Development and quality evaluation of vegan cheese prepared from red kidney bean: a plant-based dairy alternative for sustainable protein choice

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Roll No.: 0119/01

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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The Author

June, 2020

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List of Abbreviation

Abbreviations	Elaboration
%	Percentage
°C	Degree Celsius
μL	Micro Liter
mg	Milligram
mL	Milliliter
FLR	Fluorescence
HPLC	High Performance Liquid Chromatography
RKBC	Red Kidney Bean Cheese
FAO	Food and Agricultural Organization
CVASU	Chattogram Veterinary and Animal Sciences University
TPC	Total Polyphenol Content
TFC	Total Flavonoid Content

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Abstract

The Red Kidney Bean is a good source of protein among the pulses. In this study plant based (vegan) cheese of Red Kidney Bean were developed from whole, dehulled and germinated red kidney bean respectively. Novel vegan cheese was developed from red kidney bean. Proximate analysis, mineral composition, bioactive compound and amino acid profile were analyzed. The ranges of moisture, protein, ash and fat content was found (83.38-77.68)%, (11.21-7.28)%, (0.84-0.48)% and (6.69-1.32)% respectively. Among the minerals, magnesium, phosphorus, potassium, chloride, sodium, calcium, iron, copper and zinc were analyzed and the maximum values obtained for those were 6.6 mg/dL, 2.4 mg/dL, 7.8 mmol/L, 14.6 mmol/L, 41.7 mmol/L, 1.39 mg/dL, 74.1µg/dL, 50 µg/dL and 50 ppm/dL respectively. Among the bioactive compound total polyphenol content and total flavonoids were determined. The maximum values obtained were 0.853 mg GAE/g and 9.002 mg QE/g respectively. Ten essential amino acids and four non-essential amino acids were analyzed. The results were compared with the dairy cheese and soy tofu. The soy tofu and dairy cheeses contain higher amount of nutrients than the vegan cheese. But the price is of the vegan cheese is 15 times lower than the dairy cheese and almost 3 times lower than the soy tofu. So, these products could be sustainable and alternative protein-rich foods that have the potential to be a source of protein for lactose intolerant and allergic people while also being more cost-effective than regular cheese and soy tofu.

Keywords: Red Kidney Bean, Vegan Cheese, Amino Acid Profile, Proximate analysis, Bioactive compounds.

Chapter 1

Introduction

Red Kidney Bean is one of the most popular legumes. They are often called as 'Rajma' in Indian Sub-Continent. Among the beans the red kidney bean (*Phaseolus vulgaris*) contains about 17-25% protein and 50-60% carbohydrates (Hayat *et al.*, 2014; Audu and Aremu, 2018). They are also good sources of vitamins, minerals and bio active compounds (Margier *et al.*, 2018). It can be possible to prepare tofu or cheese like product from the red kidney beans.

Tofu is a well-known product obtained from pulses like soy bean, black gram, peas etc. As pulses contain higher amount of energy, dietary protein, fiber, minerals and vitamins they are widely consumed around the world. They provide different beneficial effects to the health. The protein content of pulses varies from species to species. The range of the protein percentage is 18-32% (Boye *et al.,* 2010). Soybean contains high biological value protein which is about 40% and widely used for Tofu production (Shaheen *et al.*, 2013). The black gram is also used for the production of soy free tofu. The yellow field peas (*Pisum sativum*) can also be used for the production of the pea tofu where the curd formation is done by using magnesium chloride solution. The coagulation of the milk obtained from the soy is influenced by cooking temperature, pH, solid contents; coagulant types, amount, concentration; mixing and adding methods, time etc. (Cai *et al.*, 1998). Among the coagulant's different salts like magnesium chloride, magnesium sulphate (Epsom salt), calcium sulphate etc. are used because they reduce the pH of the solution helps to coagulate the proteins (Obatolu, 2008). These salts are also used to coagulate the milk obtained from other pulses (Cai *et al.*, 2001).

Dairy milk and dairy milk products are nutritious and highly consumed around the world but many people are also allergic and have lactose intolerance to dairy milk products. Among the grains, wheat flour contains good amount of protein. Eventually they also can cause celiac diseases in a group of people. So, protein rich Products prepared from different pulses can be a better choice.

There's a huge scope of utilizing the protein portion of red kidney bean by preparing almost similar products like tofu or vegan cheese. This product obtained from red kidney bean can be beneficial for the lactating mother as well as for the other group of people (Chaudhary *et al.*, 2013). The red kidney bean also contains phytochemicals which are the non-nutrients providing different beneficial health effects. Among the phytochemicals the phenolic compounds, Phytates, flavonoids, vitexin, isovitexin etc. are notable. Some anti-nutrients may also present but they can be inactivated by heat treatment. The kidney beans also contain higher antioxidant property. (Winarsi *et al.*, 2020; Luo *et al.*, 2016).

As, the consumers are now concerned about products using GMOs, they are also searching for products obtained from different crops specially pulses (DePalma *et al.*, 2019).

In this study, vegan cheese were developed containing high biological value protein using Red Kidney Beans (*Phaseolus vulgaris*) with four different formulations. After that the proximate analysis, Mineral analysis, Bio-active compounds analysis, amino acid analysis, cost analysis was done.

1.1. Significance of the study

Protein Energy Malnutrition is one of the major nutritional emergencies. Raw or processed red kidney beans are a good source of nutrients. The products obtained from the red kidney bean can reduce the Protein Energy Malnutrition. Red kidney bean is also cheaper than other animal-based foods. So, it will be easier to get and consume for the general mass. The protein rich red kidney bean tofu/cheese will help to fulfill the demand of protein among the general mass. The study tells the shelf life of the red kidney bean tofu towards different environmental conditions. The essential amino acid profile in different formulations of the vegan cheese can be found. The agro-processors of the country may get a clear idea about the economical and flexible production methods of red kidney bean tofu utilizing our indigenous resources. Production of red kidney bean cheese can help to uplift the public health and economy of Bangladesh. Thus, it will contribute to produce good quality, highly stable and economical vegan cheese to achieve the Sustainable Development Goal (SDG) of the country.

1.2. Objectives:

The Overall objective of the study was to develop a plant based vegan cheese obtained from the Red Kidney Bean which can be a source of potential dairy alternative for sustainable protein choice. The specific aim of the study was to determine the effect of different formulation of Vegan Cheese prepared from Red Kidney Bean on the Proximate composition, Bioactive compounds, Amino Acid Profile, Minerals in the product and to compare the cost with the price of dairy cheese and soy tofu.

Chapter 2

Review and Literature

2.1. Red Kidney Bean

According to Audu and Aremu (2011); Liebenberg *et al.* (1997), Red Kidney Bean is herbaceous legume of the family leguminosae which is a good source of starch, protein, soluble and insoluble fiber, mineral (Potassium, Phosphorus, Iron, Magnesium etc.) and Vitamin B. The Red Kidney Bean is Produced in dry and tropical climate zones in the world.

Scientific Classification of Red Kidney Bean

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Fabales
Family: Fabaceae
Genus: Phaseolus
Species: *Phaseolus vulgaris*

Source: www.cabi.org

In Bangladesh red kidney bean is widely consumed as it is a good source of plant protein. But in most of the cases they are consumed by the general mass after minimal processing like drying, canning etc. As red kidney bean is high in protein, they can be used to prepare new value-added products.

2.2. Vegan Cheese from different beans

Among the protein-based vegetable products tofu is very popular which is derived from the soy bean. According to Fernández-Quintela *et al.*, (1997) and Thrane *et al.*, (2017) soybean contains about 36% protein, (15-18)% of carbohydrates, (7-15)% dietary fiber, (18-22)% fat. The curd formation of soy milk is done by coagulating the soluble protein present in the soy milk with the help of different coagulants.

According to Gebre-Egziabher and Sumner (1983), the curds formed from the field pea contains higher protein than the pea flour which is also similar to the soy curd. Apparently, the curd formed from the field peas are softer in texture than the curd formed from the soy bean. In the study it has been seen that the curdling of the milk of beans can be increased with higher concentration of the coagulant.

2.3. Anti-nutrient Property of Red Kidney Bean

Similar to other beans, the red kidney bean contains phytates, tannin, saponin, phyto-haemagglutinin, lactins, oxalate, phytic acid (as myoinositol hexakisphosphoric acid) which are responsible for anti-nutrients property (Begum *et al.*, 2020). These anti-nutrients affect the bioavailability (for example mineral), nutritional quality (for example, α amylase inhibition), interact the intestinal tract, protein digestibility and amino acid absorption etc. (Audu and Aremu, 2011; Olanipekun *et al.*, 2015; Enrico *et al.*, 2012). According to Olanipekun *et al.* (2015), the red kidney beans are less in industrial or household importance due to some difficulties in cooking and anti-nutritional activities. But these anti nutrients can be deactivated by different processing techniques.

2.4. Magnesium Chloride

Magnesium Chloride is a food additive (E-511) which is used in food as color retention agent and coagulating or firming agent. Magnesium Chlorides are colourless, odourless flakes, granules, lumps or crystals; it is very deliquescent. They are very soluble in water and can be easily soluble in ethanol. Magnesium Chloride are used as coagulant for preparing different tofu/vegan cheeses from soy milk, pea milk etc.

2.5. Agar

Agar (E-406) is authorised in a wide range of foods but most of the time they are used in a limited number of food categories. Agar is also known as Agar-Agar, Bengal, Ceylon, Chinese or Japanese isinglass, Gelose, Japan Agar, Layor Carang etc. They act as different functions in food like bulking agent, carrier, emulsifier, gelling agent, glazing agent, humectant, stabilizer, thickener etc. Agar is dried hydrophilic, colloidal substance extracted from definite marine algae falls in the class Rhodophyceae. They are basically polysaccharide, consisting primarily of D- and L-

galactose units. In their structure they contain a sulfate ester group at every tenth D-galactopyranose unit. Calcium, magnesium, potassium or sodium cations also helps functioning the polysaccharide. (Source: FAO General Standard for Food Additives).

2.6. Magnesium Chloride and Agar-Agar as coagulating agent for the kidney bean cheese

According to the Obatolu (2008), The curdling of soy milk can be done by using coagulants like $CaSO_4$, $CaCl_2$, Allum etc. which produces protein gels that traps the water, soy lipids and other compounds into the matrix forming curds. In some study citric acids, lactic acids, tartaric acid, calcium lactate etc. also used to make the curd in the soy milk (Sengupta *et al.*, 2021).

Magnesium Chloride was used as coagulating agent which is a main component of Nigari, a liquid produced by removing salt from the sea water. The Magnesium Chloride is used as coagulant to produce firm tofu from the soy bean milk. The curd from red kidney bean cheese was prepared. The red kidney bean milk cheese contains less protein than the soy milk. The red kidney beans protein has higher solubility and has better emulsifying and foaming property (Hayat *et al.*, 2014). That's why the curdling of the red kidney bean is a little bit challenging. To strengthen the curd agar-agar were also used. The curd/cheese gave a pudding like texture.

2.7. Proximate Composition of Red Kidney Bean at Different Stage

Processing steps like heating can cause to the decrease in the proximate composition of the food product. So, it is really important to know the proximate composition of the red kidney bean. According to the Ibeabuchi *et al.* (2017), the moisture%, fiber%, fat%, protein%, carbohydrate% and ash% of red kidney bean are 1.06±0.11, 4.00±0.011, 1.57±0.005, 20.31±0.011, 68.03±0.13, 5.00±0.00 respectively. Audu and Aremu, (2011) showed that the processing of the red kidney beans causes deviation (both higher and lower) in the nutrient contents. According to the study, it has been seen that the raw red kidney bean seed contains 2.4±0.23 % of moisture, 4.4±0.52% of ash, 15.8±0.10% of fat, 15.3±0.20% of crude protein, 3.6±0.50% of crude fiber and finally 49.0±0.50% of carbohydrates. Whether, the sprouted red kidney beans were slightly higher in moisture, crude protein and carbohydrates which are 3.0±1.50%, 20.1±2.30% and 59.7±0.50%. But the other contents are found to be lower than the

raw seeds where the % of Ash is 2.0±0.50, Fat% is 11.6±0.60 and crude fiber% is 3.6±0.50%. Winarsi *et al.* (2020) also reported that the protein content of the sprouted kidney bean milk is higher than the raw kidney bean milk.

Table 1: Proximate Composition of Raw Red Kidney Beans

Parameter	Raw Red Kidney Bean Seed
Moisture %	8.12 ± 0.53
Ash %	4.34 ± 0.20
Fat %	1.92 ± 0.15
Crude Protein %	25.78 ± 0.77
Crude Fiber %	6.82 ± 0.31
Carbohydrates %	53.02 ± 1.14

Source: Hayat *et al.*, (2014)

From the above studies it can be seen that proximate percentages of red kidney beans vary region to region, based on the weather, storage condition.

2.8. Mineral Content in the raw kidney bean

The mineral content in the raw kidney bean and processed kidney beans can be observed in different studies. Olanipekun *et al.* (2015) stated that the Iron content in the raw kidney bean, boiled kidney bean and roasted kidney bean are present in 23.04±0.42 ppm, 21.07±1.10 ppm and 19.92±1.04 ppm concentration respectively. And the Zinc content are in 18.32±1.20, 17.94±0.22, 15.72±1.24. It has been seen that those minerals are reduced due to the processing.

2.9. Amino Acid Profile in Raw Kidney Beans and Sprouted Red Kidney Beans

In this study the amino acid profiles of the four kidney bean cheese formulations are determined. To compare it is important to know the amino acid profile of the raw kidney bean and also in the sprouted red kidney bean. According to Mbithi-Mwikya *et al.* (2000), a good percentage of essential amino acids are present in red kidney bean. According to the study about (44.2-45.1) % of total amino acids are present in the red

kidney bean. It has been seen that the lysine content of the red kidney bean was decreased in the sprouted red kidney beans. Different types of processing like heating can also decrease the amino acid content of the red kidney bean. Red kidney bean milk was heated to form the bean cheese. So, the assumption is that the amount of amino acid would be lower than the raw kidney beans. In the raw kidney bean, it has been seen that the amino acids arginine, valine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, tyrosine are present at the concentration of 6.77±0.11g, 5.04±0.38g, 2.94±0.02g, 4.35±0.34g, 8.48±0.24g, 7.18±0.06g, 1.22±0.09g, 5.84±0.15g, 1.06±0.03g and 3.57±0.04 g respectively per 100g of dry sample. Whether in the sprouted kidney beans the compositions are 6.49±0.12 g, 4.98±0.57 g, 2.93±0.07 g, 4.13±0.48 g, 8.37±0.34 g, 6.81±0.12 g, 1.21±0.06 g, 5.83±0.08 g, 1.09±0.03 g, 3.55±0.05 g respectively per 100 g of dry sprouted kidney beans. The result clearly shows that sprouting caused the decrease of the amino acid contents.

Another study reports that different variety of red kidney bean contains different amount of essential amino acids (de Moraes and Angelucci, 1971). The ranges are close to the previous data.

Chapter 3

Materials and Methods

3.1. Site of the Study

The study was conducted in the Department of Food Processing and Engineering, Department of Physiology Biochemistry and Pharmacology, Department of Fishing and Post-Harvest Technology of Chattogram Veterinary and Animal Sciences University.

3.2. Collection of Red Kidney Bean

The Red Kidney Bean (RKB) samples were collected from different area of Chattogram. Then four types of vegan cheese were prepared by using whole RKB, dehulled RKB, almond mixed RKB and germinated RKB.

3.3. Development of Vegan Cheese

Germination of Red Kidney Bean

For the preparation of germinated kidney bean cheese, the kidney beans were allowed to soak in distilled water for overnight (around 6 hours) at room temperature. After that the water was drained off and the beans were placed is a wet cotton cloth which covers them fully and left to germinate for 4 days in a dark place at room temperature. The seeds along with the cloth were moistened at every 24 hours' interval. After 4 days the seeds were germinated and used for preparing vegan cheese.

Red Kidney Bean Milk Preparation:

The red kidney bean milk was prepared by following DePalma *et al.*, (2019) with slide modification. Whole red kidney bean, dehulled red kidney bean and germinated beans are collected and soaked for a 12 hrs. in 2500 ml beakers. After soaking the beans were grinded with addition of water. The quantity of water was calculated by following equation:

(Starting weight/200g) x 550 mL – (end weight – starting weight) = weight of water.

Here, starting weight is the weight of red kidney bean before soaking and the end weight is the weight of red kidney beans after soaking in water.

After soaking and addition of water the kidney beans (whole, dehulled and germinated) were then grinded using bender for 5-6 minutes. The grounded kidney bean transformed into slurry and the slurry was filtered using a cheese cloth. The corners of the cloth were brought together to form a bundle. The bundle was then squeezed to separate the insoluble materials. In case of Soya bean, they are called Okara. This can also be called red kidney bean milk. The filtration process was done for two times to separate the insoluble materials properly. The red kidney bean milk was then allowed to rest at refrigeration temperature (5ºC for 1 hour) so that the suspended solids become settled.

Cheese Formation

The prepared red kidney bean milks are used for curd formation for different formulations. The red kidney bean obtained from dehulled bean is divided into two and then both of them are homogenized followed by the addition of 20 mL of extra virgin olive oil. One part (375 mL) has been used to prepare curd with the addition of ground almond (125 gm). the other part (500 mL) is used to prepared regular curd. Then they were transferred to the heating vessel. The other whole red kidney bean milk and germinated red kidney bean milk were also mixed with 20 mL of extra virgin olive oil and transferred into separate heating vessels. About 500 mL of red kidney bean milk from each formulation was taken and the temperature was raised to 80ºC from 5ºC and heated for about 10-15 minutes with continuous stirring

For clear understanding the recipe of the formulations has been illustrated on **Table 2** and the processing steps are presented on **Figure 1**.

Table 2: Formulations of Red Kidney Bean Cheeses

Ingredients	F1	F2	F3	F4
Whole RKB Milk	500 mL	---	---	500mL
Dehulled RKB Milk	---	500 mL	375 mL	---
Ground Almond	---	---	125 gm	---
Agar Agar Solution	300 mL	300 mL	300 mL	300 mL
Olive Oil	20 mL	20 mL	20 mL	20 mL
Salt	5-7 gm	5-7 gm	5-7 gm	5-7 gm
Magnesium Chloride Solution	30 mL	30 mL	30 mL	30 mL

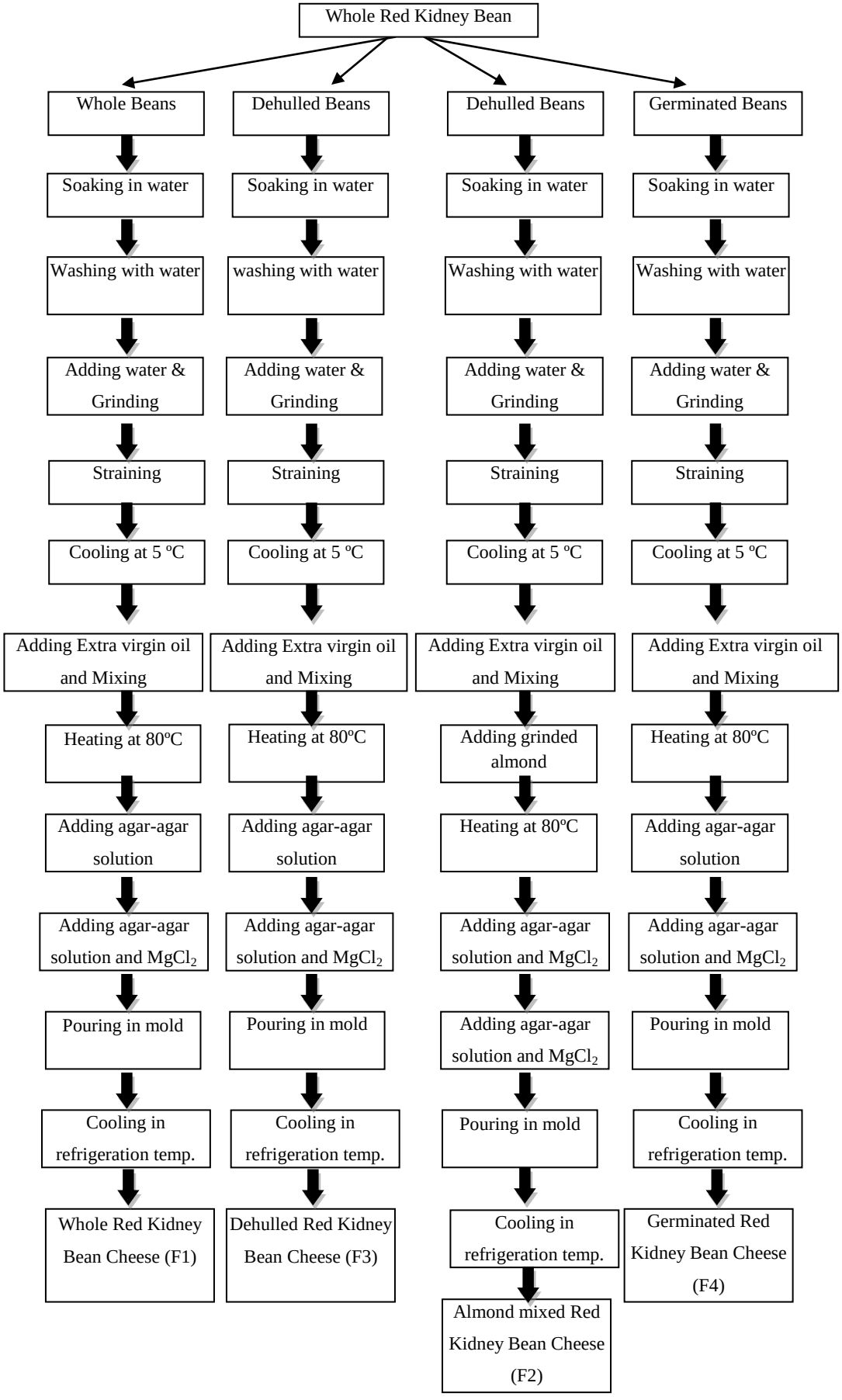

Figure 1: Flow chart of vegan cheese preparation

During heating about 300 mL of preheated Agar-Agar solutions was added to each formulation. The agar-agar solutions were prepared for each formulation by using agar-agar powder. About 3 gm of agar-agar powder was added in about 300 mL of water at room temperature. After that the powder was mixed and heated until all the powder become dissolved in the water. Agar solution was added at room temperature After that the heating was stopped and 30 mL of Magnesium Chloride solution with continuous stirring. The next stage is a little different then the soy curd or pea curd formation. In this stage the heated bean milk was poured into rectangular molds and let them cool in refrigeration temperature. After cooling the curds from different formulations were formed. Then the vegan cheeses are stored in frozen storage below -18ºC. After preparing all the samples, the proximate analysis, mineral tests, bio-active compound tests, amino acid analysis was conducted.

3.4. Cost Analysis

The Cost analysis was done by calculating the price of the raw materials according to the present market price. The local unit price of each item was taken costing for 200 grams of vegan cheese were done. This is to compare the price against dairy cheese and tofu produced locally. In most cases the cheese and tofu are sold in 150 gm to 200 gm of packet.

3.5. Proximate Analysis

3.5.1. Determination of moisture content

The moisture content was determined according to AOAC method (1965).

Every sample of bean cheese was thoroughly mixed. About 5g of the sample was transferred into pre-weighed dry crucible. The crucible and contents were weighed. After that each sample was dried to a constant weight at 95-100ºC under pressure not exceeding 100mm Hg (for about 5 hours). After completely drying each sample, the crucibles were placed in a desiccator to cool. Sample was weighed again and the loss in weight as moisture was recorded.

The percentage moisture was calculated as follows:

$$\% \text{ moisture} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where: W_1= initial weight of empty crucible

W_2= weight of crucible + samples before drying

W_3= final weight of crucible + samples after drying

3.5.2. Determination of ash content

Procedure

The ash content was determined according to AOAC method (1965). 2-5g finely grounded Samples were accurately weighed, sample moisture was evaporated in an oven at 100ºC, into a crucible. Sample was charred on a Bunsen flame. Sample was transferred into a pre-heated muffle furnace at 600ºC and left at this temperature till white or light, moisten with a small amount of water to dissolve salts, sample was dried in an oven and the process was repeated. Sample was cooled in a desiccator and reweighed.

Calculation:

$$\% \text{ Ash (dry basis)} = \frac{\text{Weight of ash}}{\text{Weight of Original Samples}} \times 100$$

$$= \frac{W3 - W1}{W2 - W1} \times 100$$

Where: W_1= initial weight of empty crucible

W_2= weight of crucible + Samples before ashing

W_3= final weight of crucible + Samples after ashing

3.5.3. Determination of protein content

Procedure

The Protein determination was done by using the Kjeldahl Method.

Step 1. Protein Digestion

0.3g of every bean cheese sample was weighed into a Kjeldahl flask**.** Then 4g of catalyst (Potassium sulphate : Copper Sulphate = 9:1). After that 5ml concentrated Sulphuric acid was added into the mixture and 5 glass beads were inserted. Then digestion tubes were placed in the digestion unit () and digested with heating for 30 minutes with occasional shaking till the solution gets a green color. The black particles present at the neck and mouth of the tubes was cleaned with distilled water. Then it was re-heated gently until the green color becomes disappeared. It was then allowed to cool. After cooling, 25mL of water was added on each. After adding water 10 mL of mixed indicator was taken into the conical flask of distillation unit. mixed indicator was prepared by adding 05 mL bromocresol green solution and 3.5 mL Methyl Red solution into 500 mL of 4% Boric Acid solution.

Step 2. Protein distillation and titration

Distillation was done with distillation apparatus (Model: UDK 129, Company: VELP Scientifica). The distillation apparatus was steamed for about 15 minutes before it was used. Under the condenser, 100mL conical flask containing 10 mL of mixed indicator was placed in a way that the condenser tip is under the liquid. 5 ml from the digest was transferred into the body of the apparatus using pipette through a small funnel aperture; the digest was washed down with distilled water followed by 25mL 35% NaOH solution. The mixture was steamed for about 3 minutes to collect adequate ammonium sulphate. The ammonium sulphate receiving flask and the condensed water were removed. Titration of the solution was done in the receiving flask against 0.2N hydrochloric acid and calculation of the nitrogen content was made and hence the protein content of the sample (bean cheeses).

$$\% \text{ of N} = \frac{mL\ of\ titrant \times Strength\ of\ HCL\ (0.2N) \times Equivalent\ of\ Nitrogen\ (0.014)}{Weight\ of\ sample} \times 100\%$$

% Protein = %N ×5.85 (Plant Origin)

3.5.4. Determination of fat content by Soxhlet extraction method

Procedure

Fat content of the products was done by following AOAC method of Analysis (1965). Clean flasks of 250 mL were dried in an oven at 105-110ºC for about 30 minutes. 2g of the sample was weighed and placed into labelled thimbles. The labelled cooled boiling flasks were weighed. About 300ml of petroleum ether (boiling point 40- 60ºC) were taken into the boiling flasks. The top of the extraction thimble was plugged with cotton wool. The Soxhlet extractor apparatus was attached and allowed to reflux for about 6 hours. In this time the petroleum ether collected the fat contents from the sample and gathered them into the flasks. The thimble was removed. The petroleum ether was separated from the flask by heating and they were collected in the top container of the setup and drained into a flask for re-use. When flask was almost free of petroleum ether, the flask was removed and dried at (105-110)ºC for 1 hour. The flask was transferred from the oven into a desiccator and allowed to cool, then weighed.

Calculation:

$$\% \text{ fat} = \frac{\text{Weight of fat}}{\text{Weight of samples taken}} \times 100$$

3.6. Mineral Tests

All the bean cheese samples were analyzed for nine minerals which are Magnesium (Mg), Phosphorus (P), Potassium (K), Chloride (Cl), Sodium (Na), Calcium (Ca), Iron (Fe), Copper (Cu) and Zinc (Zn). The concentration of Mg, P, K, Cl, Na, Ca, Fe was done by using spectrophotometer. The methods for the mineral tests are given below.

Sample Preparation

The samples for the mineral tests were done by Acid Digestion.

Apparatus required for sample preparation: Beaker, Measuring Pipettes, Volumetric flask, Analytical balance, Heating mantle/ hot plate, Filter paper (Whatman No. 541)

Reagents Required: Nitric Acid, Perchloric acid

Procedure

One gram of dry sample was weighted in a conical flask. For wet sample, 5mL HNO_3 and 1mL $HClO_4$ was added (HNO_3:$HClO_4$ = 5:1). Then the flask was placed in a hot plate at 200W for 1-2 hours until full designation. After digestion, it was cooled to room temperature. Then transferred the digested samples into 100 mL volumetric flask and diluted up to 100 marks with deionized water and mixed well. Later, the solution was filtered through whatman filter paper No. 1 and transfer to eppendorf tube for mineral quantification. The mineral contents in the digested compounds were determined by spectro photometer (Humalyzer 3000).

3.6.1. Magnesium

The quantity of magnesium was determined by following the method given by Lindstrom and Diehl (1960).

Principle:

At alkaline pH the Magnesium create a specific binding with calmagite (metallochromic indicator) resulting a complex which can be calculated by measuring the absorption of the complex at certain wavelength (520 ± 20 nm). The intensity of the cromophore formed is proportional to the concentration of magnesium in the sample.

Calmagite + Magnesium → Calmagite- Magnesium Complex (at pH 11.5)

Reagents Composition

Chromogen (R1): Calmagite 75 mmol/L, EGTA 60 mmol/L, Amino-methyl-propanol 0.2 mol/L, KCl 0.2 mol/L, surfactant 0.05% (w/v).

Calcium/Magnesium Standard (CAL): Calcium 10 mg/dL, Magnesium 2 mg/dL

Materials Required

1. Spectrophotometer capable of measuring absorbance at 520 ±20 nm
2. Micro Pipettes with disposable plastic tips
3. Disposable small plastic tubes for the test (PCR Tubes)

Procedure

At first all the samples and reagents were brought to room temperature. Then three small tubes are taken for Blank, Sample and CAL Standard respectively. For Blank tube 1 mL of R1 reagents were taken. For Sample tube 1.0 mL of R1 reagent and 10 µL of each sample were taken. Lastly in CAL standard tube 1.0 mL of R1 reagent and 10 µL of CAL standard are taken. Each sample were mixed and let to stand for 2 minutes at room temperature. After that the absorbance (A) was determined for the Samples and also for the standard at 520 nm against the reagent blank.

Calculations:

mg/dL Magnesium= $\frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times Concentration\ of\ Standard$

3.6.2. Phosphorus

The analysis for phosphorus was done by following the method given by Daly and Erthinghsausen (1972); Gamst and Try (1980).

Principle:

In the presence of sulfuric acid inorganic phosphate reacts with ammonium molybdate to form a phosphomolybdic complex which is measured at 340nm.

$PO_4^{3-} + H^+ + (NH4)_6Mo_7O_{24} \xrightarrow{Pi}$ Phosphomolybdic Complex

Reagent Composition:

Molybdate Reagent (R1): Ammonium Molybdate 0.40 mmol/L sulfuric acid 210mmol/L Xi R:36/38

Chloride/ Phosphorus Standard: Chloride 100 mEq/L or Phosphorus 5mg/L, Organic matrix based primary standard.

Material Required:

1. Spectrophotometer with a thermostatted cell compartment set at 25/ 30/ 37ºC, capable of reading at 340 nm.
2. Cuvettes with 1-cm path length.
3. Pipettes to measure reagent and samples.

Procedure:

The following procedure was done for every sample of bean cheese.

At first pre-incubate the working reagents, samples and controls to react temperature 25/30/37ºC. Then the spectrophotometer was adjusted to 0 absorbance with the reagent blank. Pipette the reagents, samples and the standards into the cuvette. For Blank, 1.0 mL R1 reagent was taken. For Sample 1.0 mL R1 reagent and 1.0 µL of samples were taken. For CAL standard 1.0 mL and 10 µL samples were taken. They were mixed properly and incubated for 5 minutes at the 25/30/37ºC. After that the absorbance (A) of the samples and standard were determined at 340 nm against the reagent blank.

Calculations:

mg/dL Phosphorus = $\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard$

3.6.3. Sodium

Principle: Sodium is precipitated as a triple salt with magnesium and uranyl acetate. The excess of uranyl ions are reacted with ferrocyanide in an acidic medium to develop a brownish colour. The intensity of the colour produced is inversely proportional to the concentration of sodium in the sample.

Uranyl ions + Mg ions + Na^+ → UranylMgNa Precipitate

Free Uranyl ions + $K_4Fe(CN)_6$ → Brown Colored Complex

Reagent Composition:

1. **Precipitating Reagent (L1):** Uranyl Acetate

   Potassium Ferrocyanide

   Magnesium ions

2. Acid Reagent
3. Na^+/K^+ Standard
4. Color Reagent

Procedure:

Step 1- Precipitation:

Two dry tubes were takes and labelled as Standard (S) and Test (T) for each sample. After that the pipetting was done by using micro pipette. Into the tube 'S' 1.0 mL of reagent L1 and 0.02 mL of Na^+/K^+ Standard were taken. Into the tube 'T' 1.0 mL of reagent L1 and 0.02 mL of samples were taken. Both of the tubes were mixed well and let stand at room temperature for 5 minutes. with shaking well intermittently they were centrifuged at 2500 to 3000 RPM to obtain a clear supernatant.

Step 2- Colour Development:

Another three tubes were taken and labelled as Blank (B), Standard (S) and Test (T). In tube 'B', 1.0 mL of Acid Reagent, 0.02 mL of precipitating reagent (L1) and 0.1mL of colour reagent were taken. In tube 'S', 1.0 mL of Acid Reagent, 0.02 mL of supernatant from step 1 and 0.1mL of colour reagent were taken. For tube 'T', 1.0mL

of Acid Reagent, 0.02mL of supernatant from step 1 and 0.1mL of colour Reagent were taken. The tubes were mixed well and incubated at room temperature for 5 minutes. Then the absorbance of the Blank, Standard and Test sample were taken using spectrophotometer at a wavelength of 530 nm (Hg 546)/Green.

Calculations:

Sodium in mmol/L= $\frac{Absorbance\ of\ B - Absorbance\ of\ T}{Absorbance\ of\ B - Absorbance\ of\ S} \times 150$

3.6.4 Potassium:

Principles: Potassium reacts with sodium tetraphenyl boron in a specially prepared buffer to form a colloidal suspension. The amount of the turbidity produced is directly proportional to the concentration of potassium in the sample.

Tetraphenyl Boron + K^+ → White Turbidity

Reagent Composition:

1. Potassium reagent (L1): Sodium Tetraphenyl Boron
2. Na^+/K^+ Standard

Procedure:

At first Three small tubes (PCR tubes) were taken and labelled as Blank (B), Standard (S), Test (T) for each sample. After that the pipetting was done by using micro pipette. In tube 'B', 1.0 mL of Potassium Reagent (L1) and deionized water 0.02 were taken. In tube 'S', 1.0 mL of Potassium Reagent (L1) and 0.02 mL of Na^+/K^+ Standard were taken. In tube 'T', 1.0 mL of Potassium Reagent (L1) and 0.02 mL of samples were taken. After that they mixed properly and incubated at room temperature for 5 minutes. Finally the absorbance of the Standard and the absorbance of the Test sample against Blank were measured using spectrophotometer at wavelength of 630 nm (Hg 623)/Red.

Calculations:

Potassium in mmol/L = $\frac{Absorbance\ of\ Test\ sample}{Absorbance\ of\ Standard} \times 5$

3.6.5. Chloride:

Principle: Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with the ferric ions to form a red brown ferric thiocyanate complex. Intensity of the colour formed is directly proportional to the amount of chloride present in the sample.

$2Cl^- + Hg(SCN)_2 \rightarrow HgCl_2 + 2(SCN)$

$3(SCN) + Fe^{3+} \rightarrow Fe(SCN)_3$

Reagent Composition:

1. **Chloride Reagent (L1):** Mercuric Thiocyanate, Ferric Nitatre, Nitric Acid, Non Reactivr stabilizers and preservatives.
2. **Chloride Standard**

Procedure:

Three dry clean PCR tubes (small plastic tubes) were taken and labelled as Blank (B), Standard (S) and Test (T) for each sample. After that the pipetting was done by using micro pipette. Into the tube Blank (B), 1.0mL of Chloride reagent (L1) and 0.01 deionized water were taken. In the tube Standard (S), 1.0 mL of Chloride reagent (L1) and 0.01 Chloride standard were taken. Into the tube Test (T), 1.0 mL of Chloride Reagent (L1) and 0.01 of samples were taken. After pipetting they were mixed well and incubated at room temperature for 2 minutes. Finally, the absorbance of the Standard and Test sample against blank were measured within 60 minutes. This was done by using spectrophotometer at a wavelength of 505 nm (Hg 546)/Green.

Calculations:

Chloride in mmol/L= $\frac{Absorbance\ of\ Test\ Sample}{Absorbance\ of\ Standard} \times 100$

3.6.6. Calcium:

Principle: Calcium ions form a violet complex with O-Cresolphthalein complexone in an alkaline medium.

Reagent Composition:

1. Calcium Standard 2.61 mmol/L or 10.45 mg/dL
2. Buffer (R1): 2-amino-2 methyl-propan-1-ol 3.5 mol/L, pH 10.7
3. Chromogen (R2): O-Cresolphthalein complexone 0.16 mmol/L

   8-Hydroxyquinoline 6.89 mmol/L

   Hydrochloric acid 60 mmol/L
4. EDTA (R3) 150 mmol/L

Procedure:

The reagents/ samples were taken into dry, clean small eppendorf tubes separately for Blank, Standard and Test Sample according to following quantity.

The Reagent Blank tube was pipetted with 25 µL of distilled water, 0.5 ml solution R1 and 0.5 mL of solution R2. For the standard tube, 25 µL of Calcium standard, 0.5mL of Solution R1 and 0.5 mL of solution R2 were pipetted. For the test sample tubes, 25 µL of sample, 0.5 mL of solution R1 and 0.5 mL of solution R2 were taken. After pipetting they were mixed well. After 5-50 minutes the absorbance of the sample and standard were measured against the Reagent blank by using spectrophotometer at wavelength 570 nm and room temperature.

Calculation:

Calcium concentration mg/dL= $\frac{Absorbance\ of\ Sample}{Absorbance\ of\ Standard} \times \text{Standard Concetration } (\frac{\text{mg}}{\text{dL}})$

3.6.7. Iron

Principle:

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with ferrozine a coloured complex.

$$\text{Transferrin } (Fe^{3+})_2 + e^- \xrightarrow{Ascorbic\ Acid} 2Fe^{2+} + \text{Transferrin}$$

$Fe^{2+} \xrightarrow{FerroZine}$ Coloured Complex

The intensity of the color formed is proportional to the iron concentration in the sample. (Perrrotta and Kaplan, 1984)

Reagent Composition:

1. Buffer (R1): Acetate pH 4.9, 100mmol/L
2. Reductant: Ascorbic Acid 99.7%
3. Color: FerroZine 40 mmol/L
4. IRON CAL: Iron aqueous primary standard 100 µg/dL

Preparation of working reagent (WR): The contents of tube R2 reductant was dissolved in one bottle of R1 Buffer. After that the capping was done properly and mixed gently to dissolve contents. The working reagent prepared is stable for 3 months at 2-8ºC or 1 month at 15-25ºC.

Equipment Required:

1. Spectrophotometer at 562 nm
2. Matched cuvettes 1.0 cm light path

Temperature of testing was room temperature.

Procedure:

At first the instrument was adjusted to zero with distilled water. After that pipetting was done into the cuvette. Pipetting was done for four cuvettes. They are Reagent Blank, Standard, Sample Blank, Sample (for all bean cheese separately). For Reagent Blank, 1.0 mL of Working reagent, 1 drop of R3 and 200 µL of Distilled water. For Standard, 1.0 mL of Working Reagent, 1 drop of R3 and 200 µL of standard (IRON CAL) were pipetted. For the Sample Blank, 1.0 mL of working reagent, and 200 µL of sample were taken. Lastly for Sample, 1.0 mL of working reagent, 1 drop of R3 and 200 µL of sample were pipetted. After pipetting they were mixed well and incubated at 37ºC for 5 minutes. Then color is formed which is stable for 30 minutes.

The absorbance of standard and sample against the reagent blank were measured by using the spectrophotometer at a wavelength 562 nm.

Calculations:

µg/dL Iron=

$$\frac{Abs.\ of\ Sample - Abs.\ of\ Sample\ Blank - Abs.of\ Reagent\ Blank}{Abs.\ of\ Standard - Abs.\ of\ Reagent\ Blank} \times 100\ (standard\ conc.)$$

3.6.8 Copper

The copper was measured by following the colorimetric method of Abe *et al.*, (1989).

Principles

Copper, released from ceruplasmin, in an acidic medium, reacts with Di-Br-PAESA to form a colored complex. Intensity of the complex formed is directly proportional to the amount of copper present in the sample.

$$\text{Copper} + \text{Di-Br-PAESA} \xrightarrow{Acidic\ Medium} \text{Coloured complex}$$

Reagent Composition:

1. Buffer Reagent (L1)
2. Colour Reagent (L2)
3. Copper Standard (S)

Reagent Preparation

Reagents were ready to use & protected from bright light. The Cold buffer were retrieved from 2-8ºC and brought to a temp. at 25ºC.

Working Reagent: Working reagent were prepared by mixing equal volume of L1 (Buffer reagent) and L2 (Colour Reagent). The working reagent is stable at 2-8ºC for at least 3 weeks.

Procedure:

Three dry small eppendorf tubes were taken and labelled as Blank (B), Standard (S) and Test (T) for each sample. After that, pipetting was done for each sample. In the Blank eppendorf tubes (B), 0.5 mL of Buffer Reagent (L1), 0.5 mL of Colour Reagent (L2) and 0.05 mL of Distilled water were taken. In the Sample tube (S), 0.5 mL of Buffer Reagent (L1), 0.5 mL of Colour Reagent (L2) and 0.05 mL of Copper

Standard were taken. In the Test sample tube (T), 0.5 mL of Buffer Reagent (L1), 0.5 mL of Colour Reagent (L2) and 0.05 sample were taken. After pipetting the tubes were mixed properly and incubated at room temperature (25ºC) for 10 minutes. Finally, the absorbance of the Standard and Test sample were measured against the by Blank within 30 minutes by using spectrophotometer at a wavelength of 580 nm.

Calculations:

Copper in µg/dL= $\frac{Absorbance\ of\ Test\ sample}{Absorbance\ of\ Standard} \times 200$

The copper content was converted into ppm. [1 ppm= 100 µg/dL]

3.6.9 Zinc

The Zinc was measured by following the colorimetric method of Abe and Yiamashita (1989).

Principle:

Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple colored complex. Intensity of the complex formed is directly proportional to the amount of Zinc present in the sample.

$$\text{Zinc + Niro PAPS} \xrightarrow{Alakline\ Medium} \text{Purple Colored Complex}$$

Reagents:

1. Buffer Reagent (L1)
2. Color Reagent (L2)
3. Zinc Standard (200 µg/dL)

Reagent Preparation:

Working Reagent:

Pour the contents of 1 bottle of L2 (color Reagent) into 1 bottle of L1 (Buffer Reagent). This working reagent is stable for at least 2 weeks when stored at 2-8ºC.

Procedure:

Three clean small eppendorf tubes were taken and labelled as Blank (B), Standard (S), Test sample (T) for each sample. For Blank tube, 1mL of Working sample, 0.05mL of Distilled water were pipetted. For Standard sample tube, 1mL of Working Reagent and 0.05mL of Zinc Standard were pipetted. for Test Sample tube, 1mL of Working Reagent and 0.05 of samples were taken for each sample. After that they were mixed well and incubated for 5 minutes. Finally, the absorbance of the standard an test sample against the blank within 20 minutes by using spectrophotometer at a wavelength 570 nm.

Calculation:

$$\text{Zinc in } \mu g/dL = \frac{Absorbance\ of\ Test\ Sample}{Absorbance\ of\ Standard} \times 200$$

3.7. Bio-Active Compound Tests

Extract preparation:

Samples were cut into small pieces with spoon. Then transferred into respective beakers added with absolute ethanol and left to shake on a shaker for 72 hours at room temperature. Solvent was strained out and separated from the residue. The filtrates were separated and stored at ambient temperature while the residues were re-extracted twice using fresh solvent. At last, all the filtrates were mixed and evaporated under lower pressure at 60°C using a rotary evaporator to collect the crude extracts. The crude extracts were weighed and stored at 4°C.

3.7.1. Determination of Total Polyphenol Content (TPC):

Total Polyphenol Content (TPC) of the Red Kidney Bean Cheese extracts were determined according to the Folin-Ciocalteu method. (Parthasarathy *et al.*, 2009; Sarwar *et al.*, 2019).

1 mg/mL stock solutions of extracts were prepared. 1.5 mL diluted FC reagent (1:10) pipetted into a cuvette and 0.3 mL of extracts were transferred using micropipette. The solutions were mixed and kept for 3 min at room temperature. Then 1.5 mL of 7.5% sodium carbonate solution was pipetted and again incubated at room

temperature for 60 min. The absorbance was measured using wavelength 765 nm using a UV-VIS Spectrophotometer (UV-2600, Shimadzu Corporation, USA). Ethanol was used as the blank in another cuvette. To determine the Total Polyphenol Content (TPC), a gallic acid standard curve was plotted with different standard solutions of gallic acid i.e., 0.02, 0.04, 0.06, 0.08 and 0.10 mg/mL. The absorbance of the red kidney bean cheese extract was compared against gallic acid standard curve. TPC was calculated and expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of extracts (mg GAE/g).

3.7.2. Determination of Total Flavonoid Content (TFC):

Total Flavonoid Content (TFC) of the bean cheese extracts was determined using the colorimetric method (Chang *et al.*, 2002; Sarwar *et al.*, 2019).

1. 1 mg/mL Stock solution of extracts were prepared and 0.5 mL of diluted extract mixed with 1.5 mL of 95% ethanol in a cuvette.
2. Then 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate and 2.8 mL of distilled water were pipetted in the cuvette.
3. The mixture left at room temperature for 30 min.
4. The absorbance was measured at wavelength 415nm in UV-visible spectrophotometer (UV-2600, Shimadzu Corporation, USA). Again, same amount of 10% aluminum chloride substituted with distilled water, cuvette containing other reagents, which was used as the blank. Quercetin dissolved in 80% ethanol to make standard solutions (0.025, 0.050, 0.075 and 0.100 mg/mL).
5. Total flavonoid content in the sample was estimated by comparing absorbance of the sample extracts against a quercetin standard curve. TFC calculated and expressed as milligrams Quercetin Equivalents (QE) per gram of extract (mg QE/g).

3.8. Amino Acid Profile

Amino acid profile of the bean cheese formulations was done by applying the Waters AccQ•Tag Amino Acid Analysis Method using High Performance Liquid Chromatography (Waters Corporation, USA, Model: e 2695).

Sample Preparation:

The Procedure for analyzing amino acids was applied to three replications of each sample. 2 grams of each sample from bean cheese formulation was taken into a 50 mL volumetric flask and labelled with three replications. Then 20 mL of 6N HCl was added to each conical flask. After that the caps of the volumetric flasks were closed properly so that they become airtight. The hydrolysis was performed by keeping each flask in the oven at 105ºC for 24 hours. When the hydrolysis was finished the conical flasks were cooled and teach solution was mixed with 20 mL of water. The solution was mixed properly and filtered with whatman filter paper. The filtrate was collected and the pH of the filtrate was adjusted to 7-10 by using 0.1 N NaOH. Then each solution was further filtered with 0.2µm filter syringe and taken into sample vial of 2mL as stock solution. For each stock solution about 50µL sample were pipetted to another vial (reagent + sample vial) and labeled according to the replications. In this vial about 350µL of AccQ-Fluor Borate Buffer were pipetted and vortexed for 10 sec. After that 50µL of reagent dilutent and 50µL of reagent powder were pipetted into each vial and vortexed for 10 sec. Finally, the solutions were heated at 55°C for 10 minutes. From each vial (reagent and sample) about 150µL of the solution pipetted into the HPLC vials containing the inner tube. Amino Acid Analysis was done on High Performance Liquid Chromatography system (Model: Waters e2695, Waters Corporation, USA) with Fluorescence Detector (FLR 2475) with the C8 column (3.9 × 150mm). The instrumental parameters were as follows: Column temperature: 37º C, injection volume 10µL, detection= Fluorescence @250-395 nm and flow rate= 1 mL/min. The method was used using the mobile phases: Aqueous Buffer, Acetonitrile, Deionized water.

3.9. Statistical Analysis

All the statistical data analysis was done using the statistical software named SPSS (Version 26). One way ANOVA test were done to determine the mean and standard deviation and the values were compared using Duncan multiple comparison post hoc test, $p<0.05$. All the tests were taken in three replications strategy.

Chapter 4

Results

4.1. Proximate composition

The results of proximate analysis of four different formulations are illustrated on Table-3. They are compared with the soy tofu and the dairy cheese.

Table-3: Proximate composition of Red Kidney Bean Cheese, Soy Tofu and Dairy Cheese.

Sample	Moisture%	Protein%	Ash%	Fat%
F1	82.41 ± 0.01^{b}	11.21 ± 0.27^{c}	0.72 ± 0.02^{c}	1.56 ± 0.23^{d}
F2	82.92 ± 0.22^{ab}	10.56 ± 0.32^{c}	0.69 ± 0.11^{c}	1.32 ± 0.12^{e}
F3	77.68 ± 0.46^{c}	8.92 ± 1.14^{d}	0.84 ± 0.04^{b}	6.69 ± 0.70^{b}
F4	83.38 ± 0.35^{a}	7.28 ± 0.83^{e}	0.48 ± 0.06^{d}	3.75 ± 0.22^{c}
Soy Tofu*	65.8 ± 0.76^{d}	12.4 ± 0.56^{b}	1.8 ± 0.01^{a}	2.1 ± 0.74^{d}
Dairy Cheese**	54.75 ± 0.05^{e}	15.11 ± 0.01^{a}	1.80 ± 0.05^{a}	11.65 ± 0.05^{a}

Results are expressed in wet weight basis as means ± standard deviations of three replicates. Different superscript letters in the same column within each fraction indicate significant difference between mean values (One-way ANOVA followed by Duncan multiple comparison post hoc test, $p<0.05$).

Here, F1= Whole Red Kidney Bean Cheese, F2= Dehulled Red Kidney Bean Cheese, F3= Almond mixed Red Kidney Bean Cheese, F4= Germinated Red Kidney Bean Cheese.

*Source: Ezeama and Dobson (2019)

**Source: Ekanem and Ojimelukwe, (2017)

Moisture Content:

Among the four samples, the range of average percentage of moisture was between (77.68-83.38) %. There is significant difference ($P<0.05$) among the samples. F4 (Germinated Red Kidney Bean Cheese) has the highest moisture content than others. And F3 has the lowest moisture content among other formulations. There is no significant difference in the moisture content between F1 and F2. The moisture of

dairy cheese was the lowest than soy tofu and red kidney bean vegan cheese sample. And the Soy tofu had higher moisture content than the dairy cheese but lower than other formulations of red kidney bean cheeses.

Protein Content:

Among the four samples, the range of average percentage of protein was between (7.28 to 11.21) %. There was significant difference ($P<0.05$) between the dairy cheese and other samples. Dairy cheese had the highest protein content and soy tofu had lower protein percentage than dairy cheese. On the other hand, there was no significant difference ($P<0.05$) between the sample F1 and F2. The protein content among them was almost similar. That means F1 and F2 was high in protein content where the F4 has the lowest protein percentage.

Ash Content:

Significant difference was present between red kidney bean cheese formulations and other dairy or non-dairy cheeses. Ash content of the soy tofu and the dairy cheese were statistically similar. Among the red kidney bean cheeses the F3 had significantly higher ash content than other formulation. F3 contained higher ash content than F1 and F2. There was no significant difference between F1 and F2.

Fat Content:

Additional olive oil was added to facilitate the coagulation process. For Red kidney Bean Cheeses, the average percentage of Fat was found between (1.32 to 6.69)%. There was significant difference ($P<0.05$) between red kidney bean cheese, soy tofu and dairy cheeses. Among the red kidney bean cheeses, there was significant difference between F3 and F4. F3 was found to have highest fat content than other formulations of red kidney beans. F1 and F2 were statistically indifferent and had the lowest among all of them. Maximum fat content was observed in the dairy cheese. The soy tofu has lower fat% than the sample F4.

4.2. Result for Minerals

Nine minerals were analyzed for the four red kidney bean cheese samples. The nine minerals are Magnesium (Mg), Phosphorus (P), Potassium (K), Chloride (Cl), Sodium (Na), Calcium (Ca), Iron (Fe), Copper (Cu) and Zinc (Zn). The most abundant mineral present in the Red Kidney Bean Cheeses was Magnesium and the lowest minerals found were Copper and Zinc.

Magnesium:

Magnesium content varied from one formulation to others. The range of the Mg content were between (2.9 to 6.6) mg/dL. Significant difference was present between the samples F3 with others. The formulation F3 (Almond Mix Red Kidney Bean Cheese) contained highest amount of magnesium and the F2 contained the lowest.

Phosphorus

Phosphorus content of the four vegan cheeses varied from (2.0-2.4) mg/dL. The significant difference ($P<0.05$) between F4 and others was observed. The highest value of Phosphorus was in the F4 (Germinated Red Kidney Bean Cheese) and the least concentration was in the F2 (Dehulled Red Kidney Bean Cheese). The sample F1 and F3 were found statistically similar.

Potassium

The concentration of Potassium present in the formulations ranged from (5.4 to 7.8) mmol/L. The highest potassium content was observed in F1 (Whole Red Kidney Bean Cheese) and the lowest concentration was the F4 (Germinated Red Kidney Bean Cheese). There was significant difference among all the samples.

Chloride

The Chlorine concentrations in the red kidney bean cheese were from (2.8-14.6) mmol/L. The highest was in the F3 (Almond Mixed Red Kidney Bean Cheese) and the lowest was in the F4 (Germinated Red Kidney Bean Cheese).

Sodium

Among the Potassium, Chlorine and Sodium, the Sodium was in high quantity. The concentration of the Sodium varied from (20.6-41.7) mmol/L. The highest sodium content was present in the F4 (Germinated Red Kidney Bean Cheese) and the lowest was in the F1 (Whole Red Kidney Bean Cheese).

Calcium

The extent Calcium concentration of the four formulations was between (0.92-1.39) mg/dL. The highest value determined was in the formulation F4 (Germinated Red Kidney Bean Cheese) and the lowest was determined in F3 (Almond Mixed Red Kidney Bean Cheese).

Iron

The iron concentrations in the four formulations were within (29.6-74.1) µg/dL. Maximum concentration was obtained in F1 (Whole Red Kidney Bean Cheese) and the minimum concentration iron was found in the F3 (Almond mixed Red Kidney Bean Cheese).

Copper

Copper was found in very little amount than other minerals. The range of the concentration was (0.25-0.50) ppm or (25-50) µg/dL. The highest value was found in both for F3 and F4. And the lowest value was found in both F1 and F2.

Zinc

Zinc concentration in those cheese formulations were also found in very little amount. The range varied from (0.25-0.50) ppm or (25-50) µg/dL. The highest Value was found in F2 and the lowest value was in F1, F3 and F4.

The results for the minerals analysis are presented in Table 4.

Table 4: Minerals Composition of Red Kidney Bean Cheese

Sample	Mg mg/dL	P mg/dL	K mmol/L	Cl mmol/L	Na mmol/L	Ca mg/dL	Fe µg/dL	Cu µg/dL	Zn µg/dL
F1	4.3 ± 0.01^{b}	2.2 ± 0.06^{b}	7.8 ± 0.09^{a}	11.0 ± 0.3^{b}	20.6 ± 0.5^{d}	1.08 ± 0.03^{c}	74.1 ± 0.3^{a}	25 ± 0.153^{b}	25 ± 0.232^{b}
F2	2.9 ± 0.03^{d}	2.0 ± 0.09^{d}	6.3 ± 0.02^{c}	10.6 ± 0.6^{b}	29.2 ± 0.7^{c}	1.17 ± 0.005^{b}	55.9 ± 0.03^{c}	25 ± 0.21^{b}	50 ± 0.55^{a}
F3	6.6 ± 0.03^{a}	2.1 ± 0.03^{bc}	7.3 ± 0.51^{b}	14.6 ± 0.4^{a}	36.8 ± 0.4^{b}	0.92 ± 0.006^{d}	29.6 ± 0.05^{d}	50 ± 0.32^{a}	25 ± 0.169^{b}
F4	3.0 ± 0.01^{c}	2.4 ± 0.05^{a}	5.4 ± 0.05^{d}	2.8 ± 0.03^{c}	41.7 ± 0.8^{a}	1.39 ± 0.005^{a}	63.2 ± 0.1^{b}	50 ± 0.43^{b}	25 ± 0.32^{b}

Results are expressed in wet weight basis as means ± standard deviations of three replicates. Different superscript letters in the same column within each fraction indicate significant difference between mean values (One-way ANOVA followed by Duncan multiple comparison post hoc test, $p<0.05$).

Here, F1= Whole Red Kidney Bean Cheese, F2= Dehulled Red Kidney Bean Cheese, F3= Almond mixed Red Kidney Bean Cheese, F4= Germinated Red Kidney Bean Cheese.

4.3. Bioactive compounds

Among the bioactive compounds total polyphenol Content and the total flavonoid Contents were analyzed. These bioactive compounds are found in the plant sources and animal sources. These compounds provide important effects on our health. The results for these compounds are given below.

4.3.1. Total Polyphenol Content

The total polyphenol content of the four red kidney bean cheese samples varied from (0.853 to 0.154) mg GAE/g. The F4 (Germinated Red Kidney Bean Cheese) had significantly higher polyphenol compounds that others. The least concentration was present in F2 (Dehulled Red Kidney Bean Cheese). The mean polyphenol content with the standard deviation is given in the Figure 2.

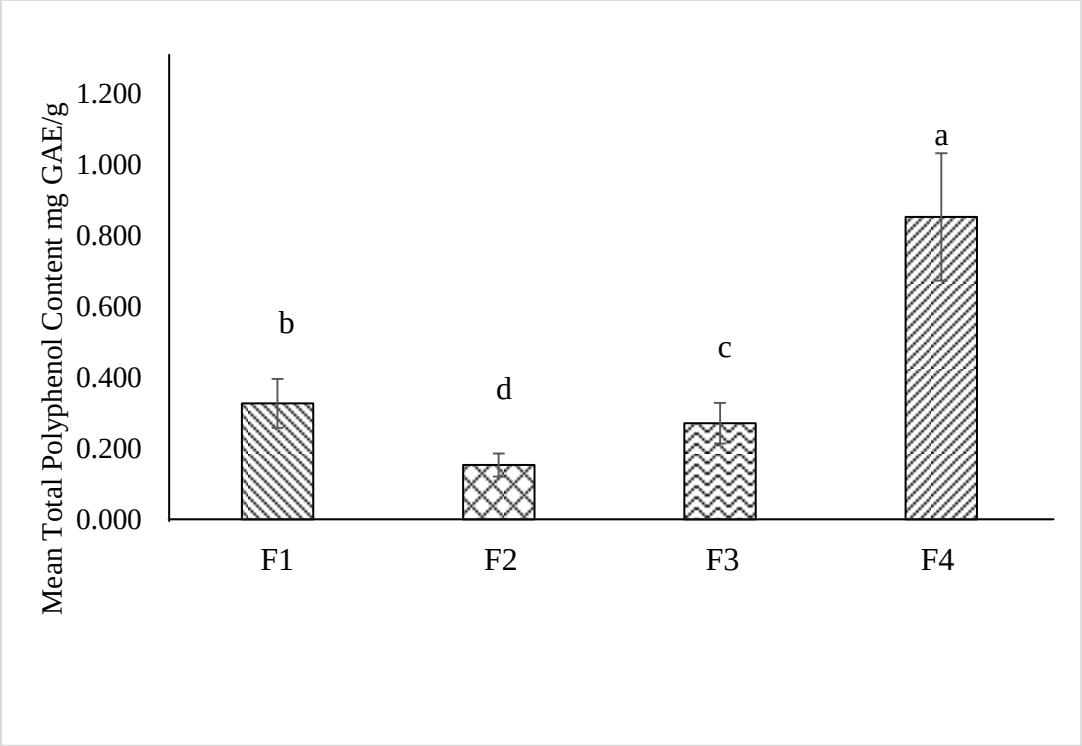

Figure 2: Mean Total Polyphenol Content in Different Red Kidney Bean Cheeses

Results are expressed as means ± standard deviations of three replicates. Different superscript letters in the bar chart within each fraction indicate significant difference between mean values (One-way ANOVA followed by Duncan multiple comparison post hoc test, $p<0.05$).

4.3.2. Total Flavonoid Content

The mean value of total flavonoid contents present in different Red Kidney Bean Cheese were ranged from (5.005 to 9.002) mg QE/g. Significant amount of total flavonoids were found in the F1 (Whole Red Kidney Bean Cheese) than other formulations/ treatments. And the lowest value for total flavonoids was observed in F2 (Dehulled Red Kidney Bean Cheese). The results for the total flavonoid content are given in Figure 3.

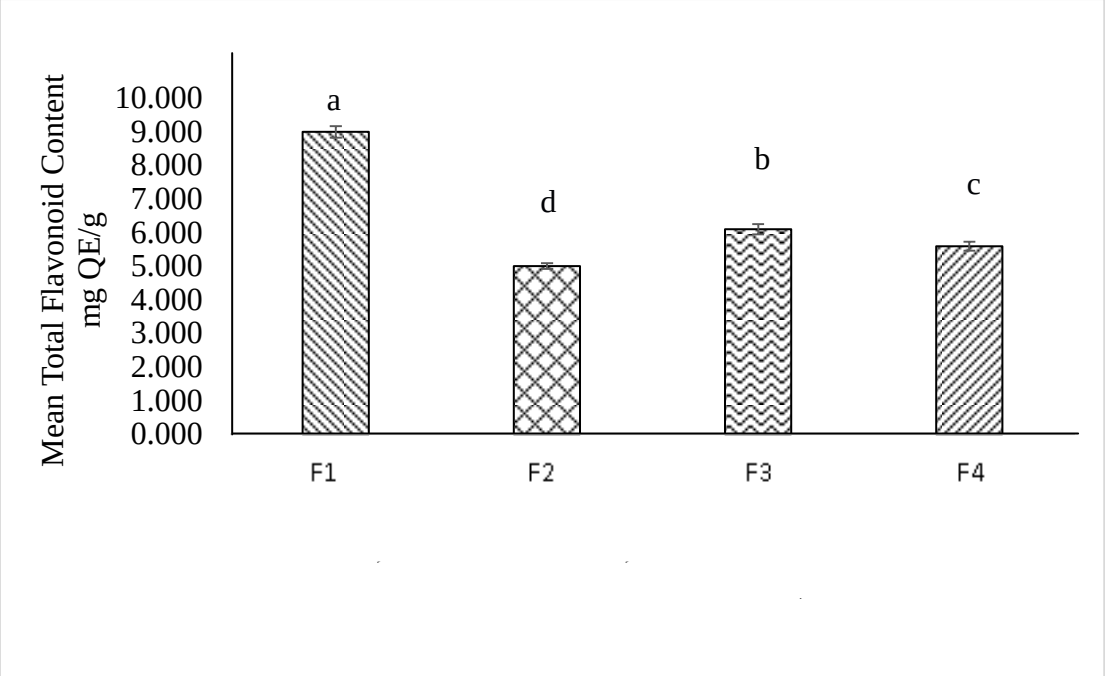

Figure 3: Mean Total Flavonoid Content of Different Red Kidney Bean Cheese

Results are presented as means ± standard deviations of three replicates. Different superscript letters in the bar chart within each fraction indicate significant difference between mean values ($p<0.05$).

4.4. Amino Acid Profile

The samples of Red Kidney Bean Cheeses were analyzed to determine the essential/non-essential or conditionally essential amino acids which are Alanine (ALA), Arginine (ARG), Asparagine (ASP), Cystine (CYS), Glutamine (GLU), Glycine (GLY), Histidine (HIS), Isoleucine (ILE), Leucine (LEU), Lysin (LYS), Methionine (MET), Phenyl Alanine (PHE), Proline (PRO), Serine (SER), Threonine (THR), Tyrosine (TYR), Valine (VAL). The results are given in Table 5.

Table 5: Mean Essential Amino Acid profile (ppm) of Red Kidney Bean Cheese samples, Dairy Cheese and Soy Tofu.

Amino Acids	Whole RKBC (F1) ppm	Dehulled RKBC (F2) ppm	Almond Mixed RKBC (F3) ppm	Germinated RKBC (F4) ppm	Dairy Cheese (30 days ripened) *	Soy Tofu**
ARG	113982±119^{b}	1830.41±27^{c}	33350.05±565^{a}	21.79±2.174^{e}	16 ± 5.0^{e}	720±3.0^{d}
HIS	43.61±4.85^{d}	456.47±36.59^{a}	450.74±4.26^{a}	177.55±8.68^{c}	72 ± 0.0^{d}	280±7.0^{b}
ILE	124.84±2.7^{e}	33250.44±11.08^{a}	306.17±3.48^{d}	9039.58±18.52^{b}	117 ± 6.0^{e}	510±1.0^{c}
LEU	17.01±2.79^{f}	202.25±11.08^{c}	113.61±3.48^{d}	48.80±18.52^{e}	280 ± 8^{b}	870±6.0^{a}
LYS	334.05±9.84^{ab}	239.88±8.92^{b}	127.32±6.73^{b}	381.34±38.74^{ab}	184 ± 0.3^{b}	690±7.0^{a}
MET	17.34±2.33^{c}	35.29±1.66^{bc}	15.92±0.367^{c}	41.49±5.61^{bc}	69 ± 5.0^{ab}	100±1.0^{a}
PHE	24.16±2.049^{f}	429.51±2.79^{a}	221.24±5.87^{d}	93.99±4.87^{e}	275 ± 9.0^{c}	360±4.0^{b}
THR	12.03±1.09^{c}	237.45±8.7^{b}	151.75±1.2^{c}	151.8±1.2^{d}	55 ± 2.0^{d}	400±5.0^{a}
VAL	15.46±5.95^{f}	291.38±4.82^{c}	164.93±8.3^{d}	75.22±3.95^{e}	389 ± 7.0^{b}	540±7.0^{a}

Results are expressed in wet weight basis as means ± standard deviations of three replicates. Different superscript letters in the same row within each fraction indicate significant difference between mean values ($p<0.05$).

*Source: Barcina *et al.,* (1995)

**Source: Wang and Chang, (1995)

The amino acid profile of the Red Kidney Bean Cheese samples was compared with the dairy cheese and soy tofu. It can be seen that there is significant difference ($P<0.05$) present between soy tofu and others.

Table 6: Mean Non-Essential or Conditionally Essential Amino Acid profile (ppm) of Red Kidney Bean Cheese, Dairy Cheese and Soy Tofu

Amino Acids	Whole RKBC (F1) ppm	Dehulled RKBC (F2) ppm	Almond Mixed RKBC (F3) ppm	Germinated RKBC (F4) ppm	Dairy Cheese (30 days ripened) *	Soy Tofu**
ALA	308.45±9.5^{d}	1970.31±6.07^{a}	07.40±0.32^{f}	1046.12±1.7^{b}	199 ± 1.0^{e}	570±5.0^{c}
ASP	63.69±0.8746^{d}	345.63±2.48^{b}	226.44±3.18^{c}	168.84±9.67^{c}	232 ± 5.0^{c}	1100±6.0^{a}
CYS	2.49±0.74^{c}	15.440±2.36^{c}	9.44±8.04^{c}	50.23±3.58^{b}	14 ± 1.0^{c}	240±3.0^{a}
GLU	60.22±9.408^{e}	433.50±12.53^{c}	188.8±7.98^{d}	101.64±7.00^{de}	902 ± 7.0^{b}	2350±5.0^{a}
GLY	5.14±1.31^{c}	70.18±7.18^{bc}	76.49±8.92^{b}	33.47±5.9^{bc}	16 ± 0.0^{bc}	500±10.0^{a}
PRO	14.76±1.51^{d}	31.90±3.55^{c}	16.27±3.44^{d}	16.09±1.30^{d}	197 ± 1.0^{b}	620±4.0^{a}
SER	352.22±4.5^{ab}	390.44±5.31^{ab}	403.86±7.68^{ab}	1143.67±4.02^{a}	107 ± 2.0^{b}	350±2.0^{ab}
TYR	10.66±7.1^{f}	165.93±10.3^{b}	84.12±1.736^{d}	36.66±14.11^{e}	104 ± 3.0^{c}	250±5.0^{a}

Results are expressed in wet weight basis as means ± standard deviations of three replicates. Different superscript letters in the same row within each fraction indicate significant difference between mean values ($p<0.05$).

*Source: Barcina *et al.,* (1995)

**Source: Wang and Chang, (1995)

The amino acid profile of the Red Kidney Bean Cheese samples was compared with the dairy cheese and soy tofu. It can be seen that there is significant difference ($P<0.05$) present between soy tofu and others.

The dairy cheese contains less amino acid than soy tofu. On the other hand, among red kidney bean cheeses the amino acid profile were less than the tofu and dairy cheese but the result indicates the presence of all essential amino acids in the developed product.

4.5. Cost Analysis

Cost analysis was done for different developed red kidney bean cheese samples and compared with the dairy cheese and soy tofu. In the Table, Formulation F3 was formed using additional ground almond. The other formulations do not contain the ingredient. That's why the price of the F3 per Kg was almost two times greater than the others.

Table 7: Production and packaging cost of red kidney bean cheese

Expenditure Sector	**Unit Price/Kg in BDT**	**Quantity in Kg**	**Cost for F1, F2, F4 in BDT**	**Cost for F3 in BDT**
Raw Materials				
Red Kidney Bean	60	0.350	21.00	21.00
Ground Almond	480	0.100	0.00	48.00
Agar Agar Solution	3000	0.003	9.00	9.00
Olive Oil	500	0.020	10.00	10.00
Salt	35	0.007	0.245	0.245
Magnesium Chloride	2500	0.010	25.00	25.00
Sub Total			**65.25**	**113.25**
Processing Cost (15% of the Raw Material)			16.31	28.31
Packaging Cost	280	0.006	1.68	1.68
GRAND TOTAL			**83.24**	**143.23**

From the result the cost of 200 gm of red kidney bean cheese can be found.

Cost of 200 gm of F1, F2 and F4 = BDT $\frac{83.24*200}{1000}$

= BDT 16.648

Cost of 200 gm of F3= BDT $\frac{143.23*200}{1000}$

= BDT 28.6

The cost for was about 15 times lower than the dairy cheese and 3 times lower than the soy tofu. The cost of 200 gm of red kidney bean cheese samples are BDT 200 to 250. The product can be a good source of nutrients at minimal price if more technologies can be introduced to extract more proteins.

Chapter 5

Discussion

5.1. Proximate Composition

Average moisture content in the red kidney bean cheeses was present between (77.68-83.38) %. Dairy Cheese contains moisture of about 66.69% where the Soy Tofu has a moisture content of almost (73-75) % (Balogun *et al*, 2016; Tripathi and Mangaraj, 2013).

Proteins content of RKB cheese samples were comparatively lower than the red kidney beans. This may occur due to the addition of extra water and other ingredients during the production of the cheese. The maximum protein percentage has been seen in the F1. In the raw kidney beans the percentage varied from 20-27% (Hayat *et al.*, 2014; Ibeabuci *et al*., 2017; Okoye *et al.*, 2008). In the different formulation red kidney vegan cheeses, the protein percentage was lower than this. The protein ranges of red kidney bean cheese are lower than traditional tofu. Tofu contains about 52% protein (Van der Riet, Wight, Cilliers and Datel, 1989). The pea (*Pisum sativum*) contains about 13.6% (coagulant CaSO4 at 54% concentration) which is a little higher than the red kidney bean samples (Gebre-Egziabher and Sumner, 1983). For those people whole has allergen issues can consume the red kidney bean cheeses.

The mean ash content of the RKB cheeses were from 0.48 to 0.72%. In the germinated RKB cheese the percentage was the lowest. In previous studies among the raw/fresh kidney beans ash% was mostly (3.9-4.34) %. Here, the ash percentage in our products is higher than the previous studies. The ash percentage was found higher in the F3. In this ground almond was mixed. So due to this reason the ash percentage can be higher. Among the other pulses the pea curd contains about 5.3% ash contents and soy tofu contains 4.3% of ash contents (Gebre-Egziabher and Sumner, 1983; Tripathi and Mangaraj, 2013). The amount of ash is higher in soy tofu. A proper extraction of RKB milk can increase the ash content.

Fat % of the formulation were found the highest in the F3. This is also because of almonds in that sample. Naturally, Almond contains some oil. which helped the formulations to be higher in the fat content. The lowest fat% was obtained in the F2.

According to the previous study the oil content of the raw kidney beans were lower. The addition of extra olive oil also caused the higher percentage of oil. In the sample F4 the fat content is about 3.75%. while soy tofu contains about 7.51% and pea curd contains 3.7% (Gebre-Egziabher and Sumner, 1983; Tripathi and Mangaraj, 2013).

By comparing the values with the cheese and soy tofu it is clear that the red kidney bean cheese contains less Moisture, Protein, Ash and Fat than those items. But with further development and latest processing technology this product can be a better choice.

5.2. Mineral composition

Among the mineral, magnesium was found in higher amount among other minerals. But the quantity is a little lower than the raw red kidney beans. Maximum Phosphorus contents were found in the F4. The milk of the red kidney beans was used that could be the reason why the mineral contents are a little lower than the whole raw red kidney beans. Anino *et al.* (2019) showed that the mineral contents like Magnesium, Phosphorus, Calcium, Iron Zinc etc. are lower in kidney bean milks than the Raw beans. The F1 has the highest conc. of Potassium and Iron. The F2 has the highest concentration of Zinc among others. Formulation F3 has the highest concentration of Magnesium, Chloride and Copper. Finally, the F4 has the highest concentration of Phosphorus, Sodium, Calcium, and Copper. It can be interpreted that the formulation F3 and F4 are better than other minerals in terms of mineral composition.

5.3. Bio active Compounds

Limón *et al.*, (2015) reported that the processing like fermentation of beans can increase the bio active compound. In our study the germination of the red kidney beans was done. As the product is made from the kidney bean milk, it contains less amount of bioactive content than the raw kidney beans. Due to germination the total polyphenol content was higher in the Formulation F4 than others. The concentration of TPC in F4 was 0.853 mg GAE/g. On the other hand, the Flavonoid Content was found highest in the Formulation F1. This may indicate that the whole red kidney bean cheese (which was not further processed like germination) has the highest flavonoid compounds than others.

5.4. Amino Acid Profile

The Amino Acid analyzed in this study has shown a wide range of results. According to Mbithi-Mwikya *et al.* (2000), it has been seen that the amino acid in the raw kidney beans and the sprouted kidney beans are slightly differ from each other. The Alanine was found highest in the F2 while F1 had the lowest. According to Thakur *et al.* (2017), Mung bean, Black gram, Lentil etc. contains Alanine about 2,300 ppm, 20,000ppm, 22,000 ppm respectively. The pea protein isolate contains about 43,400 ppm, Alanine of total protein (Pawnall *et al.*, 2010). Among the essential amino acids Arginine content was highest in the F3 and the lowest was found in the F4. According to Barcina *et al.,* (1995), Arginine content was lower in the ripened dairy cheese which is lower than he RKB cheese of F3 formulation. The reason behind the highest value may occur due to presence of high amount of arginine both in the red kidney bean and almond. In the pea protein isolate the arginine content is about 8.6% of total protein (Pawnall *et al.*, 2010). Mung bean, Black gram, Chick pea, Lentil etc. contains Arginin about 20,000 ppm, 18,290 ppm, 15,660 ppm, 21,100 ppm respectively (Thakur *et al.*, 2017). The Asparagine was found highest in the F3 and the lowest in the F1. This value is lower than the amino acids present in the soy tofu. Cystine was present in the samples in very low amount. It was found the F4 had the highest cystine concentration (50.23 mg/dL) and F1 had the lowest (2.49 mg/dL). But these values are also lower than the soy tofu. Pea protein isolates contains Cystine about 8,700 ppm (Pawnall *et al.*, 2010). Glutamine was found the highest in F2 and the lowest was found in the F1. The highest Glycine was found in F3 and the lowest concentration was in F1. On the other hand Glutamic Acid is absent in the Black gram, Chick Pea and little present in the Mung bean and Lentil (Thakur *et al.*, 2017). In Soy bean tofu the Glycine contentis higher than the other beans. This might increase the binding capacity of the tofu giving it a firm texture. Histidine was found highest in the F2 and the lowest was in F1. From Wang and Chang, (1995), Histidine was a little lower in the soy tofy in the F2 and F3. There was no significant difference between the F2 and F3. But the value was quite higher in the F3. This can be also caused due to the addition of almond in the F3. In the Pea proyen isolate the Histidine present in 17400 ppm which s higher than the F3 sample (Pawnall *et al.,* 2010). F2 contained large portion of Isoleucine while F1 had the lowest. Leucine was found highest in the F2 and lowest into the F1. Lysin was higher in the F4 but F3 contained the lowest

amount of lysine. The average Methionine was very little in those samples. The highest value was obtained from the F4 and the lowest value was in F3. The highest methionine was found in the soy tofu. Methionine was found in 1120 ppm in the pea protein isolates (Pawnall *et al.*, 2010). Phenyl Alanine was highest in the F2 while F1 had the lowest value. There is significant difference between the Phenyl Alanine content of F2 than that of others. Proline was higher in the F2 and lowest was in F1. Serine was found to be higher in the F4 which may indicate that germination can increase the serine content. The lowest serine was found in the F1. Threonine was highest in the F2 and the lowest was found in the F1. Tyrosine was higher in the F2 and lowest in the F1. Valine was found highest in the F2 and the lowest was in F1. As soy bean is good source of protein this is one of the reasons why most of the amino acids are higher in soy tofu.

Among the comparison between soy tofu, cheese and other beans, there was a significant different with the amino acid composition of Red Kidney beans. They may not substitute the cheese and soy tofu but they can be an alternative choice for certain group of people.

Chapter 6

Conclusion

The Nutritional values, mineral contents, bioactive compounds, amino acids Red Kidney Bean cheeses were determined in this study. It has been seen that the Red Kidney Bean Cheese has good amount of protein, Bio active compounds and the essential amino acids which can provide nutritional and other beneficial effects. The maximum protein content in the cheeses were 11.21%. The moisture percentage of the kidney bean cheese were found to be higher and the maximum value is 83.38%. So proper storage of this product is a requirement. The total phenolic content was higher in the Germinated RKB Cheese which is 0.853 mg GAE/g. Total Flavonoid contents were highest in the Whole RKB cheese which is about 9.002%. The presence of this bio active compounds can provide beneficial effects on health. The mineral contents also varied from formulations to formulations. Magnesium is on the top on them. About 6.6 mg/dL of magnesium present on the sample F3. The highest amino acid contents were found in Dehulled RKB Cheese. The study provides clear evidence that the Red Kidney Bean Cheese has all the essential amino acids in different amount. These might not be higher than the soy milk derived tofu but they can be one of the substitutes of that product for those who has allergen issue or trying to get nutrition from different food sources. As Red kidney beans are a good source of plant protein their products can be used to overcome the malnutrition and allergen issues. This might not substitute the dairy and other non-dairy products but this product can be a good choice to fulfill the nutritional demand.

Chapter 7

Recommendation and Future perspectives

The Quality Evaluation for the different formulations of Red Kidney Bean were done in this thesis and has provided new knowledge, in minimally processed Red Kidney bean milk. Some suggestions for continued work in this issue are the following:

1. The further development in processing like using centrifugal forces to separate the protein from the kidney bean milk can increase the protein percentage of the product. In this way the wastage can be reduced and productivity of the product can be improved.

2. The Changes in the coagulant can improve the texture of these products. Allum, $CaSO_4$ etc. could be used to improve the product.

3. The Total Anthocyanin Content and Total Antioxidant Content, Sensory Evaluation of these products can be done in future.

4. The introduction of this processed product to the low-income target groups can help to maintain the nutritional status among them.

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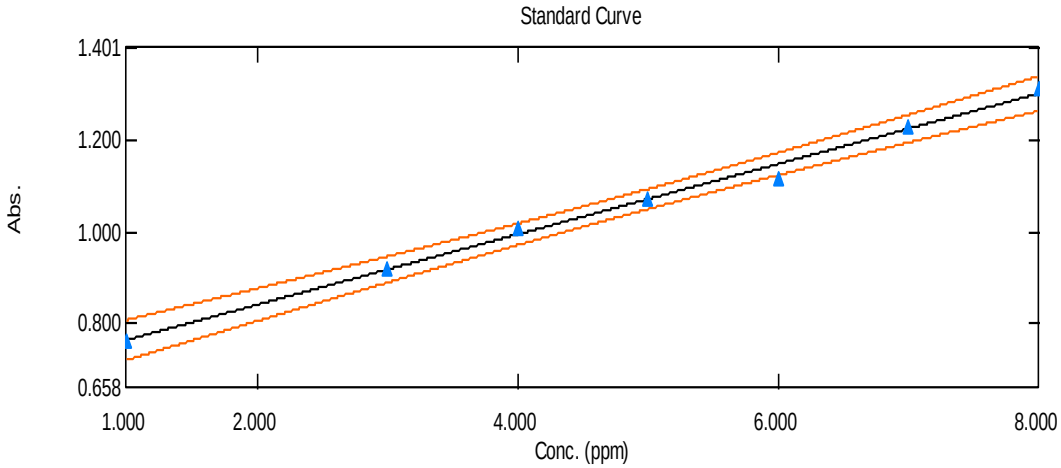
APPENDICES

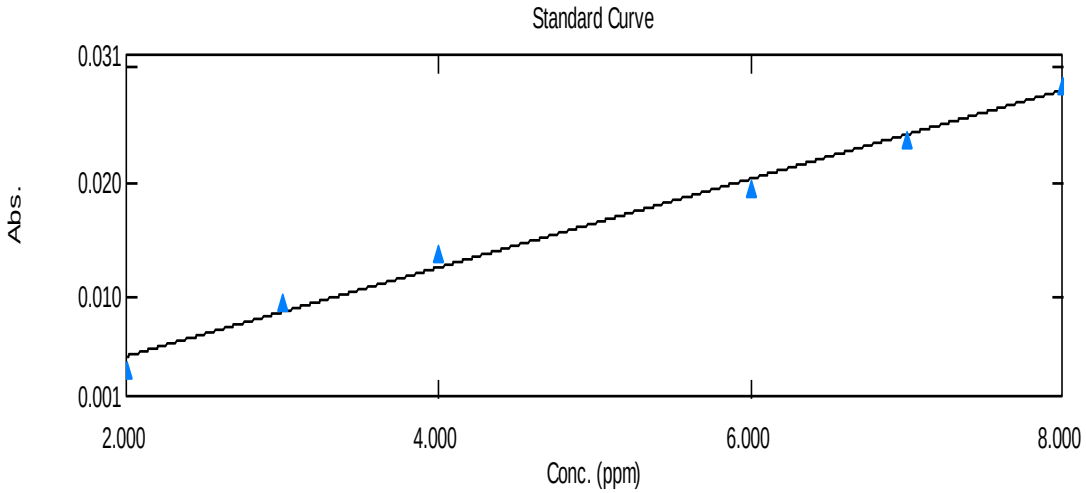
Appendix A: Bio Active Compounds Standard Curves

Standard (Gallic Acid) Curve for Total Polyphenol



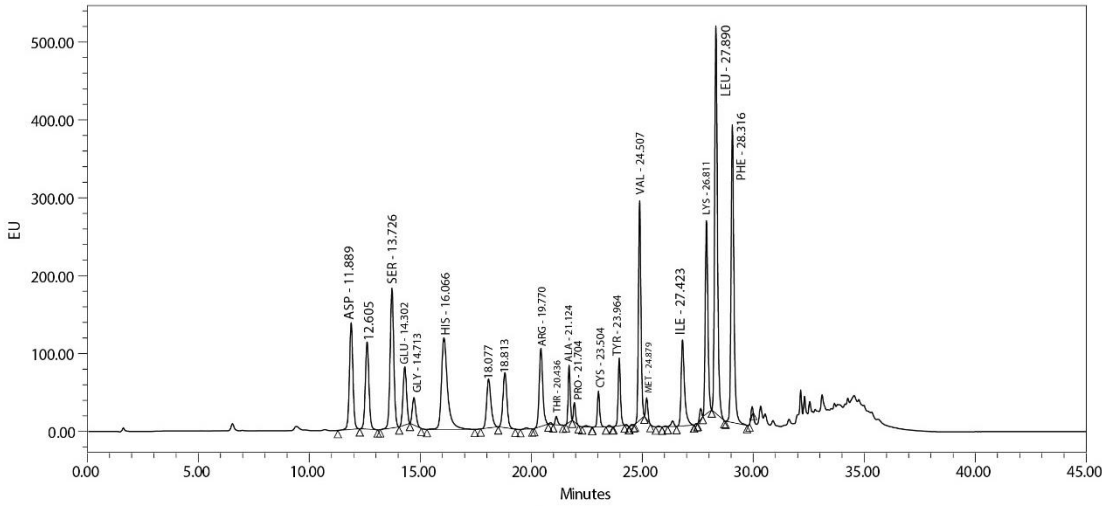
y = 0.0768527 x + 0.687090
r2 = 0.99301

Standard (Quercetin) Curve for Total Flavonoids



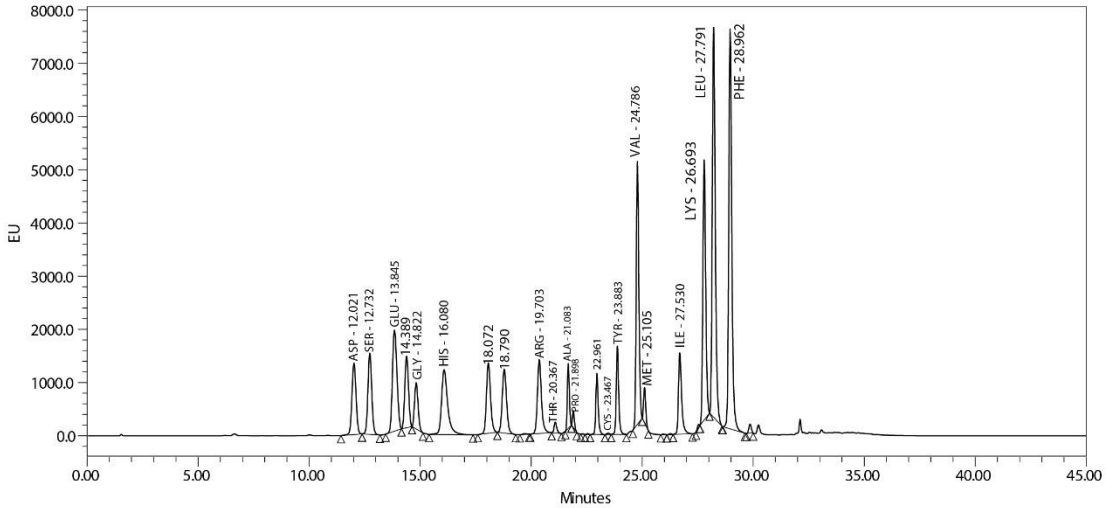
y = 0.00385110 x - 0.00271158
r2 = 0.98868

Appendix B: Amino Acid Contents of Different Formulation of Red Kidney Bean Cheese in HPLC curves



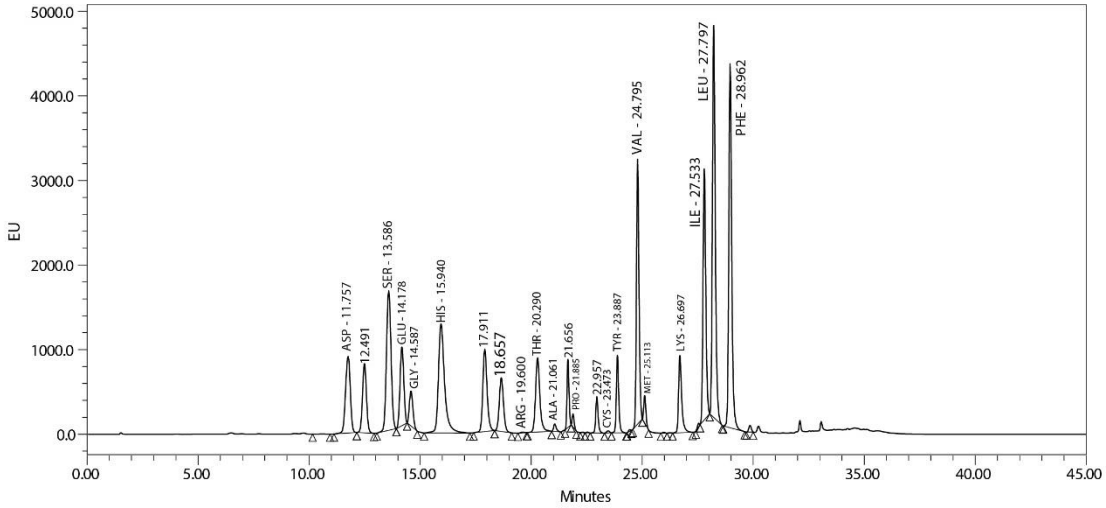
Channel: 2475ChA ex250/em395; Processed Channel: 2475ChA ex250/em395; Injection: 1; Date Acquired: 6/2/2021 1:37:09 PM BDT; Result Id: 2526; Processing Method: Amino_Acid_Process S1 F11

HPLC curves for the sample F1 replication 01



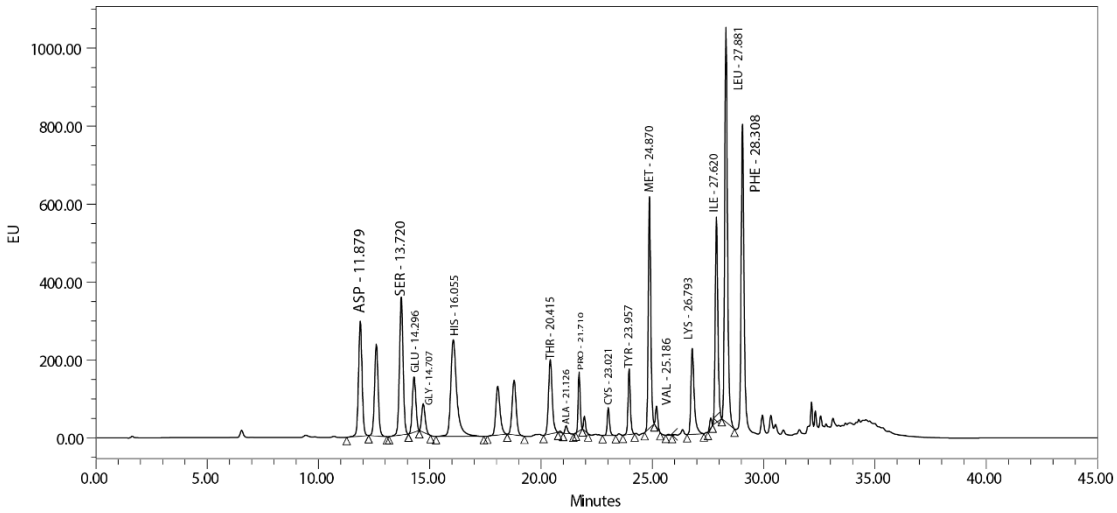
Channel: 2475ChA ex250/em395; Processed Channel: 2475ChA ex250/em395; Injection: 1; Date Acquired: 6/3/2021 12:53:29 PM BDT; Result Id: 2762; Processing Method: Amino_Acid_Process F21

HPLC Curve for the sample F2 replication 01



Channel: 2475ChA ex250/em395; Processed Channel: 2475ChA ex250/em395; Injection: 1; Date Acquired: 6/3/2021 3:29:13 PM BDT; Result Id: 2915; Processing Method: Amino_Acid_Process F32

HPLC Curve for the sample F3 replication 02



Channel: 2475ChA ex250/em395; Processed Channel: 2475ChA ex250/em395; Injection: 1; Date Acquired: 6/2/2021 2:22:53 PM BDT; Result Id: 2406; Processing Method: Amino_Acid_Process S2 F41

HPLC Curve for the sample F4 replication 01

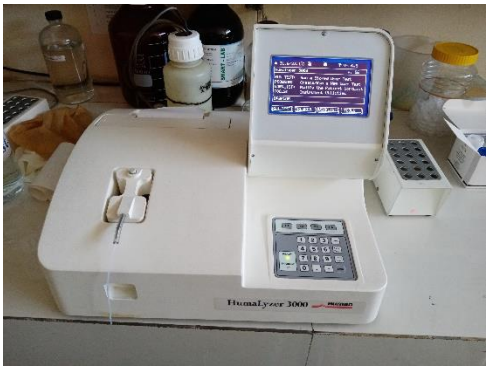
Appendix C: Collection, Processing and Analysis of different Red Kidney Bean Cheeses

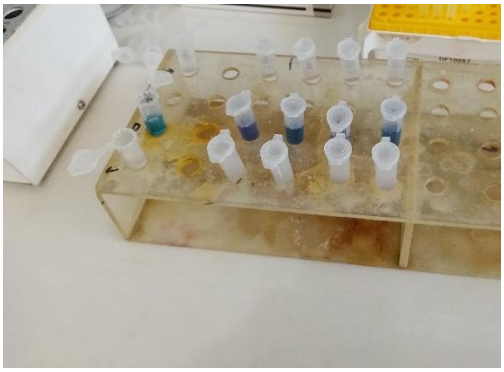
Four Formulations of Red kidney Bean Cheese
F1 (Whole Red Kidney Bean Cheese)
F2 (Dehulled Red Kidney Bean Cheese)
F3 (Almond Mixed Red Kidney Bean Cheese)
F4 (Germinated Red Kidney Bean Cheese)

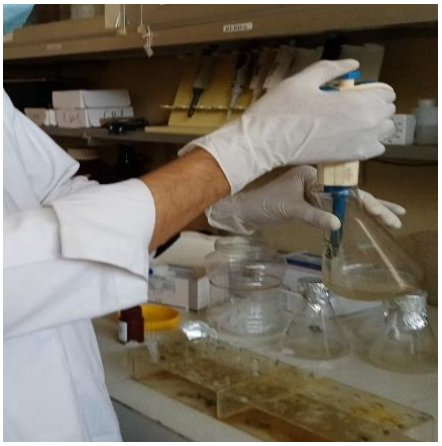
Whole, Dehulled and Germinated Red Kidney Bean

Blending, Straining and cooking with addition of other ingredients.

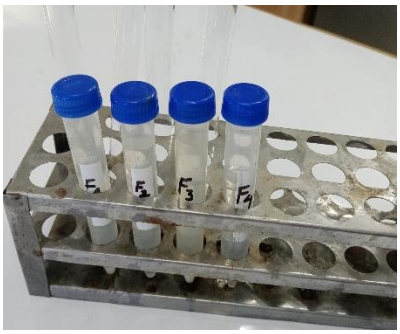
Proximate Analysis (Protein and Fat Analysis)







Mineral Analysis using Spectrophotometer.



Bioactive Compound Analyzing

Determining Amino Acid Profile using HPLC

Brief Biography

Sultan Mohd. Adnan has successfully completed the B.Sc. in Food Science and Technology under the Faculty of Food Science and Technology in Chattogram Veterinary and Animal Sciences University. He completed the Secondary School Certificate Examination in 2011 and Higher Secondary Certificate Examination in 2013. Now, he is a candidate for the degree of Master of Food Processing and Engineering under the Department of Food Processing and Engineering, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University. His research interests are new product development, food processing, food preservation, food toxicology, food safety and food safety management, innovation in food processing, public health etc.