient absorption and for the treatment of moderate acute malnutrition (MAM) (Rogers, *et al*., 2016). Mostly this product is a cooked blend of milled, heat-treated corn and soybeans and fortified with a vitamin and mineral premix. CSB is processed under conditions that permit improvements in the digestibility of starches and protein and in particular the de-activation of trypsin inhibitors in soy.

Mainly there are two techniques used in order to process SC-CSB. These are roasting and extrusion cooking processes. The Roasting process can be either batch or continuous but the higher throughputs are usually achieved with continuous systems. The soy beans and maize kernels are roasted separately to achieve optimal cooking. The energy source can be electric, gas or wood. Equal sized particles will roast equally different size particles are not used in one batch this is because that small ones will be too dark were as large ones will be too light.

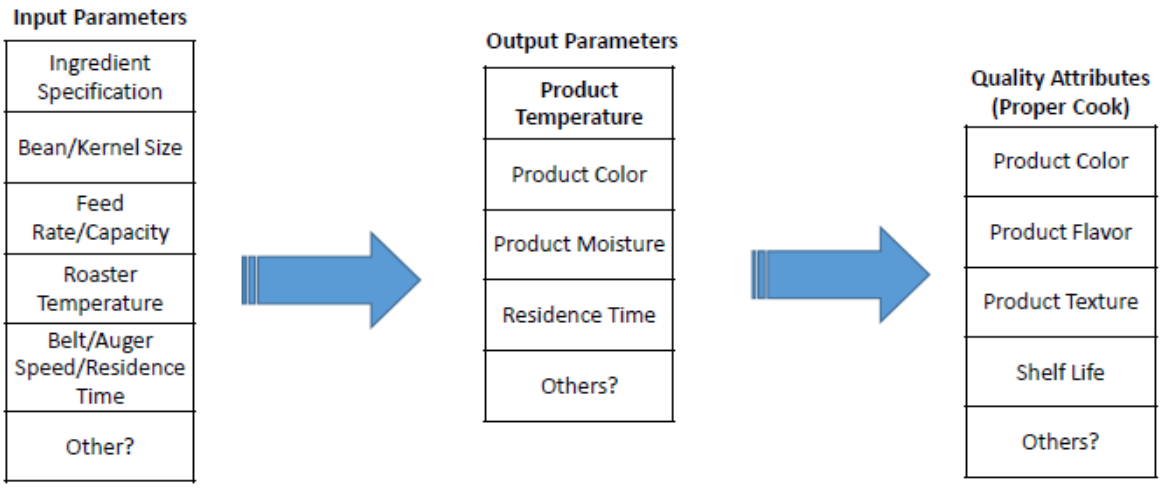


Figure: 2.3- Roasting Parameter Overview

The raw materials corn and soybean are received and stored in the storage house. The raw materials should be clean in the cleaning section before going to the roasting process. The first step is that impurities which are larger and smaller in size than maize and soya should be separated. Then by the friction principle remove impurities attached and then air resistance at machine attached with separator and removes lighter impurities. De-stoner will separate impurities denser than maize and soya bean like stone and coarse glass. Finally, in the magnetic separation section, physical hazards like metals which may cause choking and traumatic injury including perforation of tissue of mouth, tongue, throat, stomach etc. and sphere of ferrous material of diameter ≥ 2 mm should not pass through the magnet and mixed with the product.

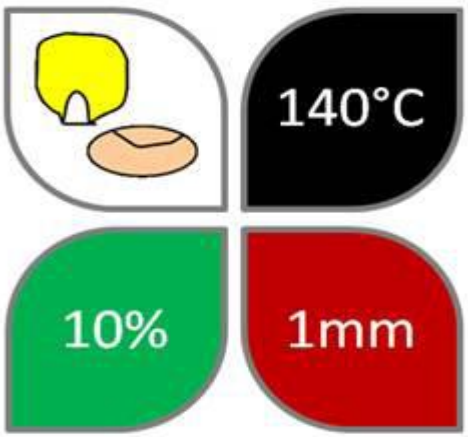


Figure:2.4- SC- CSB key numbers (Ashagrie and Paulos, 2017)

CSB must be cooked to at least 140 °C, CSB final moisture must be less than 10% and CSB final particle size must be less than 1 mm (1000 microns).

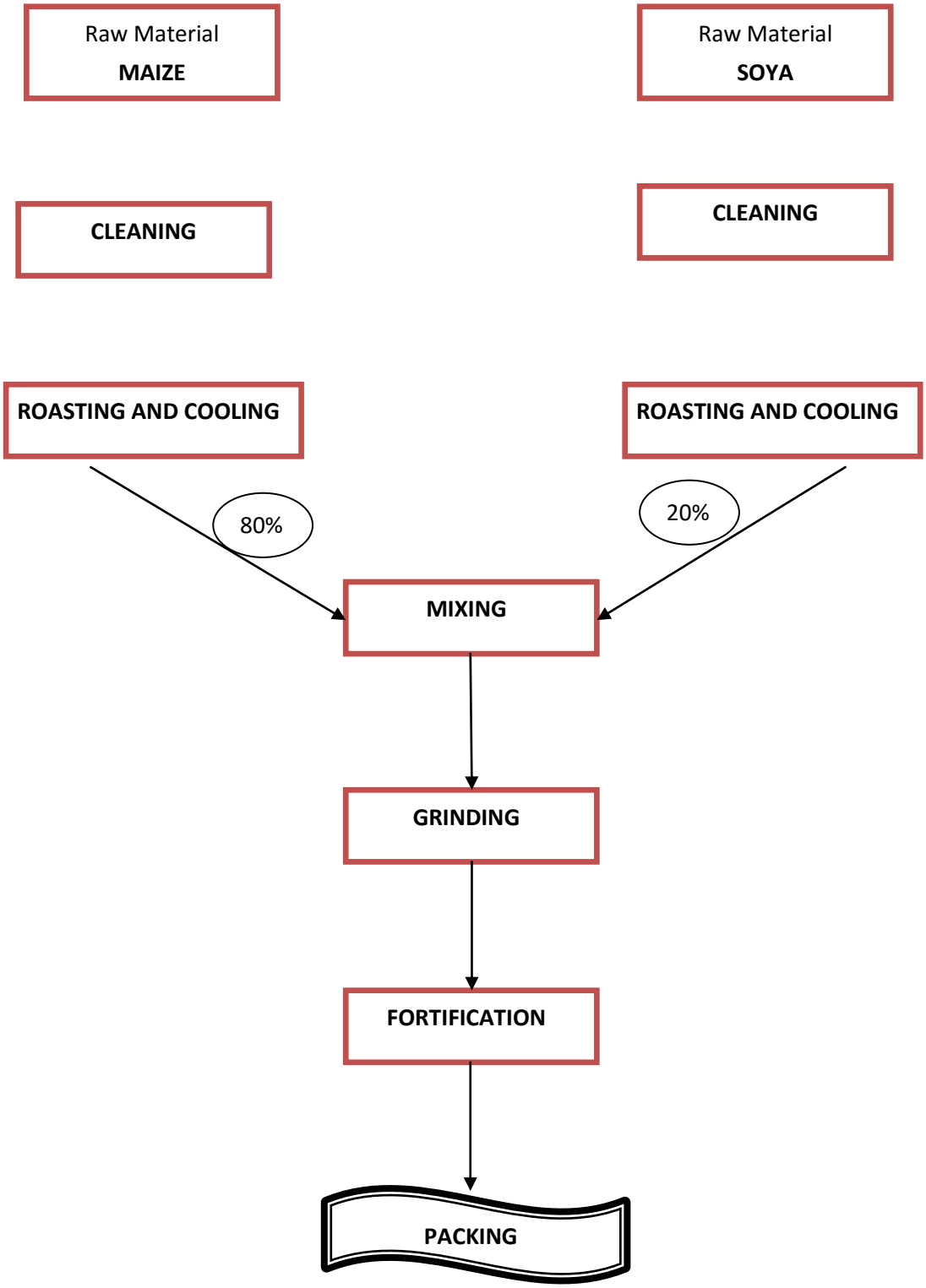


Figure:2.5- General Roasting process flow diagram

The Soya temperature must reach 140 °C to increase shelf life, improve digestibility and reduce anti-nutritional factors. The product temperature should not exceed 160 °C. Above this temperature, the nutritional value is decreased. Equal sized particles will roast equally. Do not use different size particles in one batch. Small ones will be too dark and large ones will be too light. The Roasting process conditions yielding good product when roaster temperature 225 ^{o}C

(maximum temp 250 ^{o}C), Maize: 14 minute residence time with product exit temp of 109 ^{o}C and Soya: 17 minute residence time with product exit temp 149 ^{o}C.

Figure:2.6- Show's unroasted and roasted corn and soybean

Extrusion is a process which combines several unit operations including mixing, cooking, kneading, shearing, shaping and forming. The extruder is made up of three main components, the screw, the barrel, and the die. The most important operating parameters in an extruder are: temperature, pressure, the diameter of the die apertures and shear rate.

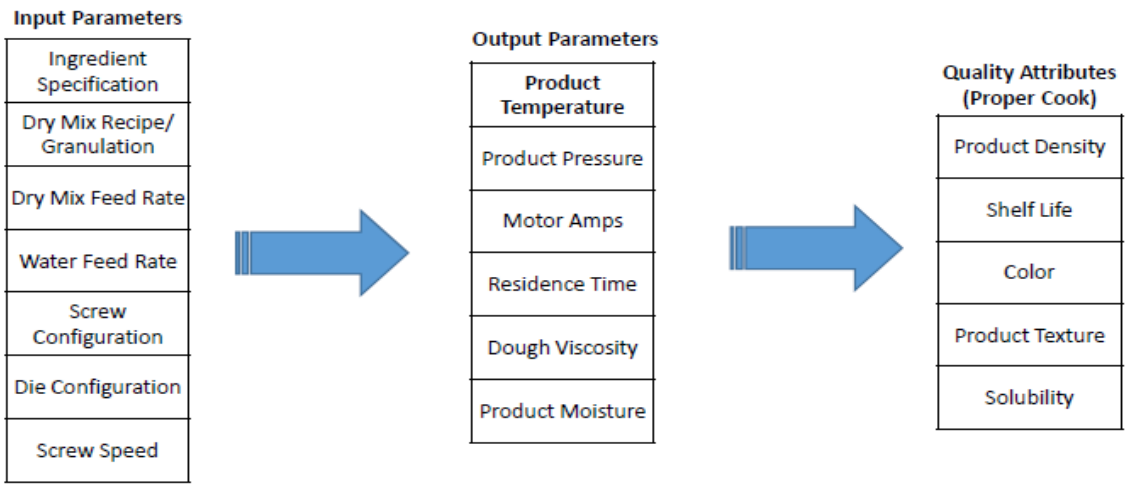


Figure:2.7- Extruder parameter overview

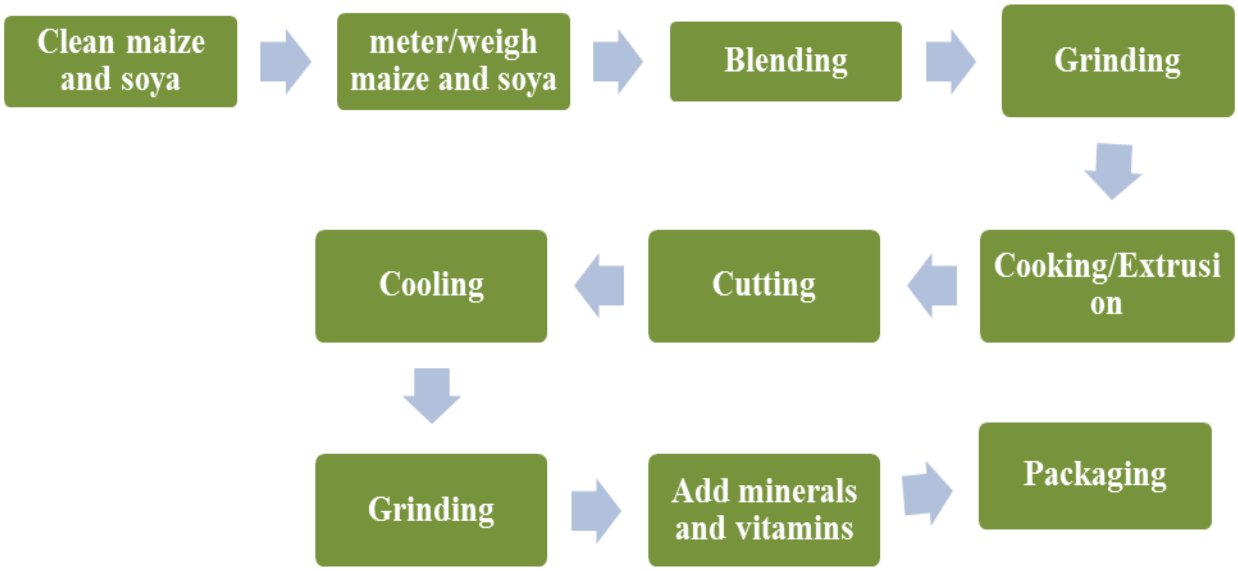


Figure:2.8- General Extrusion Cooking process flow diagram

In the extrusion process, materials to be expanded enter the feeding bin and are fed to the conditioner through the feeder. After conditioned by steam in the conditioner, materials enter the extruding chamber. Materials with certain moisture enter the extruder and flow axially by pushing of the screw. Due to the mechanical friction between the screw and materials, the machine cylinder and materials as well as the internal friction in materials, the materials are extensively extruded, stirred and cut, so that they are further pulverized and homogenized. Friction is the force resisting the movement of one particle sliding against another. When this force is overcome, heat is generated. The extruder causes friction as particles of grain rub against each other. The heat generated from this friction cooks the dough. The extruder uses steam locks, wear sleeves, and the die holes to create points of restriction which produce high levels of friction.

Since the temperature rises along with the increasing pressure, the properties of materials change under conditions of high temperature, high pressure and high shear force, that is, powdery materials become pasty materials, starch is gelatinized, protein is modified, fiber is partially degraded and fined, pathogenic bacteria are killed, hygienic indexes are improved and toxic substances are inactivated. Under the action of the great pressure difference, the pasty materials just sprayed out of the die holes are expanded, dehydrated and cooled. The expanded product is crisp with loose structure, multi-hole, good palatability and flavor. Temperature probes are essential for producing CSB to ensure the CSB is properly cooked (LaGrone, 2012).

2.3 Food Fortification

As defined by the World Health Organization and Food and Agricultural Organization of the United Nations, fortification refers to "the practice of deliberately increasing the content of an essential micronutrient; vitamins and minerals (including trace elements) in a food irrespective of whether the nutrients were originally in the food before processing or not, so as to improve the nutritional quality of the food supply and to provide a public health benefit " (Shubhangi and Dipanshu, 2014). It is also used to correct or prevent widespread nutrient intake shortfalls and associated deficiencies, to balance the total nutrient profile of a diet, to restore nutrients lost in processing, or to appeal to consumers looking to supplement their diet.

Nutritional deficiencies are common worldwide. Because of the increasing poverty and global food insecurity, in fact, the incidences of micronutrient deficiencies continue to increase so, food fortification programs to alleviate micronutrient deficiencies is a need (Wimalawansa, 2013). Because the need for and the effectiveness of fortification varies by age, sex, life stage, and genetic profile, groups that are at high risk of inadequacy and/or excess deserve special attention in all countries (Dwyer, *et al*., 2015). Challenges of fortification of food are choosing appropriate fortification vehicles, reaching target populations, avoiding overconsumption in non-target groups, and monitoring nutritional status, are relevant to all countries because they occur everywhere. There is an attempt to fortify foods to optimize intakes and nutritional status (Dwyer, *et al*., 2015).

Hidden hunger is one of the most common health problems worldwide, with vitamin and mineral deficiencies alone accounting for about 10% of the global health burden. It is a global health challenge affecting both developed and developing countries. At this time more than 2 billion people suffer worldwide from micronutrient deficiencies involving all age groups and leading to serious consequences, even death. Micronutrient malnutrition has profound implications for well-being, economic and social development, and human productivity, particularly in terms of public health costs and loss of productivity caused by human illnesses and absenteeism (Wimalawansa, 2013).

Food fortification is the most efficient and viable solution, since it is available to the poor, pregnant women, young children and the population in general, whose needs could never be fully met by the social services; it is also available to the elderly, the sick and other groups that somehow do not maintain a balanced diet (Liberato, *et al.,* 2006). According to (Shubhangi and Dipanshu, 2014) there are four main methods of food fortification or procedure that is used in order to fortify the food these are 1) Bio-fortification (i.e. breeding crops to increase their

nutritional value, which can include both conventional selective breeding, and modern genetic modification), 2) Synthetic biology (i.e. addition of pro-biotic bacteria to foods), 3) Commercial and industrial fortification (i.e. flour, rice, oils (common cooking foods) and 4) Home fortification (e.g. vitamin D drops)

A fortified food should: (a) Be commonly consumed by the target population; (b) Have a constant consumption pattern with a low risk of excess consumption; (c) Have good stability during storage; (d) Be relatively low in cost; (e) Be centrally processed with minimal stratification of the fortificant; (f) Have no interaction between the fortificant and the carrier food; (g) Be contained in most meals with availability unrelated to socio-economic status.

Selection of an appropriate vehicle is a critical step in successful fortification. In many cases identification of suitable vehicles is made difficult by the absence of reliable information on dietary habits of the target population (Food Fortification Technology, 2013). Addition of vitamins to foods must be done without health risks to any consumer group (Dwyer, *et al*., 2015).

2.4 Fortification of SC-CSB

SC-CSB is a fortified blended food (FBF) used in food aid programs and made from heat treated maize and soy beans added vitamins and minerals after the heat treatment (Ashagrie and Paulos, 2017).

Vitamins and minerals are essential for growth and metabolism. The World Health Organization estimates that more than 2 billion people are deficient in key vitamins and minerals. Groups most vulnerable to these micronutrient deficiencies are pregnant and lactating women and young children, given their increased demands. Food fortification is one of the strategies that has been used safely and effectively to prevent vitamin and mineral deficiencies (Das, *et al.*, 2013).

Vitamins are defined as organic substance required in a small amount for the maintenance and growth of living organisms. Their deficiency may lead to certain specific diseases or symptoms which can be cured by the administration of that specific vitamin only (Panchumarthy, *et al*., 2015). Vitamin A is retinol, it is also known as a xerophthol as it is used in the treatment of Xerophthalmia which means drying and thickening of the conjunctiva (Panchumarthy, *et al*., 2015). Vitamin A is a dietary compound, soluble in fat, essential for vision, growth, reproduction, cell proliferation and differentiation and integrity of the immune system (Liberato, *et al.*, 2006). It is also an organic compound required as a nutrient in tiny amounts for

the healthy maintenance and growth of a living organism; they are organic substances essential for the diet in small amounts that are involved in fundamental functions of the body (Panchumarthy, *et al.,* 2015).

Vitamin A deficiency is common in developing countries and is responsible for 1 to 2 million deaths and half a million new cases of blindness every year (Sommer, 2001). The stability of *vitamin A* in fortified cereal and cereal products is excellent. Fortification of cereal flours with vitamin A is technically feasible, and nutrient stability in the products is good. (Dary*, et al.*, (2002).

One of the most common and recommended vehicles for vitamin A fortification is SC- CSB flour (Wimalawansa, 2013). Its selection as a vehicle of choice should be guided by estimates of intakes of vitamin A and wheat flour by intended beneficiaries, levels of fortificant required to meet dietary corrective and safety goals, stability under ambient conditions, stability under usual conditions of product preparation (e.g., high temperature and humidity during cooking or baking) and product storage conditions, and comparative costs. The form of vitamin A and premix to be used in fortification should be the highest grade, appropriate for the intended food vehicle, stable under ambient conditions and for the duration of expected use, and introduced into the food supply in accordance with industry standards. In general, the provision of 15% to 50% of the RDA can be expected to meet both nutritional and safety goals (Klemm*, et al.*, 2010).

If food with a low quantity of required defined daily dose of vitamin A is consumed VAD (vitamin A deficiency) leading to liver disease, malabsorption due to the body fails to absorbs nutrients from food in small intestine causing celiac diseases, chronic liver, chronic pancreatitis, crones diseases etc., decreased mucus production, decreased immunity. Bacterial invasion of the eye, conjunctival xerosis, Bigot's spots (white triangular plaques on conjunctiva), night blindness (nyctalopia), follicular hyperkeratosis, poor growth, skin disorders (Panchumarthy, *et al*., 2015).

Based on this distinction, vitamin A fortificants can be divided into two categories: Oily forms that can be incorporated directly into fat-based foods or emulsified into water-based ones (e.g. milk) and Dry forms that can be dry mixed into foods or dispersed in water, depending on whether they are cold water dispersible or non-cold water dispersible (Alavi*, et al.,* 2008) (Kyritsi, *et al.,* 2011).

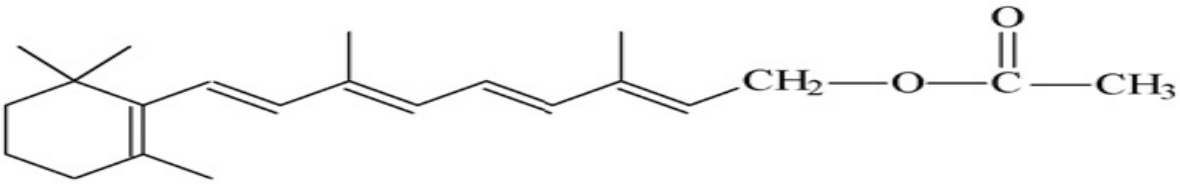


Figure: 2.9 Chemical structure of Vitamin A (Retinol)

The following recommendations are offered to guide fortification of Corn soya blend flour or other potential food vehicles with vitamin A: Vitamin A fortification should be motivated and guided by evidence of deficiency as a public health problem. This evidence should be derived from population-based findings of deficient vitamin A status and dietary inadequacy of the vitamin or its food sources. Vitamin A deficiency is a public health concern in preschool-aged children, women of reproductive age, and school-aged children and young adolescents. Fortification of food with vitamin A should be designed to correct estimated dietary inadequacy in one or more vulnerable groups, that is, to fill a dietary gap.

2.5 Criteria for Selection of Food Vehicles and Premix for Vitamin Fortification

The success of a fortification program depends, in part, on the selection of the right food vehicle. This requires knowledge of food intake patterns that show that is eating what foods among the groups at greatest risk of deficiency. Selection of an appropriate vehicle is a critical step in successful fortification. In many cases identification of suitable vehicles is made difficult by the absence of reliable information on dietary habits of the target population (Food Fortification Technology). The selection of a vehicle should be based on several key criteria: (Mokhtar, *et al.,* 2001). It must be centrally processed so that quality control can be effectively implemented, It must be consumed regularly and in predictable amounts, and be affordable by the target population, the stability and bioavailability of the micronutrients added to the food must remain high under standard local conditions of storage and use, the fortified food should not undergo changes in color, taste, or appearance as a result of adding the micronutrients, the addition of micronutrients should be economically feasible through an industrial process, the added nutrient should supply optimal amounts without a risk of excessive intake or toxic effects

A fortified food should: (a) Be commonly consumed by the target population; (b) Have a constant consumption pattern with a low risk of excess consumption; (c) Have good stability during storage; (d) Be relatively low in cost; (e) Be centrally processed with minimal stratification of the fortificant; (f) Have no interaction between the fortificant and the carrier food; (g) Be contained in most meals with availability unrelated to socio-economic status.

Selection of an appropriate vehicle is a critical step in successful fortification. In many cases identification of suitable vehicles is made difficult by the absence of reliable information on dietary habits of the target population (Food Fortification Technology, 2013).

2.6 SC- CSB Quality

Several quality authorities have defined quality in various ways considering different attributes of a product. Some definitions are: Juran: "Fitness for purpose" or" Quality is customer satisfaction.", Deming: "Quality should be aimed at the needs of customer, present and future." Crosby: "Conformance to requirement" Feigenbaum: "Total composite product and service characteristics of marketing, engineering, manufacture and maintenance through which the product and service in use will meet the expectations of the customer." Taguchi: "The loss impart to the society from the time a product is shipped." ISO 9000:2000: "The degree to which a set of inherent characteristics fulfills requirements." Others define quality to be the consistency in meeting the user's requirements. When the expected sensory attributes are observed persistently, the food item is considered to be of good quality. And when these attributes are missing or non-uniformly/non-consistently exist in a food item, it is judged to be of low quality. (D. Sarkar, 2000).

The quality of a food item can also be evaluated based on its ability to fit the intended purpose, provided that it is supplied at the right time and price. People buy items simply because they have use for them. And manufacturers produce these items just because of their demands. Quality of a commodity is a composition of attributes that determine its acceptability to the customers (Wari & Zhu, 2004).

Millions of children in Africa depend on CSB for nourishment. Energy, protein and nutrients provided by CSB are crucial for health, growth and development. Taste, odor or texture problems may cause children to reject CSB and miss a chance to meet their daily nutritional needs. Producing high quality CSB ensures children will enjoy eating their porridge and obtain the energy and nutrients they need (CSB in Ethiopia, 2015).

Figure: 2.10- Packaged SC- CSB flour

Source:- **(**World food Program: Food Fortification in Malawi, 2008 **).**

The use of soybean products in the feed and food industry has increased steadily. The world soybean production is currently 219.8 million metric tons out of which India produced 9.3 million metric tons constituting about 4% of the total world production. Out of this production, less than 10% is directly used for human consumption (Gandhi, 2006). The dominant position of soybeans and their products is primarily associated with their high nutritional quality especially with respect to protein and amino acids. While basic standards specifications for soybeans/soy meals have been established (NOPA, 1997), no official specifications exist for other soy products that are used nowadays.

Furthermore, NOPA specifications only refer to four chemical constituents. Current evaluations of soy products are based on a much larger array of tests allowing a more accurate evaluation of the nutritive value of the different products. Developments in the technological modifications of soybean products, along with a better understanding of the effects on performance and health of relatively unknown compounds, such as iso-flavones, will add value to soy products. Accurate analysis of these new compounds will be of greater importance. Hence the quality analysis of soy products is needed at all stages of the protein supply chain in the food and feed industry and quantified with the maximum and minimum limits of each desirable component (Gandhi, 2009).

Quality characteristics can be classified into four general categories: defects, shipment and storage factors and end-use related factors. End use quality factors are classified as either physical properties or chemical composition characteristics. The physical properties include germination, hilum color, seed count, seed size, hardness, seed coat cracking, and purity. Chemical composition includes moisture, protein, Nitrogen Solubility Index (NSI), 7S/11S

proteins, Protein Dispersibility Index(PDI), amino acids, lipoxygenase, Trypsin Inhibitor(TI), oil, fatty acids, fiber, sugars and iso-flavones. The level, plus presence or absence of these characteristics is generally referred to as Quality. High quality soybeans have desirable levels of certain characteristics or combination of characteristics. The physical and chemical characteristics are usually measurable by some means (AACC, 2004; AOAC, 2004; AOCS, 2004, AOSA, 2003). Other practices such as organic production practices are very difficult, if not impossible, to measure in the soybeans themselves and require a system of traceability or verification. Most of the countries are adopting the National Oilseed Processor Association (NOPA) specifications for their domestic soybeans (Gandhi, 2009).

2.7 CSB Safety

Food safety is an important attribute. The safety of flour-containing foods has been compromised over the years, not only by pathogenic bacteria, but also by fungal contamination, especially those mycotoxin-producing molds (M. Weidenbörner, *et al*., 2000).

Even though CSB is accepted for its nutritional value, there is high risk of contamination of CSB with hazardous toxins such as Aflatoxins and microorganisms mainly due to reception of unsafe raw materials (corn and soy-bean), poor handling and storage of the raw materials and during processing of the intermediate and packaging of the finished product at the CSB processing factories (Hell,2008).

Microorganisms and mycotoxins are naturally associated with grains and keeping them out from the food chain is the best approach. Several factors influence the level of contamination and/or mycotoxin in the final grain based product like ingredient storage and processing choices and parameters (John, 2010).

Most food borne illnesses are largely attributed to microbiological contamination. Not surprisingly, there has been a sharp increase in national public interest in microbial and chemical food safety recently. Mycotoxins have been implicated as causative agents of adverse health effects in humans and animals that have consumed fungus-infected agricultural products.

2.7.1 Aflatoxin

Aflatoxins are toxic metabolites produced by a variety of moulds such as *Aspergillus flavus* and *Aspergillus parasiticus*. They are carcinogenic and can be present in grains, nuts, cottonseeds and other commodities associated with human food or animal feeds. Since cereals are one of the most important sources of human nutrition. Baby food supplements mostly consist of cereals

and if these products are improperly prepared, they may get contaminated with various aflatoxin types like B_1, B_2, G_1, and G_2.

Aflatoxin B_1 is the most toxic and frequently detected form. The other types present a significant danger at a high concentration. Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Rey's syndrome and chronic hepatitis. Humans are exposed to aflatoxins by the consumption of foods that are contaminated with aflatoxin producing fungal strains during growth, harvest or storage (Enyisi, *et al.,* 2015).

The occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during pre-harvesting, storage and/or processing periods. Since crops are not yet grown under sterile conditions, the probability of mold infection and mycotoxin contamination is ever present and the carcinogenic effect of aflatoxin is well recognized. One of the most vulnerable parts of the population is children, due to, their physiology, lack of food diversity, and a higher consumption relative to their body. Therefore, the significance and potential health risk of any contaminant in foods consumed by infants are increased and special attention must be paid to this problem (Kalantari and Kalantari, 2007).

The safety and integrity of the food supply are of paramount importance and are among the drivers of safe grain storage. Bacterial contamination of stored grains may be a significant consideration for users where the grain will not undergo further processing that includes a microbiocidal step, but problems caused by moulds and mycotoxins are more usually associated with grain storage. With an increased knowledge of the origins of mycotoxins, we now understand that at least some mycotoxins in some commodities are formed either before harvest, or immediately after harvest. Mycotoxins can be formed during storage, but only if there is sufficient moisture. Fungal growth usually develops as a complex succession of species, beginning with the most drought-tolerant xerophilic species. The metabolic activity of these pioneer species raises the moisture content of the grain, which may allow growth of mycotoxigenic species and, ultimately, the formation of mycotoxins (Berghofer, *et al.,* 2003).

Ultimately, most stored grain is destined to end up as either human or animal food. Food safety and quality are driving factors in the long-term storage of grain. In the food safety area, bacterial pathogens and mycotoxins must be considered. Currently, quality issues include spoilage due to mould growth, insect and mite infestation, and general hygiene issues such as protection from rodents and birds (Wright, 2003).

Some grains and oilseeds may be contaminated with aflatoxins before, or just after, harvest. Corn plants may be colonized during the growth cycle by strains of *Aspergillus flavus* or *Aspergillus parasiticus*, both of which are capable of producing aflatoxins (Klich, 2007). Under dry land conditions, if the corn plants experience stress (e.g. drought stress) close to harvest time, then aflatoxin may be formed in the maturing corns. Aflatoxin levels may also increase immediately after harvest during field drying, particularly if in-field drying period is extended by rain. Aflatoxins may affect many other commodities during storage, as *A. flavus* and related species grow well at reduced a_w (down to about 0.80), and aflatoxins may be produced down to approximately 0.85 a_w (Gock, *et al.,* 2003).

Table 2.3 Physical Properties of Aflatoxins

Aflatoxin	**Molecular Weight**	**Molecular Formula**	**Melting Point (M.P)**
B1	312	C17H12O6	268-269
B2	314	C17H14O6	286-289
G1	328	C17H12O7	244-246
G2	330	C17H14O7	237-240
M1	328	C17H12O7	299
M2	330	C17H14O7	293

Source: Hartley, *et al.,* 1963, Nesbitt *et al.,* 1962, Sargeant, *et al.,* 1961

2.7.1.1 Chemical Structure of Aflatoxins

Aflatoxins structure consists of a bifuran ring fused to a coumarin nucleus with a pentenone ring (in B and M aflatoxins) or a six membered lactone ring in G aflatoxins. The four compounds (B1, B2, G1, and G2) are separated by the color of their fluorescence under long wave ultraviolet illumination (B=blue, G= green). Two other aflatoxins M1 and M2 were isolated from urine and milk and identified as mammalian metabolites of AFB1 and AFB2 (Patterson, *et al.,* 1978).

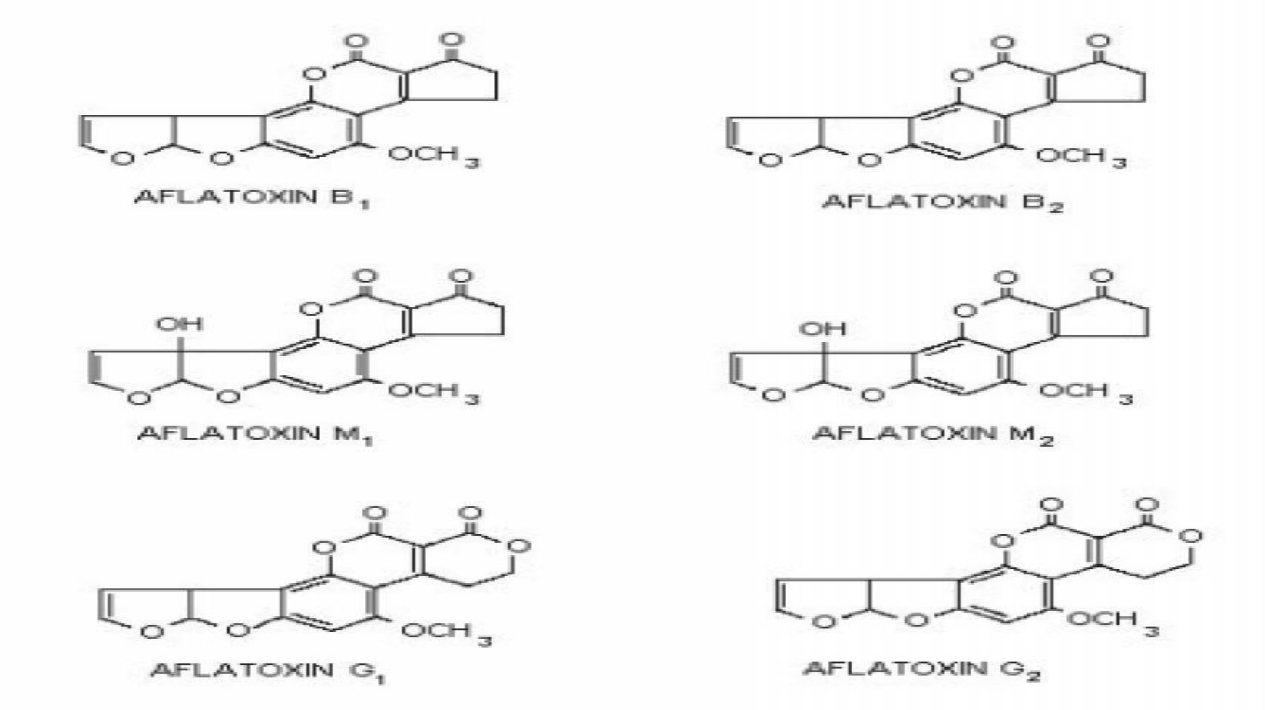


Figure: 2.11- Chemical Structure of Aflatoxins Source: Zain, 2011

2.7.1.2 Mycotoxin Contamination before Storage

The presence of mycotoxins in grain is traditionally regarded as an indicator of poor storage conditions. The corollary to this is that mouldy grain contained mycotoxins. Neither statement is necessarily true. Conversely, not all moulds those grow in stored commodities produce mycotoxins. Some of the fungi associated with grain in the field (often referred to as 'field fungi') can form mycotoxins, either immediately before, or just after harvest. *Alternaria, Fusarium, Aspergillus,* and *Penicillium* can all act as prior postharvest pathogens of grain, and may form mycotoxins. *Alternaria* and *Fusarium* do not compete strongly at reduced a_w, unlikely to form mycotoxins once the grain is dry, or during storage. Conversely, *Aspergillus* and *Penicillium* are more often considered as 'storage fungi'. They are known to form mycotoxins in stored grains, and are usually not regarded as fungi that can produce mycotoxins before harvest (Gock, *et al.*, 2003).

2.7.1.3 Mold Growth and Quality Issues

Mould growth in grains may cause deleterious changes in addition to the formation of mycotoxins. Many spoilage fungi cause loss of germination in seed grains, discolouration and darkening of the grains, reduction in protein content, musty odours, and changes in fatty acid profiles and other constituents of the grains. Mould development may also encourage mite and insect infestation (Wicklow, 1995).

Mould development during storage can be controlled or prevented by ensuring that grain is adequately dry at intake. Further protection can be provided by preventing the development of temperature and moisture gradients by cooling and/or aeration of the grain. Protection from insect infestation will also help prevent mould development in stored grains, both in bulk or bag storages.

2.7.2 Bacterial Contamination

The bacterial species that occur commonly on grain are generally non-pathogenic, though contamination with bacterial pathogens such as *Salmonella, Escherichia coli,* and *Bacillus cereus* can occur. *Salmonella* and *E. coli* are enteric bacteria, and their presence on grain is usually an indication that it birds or rodents have contaminated it. This may occur during harvesting, but more often is a result of poor hygiene in road or rail trucks during transportation, or poor pest control during storage (Wicklow, 1995).

Levels of contamination with enteric pathogens are usually very low. Most grain destined for human food is first milled into flour or other grain products such as semolina, wheat germ, and bran. The milling process may contribute to the microbiological load of the flour, but flour then usually undergoes further processing, such as baking, that will kill most bacteria. Conditioning wheat to increase the moisture content to a level suitable for milling can also increase the counts of bacteria, yeasts and moulds also the microbial contaminants are concentrated in the outer grain layers (bran and wheat germ). These are removed during milling, leaving the end product, flour, relatively clean and usually pathogen-free. Enteric pathogens such as *Salmonella* are rarely isolated from straight run flour, although *B. cereus* may be present in low numbers (Berghofer, *et al.,* 2003).

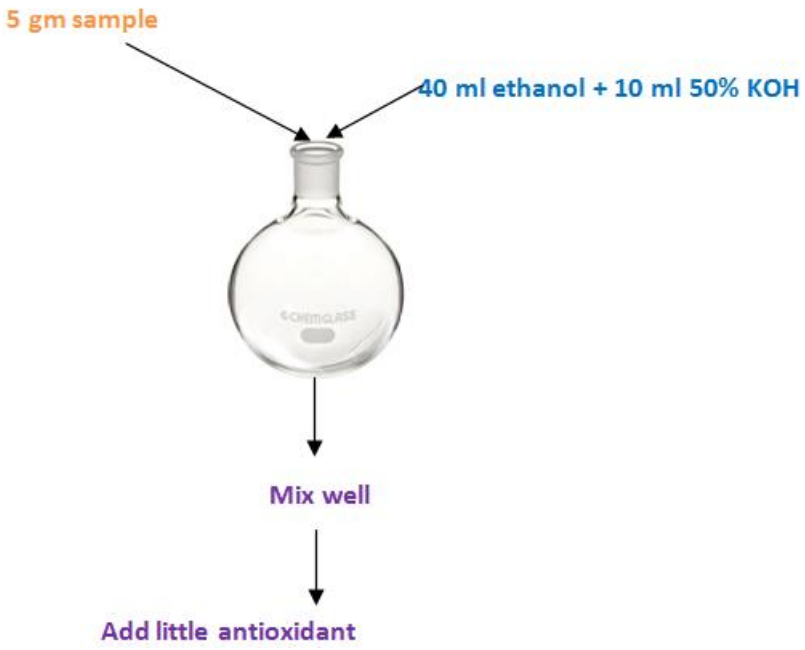
2.8 Protocol for Sample extraction and Analysis of Vitamin A with HPLC

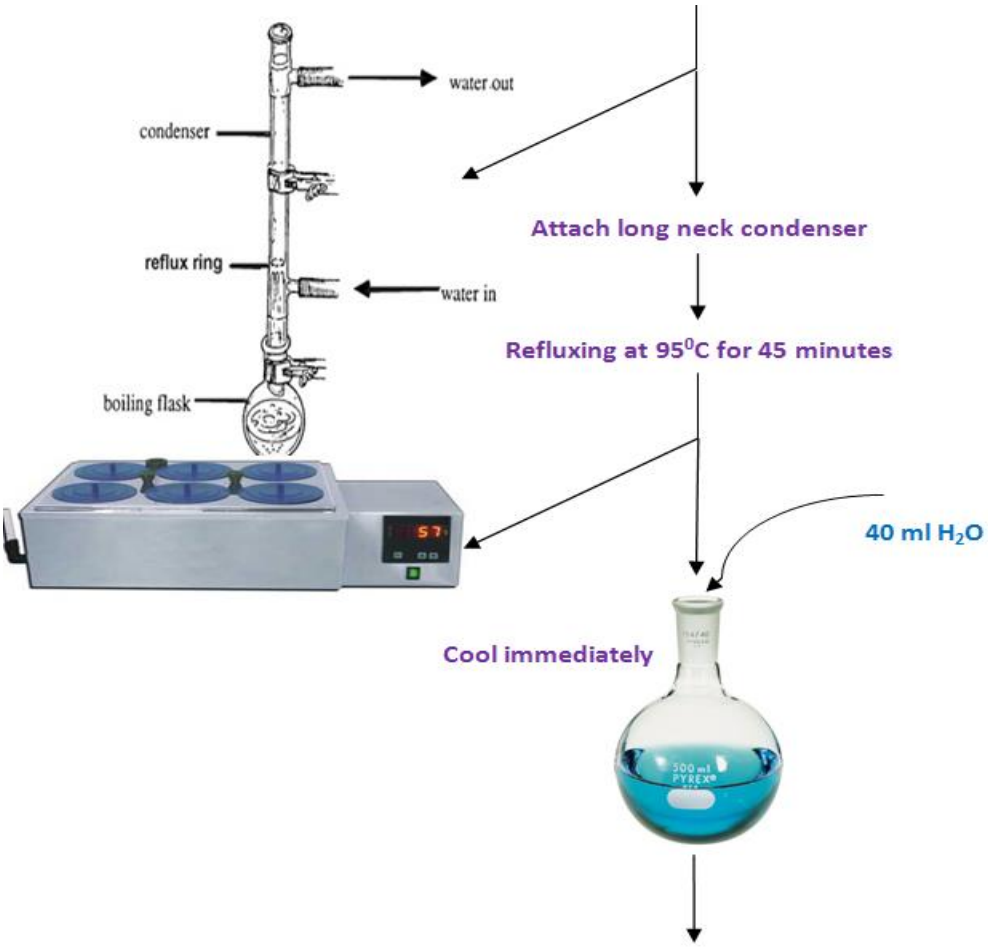
Vitamin A in flour can be analyzed by using High Performance liquid Chromatography with DAD detector. The details way of Vitamin A analysis in SC-CSB is stated below. Weigh 5gm of the sample in a round bottom flask, Add 40 ml ethanol and 10 ml 50% KOH. Mix well and add 2 gm of pyrogallol (as an antioxidant). Attach the round bottom flask to a reflux condenser. Reflux at 95ºC for 45 minutes (mix and agitate with every 10 minutes). Cool immediately using cold water. Add 40 ml deionized water. Transfer to a separatory funnel. Add 70 ml petroleum ether to the flask and rinse and then transfer to separatory funnel. Add 20 ml ethanol to the flask, rinse and transfer to the separatory funnel. Extract by shaking for 5minutes (by letting the

air out from time to time). Collect the lower layer in to the previous round bottom flask and the upper layer (ether layer) in to another 2nd separatory funnel.

Transfer the solution from the round bottom flask to the 1st separatory funnel. Add 70ml n-hexane and rinse the round bottom flask then transfer to the 1st separatory funnel. Extract by shaking for 5 minutes (by letting the air out from time to time). Collect the lower layer in to the previous round bottom flask and the upper layer (n-hexane layer) in to the 2nd separatory funnel. Transfer the solution from the round bottom flask to the 1st separatory funnel. Add 30 ml petroleum ether and 30 ml n-hexane, rinse the round bottom flask then transfer to the 1st separatory funnel. Extract by shaking for 5min (by letting the air out from time to time). Discard the lower layer and collect the upper layer (n-hexane layer) in to the 2nd separatory funnel.

Wash the collected organic layers with deionized water around five times. Check the washings are free from alkaline by taking some amount of water layer and add a drop of phenolphthalein solution. If the washings are free from alkaline discard the water (lower) layer. Filter the organic (upper) layer through a filter paper in a 2nd round bottom flask. Evaporate the collected organic layer using a Rota-vapor at 40ºC until it become around 5ml. Evaporate the rest and dry with nitrogen. Reconstitute with 10ml methanol and sonicate. Filter through 0.45 µm syringe filter in to 2 ml vial. Vitamin A (Retinol) in supper cereal – corn soya blend will be determined using High Performance Liquid Chromatography with DAD Detector (HPLC/DAD) (Katsa, *et al.*, 2016), (Landen, et al., 2016), (Devries and Silvera, 2002), (AOAC 992.06[19th] edition).





First Extraction

Petroleum ether 70 ml

and + 20 ml ethanol

Extract for 5 minutes

Collect ether layer

Second Extraction

Add 70 ml n-hexane

Extract for 5 minutes

Collect n-hexane layer

Third Extraction

Add 30 ml n-hexane and

30 ml petroleum ether

Extract for 5 minutes

Collect n-hexane layer

Washing the organic layer 5 times with de-ionized water

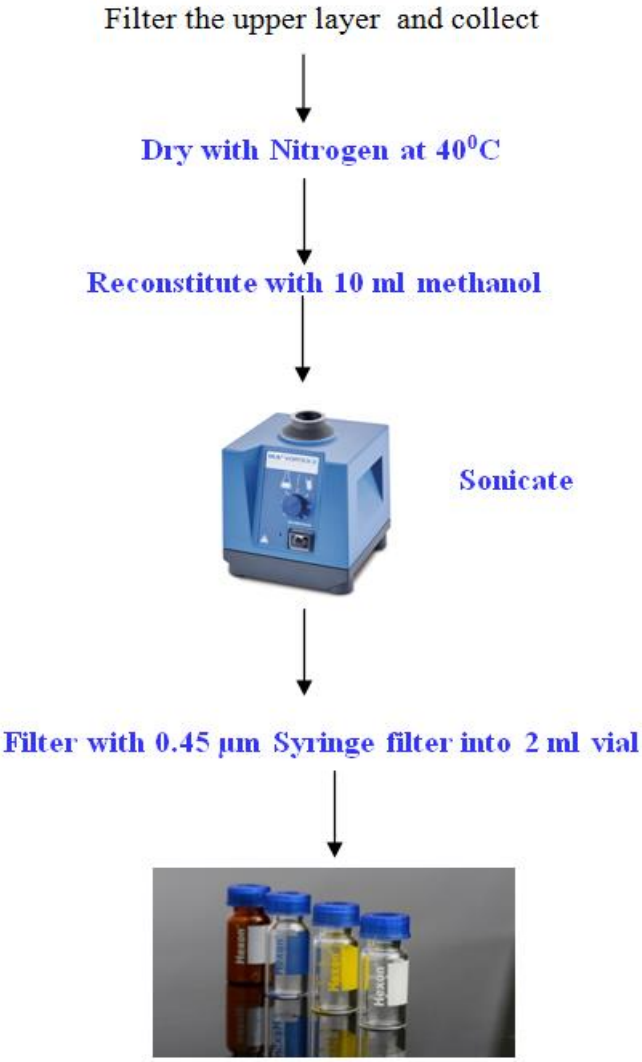


Figure 2.12:- Systematic Representation of the Vitamin A analysis procedures

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study Area

The study has been conducted in Addis Ababa, the capital city of Ethiopia and the experiments were carried out from February 2017 to June 2018 in Addis Ababa. Proximate and Aflatoxin was carried out at Addis Ababa University Center for Food Science and Nutrition, Minerals analysis at Horticop Laboratory and Vitamin A and Microbiological analysis was carried out at Ethiopian Conformity Assessment Enterprise (ECAE).

3.2 Sampling CSB Factories and Sample Collection

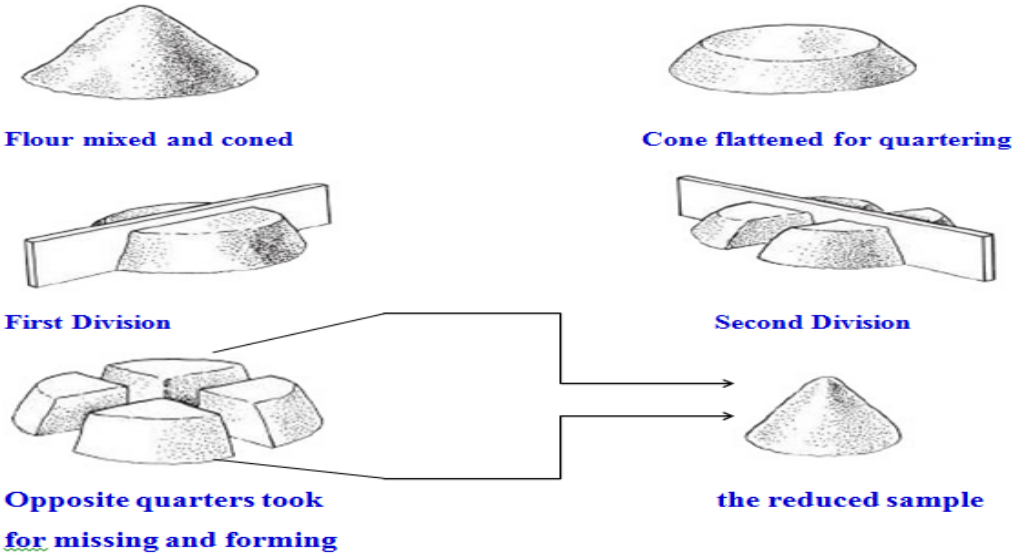
Sampling CSB Factories: From seven factories in Ethiopia, the researcher purposely selected four factories based on their cooking processing methods, two from roasting processing randomly coded R1 and R2 and two from extrusion processing randomly coded E1 and E2 Company.

Sample Collection. A total of 4 SC-CSB flour samples obtained from four sites in Addis Ababa. A 10 kg portion of each sample was immediately transported to the laboratory in chilled containers at 4-6 °C and subsequently analysed in terms of physico-chemical and microbial parameters. All chemical were analytical grade and media were obtained from Oxoide (Basingstoke, UK).

For the sake of keeping the data privacy of the companies under the study, the researcher advisor again re-coded the samples to blind the researcher himself as a result neither of them was knew which sample was from which company. Finally after the analysis was completed, the advisor returned back the code to the two treatments for comparison. This was proves that the companies' information will never be disclosed to any one or group of audience, as the target of the research is only about the problem rather than companies individual performance. By this the researcher would like to ensure that the research meets the highest degree of ethical considerations regarding company data privacy.

Sampling Technique: Before the sampling, the sampling material was sterilized and the researcher always used gloves, mouth mask and hairnet to avoid cross contamination. The sample was collected from the stores. Then, the sample was drawn from the selected filled and closed bag utilizing a sterilized stainless steel single-tube open-ended trier. The sample was divided into two equal sub-samples; one was for research analysis and the other retained as a

reserve. The weight of the incremental sample taken from a lot was 100 grams and weight from each target companies, so the aggregate sample taken was 10 kg.



The following formula was used as a guide for the sampling of lots traded in individual packs, such as sacks, bags, retail packings.

$$\text{Sampling frequency (SF) } n = \frac{\text{Weight of the lot} \times \text{Weight of the incremental sample}}{\text{Weight of the aggregate sample} \times \text{Weight of individual packing}}$$

Where: weight: in kg, sampling frequency (SF): every n^{th} sack or bag from which an incremental sample must be taken (decimal figures should be rounded to the nearest whole number).

Table: 3.1- Number of incremental samples to be taken depending on the weight of the lot CSB. (Official Journal of the European Union, 23 February 2006)

S/N	Lot weight (tons)	No. of incremental samples	Aggregate sample weight (kg)
1	≤ 0.05	3	1
2	> 0.05-≤ 0.5	5	1
3	> 0.5-≤1	10	1
4	> 1-≤3	20	2
5	> 3-≤ 10	40	4
6	> 10-≤ 20	60	6
7	> 20-≤ 50	100	10

The samples collected in the black plastic bag by taking preventive measures to avoid environmental factors, and transported to Addis Ababa University Center for Food Science and Nutrition, Food toxicology Laboratory and stored in cool dry and dark place until the analysis. The CSB was completely homogenized in dark room by using a cone shape method.

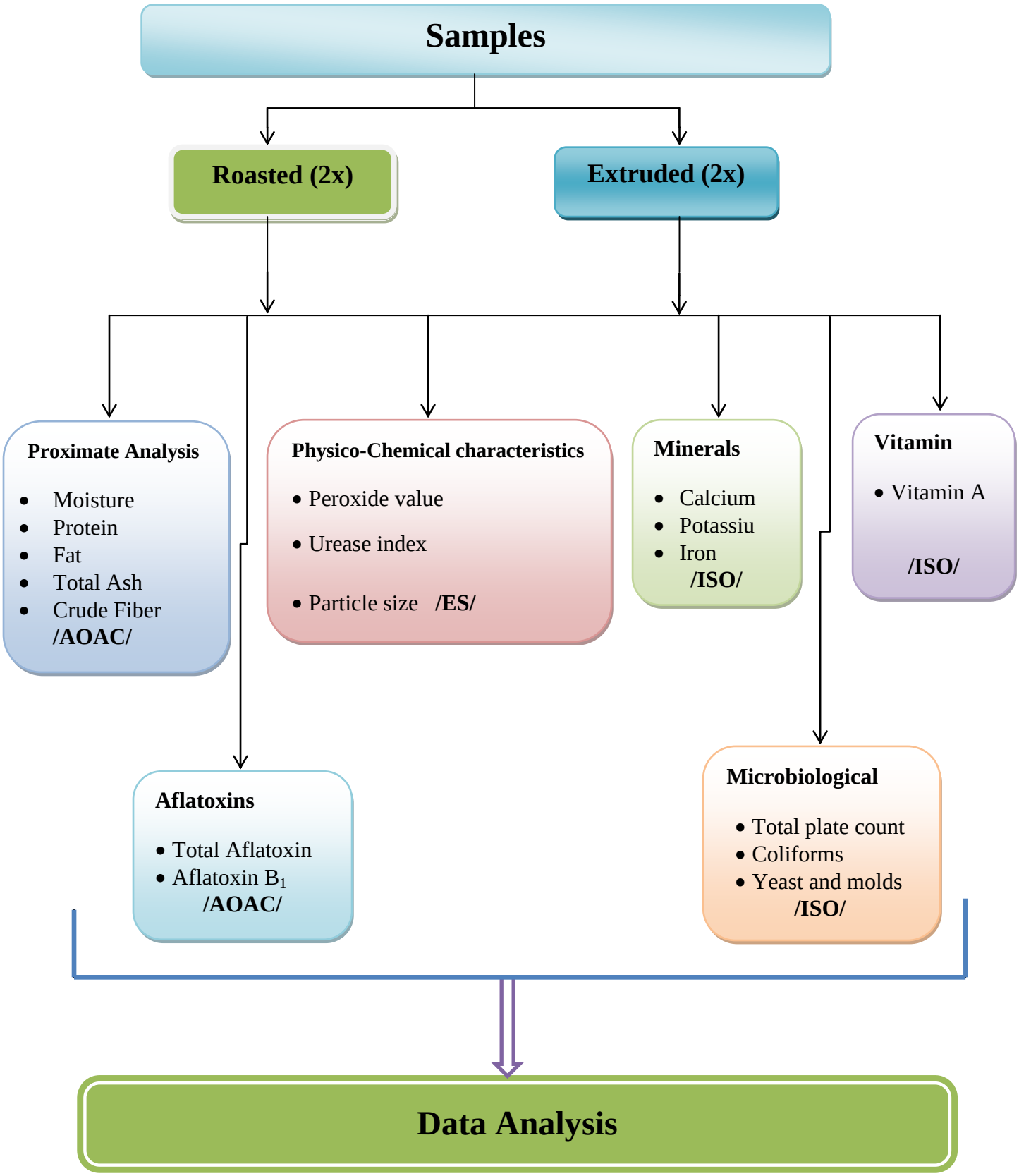


Figure: 3.1- Experimental framework

3.3 Proximate Composition of SC-CSB

3.4.1 Moisture content

Moisture content was determined by AOAC 2010 Method 925.10. The aluminum dishes used for the moisture determination were dried at 130 ^{o}C for 1hr using drying oven. The dishes were removed and kept in a desiccator for about 30 minutes. The mass of empty dishes were measured as M_1. About 5 gm of the sample was weighed using analytical balance in to the dish and record as M_2. The sample was mixed thoroughly and dried at 100 ^{o}C for 6 hrs. And it was taken and kept in a desiccator to cool. After cooling the weight was taking as M_3. Then keep in oven for another 15 minutes. Then it was removed and allowed cooling in a desiccator and again the weight was taken. Then, the moisture content has been calculated based on the mass difference.

3.4.2 Crude Fat content

Crude fat content was determined by AOAC 2000 method. The flasks used for the extraction was washed and then dried in a drying oven at 92 ^{o}C for 1hr and cooled in a desiccator. The masses of the cooled flasks were measured by analytical balance and record as **W_1.** About 5 gm of the powder sample was weighed in to each thimble line with cotton at their bottom. The thimbles with its sample content were placed in to the Soxhlet extraction apparatus. Then 70 mL of diethyl ether was added in to each flask and the extraction process was done for about 4 hrs followed by removing this flask with its content from the Soxhlet, it was placed in drying oven at 92 ^{o}C for 1hr. The flasks with their contents were then placed in a desiccator for 30 minutes. The mass of each flask together with its fat contents was measured as **W_2.** Then, the total fat content has been calculated based on the mass difference.

3.4.3 Total Ash content

Total ash content was determined by AOAC 2000 Method 923.03. Porcelain crucibles were cleaned and dried in a muffle furnace for 30 minutes at 550^{o}C. Cool the crucible in a desiccator for about 30 minutes and weighed **(M_1).** Weighed 2.0 gm of corn soya blend flour **(M_2).** Charred the sample on the hotplate until smoking was eliminated and placed the charred sample in a muffle furnace at 550 ^{o}C for 5 hours until the ash became cleaned and white in appearance. Finally, cooled the crucible with ash in a desiccator and weighed **(M_3).** Then, the total ash content has been calculated based on the mass difference.

3.4.4 Crude Protein

Crude Protein was determined by AOAC 2000 Method 979.09. About 0.5 gm of CSB powder sample was weighed on analytical balance and transferred to the tecator tube. Then 6 mL of concentrated sulphuric acid and mix immediately sample and acid carefully and 3.55 mL of 30 % H_2O_2 was added in to the digestion flask step by step. The tubes were shaked observing a violent reaction. After this violent reaction disappeared, 3 gm of the catalyst mixture (1:10 $CuSO_4$: K_2SO_4) was added to the digestion flask and stand for 5-15 minutes before digestion. The solution was then digested at 370°C for four hours and cooled by adding 50 ml of distilled water. After digestion was completed, 25 ml of 40 % sodium hydroxide was added to neutralize the acid and to make the solution slightly alkaline.

Then Borate ion was titrated with 0.1 N HCl until color changes to reddish color. Total nitrogen calculated as N x V x 14.01 x100 divided by the weight of sample taken. Then, the total nitrogen content directly converted to crude protein as total nitrogen times 6.25. Where: V = volume of hydrochloric acid consumed (ml), B = volume of hydrochloric acid consumed blank (ml), N = normality of the acid and 14 = eq. wt of nitrogen and W = Weight of the sample (mg).

3.4.5 Crude Fiber

Crude fiber was determined AOAC 2010 and ES ISO 5498 method. Determination of crude fiber has been conducted by using Fibertech^{TM} 2010 instrument. Grind the samples by using a suitable laboratory mill with a screen that was given a particle size < 1 mm. To simplify filtration 1000 + 2 mg of Celite 545 were added to the crucible before the sample. Weigh 1 g of the sample to an accuracy of + 2 mg into a pre-dried crucible **(W_1)**. If the fat content is above 10%, samples have to be defatted prior to analysis. Place crucible in the Fibertec cold extraction unit and fill crucible with 24 ml Acetone. Leave 5 minutes and filter. Repeat three times. Place crucible in the Fibertec Hot extraction Unit and 1550 ml of hot 0.640 N H_2SO_4 was added. 2-4 drops of n-Octanol to prevent foaming and heat to boiling. Adjust heater and let it boil for 10 minutes. Boiling time was measured from the time when the solution has reached the boiling point. It was washed three times with 30 ml of hot deionized water.

150 ml of 0.556 N KOH was added. 2-4 drops of n-Octanol to prevent foaming and heat to boiling was added. Adjust heater and let boil for 10 minutes. Boiling times was measured from the time when the solution has reached the boiling point and washed three times with hot deionized water. 30 ml portions of water was used and sucked as dry as possible between washings. Make sure that no residue remains on the inside wall of the column. Place crucible in the Fibertech cold extraction unit and filled the crucible with 25 ml acetone. The digested

solution was filtered and the above filtration was repeated three times. Evaporated the solvent and dried the crucibles at 130 ± 2 ^{o}C for 2 hrs or at 105 ± 2 ^{o}C for at least 5 hrs. Cooled to room temperature in a desiccator and weighed **(W_2).** Ash the sample in the crucibles at 525 ± 15 ^{o}C for at least 3 hrs. Cool to room temperature in a desiccator and weighed **(W_3).** % crude fiber = W2-W3/W1 x 100 %**.** Where W_1= Sample weight, W_2= Crucible + Residue and W_3= Crucible + ash residue.

3.4 Minerals (Fe, K and Ca) in CSB

Mineral analysis of Calcium, Iron and Potassium was determined by AOAC (2001) 968.08 using ICP-OES. A porcelain crucible was cleaned and dried in a muffle furnace for 30 minutes at 550 ^{o}C. The crucible was cooled in a desiccator for about 30 minutes**.** 2.0 gm of corn soya blend flour was weighed. The sample was charred on the hotplate until smoke was eliminated and placed in a muffle furnace at 550 ^{o}C for 5 hours. The dish was removed from the furnace and cooled. Ash should be white and free from Carbon. If ash contains Carbon particles (i.e., it is gray), wet with H_2O and add 0.5 to 3 ml HNO_3. Dry on hot plate or steam bath and return dish to 550 °C furnace 1 to 2 hrs. Finally, the crucible with ash was cooled in a desiccator.

The ash in 5 ml of 1M HNO_3 was dissolved, warming on steam bath or hot plate 2 to 3 minute to aid in the solution. The solution with medium size filter paper was filtered in to 50 ml volumetric flask and repeated with 2 additional portions of 1M HNO_3. Then diluted to 50 ml with 1M HNO_3.

Transferred the above prepared calibration standards and unknown sample to ICP-OES sample holder and put it in the auto-sample rack. C=A x (50 ml/B), Where, A = concentration (µg/ml) of element as determined by ICP-OES, B = volume or weight of test portion as ml or g; C = elemental concentration in test solution, µg/ml or µg/g, depending on value of B. **y = ax + b**, y= instrument response (Peak area), a= slope, x= calibration point, known concentration, b= y-intercept.

3.5 Vitamin A in CSB

3.6.1 Materials and Chemicals

Chemicals and Reagents

Deionized water, Methanol HPLC grade, Ethanol absolute, Potassium hydroxide, Pyrogallol, Petroleum ether, n-hexane, Nitrogen gas, Vitamin A standard (Retinol)

Equipments

Liquid chromatography with UV detector and integrator, Column: SB C18, 3 x 250mm, 5µm, Syringe, Syringe filter 0.45 µm, Sample Vial with cap, Separatory funnel 250ml capacity, Filter paper 541 por, Reflux condenser, Round bottom flask 250ml capacity, Cylinders 100ml, 10ml, Beakers

Chromatographic Condition

Detection – UV, 325nm, Column type – SB C18 3 x 250mm, 5µm, Mobile phase composition – 98:2, MeOH:H_2O, Flow rate – 1ml/min, Injection volume – 10µl, Run time – 5 min, Column Temperature: 25 ^oC.

3.6.2 Extraction

About 5 gm of sample was weighed in a round bottom flask, 40 ml ethanol and 10 ml 50 % KOH was added. Mix well and 2 gm of pyrogallol (as an antioxidant) was added. The round bottom flask to a reflux condenser was attached. Reflux at 95ºC for 45 minutes (mix and agitate with every 10 minutes). Cool immediately using cold water. 40 ml deionized water was added and transferred to a separatory funnel.

Added to the flask and rinsed with 70 ml petroleum ether then transfer to separatory funnel. 20 ml ethanol to the flask was added, rinsed and transferred to the separatory funnel. Extract by shaking for 5 minutes (by letting the air out from time to time). The lower layer was collected in to the previous round bottom flask and the upper layer (ether layer) in to another 2nd separatory funnel.

Transfer the solution from the round bottom flask to the 1st separatory funnel was transferred. About 70 ml n-hexane was added and rinsed the round bottom flask then transferred to the 1st separatory funnel. Extract by shaking for 5 minutes (by letting the air out from time to time). The lower layer was collected in to the previous round bottom flask and the upper layer (n-hexane layer) in to the 2nd separatory funnel.

The solution from the round bottom flask was transferred to the 1st separatory funnel. 30ml petroleum ether and 30 ml n-hexane was added, rinsed the round bottom flask then transfer to the 1st separatory funnel. Extract by shaking for 5 minutes (by letting the air out from time to time). Discarded the lower layer and collected the upper layer (n-hexane layer) in to the 2nd separatory funnel.

The collected organic layers were washed with deionized water around five times. The washings are free from alkaline was checked by taking some amount of water layer and added a drop of phenolphthalein solution. If the washings are free from alkaline discard the water (lower) layer. Filter the organic (upper) layer through a filter paper in a 2nd round bottom flask.

The collected organic layer was evaporated using a Rota-vapor at 40 ºC until it become around 5ml. Evaporated the rest and dried with nitrogen. Reconstitute with 10 ml methanol and sonicate. Filtered through 0.45 µm syringe filter in to a 2ml vial.

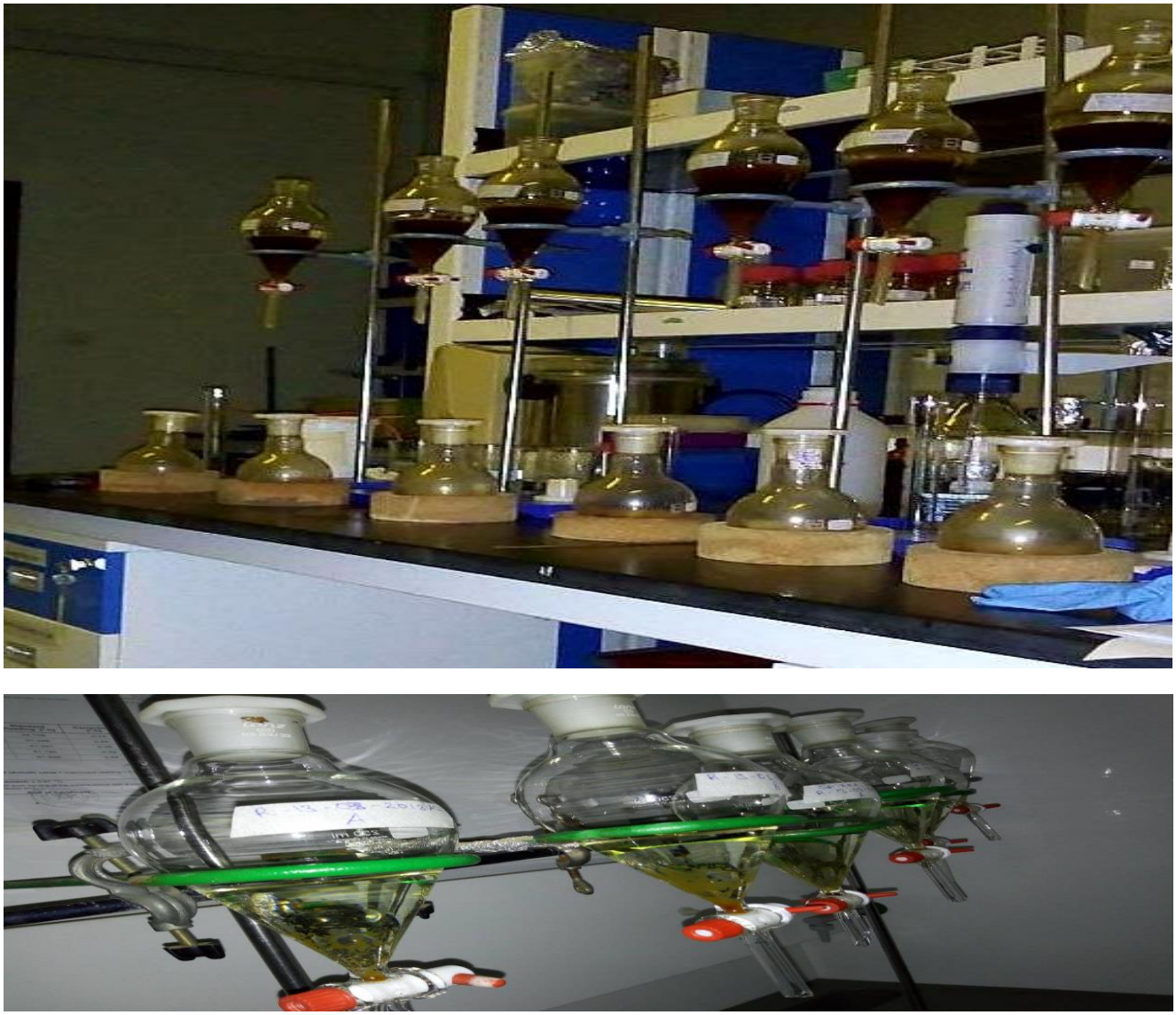


Figure: 3.2- Sample preparation and cleanup for Vitamin A (Retinol)

3.6.3 Analysis of Vitamin A using HPLC/DAD

Vitamin A (Retinol) in supper cereal – corn soya blend has been determined using High Performance Liquid Chromatography with DAD Detector (HPLC/DAD). The concentration of vitamin A has been calculated by comparing the corresponding peak area of the sample and those of the standard solutions, taking into consideration the dilution. There is a linear relationship between the concentration and the area of the vitamin A peak. Results were expressed in mg/kg and converted to IU/100g, to 1 decimal place. y= mx+b, Where, y= is the area of sample, m= is the slope found on the calibration graph, x = is the concentration of vitamin read on the instrument, b= is the y-intercept from the graph. X=y-b/m, concentration of vitamin A=x * dilution volume/test portion.

3.6.4 Vitamin A Method Validation

Linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) was determined to test the validity of the HPLC procedure used for Vitamin A. Identification of Vitamin A was determined by retention time of 2.5 ppm and 10 ppm Vitamin A standards injecting at the same condition and its precision determined by percent relative standard deviation (%RSD) less than 2% (FDA, 2000). Accuracy is examined by the determination of the recoveries of the Vitamin A. The recoveries were ascertained by the addition of 2 mg/kg and 10 mg/kg of Vitamin A (Retinol) to the clean samples.

Precision was established by performing multiple analysis of 10 ppm vitamin A standard and was expressed by the mean ± relative standard deviation (RSD). The limit of detection (LOD) defined as the concentration at which the signal to noise ratio equals 3. The limit of quantification (LOQ) defined as the concentration where the signal to noise ratio equals 10.

3.6 Physico-Chemical Characteristics of CSB

3.7.1 Urease Index

Weighed 0.200 gm (± 0.001 gm) of the sample in to a test tube and 10 ml of the buffered urea solution was added. Stopper, mix and place in water bath at 30^{o}C. The tube during the process of mixing was not invert. Note time (test) – 30 minutes. Prepared a blank by weighing 0.200 g (± 0.001 gm) sample in to a test tube and 10 ml of the phosphate buffer solution was added. Stopper, mix and place a water bath at 30 ^{o}C. Note (time) blank allow a 5 – 6 minutes. interval between the preparation of the test and the blank.

In both test and blank runs, mix contents of tubes every 5 minutes during the digestion period. The test and blank tubes was removed from the water bath after 30 minutes. Transfer only the supernatant liquids to a small beaker (5 – 25 ml size) maintaining the 5 minutes interval between the test and the blank. PH of the supernatant liquids EXACTLY 5 minutes after removal from the bath was determined. The difference between the PH of the test and the PH of the blank is an index of Urease activity. ΔPH = (PH of test sample) – (PH of blank).

3.7.2 Peroxide Value

About 100 g of the prepared sample in 150 ml chloroform in a 1 liter beaker was soaked for 1hr. Filtered through a dried, fluted filter paper rejecting the first few milliliters and keeping the filtrate in a stoppered flask. Oil content in 25 ml of chloroform extract: pipette 25 ml of the filtrate into a tarred evaporating dish. Evaporate off the chloroform on water-bath and then dried in an air oven at 100 ^{o}C for 3 hrs. Cool the dish in the desiccator's before weighing.

Then, 25 ml of filtrate was pipetted into a 150 ml conical flask. 35 ml of glacial acetic acid and 0.5 ml of saturated potassium iodide was added and shaked for 1 minute. 30 ml of water was added and titrated with 0.1 N sodium thiosulphate using starch as indicator. Vigorous stirring has been done to remove the last traces of iodine from the layer of chloroform.

Calculation of oil content in 25 ml of chloroform extract was as follows: $M_0 = M_1 – M_2$. Where M_0 is oil content in 25 ml of chloroform extract, in grams; M_1 is the mass in grams of dish and oil contents; and M_2 is the mass of the sample. Peroxide value, in mill equivalents of peroxide oxygen per kg of oil shall be calculated as (V_2 x N x1000) divided by M_0. Where, $\mathbf{V_2}$ is the volume in milliliters of sodium thiosulphate solution used; N is the normality of sodium thiosulphate solution used; and M_0 is the mass in grams of oil in 25 ml of chloroform extract.

3.7.3 Particle size

About 200 gm of homogenized sample was weighed in the beaker and transferred to the 1mm sieve and 600 micron sieve and put it in the mechanical shaker. Shake the sample with sieve for about 15 minutes. Weighed carefully the sample retained on and passed through 1mm separately. And also weighed the sample retained on and passed through 600 micron sieve. Calculate the percentage of the particle size based on sieve size. If the processing of corn soya blend is with the standard, the particle size should be 95% must pass through a 600 micron sieve and 100% must pass through a 1000 micron sieve (1 mm).

Figure: 3.3- Sieve (Ashagrie and Paulos, 2017)

3.7 Aflatoxins in CSB

3.8.1 Materials and Chemicals

Reagents:

Acetonitrile, methanol, Sodium chloride, ultrapure water and phosphate buffered saline (PBS: NaCl 8g l-1, KCl 0.2 g l-1, Na2HPO4 1.15 g l-1, KH2PO4 0.2 g l-1; and adjust pH to 7.2 using 0.1M HCl or 0.1M NaOH) were purchased. Reagents for HPLC separation were suitable for UPLC filtered through a 0.2µm membrane (Merck Chemicals, Darmstadt, Germany). All other chemicals and reagents used were of analytical grade.

Immunoaffinity columns:

Immune affinity column which contains antibodies against B1, B2, G1 and G2 with a capacity greater than 100 ng for aflatoxin B1 and recovery (70%-110%) was used for sample clean up.

Mobile phase:

The mobile phase used for aflatoxin analysis was a mixture of a de-ionized water-methanol-acetonitrile (60:25:15, v/v/v) at flow rate 1.0 ml/min. The mobile phase was sonicated for 30 minutes to remove bubbles and Isocratic method was applied for analysis for better resolution. De-ionized water was used throughout mobile phase preparation.

Standards:

Aflatoxin B1, aflatoxin B2, aflatoxin G1 and aflatoxin G2 and mixed Aflatoxin standards were purchased from Sigma Aldrich (St. Louis, MO, USA).

Apparatus:

Immune affinity column (Aflatest®,1ml), laboratory stand with clump, Graduated pipettes (1ml, 5ml, 10ml, 25ml and 50ml), volumetric flask (10ml, 25ml, 50ml, 100ml, 500ml and 1000ml), Measuring cylinder (50ml and 100ml), Beaker (50ml, 100ml and 500ml), conical flask(250, 500

and 1000ml),Hot air oven for moisture analysis, Mixer, Stirrer, Ultra bath sonicator, ultrapure water machine (Vivagen korea) Wash bottle, Micropipettes, Micropipette tips, Syringe Filter (0.45µm pore size), Electronic balance type AX120 (capacity, readability 0.1mg, shimadzu corporation), syringes (5ml and 10ml), Paraffin, Sample label, Vials with screw cap. HPLC system setup contains auto sampler, injector, oven, column (Eclipse Plus C18 column 3.5µm ×4.6 x 100mm,), Link, Degasser, fluorescence detector and desktop computer with shimadzu LC software.

Chromatographic condition:

Chromatographic separation and detection was carried out using Shimadzu USA, HPLC instrument with LC software and fluorescence detector were used for analysis. Separation was achieved with Eclipse Plus C18 reversed phase column (3.5µm x 4.6 x 100mm). The operating condition was as follows: column temperature at 25°c temperature; flow rate 1.0 ml min-1; 10 minutes running time, 20µl injection volume; detection wavelength: excitation wavelength 365 nm/emission wavelength 440 nm; diluent methanol and Needle wash (Water: Methanol: Acetonitrile 60:25:15 v/v)

3.8.2 Sample Preparation and Clean Up

The SC-CSB was completely homogenized in dark room by using a cone shape method. About 20 gm of SC-CSB was extracted with 100 ml (80 % v/v HPLC grade methanol and 20% v/v Ultra-pure water) in blender jar at high speed for 5 minutes. The extract was passed through a (Whatman® No.4) filter paper. About 10 ml of the purified extract was added to 40 ml PSB buffer (PH=7.2: NaCl 8gm l^{-1}, KCl 0.2 gm l^{-1}, Na_2HPO_4 1.15 gm l^{-1}, KH_2PO_4 0.2 gm l^{-1}). The sample was passed through 0.45 µl syringe to remove residual turbidity. 25 ml of the diluted extracted sample was taken and passed through the column.

10 ml of deionized water was passed through the column to make it clean and removed the water carefully. Aflatoxin was slowly eluted with methanol (1 ml) into amber glass vial and injected (20 µl) in to the instrument. All eluted residues were collected by pressing air thoroughly through the column.

Figure: 3.4- Sample preparation and cleanup for Aflatoxins

3.8.3 Analysis of Aflatoxins using HPLC/FLD

The toxin was quantified using HPLC-fluorescence detector (Shimadzu LC RF-20A prominence L20495273405, US), equipped with Eclipse Plus C18 reversed phase column (3.5µm x 4.6 x 100mm, UK).

The operating condition was as follows: Column temperature at 25°C temperature; Flow rate 1.0 ml min-1 isocratically; 12 minutes running time per analysis, Injection volume; 20µ l Detection wavelength: excitation wavelength 365 nm, Emission wavelength 440 nm.

Concentrations of aflatoxins were quantified with reference to AFs standards. The calibration curve for the standards was established with a concentration range from 2ppb - 50 ppb.

The toxin level was calculated with the formula total AF,

$$\text{ng/g} = \frac{\text{Csamp x solvent x Elution}}{\text{wt x Aliquot}} \left[\frac{\text{ngxmlxml}}{\text{ml x g x ml}}\right]$$

Where: Wt (g) = sample material taken for analysis; Solvent (mL) = solvent taken forextraction; Aliquot (mL) = aliquot taken for immunoaffinity clean-up; Elution (mL) = final volume collected after elution from IAC; Csmp (ng/mL) = concentration of aflatoxin calculated from linear regression; Contam (ng/g) = contamination of sample material with aflatoxin; Signal smp (units) = area of aflatoxin peak obtained from the measured solution.

3.8.4 Aflatoxin Method Validation

Method validation is the process by which a method is tested by the developer or user for reliability, accuracy, and preciseness of its intended purpose. It provides documented evidence, and a high degree of assurance, which an analytical method employed for a specific test, is suitable for use. Therefore, method validation should be an essential component of the measurements that a laboratory makes to allow it to produce reliable analytical data (FDA, 2000).

Identification:

Identification of Aflatoxins determined by retention time of individual and mixed Aflatoxins (AFG2, AFG1, AGB2, and AFB1) injecting at the same condition and its precision determined by percent relative standard deviation (%RSD) less than 2% (FDA, 2000).

Limit of Detection and Quantitation (LOD and LOQ):

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) tests for the procedure is performed on samples containing very low concentrations of analyte. LOD is defined as the lowest amount of analyte that can be detected above baseline noise; typically, three times the noise level S/N > 3. LOQ is defined as the lowest amount of analyte which can be reproducibly quantitated above the baseline noise, that gives S/N >10 (FDA, 2000).

Precision:

Precision is the measure of the degree of repeatability of an analytical method under normal operation, and is normally expressed as the percent relative standard deviation for a statistically significant number of samples. It should be determined from a minimum of seven determinations covering the specified range of the procedure. A precision criterion the instrument precision (repeatability) will be ≤ 5% RSD. Documentation in support of precision studies should include the standard deviation, relative standard deviation, coefficient of variation, and confidence interval (FDA, 2000).

Linearity and Working Range:

The linearity of the method should be tested in order to demonstrate a proportional relationship of response versus analyte concentration over the working range. The linearity range for evaluation depends on the purpose of the analytical test method. The International Conference on Harmonization (ICH) guidelines specified a minimum of five concentration levels, along with certain minimum specified ranges. Acceptability of linearity data is often judged by examining the correlation coefficient and y-intercept of the linear regression line for the response versus concentration plot. The regression coefficient (r^2) is > 0.997 is generally considered as evidence of acceptable fit of the data to the regression line. The y-intercept should be less than a few percents of the response obtained for the analyte at the target level. The range is normally expressed in the same units as the test results obtained by the method (FDA, 2000).

Accuracy and Recovery

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy is usually determined in one of four ways. First, accuracy can be assessed by analyzing a sample of known concentration (reference materials), and comparing the measured value to the true value. The second approach is to compare test results from the new method with results from mean recovery: 99.8 %; RSD 0.09 % to be accurate.

The third approach is based on the recovery of known amounts of analyte. This is performed by spiking analyte in blank matrices and the percent recovery should then be calculated. The fourth approach is the technique of standard additions, which can also be used to determine recovery of spiked analyte. This approach is used if it is not possible to prepare a blank sample matrix without the presence of the analyte. Accuracy criteria for an assay method (FDA) are that the mean recovery will be 100 ± 20% at each concentration over the range of 80-120% of the target concentration (FDA, 2000).

3.8 Microbial Quality in CSB

3.9.1 Total Plate Count

Total Aerobic plate count was determined as per international standard, ISO 4833 method for the enumeration of microorganisms, by counting the colonies growing in a solid medium after aerobic incubation at 30°C. The plates were aerobically incubated at 30 °C for 72 hrs. The number of microorganisms per gram of sample has been calculated from the number of colonies obtained on selected plates.

Inoculation and incubation: Take two sterile Petri dishes. Transfer to each dish, by means of a sterile pipette 1 ml of the initial suspension (10−1 dilution). Take two other sterile Petri dishes. Transfer to each dish, by means of another sterile pipette 1 ml of the 10−2 dilution. Pour about 15 ml of the plate count agar at 44°C into each Petri dish. The time elapsing between the end of the preparation of the initial suspension and the moment when the Plate count agar (PCA) medium is poured into the dishes shall not exceed 45 min. Carefully mix the inoculum with the medium by rotating the Petri dishes and allow the mixture to solidify by leaving the Petri dishes standing on a cool horizontal surface. After complete solidification, and only in the case where it is suspected that the product under examination contains microorganisms whose colonies will overgrow the surface of the medium, pour about 4 ml of the overlay medium at 44°C onto the surface of the inoculated medium. Allow to solidify as described above. Invert the prepared dishes and place them in the incubator at 30°C for 72 h ± 3 h. Do not stack the dishes more than six high. Stacks of dishes should be separated from one another and from the walls and top of the incubator.

Counting of colonies: After the specified incubation period, count the colonies on the plates using the colony counting equipment. Examine the dishes under subdued light. Examine doubtful objects carefully, using higher magnification where required, in order to distinguish colonies from foreign matter.

3.9.2 Coliforms

Coliforms were determined according to international standard, ISO 4832 method for the enumeration of microorganisms, by counting the colonies growing in a solid medium after incubation at 37^{o}C for 24 hrs. The characteristic colonies were counted. The number of coliforms per gram of sample has been calculated from the number of characteristic colonies obtained in the plates.

Inoculation and incubation: Prepare two sterile Petri dishes. Transfer, with a sterile pipette of dilutions to the center of each dish. Use another sterile pipette to inoculate each dilution into the dishes. Pour about 15 ml of the VRBL medium, at 42 ^{o}C, into each Petri dish. The time elapsing between the end of the preparation of the initial suspension and the moment when the medium is poured into the dishes shall not exceed 15 min.

Carefully mix the inoculum with the medium and allow the mixture to solidify, with the Petri dishes standing on a cool horizontal surface. Also prepare a control plate with 15 ml of the medium for checking its sterility. After complete solidification, pour about 4 ml of the Crystal violet neutral red bile lactose (VRBL) agar medium, at 42 ^{o}C, onto the surface of the inoculated medium. Invert the prepared dishes and incubate them in the incubator set at 37 ^{o}C for 24 hr ± 2hr.

Enumeration: Count, using the colony-counting equipment, the purplish red colonies with a diameter of at least 0.5 mm (sometimes surrounded by a reddish zone of precipitated bile). These are considered as typical colonies of coliforms and do not require further confirmation.

Confirmation: Inoculate five colonies of each atypical type into tubes of brilliant green lactose bile broth. Incubate the tubes in the incubator set at 37 ^{o}C for 24 hr ± 2hr. Consider as coliforms colonies that show gas formation in the Durham tube. Take the results into account in the calculation.

3.9.3 Yeast and Molds

Yeasts and mold were determined according to international standard ISO 7954 in the flour by means of the colony count technique at 25 ^{o}C. The preparation of poured plates using a specified selective culture medium and a specified quantity of the test sample of an initial suspension. Aerobic incubation of the plates at 25 ^{o}C for 3, 4 or 5 days. Calculation of the number of yeasts and molds per gram of sample from the number of colonies obtained on plates chosen at dilution levels so as to give a significant result.

Inoculation and incubation: Take two sterile Petri dishes. Transfer to each dish, by means of a sterile pipette 1 ml of the initial suspension (10−1 dilution). Take two other sterile Petri dishes. Transfer to each dish, by means of another sterile pipette 1 ml of the 10−2 dilution. Pour about 15 ml of the Yeast Extract-dextrose chloramphenicol agar medium at 45 ± 1 °C in water bath, from the culture bottle into each Petri dish. The time elapsing between the end of the preparation of the initial suspension and the moment when the medium is poured into the dishes shall not exceed 45 min. Carefully mix the inoculum with the medium by rotating the Petri dishes and allow the mixture to solidify by leaving the Petri dishes standing on a cool horizontal

surface. Also prepare a control plate with 15 ml of the medium for checking its sterility. Invert the prepared plates and place them in the incubator set at 25 ± 1 ^{o}C.

Enumeration: Counts after 3, 4 and 5 days of incubation. After 5 days, retain those plates containing fewer than 150 colonies

3.9 Experimental Design and Statistical Analysis

Completely Randomized Experimental Design was followed to see the assessment of quality and safety of corn soy blend flour for four Ethiopian CSB factories. Data obtained during assessment studies to identify possible difference among factories was analyzed with analysis of variance (ANOVA) and t-test. When P values ($P < 0.05$) were found significant, the means of each parameter were compared using the least significant differences (LSD) procedures of the SPSS, version 15.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Proximate composition

A total of four Ethiopian super cereal corn soya blend flour manufacturing companies were studied of which two of them from roasting and the other two from extrusion processing industry. The sample size taken from each company was 10kg. The proximate composition and mineral level of super cereal corn soya blend flour are illustrated in table 4.1.

Table 4.1 Proximate composition of SC-CSB, g/100g

Processing	Sample Code	Parameters, g/100g				
		Moisture	Fat	Ash	Protein	Fiber
Roasting	R-12 -03-2018 AZ	4.62± 0.12 ^{a}	9.01± 0.19 ^{b}	3.20± 0.06 ^{b}	12.95± 0.21 ^{a}	2.86± 0.14 ^{b}
	R-13 -03-2018 AZ	5.92± 0.03 ^{c}	7.89± 0.02 ^{b}	3.08± 0.02 ^{b}	12.97± 0.41 ^{a}	2.34± 0.13 ^{a}
Extrusion	E-12 -03-2018 AZ	7.66± 0.13 ^{d}	6.61± 0.15 ^{a}	2.30± 0.18 ^{a}	14.08± 0.22 ^{b}	3.21± 0.09 ^{c}
	E-13 -03-2018 AZ	5.69± 0.01 ^{b}	6.83± 0.03 ^{a}	3.42± 0.01 ^{c}	14.46± 0.38 ^{b}	3.75± 0.18 ^{d}

Mean value + standard deviation, n=3.

Means in the same column with different letters are significantly different (P<0.05)

Key1: a, b, c, d, e, f ….. are superscripts given to show the significant difference between means

Moisture Content: - Moisture is an important parameter in flour that significantly affects shelf life and growth of microbial contaminants. The moisture content of SC-CSB flour samples in the two cooking processes was 4.62, 5.92, 7.66 and 5.69% for roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) respectively. According to table 4.1, there is a significant (P<0.05) in moisture content. Both WFP, (2014) and CES 139, (2015) has stated that the moisture content of the super-cereal corn soya blend in (max %, m/m) need to be 10 % and based on the results in this study its notable that the moisture content is in the range or has fulfilled both international and national compulsory standard requirements.

Fat content:- According to table 4.1, the fat content of SC-CSB flour samples were 9.01, 7.89, 6.61 and 6.83 in roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) respectively. In the two different cooking processes (roasting and extrusion) shows significant ($P<0.05$) different, but the crude fat content among the samples in each process is not significantly differ ($P>0.05$), R-12 -03-2018 AZ and R-13 -03-2018 AZ in roasting and also E-12 -03-2018 AZ and E-13 -03-2018 AZ extrusion. Both WFP, (2014) and CES 139, (2015) has stated that the minimum fat content of SC-CSB flour samples should be 6.0 g/100g and all the values in this study for crude fat content are greater than 6 g/100g, therefore these values have fulfilled both international and national compulsory standard requirements.

Ash content:- The total ash content of SC-CSB flour samples in the two cooking processes were 3.20, 3.08, 2.30 and 3.42 % for roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) respectively. According to table 4.1, there is a significant ($P<0.05$) different in the samples of E-12 -03-2018 AZ and E-13 -03-2018 AZ goes via extrusion, in roasted samples of (R-12 -03-2018 AZ and R-13 -03-2018 AZ) there is no significant ($P>0.05$) difference. The international and national compulsory standard requirements WFP, (2014) and CES 139, (2015) both has stated that the total ash content in SC-CSB flour samples must have a maximum value of 4.1 g/100g and values of the total ash content in this study not maximum from the two standards.

Protein content:- According to table 4.1, the protein content of SC-CSB flour samples in the two cooking processes were 12.95, 12.97, 14.08 and 14.46 for roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) respectively. Just like the crude fat content, the crude protein content in the two different cooking processes (roasting and extrusion) have significant ($P<0.05$) difference, but among the samples in each process is protein content values not significantly differ ($P>0.05$), R-12 -03-2018 AZ and R-13 -03-2018 AZ in roasting and also E-12 -03-2018 AZ and E-13 -03-2018 AZ extrusion. Both WFP, (2014) and CES 139, (2015) has stated that the crude protein content of the super-cereal corn soya blend flour samples need to be a minimum of 14.0 g/100g and based on the results among the four samples only SC-CSB flour samples under Extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) fulfill the value of crude protein in standards, the remaining two SC-CSB flour samples under the roasting process (R-12 -03-2018 AZ and R-13 -03-2018 AZ) is lower than the required crude protein amount in standards.

Fiber content:- Crude fiber content of SC-CSB flour samples in this study were 2.86, 2.34, 3.21 and 3.75 for roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) respectively. Based on Table 4.1, crude fiber content in both processing types show a significant ($P<0.05$) different, and both WFP, (2014) and CES 139, (2015) has stated that the crude fiber in SC-CSB flour samples need to have a maximum value of 4.0 g/100g, therefore fiber values in this study is not higher than the stated values in the standards so, this study has fulfilled both international and national compulsory standard requirements.

4.2 Minerals Content (Fe, Ca and K) in SC-CSB

The mineral levels of the two treatments of roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) are presented in table 4.2. The analyzed minerals were Fe, Ca and K.

Table 4.2 Minerals (Fe, Ca and K) content of SC-CSB, mg/kg

Processing	Sample Code	Parameters		
		Ca, mg/100g	K, mg/100g	Fe, mg/100g
Roasting	R-12 -03-2018 AZ	**446.49± 1.68 ^{c}**	**592.99± 0.28 ^{a}**	**10.2± 0.14 ^{a}**
	R-13 -03-2018 AZ	**353.3± 2.77 ^{a}**	**649.0± 0.64 ^{b}**	**10.6± 0.45 ^{a}**
Extrusion	E-12 -03-2018 AZ	**377.2± 1.27 ^{b}**	**680.8± 0.42 ^{c}**	**12.11± 0.27 ^{b}**
	E-13 -03-2018 AZ	**355.1± 0.00 ^{a}**	**775.6± 0.00 ^{d}**	**13.4± 0.95 ^{b}**

Means in the same column with different letters are significantly different ($P<0.05$)

Key1: a, b, c, d, e, f ….. are superscripts given to show the significant difference between means

Calcium: Calcium is the most abundant mineral in the body. It needs more emphasis in our meal because an adequate intake helps grow a healthy skeleton in early life and minimize bone loss in late life. The main dietary source of calcium was dairy foods and products in all countries (range: 33–62.4% in men and 38.7–61.8% in women), with the exception of men in Greece, where more calcium was supplied by cereals and cereal products. The variability in the amount of calcium supplied by dairy foods was smaller than that for cereals and cereal products (Members, *et al.*, 2017).

The calcium contents of the two cooking processes were 446.49, 353.3, 377.2 and 355.1 mg/100g for roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018

AZ and E-13 -03-2018 AZ) respectively. According to one way ANOVA, there is significance difference (P< 0.05) between Roasting cooking (R-12 -03-2018 AZ) and extrusion cooking (E-12 -03-2018 AZ). This difference might be due to the addition of fortificant concentration. But the other treatments R-13 -03-2018 AZ and E-13 -03-2018 AZ have no significance difference (P> 0.05) in the level of calcium.

WFP, (2014) has stated that the calcium content of the super-cereal corn soya blend in the range of 340 – 510 mg/100 gm. According to CES 139, (2015), Compulsory Ethiopian Standard requirement table the calcium content of the super-cereal corn soya blend was 340 - 510 mg/100 gm. The result of the current study has fulfilled both international and national compulsory standard requirements.

Potassium: Potassium plays a major role in maintaining fluid and electrolyte balance and cell integrity. Controlling potassium distribution is a high priority for the body because it affects many aspects of homeostasis, including a steady heartbeat. Its deficiency is characterized by an increase in blood pressure, salt sensitivity, kidney stones and bone turnover (Welch, *et al.*, 2009).

The Potassium contents of the two cooking processes were 592.99, 649.0, 680.8 and 775.6 mg/100g for roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) respectively. According to the result illustrated on table 4.9, there is significance difference (P <0.05) between Roasting cooking (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion cooking (E-12 -03-2018 AZ and E-13 -03-2018 AZ). The difference in the Potassium content of SC-CSB samples with the two cooking processes might be due to the variation in concentration of fortificant addition in each CSB flour processing industries on this study.

WFP, (2014) has stated that the calcium content of the super-cereal corn soya blend in the range of 580 - 870 mg/100 gm. According to CES 139, (2015), Compulsory Ethiopian Standard requirement table also the potassium content of the super-cereal corn soya blend was 580 - 870 mg/100 gm and based on this two international and national compulsory standard requirements the result on this study is within the range of Potassium content limit.

Iron: The iron content of the CSB flour samples were 10.2, 10.6, 12.11 and 13.4 mg/100g for roasting (R-12-03-2018 AZ and R-13-03-2018 AZ) and extrusion (E-12-03-2018 AZ and E-13-03-2018 AZ) samples respectively. One way ANOVA revealed that there was significance difference (P<0.05) between the two cooking processes in levels of Iron contents.

WFP, (2014) has stated that the iron content of the super-cereal corn soya blend in the range of 9.4 - 14.1 mg/100 gm. The result of the current study is within the given range and /or up to the standard. Similarly, CES 139, (2015) on Compulsory Ethiopian Standard requirement table stated that the iron content of the super-cereal corn soya blend was 9.4 - 14.1 mg/100 gm. Iron content was reduced on roasting cooking as compared to extrusion cooking processes in all the treatments.

From a nutritional point of view Fe is one of the most important elements, and its deficiency affects about one third of the world's population. The best way to prevent this problem is through the Fe fortification of food (Schrooyen, *et al.*, 2001). Iron (Fe) is an essential element in human being and plays a vital role in the formation of hemoglobin, oxygen and electron transport in human body (Kalagbor and Diri, 2014). Iron was the other mineral investigated in this study.

In EHNRI, (1997) food composition table is shown reduction of iron content on boiling and roasting treatments. The reduction of minerals on soaking process could arise because all the water soluble minerals are often lost with the steeping medium and rinsing process. A similar explanation was given by (Bau, *et al.*, 2000).

4.3 Vitamin A (Retinol) Content in CSB

The vitamin A levels of the two treatments of roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) are presented in table 4.3.

Table 4.3:- The level of Vitamin A (Retinol) in different factory SC-CSB

Processing	**Sample Code**	**Parameters**
		Vitamin A, mg/Kg
Roasting	R-12 -03-2018 AZ	**32.6± 0.79** ^{b}
	R-13 -03-2018 AZ	**40.9± 0.30** ^{c}
Extrusion	E-12 -03-2018 AZ	**21.8± 1.53** ^{a}
	E-13 -03-2018 AZ	**38.7± 1.69** ^{c}

Mean value + standard deviation, n=3. **Means in the same column with different letters are significantly different (P<0.05)**

Key1: a, b, c, d, e, f ….. are superscripts given to show the significant difference between means

Vitamin A contents of CSB, mg/kg For the sake of keeping the standards free from being degraded, freshly prepared standards were used and all the analysis was conducted in dark room

because vitamins are very light sensitive. The sample was determined by supper imposing sample peak with standard and conducted in triplicate as per the method and specification.

Vitamin A Method Validation of the Chromatographic

i. Identification

Peak identification results are as shown in table 4.4. The retention time of Vitamin A gives a good precession having 0.29 % RSD, which is acceptable according to FDA standard which is less than 2 % RSD. The retention time for Vitamin A was 2.223. In addition to the retention time of chromatographic result for Blank (diluent) and vitamin A shown in Annex C demonstrates that the qualitative aspect of the identification test is more defined and acceptable.

Table 4.4: Statistics for Vitamin A retention time identification

Vitamin A (Retinol)	Vitamin A injection retention time (Min)		N	Mean	Std. deviation	% RSD
	2.5 ppm	10 ppm				
	2.227	2.218	2	2.223	0.0064	0.29

ii. Limit of Detection and Quantification (LOD and LOQ)

Detection performance of the HPLC was determined by the limit of detection and was found to be 0.5 parts per million for vitamin A. LOD was determined by the amount of analyte that can be detected above baseline noise; typically, three times the noise level S/N > 3 as shown in table 4.5 below.

Table 4.5: Limit of Detection (LOD) and Limit of Quantification (LOQ)

Vitamin A (Retinol)	LOD		LOQ	
	ppm	Signal to Noise Ratio, (S/N > 3)	ppm	Signal to Noise Ratio, (S/N > 10)
	0.5	3.1	1.5	10.90

On the other hand, the limits of quantification for vitamin A was 1.5 parts per million. LOQ was determined based on the amount of analyte which can be reproducibly quantitated above the baseline noise, that gives S/N >10.

iii. Precision

As verified in Table 4.6 the precision was evaluated through the repeatability of the method by assaying ten replicate injections of vitamin A standard at the same concentration (10ppm) during the same day, under the same experimental conditions. It shows an acceptable % RSD which had a value of < 0.62 % and < 0.14 % for the retention time and peak area respectively. Precision criteria the instrument precision (repeatability) and is normally expressed as the percent relative standard deviation for a statistically significant number of samples should be ≤ 5 % RSD in FDA standard.

Table 4.6: Descriptive statistics for the precision check (Repeatability)

***Vitamin* A (Retinol)**	***Injection Concentration***	***N***	***Descriptive statistics of Peak area***			***Descriptive statistics of Retention time***		
			Mean	***STD***	***% RSD***	***Mean***	***STD***	***% RSD***
	10 ppm	10	317.6	0.4341	0.14	2.225	0.0137	0.62

iv. Linearity and Working Range

Linearity was studied by selecting seven concentrations (05, 2.5, 5, 10, 15, 30, 40 and 50) ppm in order to demonstrate a proportional relationship of peak area versus analyte concentration over the working range. The International Conference on Harmonization (ICH) guidelines specified a minimum of five concentration levels, along with certain minimum specified ranges. The regression equation was found by plotting the peak area (y) versus the Vitamin A concentration (x) expressed in ppb as presented in Anex B.

Table 4.7: Descriptive statistics for Calibration graph data's

Vitamin A (Retinol)				
S/N	**Points, ppm**	**Calibration I**	**Calibration II**	**Mean**
1	0.5	13	13.6	12.4
2	2.5	70	69.9	70.1
3	5	140	139.9	140.1
4	10	277.25	277.2	277.3
5	15	414.5	414.9	414.1
6	30	818.3	821.7	814.9
7	40	1092.55	1098.9	1086.2
8	50	1359.7	1365.9	1353.5

Acceptability of linearity data is often judged by examining the correlation coefficient and y-intercept of the linear regression line for the peak area versus concentration plot. As revealed in Table 4.6 the demonstration coefficient (R^2) obtained for the regression line demonstrates the excellent relationship between peak area and concentration of vitamin A. The regression coefficient (r^2) equal to 1.0000 is generally considered as evidence of acceptable fit of the data to the regression line.

Table 4.8: Linearity check

Vitamin A (Retinol)	**N (Point)**	**Calibration Curve Equation**	**R^2**
	8	y = 27.0378x + 3.9772	1.0000

Table 4.9: Working Range

Calibration Point	**Vitamin A (Retinol) (ppm)**
1	0.5
2	2.5
3	5
4	10
5	15
6	30
7	40
8	50

The working range was set by considering the Ethiopian national standard and expected performance variance depending on the pre-analysis personal observation of the production process as there are no related empirical studies.

v. Accuracy and Recovery

As illustrated in Table 4.8, the concentration of 2 and 10 ppm vitamin A standard spiked in **R-13-03-2018 AZ** SC-CSB sample. A percent recovery of response factor (area/concentration) was calculated. An accuracy criterion for an assay method (FDA, 2000) is that the mean recovery is 100 ± 20% at each concentration over the range of 80-120% of the target concentration. The results of recovery and accuracy studies shown in the range between (95 -105) % and it is evident that the method is accurate within the desired recovery range.

Table 4.10: Statistics for Vitamin A (Retinol) recovery and accuracy check

Vitamin A (Retinol)	Spike Concentrations ***(2 and 10 ppm)***	**% Recovery**		**N**	**Mean**	**Std. Deviation**	**% RSD**
		2 ppm	**10 ppm**				
		105	95	**2**	100	7.07	7.07

The Vitamin A content of the SC-CSB flour samples were 32.6, 40.9, 21.8 and 38.7 mg/100g for roasting (R-12-03-2018 AZ and R-13-03-2018 AZ) and extrusion (E-12-03-2018 AZ and E-13-03-2018 AZ) samples respectively. According to one way ANOVA, there is significance difference (P> 0.05) between Roasting cooking (R-12 -03-2018 AZ) and extrusion cooking (E-12 -03-2018 AZ). But the other treatments R-13 -03-2018 AZ and E-13 -03-2018 AZ have no significance difference (P> 0.05) in the level of vitamin A. The difference in Vitamin A content in (R-12-03-2018 AZ and E-12-03-2018 AZ) could be due to higher concentration of Vitamin A fortificant addition during the processing of SC-CSB in each industry.

WFP, (2014) and CES 139, (2015) on Compulsory Ethiopian Standard requirement table have stated that vitamin A content of the super-cereal corn soya blend in the range of 8.31 - 12.48 mg/kg. The result of this study is out of the given range of the standard. Even though vitamins have significant role for our body functioning but the level of vitamins present in our food beyond the recommended level particularly children are at risk of side effects because smaller bodies are less able to process excessive amounts, they are more susceptible to over dose. Intake of foods containing too much vitamin A can lead to liver damage and skeleton problems. In adults, high vitamin A intake has been linked to hip fractures and in pregnant women too much vitamin A can harm a foetus (US Environmental Working Group (EWG), 2014).

4.4 Physico-Chemical Composition of SC-CSB

Physico-chemical Characteristics of SC-CSB are peroxide value, Urease index and particle size which are tested under this research paper. According to the analysis done on the laboratory, the results were illustrated below under table 4.11.

Table 4.11 Physico-Chemical characteristics of CSB, g/100g

Processing	Sample Code	Parameters, g/100g			
		Peroxide value, meq/kg	Urease index, pH unit	Particle size passed through 1mm	Particle size passed through 600µm
Roasting	R-12 -03-2018 AZ	ND	ND	99.92 ± 0.02^{c}	99.4 ± 0.02^{b}
	R-13 -03-2018 AZ	ND	ND	98.2 ± 0.09^{b}	99.1 ± 0.01^{b}
Extrusion	E-12 -03-2018 AZ	ND	ND	99.98 ± 0.01^{c}	99.8 ± 0.04^{c}
	E-13 -03-2018 AZ	ND	ND	97.4 ± 0.09^{a}	98.2 ± 0.25^{a}

ND= Not detected, Mean value + standard deviation, n=3.

Means in the same column with different letters are significantly different (P<0.05)

Key1: a, b, c, d, e, f ….. are superscripts given to show the significant difference between means

Urease Index: This quality parameter indicates that the degree of cooking the corn soya blend flour. And it used to evaluate the efficiency of cooking processes (roasting and extrusion). The results of Urease index of SC-CSB for all cooking process roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) were not detected. As per the findings, the results of Urease index of SC-CSB flour for both cooking processes (roasting and extrusion) has fulfilled the standard requirements of both Compulsory Ethiopian standard and WFP.

Peroxide value: All the SC-CSB flour samples in Table 4.11 met with the Compulsory Ethiopian Standard, CES 139/ WFP/ requirements which recommend a maximum peroxide value of 10 meq O_2/kg oil. Thus, the results of Peroxide value for all cooking process roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) were not detected. In this finding, peroxide value of SC-CSB flour has fulfilled the standard requirements of both Ethiopian and WFP standards.

Peroxide value is used to assess the stability or rancidity of fats by measuring the amount of lipid peroxides and hydro-peroxides formed during the initial stages of oxidation and thus, estimate to which extent spoilage of dietary oil (expressed by the level of rancidity) has

advanced. Beside these visible harmful effects on the sensory quality of the oil, per-oxidation also makes the oil dangerous for human health, as the free radicals generated by this process are proven to be carcinogenic (Rossel, 2009), (Albert, *et al.*, 2011).

Peroxide value is used as a measured of the extent to which rancidity reaction have occurred during storage. The value obtained in the study is below the standards sated by CES/FAO/WHO (1994) value which is ≤10.

Particle Size: Particle Size (sieve analysis) was used to measure flour particle size distributions as percent weight, among different SC-CSB cooking processing methods.

The results of Particle size passed through 1 mm for all cooking process roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) were 99.92%, 98.2 %, 99.98 % and 97.4 % respectively. According to the Compulsory Ethiopian standard (CES 139) and WFP, the standard requirements for particle size passed through 1mm is not less than 98 %. The result of the current study for Roasting cooking (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion cooking (E-12 -03-2018 AZ) has fulfilled both international and national compulsory standard requirements. But one of the extrusion cooking (E-13 -03-2018 AZ) samples for particle size passed through 1 mm has not fulfilled the standard requirements of CES 139 and WFP standards which implies that the grinding efficiency of extrusion cooking process is low as compared to roasting cooking process.

According to one way ANOVA, there is significance difference (P> 0.05) between Roasting cooking (R-13 -03-2018 AZ) and extrusion cooking (E-13 -03-2018 AZ) of particle size passed through 1 mm in SC-CSB. But the other treatments R-12 -03-2018 AZ and E-12 -03-2018 AZ have no significance difference (P > 0.05) in the level of particle size passed through 1 mm.

The results of Particle size passed through 600µm for all cooking process roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) were 99.4%, 99.1 %, 99.8 % and 98.2 % respectively. According to the Compulsory Ethiopian standard (CES 139) and WFP, the standard requirement for particle size passed through 600µm is not less than 95 %. The result of the current study for Roasting cooking (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion cooking (E-12 -03-2018 AZ and E-13 -03-2018 AZ) has fulfilled both international and national compulsory standard requirements of SC-CSB samples for particle size passed through 600µm.

According to one way ANOVA, there is significance difference (P> 0.05) between Roasting cooking samples E-12 -03-2018 AZ and E-13 -03-2018 AZ) of particle size passed through 600µm in SC-CSB flour. But the other treatments R-12 -03-2018 AZ and R-13 -03-2018 AZ

have no significance difference (P > 0.05) in the level of particle size passed through 600µm. In this finding, still grinding efficiency of roasting cooking processes is better than extrusion cooking processes for particle size passed through 600µm of SC-CSB flour.

4.5 Level of aflatoxins in CSB

The four factories of SC-CSB with different processing system which are listed in table 4.19 below coded as (*R-12-03-2018 AZ and R-13-03-2018 AZ*) for Roasting processing factory, (*E-12-03-2018 AZ* and *E-13-03-2018 AZ*) for the extrusion processing factory were conducted aflatoxin B1 and total aflatoxin for each selected sample . Each sample was run in triplicates according to the procedure and specification.

Aflatoxin Validation of the Chromatographic Method

A. Identification

Peak identification results are as shown in Table 4.12. The retention time of individual and mixed aflatoxins gives a good precession having a range between (0.05 - 0.27) % RSD, which is acceptable according to FDA standard which is less than 2% RSD. The elution order of individual Aflatoxins was in the order of AFG2, AFG1, AGB2 and AFB1 with 4.397, 5.434, 6.310 and 7.907 retention times respectively. In addition to the retention time chromatographic result for Blank (diluent), individual (AFG2, AFG1, AGB2 and AFB1) and mixed aflatoxins shown in Annex D demonstrate the qualitative aspect of an identification test is more defined and acceptable.

Table 4.12:- Statistics for aflatoxin retention time identification

Aflatoxin	**Aflatoxin injection retention time (Min)**		**N**	**Mean**	**Std. deviation**	**% RSD**
	For Single run (10ppb)	**For mixed run (5, 20, 5, 20 ppb resp.)**				
AFG2	4.397	4.392	2	**4.395**	**0.004**	**0.08**
AFG1	5.434	5.428	2	**5.431**	**0.004**	**0.08**
AFB2	6.31	6.286	2	**6.298**	**0.017**	**0.27**
AFB1	7.907	7.901	2	**7.904**	**0.004**	**0.05**

Annex E shows that the mixed standards peaks were supper imposed perfectly; this confirms that the retention time for each aflatoxin was almost constant. All the samples were determined by supper imposing with the standard peak and the level of aflatoxin calculated in ppb according to the formula.

B. Limit of Detection and Quantification (LOD and LOQ)

LOD is the lowest amount (concentration) of the analyte in the sample that can be detected while LOQ is the smallest amount (concentration) of analyte in the sample that can be quantitated. Detection performance of the HPLC was determined by the limit of detection and was found to be 0.05, 1.5, 0.02 and 0.5 ppb for aflatoxin AFG2, AFG1, AGB2, and AFB1 respectively. Typically, three times the noise level S/N > 3 implies LOD as shown in Table 4.13 below.

Table 4.13: Limit of Detection (LOD) and Limit of Quantification (LOQ)

Aflatoxin	**LOD**		**LOQ**	
	ppb	**Signal to Noise Ratio, (S/N > 3)**	**ppb**	**Signal to Noise Ratio, (S/N > 10)**
AFG2	0.05	5.25	0.1	11.32
AFG1	1.5	4.48	3	10.47
AFB2	0.02	4.73	0.1	14.84
AFB1	0.5	4.66	1	10.13

On the other hand, the limits of quantification for individual aflatoxin (AFG2, AFG1, AGB2 and AFB1) were 0.1, 3.0, 0.1 and 1.0 parts per billion respectively. The limits of quantification were in the range of (0.1 - 3.0) μg/L. Typically, ten times the noise level S/N > 10 implies LOQ as shown in Table 4.13 above.

C. Precision

As verified in table 4.14 the precision was evaluated through the repeatability of the method by assaying ten replicate injections of mixed aflatoxin standard at the same concentration (5 ppb, 20ppb, 5ppb, and 20ppb resp.), during the same day, under the same experimental conditions. It shows an acceptable % RSD which had a values of <0.50% and < 3.0% for the retention time and peak area respectively. Precision criteria the instrument precision (repeatability) and is normally expressed as the percent relative standard deviation for a statistically significant number of samples should be ≤ 5% RSD in FDA standard.

Table 4.14: Descriptive statistics for the precision check (Repeatability)

Aflatoxin	*Injection Concentration*	*N*	*Descriptive statistics of Peak area*			*Descriptive statistics of Retention time*		
			Mean	*STD*	*% RSD*	*Mean*	*STD*	*% RSD*
AFG2	5 ppb	10	**45573**	1112	2.44	**4.387**	0.020	0.46
AFG1	20 ppb	10	**11098**	329	2.97	**5.427**	0.029	0.53
ADB2	5 ppb	10	**174023**	4485	2.58	**6.302**	0.026	0.42
AFB1	20 ppb	10	**32494**	827	2.54	**7.903**	0.039	0.49

D. Linearity and Working Range

Linearity was studied by selecting seven concentrations (2, 5, 10, 20, 30, 40 and 50) ppb in order to demonstrate a proportional relationship of peak area versus analyte concentration over the working range. The International Conference on Harmonization (ICH) guidelines specified a minimum of five concentration levels, along with certain minimum specified ranges. The regression equation was found by plotting the peak area (y) versus the aflatoxins concentration (x) expressed in ppb as presented in Annex F.

Table 4.15: Descriptive statistics for Calibration graph data for each Aflatoxins

Aflatoxin G2			**Aflatoxin G1**			**Aflatoxin B2**			**Aflatoxin B1**		
Cal. I	**Cal. II**	**Mean**	**Cal. I**	**Cal. II**	**Mean**	**Cal. I**	**Cal. II**	**Mean**	**Cal. I**	**Cal. II**	**Mean**
3142	3001	**3072**	453	548	**501**	10543	10121	**10332**	1140	1266	**1203**
5597	6321	**5959**	1010	1095	**1053**	19778	22430	**21104**	2864	3125	**2995**
10486	9997	**10242**	1948	2000	**1974**	37066	35755	**36411**	5923	5613	**5768**
18943	18908	**18926**	3806	3916	**3861**	69347	68331	**68839**	11079	11231	**11155**
26607	27559	**27083**	5434	5731	**5583**	98168	99962	**99065**	16007	16644	**16326**
35827	36540	**36184**	7472	7702	**7587**	133262	133889	**133576**	21664	22330	**21997**
45260	44942	**45101**	9498	9625	**9562**	168345	168354	**168350**	27440	27351	**27396**

Acceptability of linearity data is often judged by examining the correlation coefficient and y-intercept of the linear regression line for the peak area versus concentration plot. As revealed in

table 4.16 the demonstration coefficient (R^2) obtained for the regression line demonstrates the excellent relationship between peak area and concentration of aflatoxin and its coefficient of correlation (R^2) sited in between 0.9995 and 0.9999. The regression coefficient (r^2) is > 0.9995 is generally considered as evidence of acceptable fit of the data to the regression line on the FDA standard.

Table 4.16: Linearity check

Aflatoxin	**N (Point)**	**Calibration Curve Equation**	**R^2**
AFG2	7	y = 2,172.2x + 1,449.7	0.9998
AFG1	7	y = 469.1x + 94.2	0.9996
AFB2	7	y = 8,143.9x + 3,748.7	0.9995
AFB1	7	y = 1,357.2x + 229.8	0.9999

The range was derived from linearity studies, and depends on the intended application of the test method. The range is normally expressed in the same units as the test results obtained by the method. As shown in table 4.17 the data obtained during the linearity studies was used to assess the range of the assay method.

Table 4.17: Working Range

Aflatoxin Sum (ppb)	**Aflatoxin (ppb)**			
	AFG2	**AFG1**	**AFB2**	**AFB1**
2	0.2	0.8	0.2	0.8
5	0.5	2	0.5	2
10	1	4	1	4
20	2	8	2	8
30	3	12	3	12
40	4	16	4	16
50	5	20	5	20

AFG2=Aflatoxin G2, AFG1=Aflatoxin G1, AFB2=Aflatoxin B2, AFB1=Aflatoxin B1

E. Accuracy and Recovery

The accuracy of this analytical method was obtained by standard additions, which can also be used to determine recovery of spiked analyte. This approach was used due to the difficulty to obtain a blank sample matrix without the presence of the analyte. As illustrated in table 4.18, the concentration of 8 and 40ppb aflatoxin standard spiked in SC-CSB sample and injected in duplicate. A percent recovery of response factor (area/concentration) was calculated. Accuracy criteria for an assay method (FDA, 2000) is that the mean recovery will be 100 ± 20% at each concentration over the range of 80-120% of the target concentration. The results of recovery and accuracy studies shown in the range between (80.0 -103.5) % and it is evident that the method is accurate within the desired recovery range.

Table 4.18: Statistics for Aflatoxin Recovery and Accuracy check

Aflatoxin	Spiking Concentrations		% Recovery				N	Mean	Std. Deviation	% RSD
	8 ppb	40 ppb	8ppb		40 ppb					
AFLG2	2 ppb	10ppb	**98**	**99**	**98**	**104**	4	**99.8**	**2.872**	**2.88**
AFLG1	2 ppb	10ppb	**107**	**104**	**91**	**93**	4	**98.8**	**7.932**	**8.03**
AFLB2	2 ppb	10ppb	**70**	**68**	**89**	**93**	4	**80.0**	**12.832**	**16.04**
AFLB1	2 ppb	10ppb	**112**	**108**	**94**	**100**	4	**103.5**	**8.062**	**7.79**

Table 4.19:- The Level of aflatoxin in different factory CSB, mg/Kg

Processing	Sample Code	Parameters, µg/Kg			
		Aflatoxin G_2	Aflatoxin G_1	Aflatoxin B_2	Aflatoxin B_1
Roasting	R-12 -03-2018 AZ	**2.12 ± 11**^{b}	**4.22 ± 18**^{a}	**1.48 ±13**^{b}	**4.66± 0.22**^{b}
	R-13 -03-2018 AZ	**1.26± 0.52**^{a}	**7.02± 0.06**^{b}	**3.07± 0.64**^{c}	**2.49± 0.32**^{a}
Extrusion	E-12 -03-2018 AZ	**1.71 ± 0.20**^{a}	**3.15 ±0.62**^{a}	**0.7 ± 0.11**^{a}	**2.63± 0.21**^{a}
	E-13 -03-2018 AZ	**< 1 (LOQ)**	6.47 **±0.94**^{b}	**< 1 (LOQ)**	**< 1 (LOQ)**

Mean value + standard deviation, n=3.

Means in the same column with different letters are significantly different (P<0.05)

Key1: a, b, c, d, e, f ….. are superscripts given to show the significant difference between means

Samples were analyzed in triplicate for AFG2, AFG1, AFB2 and AFB1 contamination and determined at parts per billion (µg/kg) levels in CSB by immunoaffinity column cleanup and reversed-phase liquid chromatography with fluorescence detection. The samples showed that the mean total aflatoxin level of 12.49, 13.84, 8.19 and 6.47 µg/kg for roasting (R-12-03-2018

AZ and R-13-03-2018 AZ) and extrusion (E-12-03-2018 AZ and E-13-03-2018 AZ) respectively.

According to one way ANOVA, there is significance difference ($P<0.05$) within each processing of SC-CSB industries, Roasting cooking (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion cooking (E-12 -03-2018 AZ and E-13 -03-2018 AZ).

The maximum level of total aflatoxin as per Ethiopian compulsory standard requirements for CSB is 10 µg/kg. The result of total aflatoxin level for roasting cooking processing industries (R-12 -03-2018 AZ and R-13 -03-2018 AZ) exceeds these limits. But according to World Food Program, (WFP, 2014), the maximum level of total aflatoxin for CSB is 20 µg/kg. So that Roasting cooking processing (R-12 -03-2018 AZ and R-13 -03-2018 AZ) of CSB has fulfilled the standard requirement of WFP but not Ethiopian national compulsory requirements. These implies that Extrusion cooking processing have significant effect on reduction of aflatoxin from contaminated crop than roasting cooking processing. CSB, which is produced by roasting cooking processing, has low chance to reduce aflatoxin contamination and have high risk and impact on the health of end-users. The food product which is contaminated with aflatoxin has a chronic effect of health of consumers alike can inhibit protein and DNA synthesis, liver damage, Mental impairment, Edema and even death (Lizárraga-Paulín, *et al.*, 2011)

Once the food product is contaminated with aflatoxin, it should never be used by humans especially infants and children. As per the findings of this research, extrusion cooking (E-12 -03-2018 AZ and E-13 -03-2018 AZ), the examined samples have shown that there's aflatoxin contamination in the CSB but the results are below both the International and National standards. It should be noted that, Even though the total amount of aflatoxin presented in the SC-CSB is below the national and International standard limits, aflatoxin contamination may increase because of improper handling, storage and transportation of CSB.

In this study, Aflatoxin B_1 contamination level of the SC-CSB flour samples were 4.66, 2.49, 2.63 and <1 mg/100g for roasting (R-12-03-2018 AZ and R-13-03-2018 AZ) and extrusion (E-12-03-2018 AZ and E-13-03-2018 AZ) samples respectively. There is significance difference ($P >0.05$) within each cooking processing. The maximum permissible level of Aflatoxin B1 level for SC-CSB as per Ethiopian Compulsory standard Requirement table is 2.0 µg/kg.

The results of AFL B1 in this study, Aflatoxin B_1 contamination level of the CSB for R-12-03-2018 AZ, R-13-03-2018 AZ and E-12-03-2018 AZ are exceeded the limit of Ethiopian Compulsory standard requirements. Aflatoxin B1 is the most toxic and carcinogenic as

compared to other aflatoxin types. Due to this fact, Aflatoxin B1 contamination level in the above three CSB flour exceeds the standard limits mean that it is not safe for human consumptions. One of the extrusion cooking processing samples of CSB, E-13-03-2018 AZ, aflatoxin B1 level is less than LOQ (< 1). On the other hand, aflatoxin B1 is detected but not quantified in E-13-03-2018 AZ which has the result between the limit of detection and quantification.

4.6 Microbiological Quality of CSB

Flour is susceptible to spoilage especially when stored improperly (in humid conditions that encourage moisture absorption) or for too long. Under such conditions, flours have been shown to develop off flavors that could result in a low quality product when used for cooking. Higher Coliforms count, Total Aerobic Plate Counts, and E. coli counts more than the legal limits indicate poor sanitation and/or problems with the process control and handling of the raw materials and their products.

Microbiological safety parameters were conducted for each research sample by considering all microbiological precautions in triplicate and the result is presented in table 4.20.

Table 4.20:- Microbiological quality of CSB

Processing	Sample Code	Parameters		
		Total Aerobic Plate count/gm of the sample at 30^{o}C for 72 hrs	Yeasts and Molds/gm of the sample at 25 ^{o}C for 5 days	Total Coliforms/gm of the sample at 37^{o}C for 48 hrs
Roasting	R-12 -03-2018 AZ	1.6 x 10^4 cfu/gm	1.77 x 10^3 cfu/gm	4.3 x 10^1 confirmed coliforms/gm
	R-13 -03-2018 AZ	4 x 10^3 cfu/gm	1.54 x 10^3 cfu/gm	3.9 x 10^1 confirmed coliforms/gm
Extrusion	E-12 -03-2018 AZ	1.5 x 10^3 cfu/gm	<10cfu/gm	9 confirmed coliforms/gm
	E-13 -03-2018 AZ	6.3 x 10^3 cfu/gm	1.41 x 10^3 cfu/gm	2.3 x 10^1 confirmed coliforms/gm

Total aerobic plate count: Total aerobic plate counts indicate general microbiological quality and hygienic status of CSB flour sample. According to WFP (2014) and Ethiopian

Compulsory standard, CES 139 (2015), the recommended limits of Mesophyllic aerobic Bacteria (cfu/g) in SC-CSB are < 100,000 cfu/g and < 10,000 cfu/g respectively. As illustrated in table 4.20, the total aerobic plate count were 1.6 x 10^4 cfu/gm, 4 x 10^3 cfu/gm, 1.5 x 10^3 cfu/gm and 6.3 x 10^3 cfu/gm in the two process roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) respectively.

It was found that both roasting cooking processing R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion cooking (E-12 -03-2018 AZ and E-13 -03-2018 AZ) flour samples harbored Mesophyllic aerobic bacteria below the national acceptable limit (CES 139, 2015) except one sample from roasting cooking process (R-12 -03-2018 AZ). According to WFP, (2014), all CSB flour samples have fulfilled the international standard requirement for total aerobic plate count. This may be due to the environmental contamination or erroneous handling and storage of raw materials used for production of CSB. As the results, Extrusion cooking processing has a positive impact on the reduction of total microbiological contamination as compared to roasting cooking processing.

Yeasts and Molds: According to WFP (2014) and CES 139 (2015), the recommended limit for yeast and mold in SC-CSB flour is 1.0 x 10^3 cfu/gm. Molds can produce mycotoxin and higher concentration of these organisms can cause deterioration of food and food borne illness (Oi-Wah Lau, *et al*, 2000). AS illustrated in table 4.20, the results were 1.77 x 10^3 cfu/gm, 1.54 x 10^3 cfu/gm, <10cfu/gm and 1.41 x 10^3 cfu/gm for process of roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018 AZ and E-13 -03-2018 AZ) respectively. Yeast and mold counts were found to lie above the recommended limit in all samples except one sample which showed < 10 cfu/g of yeast and mold in Extrusion cooking processing flour sample (E-12 -03-2018 AZ).

Total Coliforms: Members of Coliforms group are indicator microorganisms which provide evidence about the hygiene standard maintained during production. Presence of Coliforms in flour sample suggests the presence of other enteric pathogenic bacteria samples contaminated and may pose risk to public health. Coliforms bacteria are present in the environment and feces of all warm-blooded animals and humans. Coliforms bacteria are unlikely to cause illness. Infants, young children, and immuno-compromised individuals are at most risk for health concerns with Coliforms.

The recommended limits of total Coliforms in SC-CSB according to WFP (2014) and Ethiopian Compulsory standard, CES 139 (2015) are <100 and < 10 respectively. Thus, the results for the two process roasting (R-12 -03-2018 AZ and R-13 -03-2018 AZ) and extrusion (E-12 -03-2018

AZ and E-13 -03-2018 AZ) were 4.3 x 10^1, 3.9 x 10^1, 9 and 2.3 x 10^1 confirmed Coliforms/gm respectively. All samples have fulfilled the requirements of WFP (2014), but have not fulfilled the national compulsory standard requirements except one extrusion sample (E-12 -03-2018 AZ). Higher frequency and a higher load of Coliforms bacteria present in Roasting cooking processing SC-CSB flour indicate ineffective and inefficient cooking processes of the product as compared to extrusion cooking processing. So that the three samples (R-12 -03-2018 AZ, R-13 -03-2018 AZ and E-13 -03-2018 AZ) indicate that the samples were contaminated with disease-causing organisms (pathogens) could be in the sample which comes from the feces of humans or animals.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, attempts were made to evaluate and assess the quality and safety of corn soya blend flour on the basis of two cooking methods (roasting and extrusion) processed at industry level in Ethiopia. The data obtained in this study showed that, extrusion cooking methods have good performance in reduction of toxic contaminants and keep the most important fortified micronutrients and other nutritional properties of the product in required manner as compared to roasting cooking methods.

Proximate composition (moisture, fat, ash, and protein) of SC-CSB in roasting cooking processing method was different from extrusion processing method. In order to satisfy the amount of crude fiber intake, roasting cooking processing method was highly recommended as compared to extrusion cooking methods.

The concentration of each minerals (Fe, Ca and K) in the CSB samples for both cooking processing methods were up to the standard requirements which implies that addition of mineral fortificant is proper and standardized. The vitamin A amount found in both extrusion and roasting cooking methods is almost three times of the standard which implicates that the amount of vitamin A fortificant added in the process is not in the demanded amount and proper attention should be given during the addition of vitamin A fortificant.

The peroxide value and Urease index of CSB have no problem on the two cooking processing methods and also has fulfilled the standard requirements of WFP/WHO/CES. Particle sizes are under the range but still, the better proximity to the standards is found in extrusion processing.

As compared to CES/WHO/WFP criteria, the finding of this study revealed that the corn-soya blend flour was moderately affected by microbiological contaminants and aflatoxin. In addition, the relative proportion of vitamin A was too high as per CES/WHO/WFP standard requirements.

The amount of total aflatoxin, aflatoxin B1 and particle sizes found in products processed by extrusion are within the standards limits but in case of roasting the amount of total aflatoxin and aflatoxin B1 are out of the standard requirements. Extrusion cooking method have better processing efficiency in reduction of those biological contaminants (total plate count, total Coliforms, and yeasts & molds) as compared to roasting cooking process.

Generally, all results of this study, from the two cooking processing methods used to select, samples goes via extrusion cooking processing method has shown a better results in all tests with exceptional result in the value of vitamin A content which is an increased amount in both cooking methods has compared to the standards.

5.2 Recommendations

Based on the study results the following recommendations are forwarded.

- Though crude fiber is less in roasting and more available in extrusion and it is demanded in small amount by infants still the researcher recommends extrusion method because important proteins are abundant in a better amount in extrusion method because protein are very vital for all age groups and both sexes.

- All factories have to take care of the amount of vitamin A fortificant being added to the product because the amount of vitamin found in the final products were almost 3 times of the international standard (WHO/WFP/CES) requirements.

- Extrusion cooking process has better capacity on reduction of microbiological contaminants and aflatoxin during processing as compared to roasting cooking processing. So that the researcher highly recommends the SC-CSB factories to shift their production method from roasting to extrusion so as to minimize the abundance of toxic microorganisms and aflatoxin in the products.
- To minimize Aflatoxins and toxic microbiological contaminants, the industries have to purchase the raw materials which are free from grains attacked with pests/insects.
- The SC-CSB factories better to undertake HACCP based system to improve the existing problem related to mycotoxins. Furthermore, better to check the level of aflatoxin in the raw materials and final product periodically.
- The government should establish total aflatoxin and aflatoxin B1 surveillance and regular monitoring programs by capacitating of food control laboratories, training of inspectors and supporting researchers to have more research findings to rely on in alleviating the existing problem.
- Once aflatoxins happen on SC-CSB, it is difficult to eliminate the toxins through processing. So that industries should be focus on the selection of raw materials during purchasing. Aflatoxins check before going to processing for corn in the independent laboratory is highly recommended.

6. REFERENCES

Abboud, R., Abdallah, J., Abdul-Hussein, M., Abed, E., Abou Mrad, R., Abouljoud, M., and Adams, W. (2014). *American Journal Gastroenterol*, *109*, S681-S715.

Abiose, S. H., Ikujenlola, A. V., & Abioderin, F. I. (2015). Nutritional quality assessment of complementary foods produced from fermented and malted quality protein maize fortified with soybean flour. *Polish Journal of Food and Nutrition Sciences*, *65*(1), 49-56.

Adelekan, D. A. (2003). Childhood nutrition and malnutrition in Nigeria 1 1 This paper was published previously in the South African Journal of Clinical Nutrition. *Nutrition*, *19*(2), 179.

Alavi, S., Bugusu, B., Cramer, G., Dary, O., Lee, T. C., Martin, L., and Wailes, E. (2008). Rice fortification in developing countries: a critical review of the technical and economic feasibility. *A2Z Project, Academy for Educational Development.* Washington, DC, 2008.

Albert, M. M. E., Laverdure, D. E. E., & Paul, K. (2011). Assessment of the quality of crude palm oil from smallholders in Cameroon. *Journal of Stored Products and Postharvest Research*, *2*(3), 52-58.

Alemayehu, D., Desse, G., Abegaz, K., Desalegn, B. B., & Getahun, D. (2016). Proximate, Mineral Composition and Sensory Acceptability of Home Made Noodles from Stinging Nettle (Urtica simensis) Leaves and Wheat Flour Blends. *International Journal of Food Science and Nutrition Engineering*, *6*(3), 55-61.

Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A., Behjati, S., Biankin, A. V., and Boyault, S. (2013). Signatures of mutational processes in human cancer. *Nature*, *500*(7463), 415.

Allen, A. P., & Gillooly, J. F. (2006). Assessing latitudinal gradients in speciation rates and biodiversity at the global scale. *Ecology Letters*, *9*(8), 947-954.

Ashagrie Zewdu and Paulos Getachew: CSB Quality Control and Management Manual, HACCP SOPS to Ensure Safety of Corn Soy Blend (CSB) Processed at Factory Level: published by Center For Food Science and nutrition in collaboration with Techno serve, 2017.

Batool, S. A., Rauf, N., Tahir, S. S., & Kalsoom, R. (2012). Microbial and Physico-chemical contamination in the wheat flour of the twin cities of Pakistan. *International Journal Food Safety, 14*(6), 75-82.

Bau, H. M., Villaume, C., Nicolas, J. P., & Méjean, L. (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (Glycine max) seeds. *Journal of the Science of Food and Agriculture*, *73*(1), 1-9.

Belayneh Zeleke, (June 2011)., The role of targeted supplementary feeding (tsf) on child nutrition under the age of five in south nation nationalities people region case of

mareko worda, addis ababa univeristy school of gradute studies college of development studies department of food security.

Berghofer, L. K., Hocking, A. D., Miskelly, D., & Jansson, E. (2003). Microbiology of wheat and flour milling in Australia. *International Journal of Food Microbiology*, *85*(1-2), 137-149.

Bullerman, L. B., & Bianchini, A. (2007). Stability of mycotoxins during food processing. *International Journal of Food Microbiology*, *119*(1-2), 140-146.

Carnaghan, R. B. A., Hartley, R. D., & O'kelly, J. (1963). Toxicity and fluorescence properties of the aflatoxins. *Nature*, *200*(4911), 1101.

Carvalho, A. W. D., Natal, D. I. G., Silva, C. O. D., Dantas, M. I. D. S., Barros, E. G. D., Ribeiro, S. M. R., and Martino, H. S. D. (2013). Heat-treatment reduces anti-nutritional phytochemicals and maintains protein quality in genetically improved hulled soybean flour. Food Science and Technology, 33(2), 310-315.

CES 139: 2015: Prepackaged and prepared foods – Super cereal corn soya blend (SC - CSB) – Specifications

D. Sarkar, Handbook for Total Quality Management, Infinity Books, New Delhi India, 2000.

Dary, O., & Mora, J. O. (2002). Food fortification to reduce vitamin A deficiency: International Vitamin A Consultative Group recommendations. *The Journal of Nutrition*, *132*(9), 2927S-2933S.

Das, J. K., Salam, R. A., Kumar, R., & Bhutta, Z. A. (2013). Micronutrient fortification of food and its impact on woman and child health: a systematic review. *Systematic Reviews*, *2*(1), 67

De Aquino, A. C., Lima, C. D. S., Feitosa, F. X., Gallão, M. I., Pinto, G. A. S., & De Sant'ana, H. B. (2014). Chromatographic, rheological and cytochemistry evaluation of “bacuri” pulp (Platonia insignis MART.). *Boletim do Centro de Pesquisa de Processamento de Alimentos*, *32*(1).

De Pee, S., van den Briel, T., van Hees, J., & Bloem, M. W. (2010). Introducing new and improved food products for better nutrition. *WFP: Revolution: From Food Aid to Food Assistance. WFP: ROME*

Desalegn, B. B., Abegaz, K., & Kinfe, E. (2015). Effect of blending ratio and processing technique on physicochemical composition, functional properties and sensory acceptability of quality protein maize (QPM) based complementary food. *International Journal of Food Science and Nutrition Engineering*, *5*(3), 121-129.

Devero, A. (1999). Aflatoxins: The effects on human and animal health. *Biol*, *4900*.

Devries, J. W., & Silvera, K. R. (2002). Determination of vitamins A (retinol) and E (alpha-tocopherol) in foods by liquid chromatography: collaborative study. *Journal of AOAC International*, *85*(2), 424-434.

Doster, M. A., & Michailides, T. J. (1997, June). Susceptibility of maturing Calimyrna figs to decay by aflatoxin-producing fungi in California. In *I International Symposium on Fig 480* (pp. 187-192).

Dwyer, J. T., Wiemer, K. L., Dary, O., Keen, C. L., King, J. C., Miller, K. B., and Jarvis, A. B. (2015). Fortification and Health: Challenges and Opportunities–. *Advances in Nutrition*, *6*(1), 124-131.

Enyisi, S., Orukotan, I., Ado, A., & Adewumi, A. A. J. (2015). Total aflatoxin level and fungi contamination of maize and maize products. *African Journal of Food Science and Technology*, *6*, 229-233.

Ethiopian Health and Nutrition Research Institute, (1997), Food Composition Table Use In Ethiopia, Part III.

Ethiopia's demand for Corn-Soya Blend expands due to drought, 2016. USDA Foreign Agricultural Service, 1-19.

(https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Ethiopia%E2%80%99s%20Demand%20for%20CornSoya%20Blend%20Expands%20Due%20to%20Drought_Addis%20Ababa_Ethiopia_1-19-2016.pdf)

European Commission Regulation (EC) No 401/2006, SOP- Sampling instructions for assessing microbiology and/or toxin of stacked 'flour' foods (i.e. flours, meal, and FBF), 2010

European Commission. (2006). Commission Regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of Eurpean Union*, *70*, 12-34.

Gandhi, A. P. (2009). Quality of soybean and its food products. *International Food Research Journal*, *16*(1), 11-19.

Gock, M. A., Hocking, A. D., Pitt, J. I., & Poulos, P. G. (2003). Influence of temperature, water activity and pH on growth of some xerophilic fungi. *International Journal of Food Microbiology*, *81*(1), 11-19.

Guidance, F. M. R. (2011). A Guide for Grain Elevators. *Feed Manufacturers, Grain Processors and Exporters*.

Gwirtz, J. A., & Garcia-Casal, M. N. (2014). Processing maize flour and corn meal food products. *Annals of the New York Academy of Sciences*, *1312*(1), 66-75.

Hartley, R. D., Nesbitt, B. F., & O'kelly, J. (1963). Toxic metabolites of Aspergillus flavus. *Nature, London*, *198*(4885).

Hell, K., Fandohan, P., Bandyopadhyay, R., Kiewnick, S., Sikora, R., & Cotty, P. J. (2008). Pre- and post-harvest management of aflatoxin in maize: An African perspective. *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade*, 219-229.

Hocking, A. D. (2003). Microbiological facts and fictions in grain storage. *In Proceedings of the Australian Postharvest Technical Conference* (pp. 55-58). Canberra: CSIRO.

Ikujenlola, A. V., & Adurotoye, E. A. (2014). Evaluation of quality characteristics of high nutrient dense complementary food from mixtures of malted quality protein maize (Zea mays l.) and steamed cowpea (Vigna unguiculata). *Journal of Food Processing & Technology*, *5*(1), 1.

Ikujenlola, V. A., & Fashakin, J. B. (2005). The physico-chemical properties of a complementary diet prepared from vegetable proteins. *Journal of Food Agriculture and Environment*, *3*(3/4), 23.

International Organization for Standardization, ISO 24333 (2009): Cereals and cereal products — Sampling.

Inyang, C. U., & Idoko, C. A. (2006). Assessment of the quality of ogi made from malted millet. *African Journal of Biotechnology*, *5*(22).

Iwe, M. O. (2003). The science and technology of soybean. *Rojoint Communication Services Ltd, Uwani, Enugu, Nigeria*, 84-110.

Johnson Q, Mannar V, Ranum P. Fortification handbook Vitamin and mineral fortification of wheat flour and maize meal. Ottawa: Micronutrient Initiative, 2004.

Johnston, K., Ramos, B., & Weissenborn, E. (2011). Fortified Blended Foods.

Kalagbor, I. A., Barisere, V., Barivule, G., Barile, S., & Bassey, C. (2014). Investigation of the presence of some heavy metals in four edible vegetables, bitter Leaf (Vernonia amygdalina), scent Leaf (Ocimum gratissimum), water leaf (Talinum triangulare) and fluted pumpkin (Telfairia occidentalis) from a cottage farm in Port Harcourt. *Research Journal of Environmental and Earth Sciences*, *6*(1), 18-24.

Kalantari, H., & Kalantari, G. H. (2007). Evaluation of Aflatoxins Contamination In Baby Food Supplements (Mamana & Ghoncheh). *Jundishapur Journal of Natural Pharmaceutical Products*, 2011(01, Winter), 42-50.

Karakochuk, C., van den Briel, T., Stephens, D., & Zlotkin, S. (2012). Treatment of moderate acute malnutrition with ready-to-use supplementary food results in higher overall recovery rates compared with a corn-soya blend in children in southern Ethiopia: an operations research trial–. *The American journal of clinical nutrition*, *96*(4), 911-916.

Katsa, M., Proestos, C., & Komaitis, E. (2016). Determination of Fat Soluble Vitamins A and E in Infant Formulas by HPLC-DAD. *Current Research in Nutrition and Food Science Journal*, *4*(Special Issue Nutrition in Conference October 2016), 92-96.

Kikafunda, J. K., Tumwine, J. K., & Walker, A. F. (2003). Weaning foods and practices in central Uganda: *A cross-sectional study*.

Kinyuru, J. N., Konyole, S. O., Onyango-Omolo, S. A., Kenji, G. M., Onyango, C. A., Owino, V. O., and Roos, N. (2015). Nutrients, functional properties, storage stability and

costing of complementary foods enriched with either termites and fish or commercial micronutrients. *Journal of Insects as Food and Feed*, 1(2), 149-158.

Klemm, R. D., West Jr, K. P., Palmer, A. C., Johnson, Q., Randall, P., Ranum, P., & Northrop-Clewes, C. (2010). Vitamin A fortification of wheat flour: considerations and current recommendations. *Food and Nutrition Bulletin*, *31*(1_suppl1), S47-S61.

Klich, M. A. (2007). Aspergillus flavus: the major producer of aflatoxin. *Molecular plant pathology*, *8*(6), 713-722.

Kumwenda, G., Nhlema, B., Maganga, G., Rogers, B., Walton, S., & JWP, B. (2016). Feasibility and acceptability study of preparing corn soy blend with fortified vegetable oil in Malawi: formative research, report to USAID. *Boston: Tufts University*.

Kyritsi, A., Tzia, C., & Karathanos, V. T. (2011). Vitamin fortified rice grain using spraying and soaking methods. *LWT-Food Science and Technology*, *44*(1), 312-320.

LaGrone, L. N., Trehan, I., Meuli, G. J., Wang, R. J., Thakwalakwa, C., Maleta, K., & Manary, M. J. (2012). A novel fortified blended flour, corn-soy blend "plus-plus," is not inferior to lipid-based ready-to-use supplementary foods for the treatment of moderate acute malnutrition in Malawian children. The *American journal of Clinical Nutrition*, 95(1), 212-219.

Landen Jr, W. O., Eitenmiller, R. R., & Ye, L. (2016). *Vitamin analysis for the health and food sciences*. CRC press.

Lau, O. W., & Wong, S. K. (2000). Contamination in food from packaging material. *Journal of Chromatography A*, *882*(1-2), 255-270.

Liberato, S. C., & Pinheiro-Sant'Ana, H. M. (2006). Fortification of industrialized foods with vitamins. *Revista de Nutrição*, *19*(2), 215-231.

Lizárraga-Paulín, E. G., Moreno-Martínez, E., & Miranda-Castro, S. P. (2011). Aflatoxins and their impact on human and animal health: An emerging problem. In *Aflatoxins-Biochemistry and Molecular Biology*. InTech.

Magan, N., & Aldred, D. (2007). Post-harvest control strategies: minimizing mycotoxins in the food chain. *International Journal of Food Microbiology*, *119*(1-2), 131-139.

Manary, M. J., Sandige, H. L., & Maleta, K. (2009).

Mark Manary MD, Cindy Y. Chang. (2012). Comparing Milk Fortified Corn-Soy Blend (CSB++), Soy Ready-to-Use Supplementary Food (RUSF), and Soy/Whey RUSF (Supplementary Plumpy®) in the Treatment of Moderate Acute Malnutrition.

Members, W. G., Benjamin, E. J., Blaha, M. J., Chiuve, S. E., Cushman, M., Das, S. R., ... & Gillespie, C. (2017). Heart disease and stroke statistics—2017 update: a report from the American Heart Association. *Circulation*, *135*(10), e146.

Mokhtar, N., Elati, J., Chabir, R., Bour, A., Elkari, K., Schlossman, N. P., ... & Aguenaou, H. (2001). Diet culture and obesity in northern Africa. *The Journal of Nutrition*, *131*(3), 887S-892S.

M. Weidenbörner, C. Wieczorek, S. Appel, B. Kunz. 2000. Whole wheat and white wheat - The mycobiota and potential mycotoxins. Food Microbiology, 17: 103-107.

Ndekha, M. J., van Oosterhout, J. J., Zijlstra, E. E., Manary, M., Saloojee, H., & Manary, M. J. (2009). Supplementary feeding with either ready-to-use fortified spread or corn-soy blend in wasted adults starting antiretroviral therapy in Malawi: randomised, investigator blinded, controlled trial. *Bmj*, *338*, b1867.

Nesbitt, B. F., O'kelly, J., Sargeant, K., & Sheridan, A. (1962). Toxic metabolites of Aspergillus flavus. *Nature, London*, *195*(4846).

NGFA (2011). FDA mycotoxin regulatory guidance: A guide for grain elevators, feed manufacturers, grain processors and exporters. Washington, D.C.: *National grain and feed association.*

Oğuz, H., Nizamlıoğlu, F., Dinc, I., Üney, K., & Aydın, H. (2011). Determination of aflatoxin existence in mixed feed, wheat flour and bulgur samples. *Eurasian Journal of Veterinary Sciences*, *27*(3), 171-175.

Okoye, J. I., Nkwocha, A. C., & Agbo, A. O. (2008). Nutrient composition and acceptability of soy-fortified custard. *Continental Journal of Food Science and Technology*, *2*, 37-44.

Ottaway, P. B. (2010). Stability of vitamins during food processing and storage. In *Chemical Deterioration and Physical Instability of Food and Beverages* (pp. 539-560).

Palić, D. V., Lević, J. D., Sredanović, S. A., & Đuragić, O. M. (2008). Quality control of full-fat soybean using urease activity: critical assessment of the method. *Acta Periodica Technologica*, (39), 47-53.

Panchumarthy, R. K. (2015). Direct Solutions to Perceptual Organization Problems.

Patterson, D. S. P., Glancy, E. M., & Roberts, B. A. (1978). The estimation of aflatoxin M1 in milk using a two-dimensional thin-layer chromatographic method suitable for survey work. *Food and Cosmetics Toxicology*, *16*(1), 49-50.

Pérez-Expósito, A. B., & Klein, B. P. (2009). Impact of fortified blended food aid products on nutritional status of infants and young children in developing countries. *Nutrition Reviews*, *67*(12), 706-718.

Rees, D. P., Starick, N., & Wright, E. J. (2003). Current status of the warehouse beetle Trogoderma variabile (Coleoptera: Dermestidae) as a pest of grain storage in Australia. *Wright, EJ, Webb, MC & Highley, E*, 119-121.

Rogers B, Maganga G, Walton S, Jayson L, Passarelli S, Suri D, Langlois B, Chui KK, Boiteau J, Ignowski E,mRosenberg I, Vosti S, Webb P. (2015) Feasibility and Acceptability

Study of Corn Soy Blend and Fortified Vegetable Oil in Malawi. *Report to USAID from the Food Aid Quality Review*. Boston, MA.

Rogers, B. L., Wilner, L. B., Maganga, G., Walton, S. M., Suri, D. J., Langlois, B. K., and Webb, P. (2017). Program changes are effective and cost-effective in increasing the amount of oil used in preparing corn soy blend porridge for treatment of moderate acute malnutrition in Malawi. *Maternal & Child Nutrition*, *13*(4), e12393.

Rossel JB (1999). Measurement of rancidity. In Rancidity in foods. Ed By Allen JC and Hamilton RJ, UK. *Aspen Publishers*, pp. 22-51.

Rowe, J. P., Brodegard, W. C., Pike, O. A., Steele, F. M., & Dunn, M. L. (2008). Storage, preparation, and usage of fortified food aid among Guatemalan, Ugandan, and Malawian beneficiaries: a field study report. *Food and Nutrition Bulletin*, *29*(3), 213-220.

Sargeant, K., Sheridan, A., O'kelly, J., & Carnaghan, R. B. A. (1961). Toxicity associated with certain samples of groundnuts. *Nature*, *192*(4807), 1096.

Schrooyen, P. M., van der Meer, R., & De Kruif, C. G. (2001). Microencapsulation: its application in nutrition. *Proceedings of the Nutrition Society*, *60*(4), 475-479.

Shubhangi Pant and Dipanshu Chinwan, (2014). Food Fortification and Enrichment, *International Journal of Basic and Applied Biology*, 166-168.

Sommer, A., West, K. P., Ross, A. C., & Olson, J. A. (1996). Vitamin A deficiency: health, survival, and vision. *Oxford University Press*, USA.

Sopov, M. et al., 2015, Business Opportunities Report Soy #9 in the series written for the "Ethiopian Netherlands business event 5-6 November 2015, Rijswijk, The Netherlands"

Stoloff, L. (1989). Aflatoxin is not a probable human carcinogen: the published evidence is sufficient. *Regulatory Toxicology and Pharmacology*, *10*(3), 272-283.

Stössel, P. (1986). Aflatoxin contamination in soybeans: role of proteinase inhibitors, zinc availability, and seed coat integrity. *Applied and Environmental Microbiology*, *52*(1), 68-72.

Tegegne, W. A. (2015). Assessment of some heavy metals concentration in selected cereals collected from local markets of Ambo City, Ethiopia. *Journal of Cereals and Oilseeds*, *6*(2),8-13.

Temba, M. C., Njobeh, P. B., & Kayitesi, E. (2017). Storage stability of maize-groundnut composite flours and an assessment of aflatoxin B1 and ochratoxin A contamination in flours and porridges. *Food Control*, *71*, 178-186.

Tufa MA, Urga K, Weledesemayat GT, Mitiku BG (2016). Development and Nutritional Assessment of Complementary Foods from Fermented Cereals and Soybean. *Journal Food Science and Nutrition* 2: 014.

UNHCR, (2014). UNHCR Operational Guidance on the use of Fortified Blended Foods in Blanket Supplementary Feeding Programmes.

US Environmental Working Group (EWG), June 25/2014: htpps://www.independent.co.uk/life-style/health-and-families/health-news/fortified-cereals can lead to dangerous vitamin overdose in children scientists warn 6562937.html.

USAID, (2016). Commodity Specification: Super Cereal *Plus* for use in, *International Food Assistance Programs*.

USDA, (2014). Commodity Requirements: CSBP2 Corn Soy Blend Plus for use in, *International Food Aid Programs*.

USDA. (1996) Fact Sheet: Corn Soy Blend.

Vitamin A: AOAC 992.06^{19th} edition Determination of vitamin A in milk based infant formula By HPLC.

Wari, E., & Zhu, W. Ezra Tsegaye Wari, (2004). Quality Management System in Ethiopian.

Welch, A. A., Fransen, H., Jenab, M., Boutron-Ruault, M. C., Tumino, R., Agnoli, C., and Lund, E. (2009). Variation in intakes of calcium, phosphorus, magnesium, iron and potassium in 10 countries in the European Prospective Investigation into Cancer and Nutrition study. *European Journal of Clinical Nutrition*, *63*(S4), S101.

WFP. (2010). Technical Specifications for the manufacture of: Corn Soya Blend For Young Children And Adults – Csb *Plus*.

Wheeler, K. A., Hurdman, B. F., & Pitt, J. I. (1991). Influence of pH on the growth of some toxigenic species of Aspergillus, Penicillium and Fusarium. *International Journal of Food Microbiology*, *12*(2-3), 141-149.

Wicklow, D. T. (1995). The mycology of stored grain: an ecological perspective. *Stored Grain Ecosystems*, 197-249.

Wicklow, D. T., Weaver, D. K., & Throne, J. E. (1998). Fungal colonists of maize grain conditioned at constant temperatures and humidities. *Journal of Stored Products Research*, 34(4), 355-361.

Wimalawansa, S. J. (2013). Rational food fortification programs to alleviate micronutrient deficiencies. J*ournal of Food Processing Technology*, *4*, 257-267.

Wood, J., & Sibanda-Mulder, F. (2011). Improvement of fortified blended foods CSB products and Unimix. *PowerPoint Presentation. Accessed August*, *8*.

World Bank, (2005). Ethiopia Country Status Report on Health and Poverty. Volume II: Main Report.

World food Program, (2008), Food Fortification in Malawi.

World Food Programme. (2002). Fortified Blended Foods: Recipes, Facts and Practical Uses. Pp. 58.

World Health Organization. (2012). Supplementary foods for the management of moderate acute malnutrition in infants and children 6–59 months of age.

Wright, E. J., Webb, M. C., & Highley, E. (2003). Stored grain in Australia 2003. In *Australian Postharvest Technical Conference (2003: Canberra, ACT). Stored Grain Research Laboratory, CSIRO Entomology.*

Wu, F. (2007). Measuring the economic impacts of Fusarium toxins in animal feeds. *Animal Feed Science and Technology*, *137*(3-4), 363-374.

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, *15*(2), 129-144.

Annex A

Specification of Super Cereal Corn Soya Blend (SC -CSB)

CES 139:2015

Table: 6.1 Physico-chemical Characteristics and Functional properties of CSB

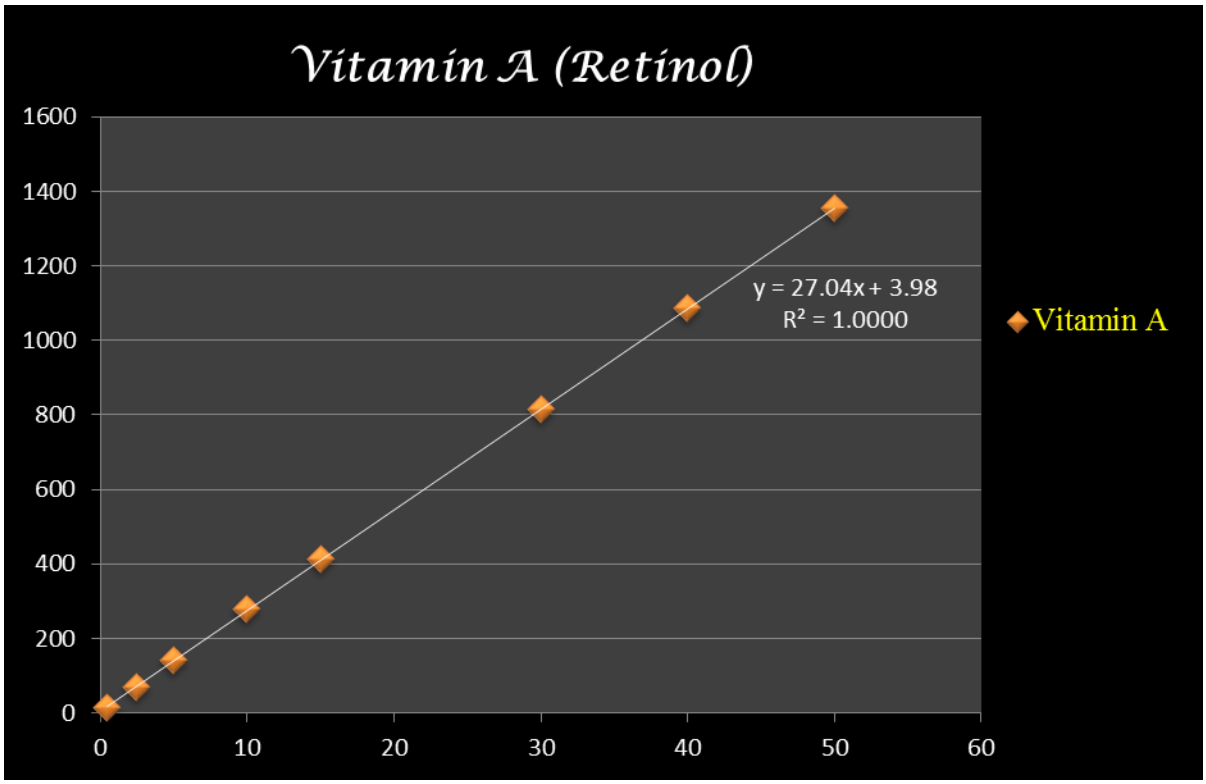
S/N	Quality Parameters	level
Main composition		

1	Moisture	10 g/100g (maximum)
2	Protein	14 g/100g (N x 6.25) (minimum)
3	Total Ash	4.1 g/100g (maximum)
4	Fat	6 g/100g (minimum)
5	Crude Fiber	4 g/100g (maximum)
6	Energy	380 Kcal/100g (minimum)
Physico-chemical characteristics of SC-CSB		
7	Peroxide value	10 meq/kg fat, (maximum)
8	Urease index	0.01-0.20 pH units
9	Particle size	- 95% must pass through a 600 microns sieve. - 98% must pass through a 1,000 microns sieve
Vitamins in SC-CSB		
10	Vitamin A	2770 - 4160 IU/100g
Minerals in SC-CSB		
11	Iron	9.4 – 14.1 mg/100g
12	Calcium	340 - 510 mg/100g
13	Potassium	580 – 870 mg/100g
Mycotoxins in SC-CSB		
14	Aflatoxin (total)	10 ppb (B1+B2+G1+G2) (maximum)
15	Aflatoxin B1	2.0 ppb (maximum)
Microorganisms in SC-CSB		
16	Aerobic Plate count	<10,000 cfu/g
17	Coliforms	<10 cfu/g
18	E. coli	Nil
19	Yeasts and moulds	<1000 cfu/ g

Source: **(**Ethiopian National Compulsory standard, CES 139:2015 for CSB**)**.

Annex B

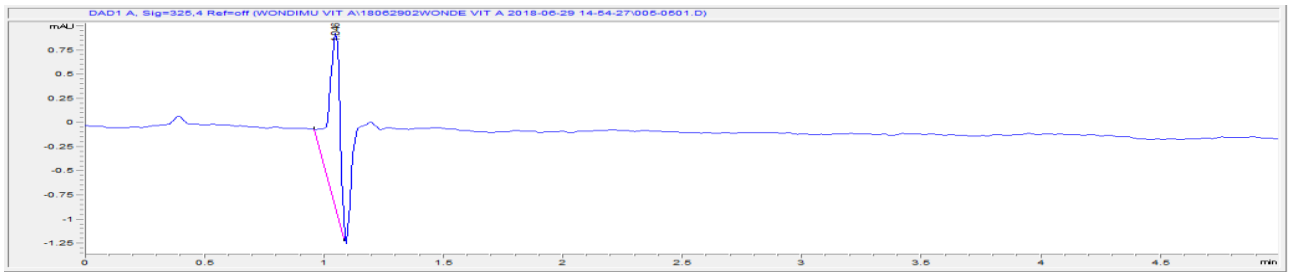
Regression equation vs. Vitamin A (Retinol) concentration



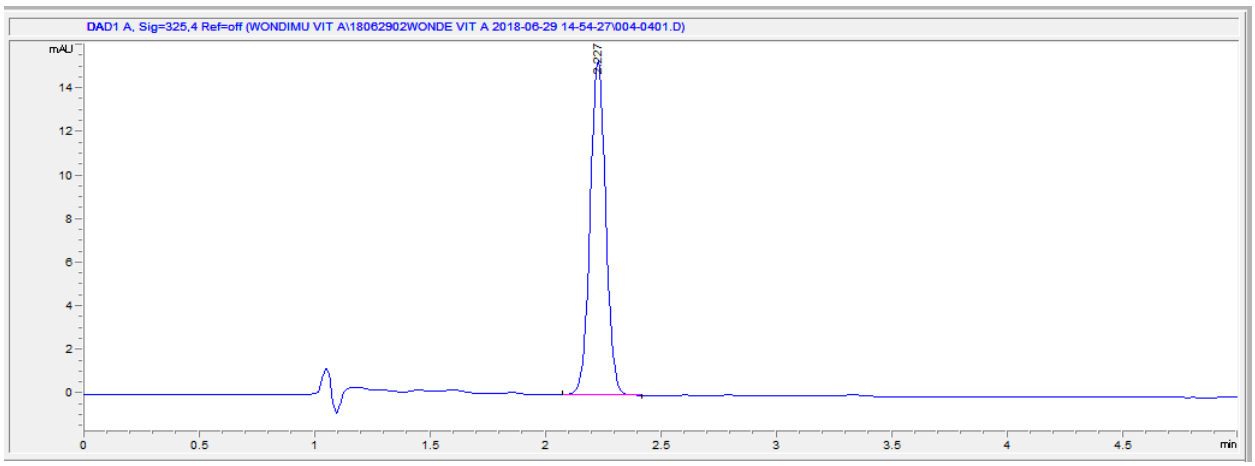
Annex C

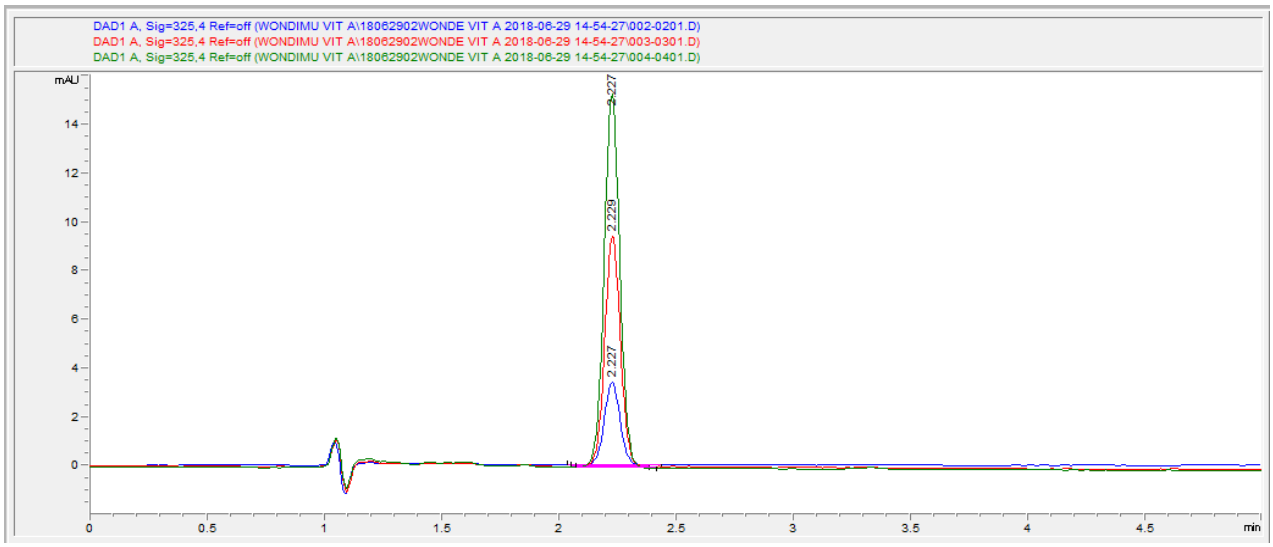
Chromatographic retention time for Vitamin A and Overlay of chromatograms

Blank Run



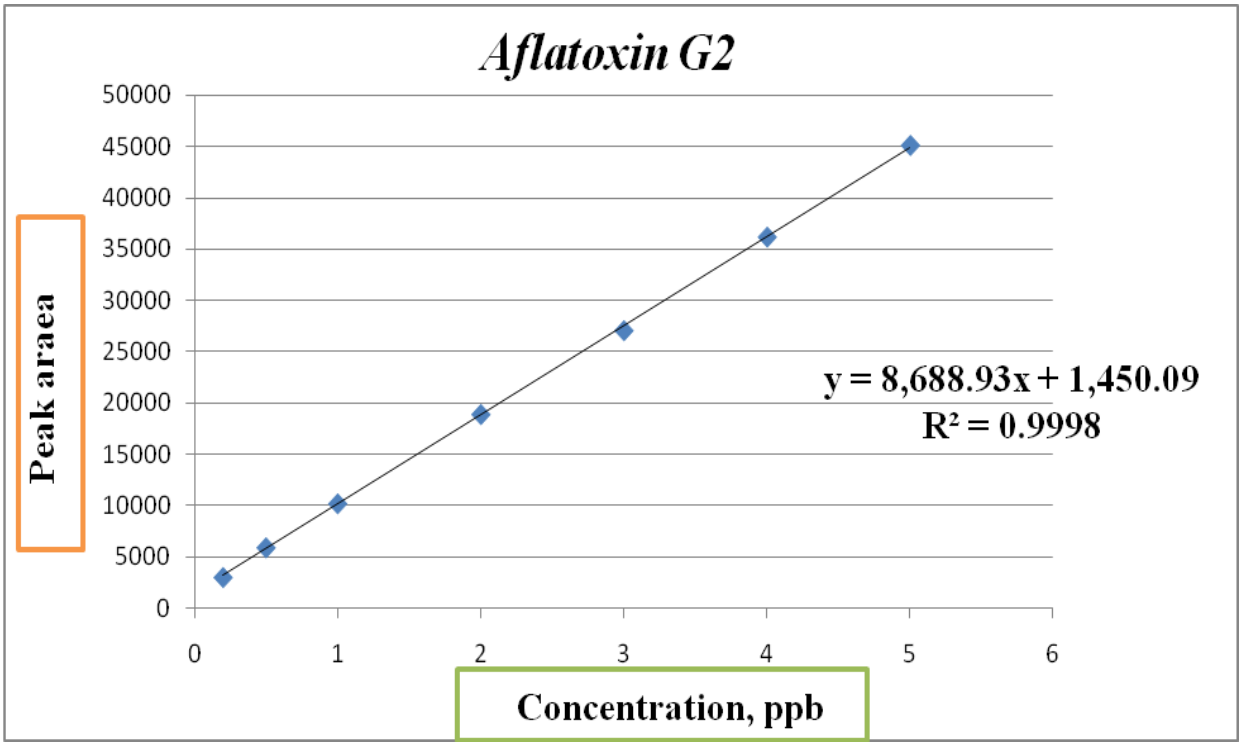
Vitamin A

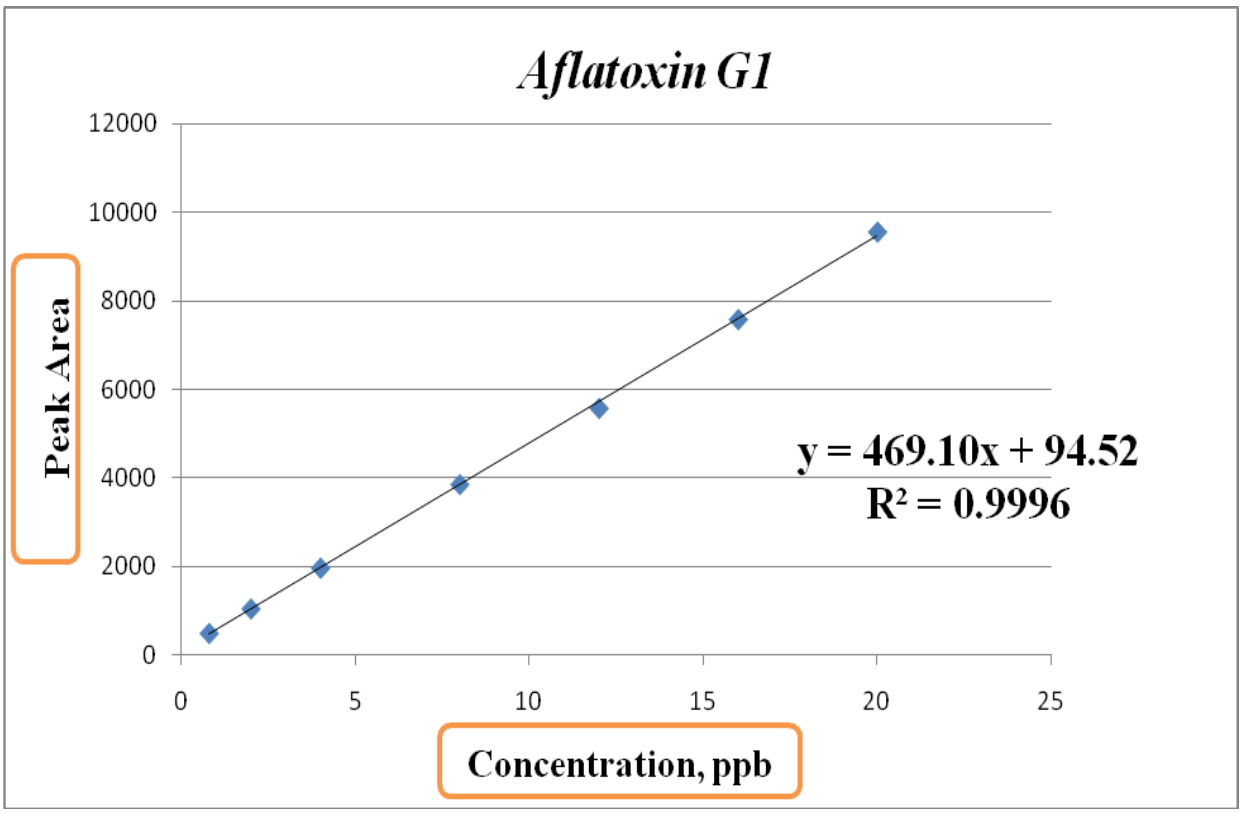


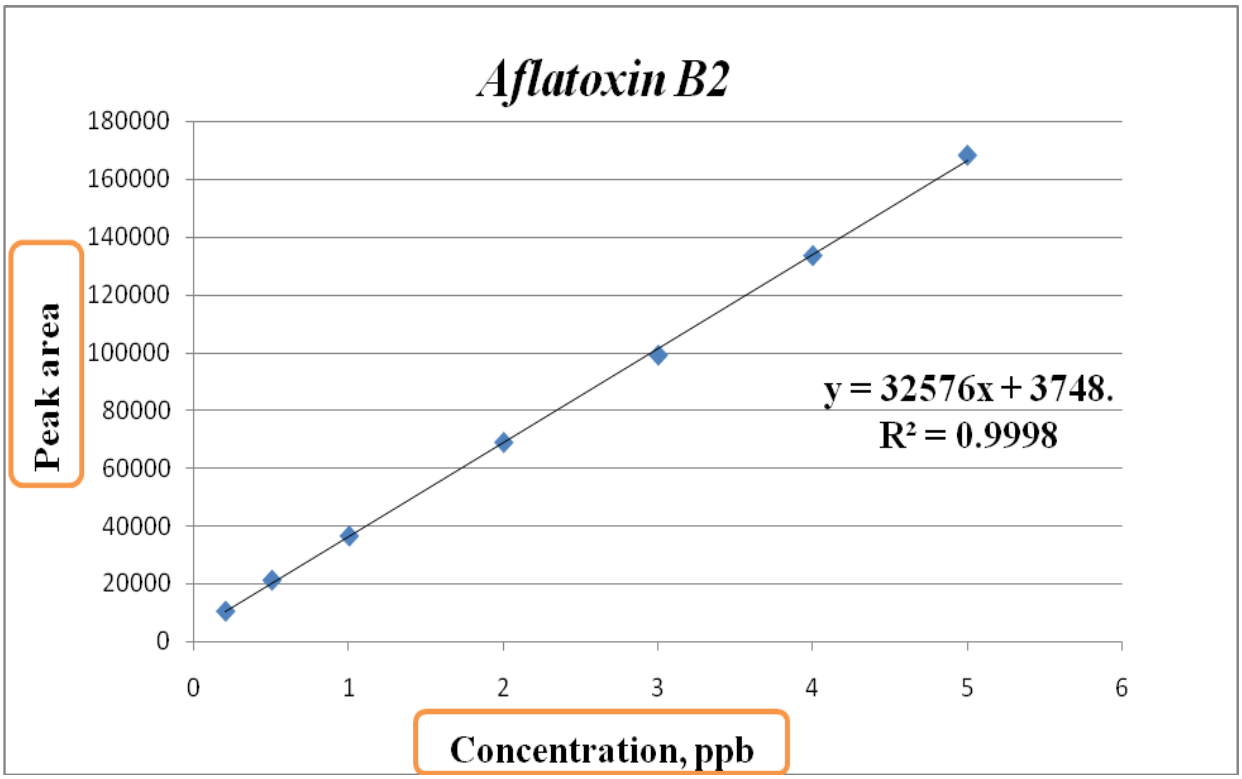


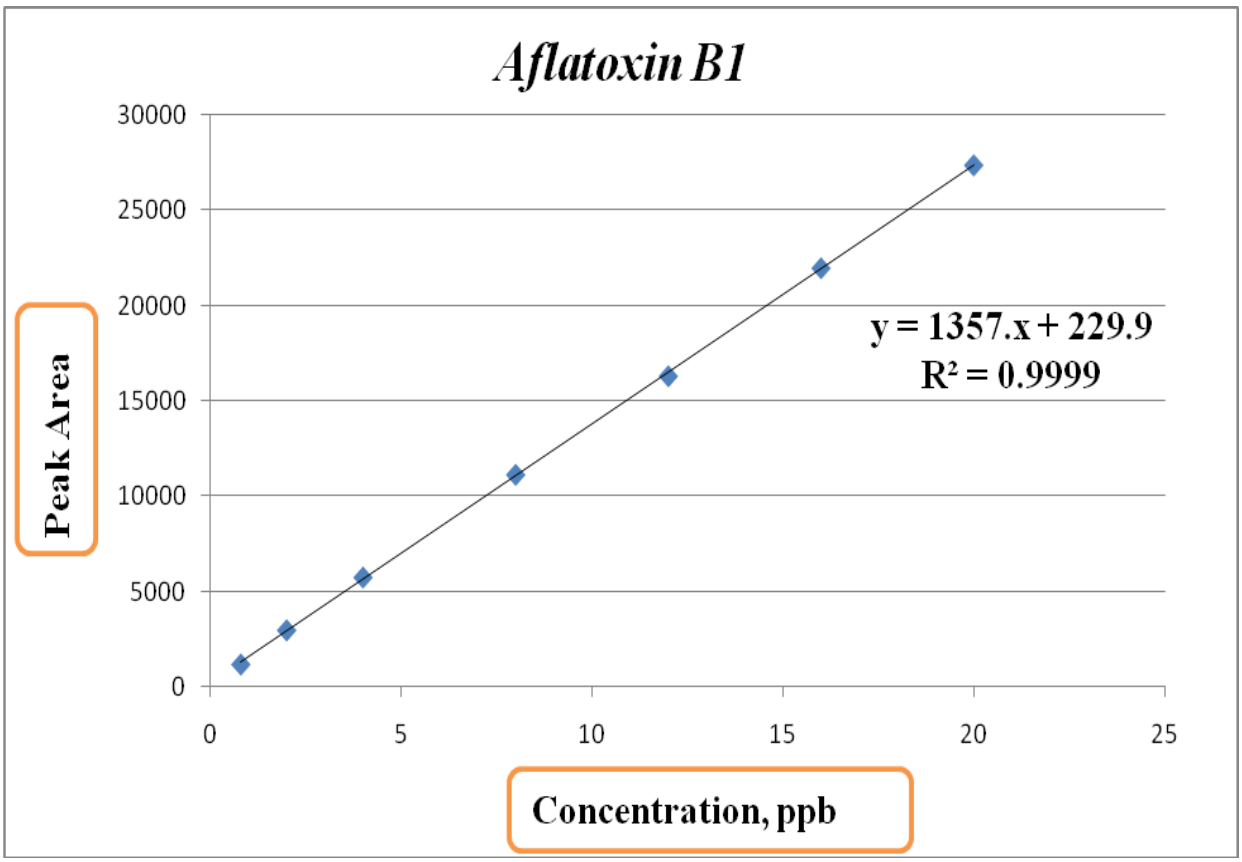
Annex D

Calibration Curve for Aflatoxin G2, G1, B2 and B1


Aflatoxin G2
Peak araea
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
1
2
3
4
5
6
y = 8,688.93x + 1,450.09
R² = 0.9998
Concentration, ppb


Aflatoxin G1
Peak Area
12000
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
y = 469.10x + 94.52
R² = 0.9996
Concentration, ppb

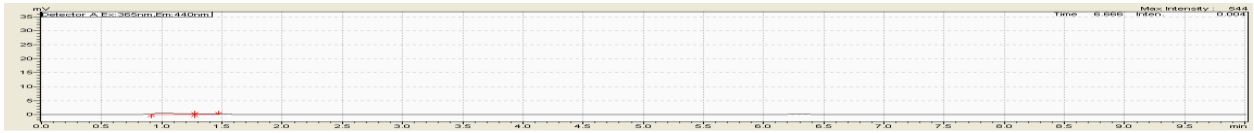

Aflatoxin B2
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Peak area
y = 32576x + 3748.
R² = 0.9998
0
1
2
3
4
5
6
Concentration, ppb


Aflatoxin B1
30000
25000
20000
15000
10000
5000
0
Peak Area
y = 1357.x + 229.9
R² = 0.9999
0
5
10
15
20
25
Concentration, ppb

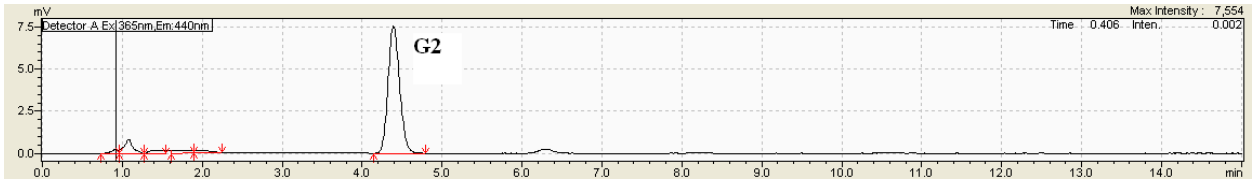
Annex E

Chromatographic retention time (Aflatoxin)

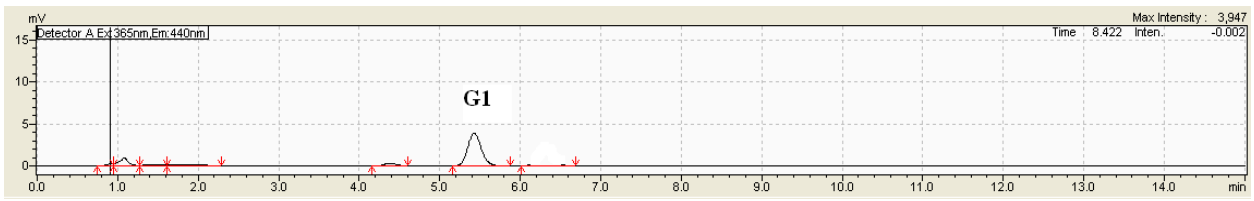
Blank run



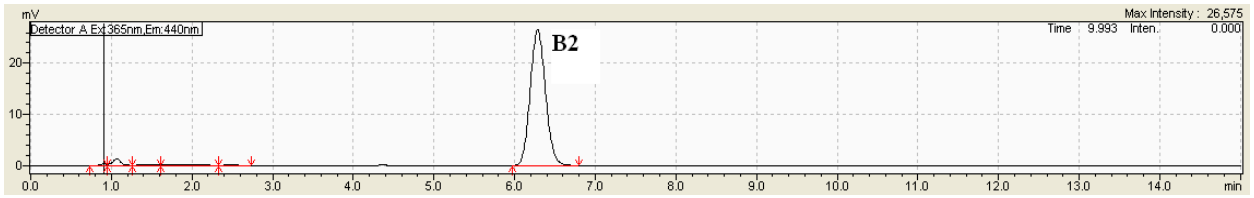
Aflatoxin G2



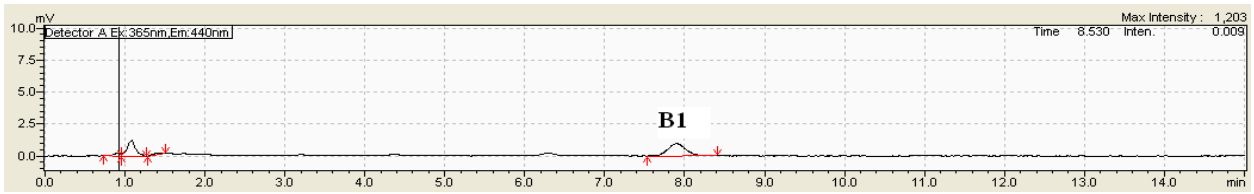
Aflatoxin G1



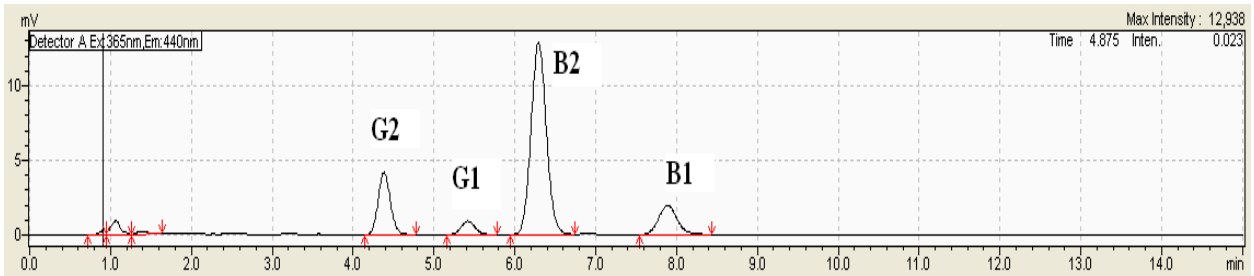
Aflatoxin B2



Aflatoxin B1

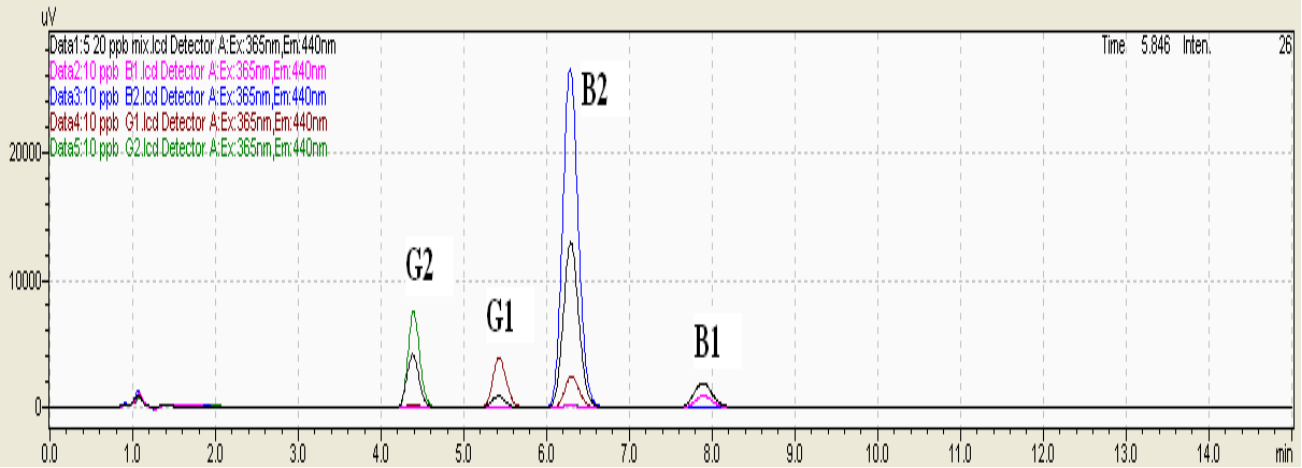


Aflatoxin Mixed Standard



Annex F

Overlay of chromatograms for mixed standards (Aflatoxins)



Annex G

Regression equation vs. the Aflatoxins concentration