ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Physico-chemical characteristics of sweet lupin-based yoghurt like product made using folate producing lactic acid bacteria

A thesis Submitted to the College of Natural and Computational Sciences of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Master of Science Food Science and Nutrition

By: Betelhem Shemelse

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Addis Ababa, Ethiopia

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Abstract

*Lupins are important crops as rich sources of protein, minerals, dietary fiber and so on. Among the legumes lupin is underutilized due to the presence of bitter and toxic alkaloids. In recent years, genetically improved sweet lupin variety with low alkaloid level is released. Yet, the consumption of sweet lupin is very low. Specially in developing countries due to lack of food products made with sweet lupin, it still remains being a neglected crop. Therefore, this study aimed to develop yoghurt like product from sweet lupin (Lupin luteus) using folate producing lactic acid bacteria. Cleaned sweet lupin seeds were soaked in water (1:9) for 12hrs. Then, the seeds were grounded into a slurry and mixed with water (1:1) followed by pasteurization at 90^{o}C for 15min. The pasteurized slurry was subjected to fermentation using highly folate producing Lactobacillus plantarum strain with 3% of inoculum for 48hrs. Then, the proximate, mineral and folate composition of the formulated sweet lupin-based yoghurt like product was assessed. Also, physico-chemical characteristics, sensory quality of the product were evaluated. Accordingly, the hull weight of the seed was 20.60% and the water holding capacity of the seed and flour were 460 and 310%, respectively. The foaming capacity and stability of the flour were 12 and 10 %, respectively with bulk density of 0.779 g/ml. The raw sweet lupin had a moisture, crude protein, crude fat, crude fiber, total ash, total carbohydrate and gross energy contents were 8.10, 35.28, 9.36, 3.61, 3.03, 44.23 %, and 402.28 (Kcal/100gm), respectively. The yoghurt-like product had percentage value of 19.87%, 36.39, 8.95, 2.63, 2.07, 32.13 and 354.76 (Kcal/100gm) of the aforementioned nutrients, respectively. The phytate, alkaloid and tannin concentrations in the raw sweet lupin were 0.60, 0.12 and 0.05 %, respectively. While in fermented product the concentrations of the anti-nutrients were 0.16, 0.02 and 0.01 %, respectively ($p \leq 0.05$). The concentration of the minerals sodium, potassium and calcium in the raw sweet lupin seed were 10.36, 4.74 and 27.78 mg/100gm on dry basis, respectively. Whereas concentrations of the same minerals in the fermented yoghurt like product were 3.11,1.75 and 15.75 mg/100gm, respectively ($p \leq 0.05$). The concentration of the minerals iron, zinc and manganese in the raw seed and fermented product were 7.71,0.21, 0.84,0.46 and 4.4,3.75 mg/100g, respectively. The pH and titratable acidity of the raw slurry and fermented product were 5.8,0.66% and 4.8,0.99%, respectively. Folate content in raw seed was 83.02 µg/100g and was reduced to 19.45 µg/100g, during pasteurization of the slurry at 90^{o}C for 15min. The final fermented sweet lupin-based yoghurt like product had a folate concentration of 35.96µg/100g. The viscosity of the fermented product was 309.29 (mPa^{-s}) and the color evaluated using L*a*b* was 112.09, 96.9 and 34.81, respectively. As a new product the sensory quality of the yoghurt like product was evaluated. Accordingly, there was no significant difference in sensory attributes of texture and overall acceptability between sweet lupin yoghurt like product with sweet lupin yoghurt like product with vanilla ($p \geq 0.05$). Vanilla (flavoring agent) incorporated (1%)of fermented product had a higher sensory score in attributes of mouthfeel (consistency), color, flavor and taste, than the sweet lupin-based yoghurt like product. In conclusion, this study demonstrated the potential of sweet lupin to produce fermented yoghurt like product with comparable sensory acceptability providing vital nutrients such as folate and reduced anti-nutritional factors. Future studies on optimizing the processing to formulate a more acceptable product along with shelf life are recommended.*

Keywords: *sweet lupin, yoghurt-like, fermentation, folate, anti-nutrients, mineral*

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List of abbreviations and acronyms

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CIE	Commission Internationale d'Eclairage
FAOSTAT	Food and Agricultural Organization Statistical Database
GI	Gastrointestinal
ISO	International Standard Organization
LDL	Low Density Lipoprotein
Lb.	Lactobacillus
RGB	Red, Green, and Blue
SD	Standard Deviation
SMP	Significant market power
SWF	Sweet Lupin Flour
WA	Western Australia
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background and justification

Milk is the normal secretion of the mammary glands of all mammals and provides the sole source of nourishment during the period directly after birth for a newborn mammal of the particular species of animal (Potter and Hotchkiss, 1996). The high nutritional value makes human milk a complete food for babies, young children and adults (Potter and Hotchkiss, 1996). Milk is a major source of dietary energy, protein and fat, contributing on average 134 kcal of energy, 8 g of protein and 7.3 g of fat/capita per day (FAOSTAT, 2012). It plays an important role in food security for both highland and pastoral communities. Milk is consumed worldwide and the per capita production is low in developing countries. Similarly, the per milk consumption in Ethiopia is 19 liters per year, which is much lower than the global per capital milk consumption of 75 liters annually. Besides, the average price of milk in present year is 26 Ethiopian birr which is difficult to acquire especially for low income people (FAO, 2011). Furthermore, the problem of allergy poses a health problem especially with the lactose malabsorbers/intolerant individuals and vegetarians (Elizabeth, 2002).

To address these problems, several legume-based milk and milk products have been developed, especially in areas where milk is in short supply (a shift from cholesterol to phytosterole). Since legumes are important sources of relatively inexpensive protein, introduction of imitation milk products from legumes may contribute to the alleviation of protein malnutrition. With this regard, (Rao et al, 1988) reported that the potential of legumes like cowpeas and mung beans as excellent raw material for the development of dairy like products. Nowadays, soy-based products like milk and yogurt alternatives or tofu are widely used. However, other plant raw materials should be considered to broaden the variety of plant derived dairy alternatives. Lupin, another protein-rich legume belonging

to the genus Lupinus (family of Fabaceae) is a promising candidate. Besides to the high protein content, sweet lupin seeds are characterized by a high content of fiber, as well as a low fat and starch content (Lampart-Szczapa et al, 2003). Lupin seeds in contrast to soybeans are free from trypsin inhibitors (van Barneveld, 1999). Moreover, beneficial cardiovascular effects can be attributed to lupin, like lowering serum cholesterol levels (Pilvi et al, 2006). Further, lupin tolerates nutritionally poor soils (Trinick, 1977) and no genetically modified varieties are commercially available (Eapen, 2008). Therefore, formulating milk analogues with underutilized, protein rich legumes like sweet lupin will bring their potentials to attention. In Ethiopia white lupin is a traditional crop, grown in the north-western part of the country (Francis, 1999). (Yeheyis et al, 2010) reported that under traditional management systems the average grain yield potential of the crop is 1.2 t/ha.

However, the use of the crop as human food and as livestock feed is limited due to its bitter taste attributed to its relatively high alkaloid content (Yeheyis et al, 2010). In recent years, sweet lupins with fewer amounts of alkaloids are developed by breeders. In Ethiopia there is a high potential to cultivate sweet lupin in the traditional lupin growing areas. On the other hand, development of leguminous dairy substitutes like yogurt alternatives is a difficult task as most of these plant materials comprise disadvantages like beany off-flavors, contents of antinutritives causing flatulence etc. To overcome beany off-flavors and to reduce antinutritives, fermentation can be applied (Hickisch et al, 2016). Hence, lactic fermentation of legume-based milks has been used as one of the approaches to prolong the shelf-life of the products and also to improve the nutritional value (Rao et al, 1988).

Fermentation is one of the oldest and widely used forms of food preservation, which is estimated to contribute about one third of the diet worldwide. Fermentation is globally applied in the preservation of a range of raw agricultural materials (cereals, roots, tubers, fruits and vegetables, milk, meat,

fish etc.). Certain microorganisms associated with fermented foods, such as strains of the *Lactobacillus* species are probiotic (Madsen et al, 2001). Probiotics are bacterial cultures comprising of potentially beneficial bacteria or yeast. Their health benefits include prevention of gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, antimutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, improvement in inflammatory bowel disease and suppression of *Helicobacter pylori* (Gomes and Malcata 1998); (Agerholm-Larsen et al, 2000); (Gotcheva et al, 2002); (Nomoto, 2005). Among the probiotic microorganisms, lactic acid bacteria (LAB) are the most common microbes used in food products. Application of LAB in food fermentation not only provides characteristic sour taste, but also acts as a preservative by lowering the pH and creating less room for spoilage organisms to grow. One of LAB fermented popular food item is Yoghurt. Yoghurt is a nutritious product of milk fermentation by starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) (Fisberg et al, 2015).

Probiotic organisms like LAB, besides the mentioned health benefits, can also produce essential micronutrients like folate. In a study conducted at Center for Food Science and Nutrition of Addis Ababa University, three strains of LAB with highest capacity of producing folate (Vitamin B9) from tef fermentation were isolated. This folate is vital to help the body form red blood cells and aids in the formation of genetic material within every body cell. It is important for the nervous system, which includes the brain and spinal cord. The vitamin also helps limit homocysteine levels, which may protect against heart disease and cancer. Women need folate before and during pregnancy to protect their babies from birth defects, such as: Spina Bifida and Anencephaly. Therefore, it will be a value addition if folate producing LAB are used in developing fermented food products like legume-based yoghurt.

In Ethiopia, fermented foods like nondairy probiotic yoghurt analogues are not well developed and the awareness towards such products is poor. In contrast, the development of such dairy analogue products will provide a remedy for the problems including, scarcity of animal based dairy products, potential uses as probiotics, improved shelf-life (reduce spoilage), and provide micronutrients such as folate rather than being dependent on cancer susceptible synthetic supplementation. With this regard, efforts are currently made to develop legume-based milk-like products from under-utilized plant sources having different health benefits. The development of acceptable milk analogues (yoghurt analogue) would improve the utilization of underutilized legumes such as sweet lupin. Thus, the main objective of this study was to develop yoghurt like product form underutilized sweet lupin through fermentation using folate producing LAB.

1.2 Statement of the problem

Milk is a complete food providing high nutritional values for babies, young children and adults. Rapidly increasing population with a growing rate of urbanization is resulting in a shift in demand for dairy products. However, there is a scarcity of cow milk to satisfy the growing demand of the population (Tefera et al, 2010). As a result, the per capita production and consumption of milk is low in developing countries. For instance, in Ethiopia the per capital milk consumption is 19 liters per year, which is much lower than the global per capital milk consumption of 75 liters annually. Besides, the average price of a liter of milk is 26 Ethiopian Birrs, yet difficult to buy for the significant low-income majority. Though not studied well, for some of the milk consumers the problem of intolerance forced them to avoid dairy from their menu (Elizabeth, 2002). To address these problems, several legume-based milk and milk products were developed. Legumes are potential candidates to develop dairy mimic products due to their high/inexpensive protein and other nutritional benefits. As a result, nowadays, soy-based products like milk and yogurt alternatives or tofu

are widely used. However, other plant raw materials should be considered to broaden the variety of plant derived dairy alternatives.

With this regard, lupin which is highly underutilized in Ethiopia is a potential protein-rich candidate. Its alkaloid induced bitterness was one of the major reasons for its underutilization. However, plant breeders developed a new lupin species with reduced alkaloid level (i.e. less bitter and safe). Besides to the high protein content, sweet lupin seeds are characterized by a high content of fiber, as well as a low fat and starch content (Lampart-Szczapa et al, 2003). These seeds are free from trypsin inhibitors (Van, 1999) and have beneficial cardiovascular effects (Pilvi et al, 2006). Above all, lupin can grow on marginal land (Trinick, 1977), which makes it ideal crop for food insecure nations. But to advocate all its agronomic, nutritional and health benefits, food products should be developed from the seeds. Therefore, formulating milk analogues with underutilized, protein rich legumes like sweet lupin will bring their potentials to attention.

In fact, development of sweet lupin-based yogurt-like products is difficult due to the beany flavor in legumes and anti-nutrients causing flatulence etc. Yet, these problems can be solved through food processing techniques such as fermentation. The starter culture for the fermentation is commonly LAB. LAB as a probiotic provides many health benefits. Additionally, some LABs have a potential to provide essential micronutrients such as folate. Since folate is very essential, its synthetic form supplementation is highly advocated these days. Yet again, such supplementations are related with some types of cancer (Aynadis et al, 2018).

Therefore, it will be a value addition if folate producing LAB are used in developing fermented sweet lupin-based yoghurt. For long time, soybean has been used in the formulation of soymilk (Nsofor, 1996); (Ihekoronye, 1999). In countries like Nigeria yoghurt analogue legume and cereal based products were developed (Khan et al, 1989). Yoghurt-like product from cowpeas and mung

beans also conducted by (Rao et al, 1988), also yoghurt like beverage from oat flakes fermentation was done by (Luana et al, 2014), lupin-based milk alternatives by (Hickisch et al, 2016). Furthermore, studies have tried to develop yoghurt-like product from cereals and legumes which grow across the world. In Ethiopia, fermented foods like nondairy probiotic yoghurt analogues are not well developed and the awareness towards as such products is poor. In fact, as to our knowledge there was no study conducted to develop sweet-lupin based yoghurt like product in Ethiopia. Thus, the main objective of this study was to develop yoghurt like product from underutilized sweet lupin through fermentation using folate producing lactic acid bacteria.

1.3 Objectives

1.3.1 General objective

To develop sweet lupin-based yoghurt like product using folate producing lactic acid bacteria.

1.3.2 Specific objectives

The specific objectives were to:

- formulate sweet lupin-based yoghurt like product with high folate concentration
- evaluate physico-chemical characteristics (pH, titratable acidity, colour and viscosity) of the new product
- evaluate the consumer preference of the new product

CHAPTER TWO

LITERATURE REVIEW

2.1. Dairy products

Dairy products are milk and of the food made from milk, including butter, cheese, ice, cream, yoghurt and condensed and dried milk. Milk has been used by humans since the beginning of recorded time to provide both fresh and storable nutritious foods. Milk can be consumed as fresh, pasteurized, low-fat, or skimmed milk. However, most milk is manufactured into more stable dairy products such as butter, yoghurt, cheese, dried milks, ice cream, and condensed milk. Cow milk (bovine species) is by far the principal type used throughout the world. Other animals utilized for their milk production include buffalo (in India, China, Egypt, and the Philippines), goats (in the Mediterranean countries), reindeer (in northern Europe), and sheep (in southern Europe). In many developing countries, the milk yield is at a very low base and the increase in productivity will remain small.

India is expected to have the largest growth in milk production, outpacing the European Union to become the largest milk producer in the world. Pakistan will have the second largest increase in milk production, with an average growth rate of 3.4% p.a. In both cases, the vast majority of production is consumed domestically in its fresh form and does not imply an increase in processed dairy products (Founou et al, 2016).

Conversely, China is a much smaller producer and consumer of milk and dairy products yet is more important for international dairy markets. China's imports of dairy products have decreased over the last two years. It is expected that China's import demand will grow at a considerably lower rate, from over 20% p.a. for all major dairy commodities in the last decade, to between 7.3% p.a.

(cheese) and 2.5% p.a. (SMP) in the next decade, although this growth is from a higher base level (Founou et al, 2016).

Ethiopia is the second most populous country in Sub-Saharan Africa, rapidly increasing population size with a growing urban population is resulting in a growing demand for dairy products. The per capital milk consumption in Ethiopia is 19 liters per year, which is much lower than the global per capital milk consumption of 75 liters annually. Besides, the average price of milk in the present year is 26 Ethiopian birr which is difficult to acquire especially for low income people (FAO, 2011). A combination of cultural and economic factors are the main reasons of the low consumption level. Also, the demand of milk and other dairy products has been rising due to urbanization, transformation of habits and population growth. Nutritional value of milk and dairy products is listed below (Table 1).

Table 1. Nutritional value of milk and milk products from different animal sources

	Energy (Kcal)	Moisture (g)	Protein (g)	Fat (g)	Mineral (g)	Fiber (g)	CHO (g)	Ca (mg)	P (mg)	I (mg)
Milk, buffalo	117	81	4	6	1	_	5	210	130	0
Milk, cow	67	87	3	4	1	_	4	120	90	0
Milk, goat	72	87	3	4	1	_	5	170	120	0
Milk, human	65	88	1	3	0	_	7	28	11	_
Cruds-cow's milk	60	89	3	4	1	_	3	149	93	0
Buttermilk	15	97	1	1	0	_	0	30	30	0
Skimmed milk, liquid	29	92	2	0	2	_	5	120	90	0
Channa, cow's milk	265	57	18	21	3	_	1	208	138	_
Channa, buffalo milk	292	54	13	23	2	_	8	480	277	_
Cheese	348	40	24	25	4	_	6	790	520	2
Khoa whole buffalo milk	421	31	15	31	3	_	20	650	420	6
Khoa skimmed milk	206	46	22	2	4	_	26	990	650	3
Khoa, whole cow milk	413	25	20	26	4	_	25	956	613	_
Skimmed milk powder	357	4	38	0	7	_	51	1370	1000	1
Whole milk powder	496	3	26	27	6	_	38	950	730	1

Source: (Lock et al, 2004)

The high nutritional value makes cow's milk a complete food for babies, young children and adults (Potter and Hotchkiss, 1996). It plays an important role in food security for both highland and pastoral community.

2.2 Yoghurt

One of the most popular and consumed dairy product is yoghurt. It is a fermented product in which milk is fermented and acidified with viable and well-defined bacteria creating a thickened, often flavored, product with an extended shelf life. Yogurt can also be produced from rice, soy, or nuts. Yoghurt could also be described as milk product with a characteristic acidic taste possessing titratable acidity of 0.85-0.95% and pH 4.0-4.5 (Ihekoronye, 1999). It's fermented in the temperature range of 40-50^{0}C (Ihekoronye and Ngoddy, 1985). The nutritional composition of the yoghurt depends on the raw milk and processing condition. Yoghurt has been generally considered as excellent source of high quality of protein, calcium, potassium, phosphorous, magnesium, zinc, and B vitamins (Table 2).

The standard definition of yoghurt is a coagulated milk product obtained by lactic acid fermentation of milk through the action of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The real 'live' yoghurt must contain live organisms (i.e. friendly bacteria or probiotic bacteria) at the time of consumption (Dave and Shah, 1997).

2.2.1 Yoghurt processing methods and type of yoghurt products

With the development of processing technologies and the growing competition in the food market, the urge to provide nutritious food with appealing vitamins, minerals, and flavors has increased. Yoghurt is one of the popular fermented milk products having different names and forms (Tamime and Robinson, 2007).

It is a mixture of milk (whole, low-fat, or nonfat) and even cream fermented by a culture of lactic acid-producing bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Other bacteria may be added to the culture. The generalized process of yogurt production is summarized in [Figure 1] below.

Table 2. Nutritional value of yoghurt

Major constituents(g)	Amount
Energy value (KJ)	220.00
Protein (g/100g)	5.00
Fat (g/100g)	1.00
Lactose (ºB)	5.00
Galactose (ºB)	1.50
Lactic acid (µg/100g)	1.00
Citric acid (µg/100g)	0.30
Potassium (mg/100g)	0.24
Calcium (mg/100g)	0.18
Phosphorous (mg/100g)	0.14
Vitamins (per 100g)	
Vitamin A (I.U)	70-130
Vitamin B1/thiamine (g)	37-50
Vitamin B2/Riboflavin (g)	220-260
Vitamin B6/Pyridoxine (g)	.40-54
Vitamin / B12/Cyanocobalamin (g)	0.1-0.35
Vitamin C/ Ascorbic acid (mg)	0.1-1.0
Vitamin E / Tocopherol (g)	30
Folic acid (g)	4
Nicotinic acid (g)	120-130
Choline (mg)	0.6

Source: (Buttriss, 1997)

As having so many milk products from different animals and plants so as yoghurt also can be made. Most popular plant-based yoghurt types are soy-based yoghurt. The fermented soy products represent an interesting alternative to the

fermented milk products. The demands of consumers for cereal and legume-based yoghurt with high acceptance and functionality is increasing (Martensson et al, 2002).

As such products are literally expected to provide functional compounds such as antioxidants, dietary fiber, minerals, prebiotics, probiotics and vitamins (Kreisz et al, 2008). With this understanding the present study aimed to formulate a new type of sweet lupin –based yoghurt like product using folate producing LAB.

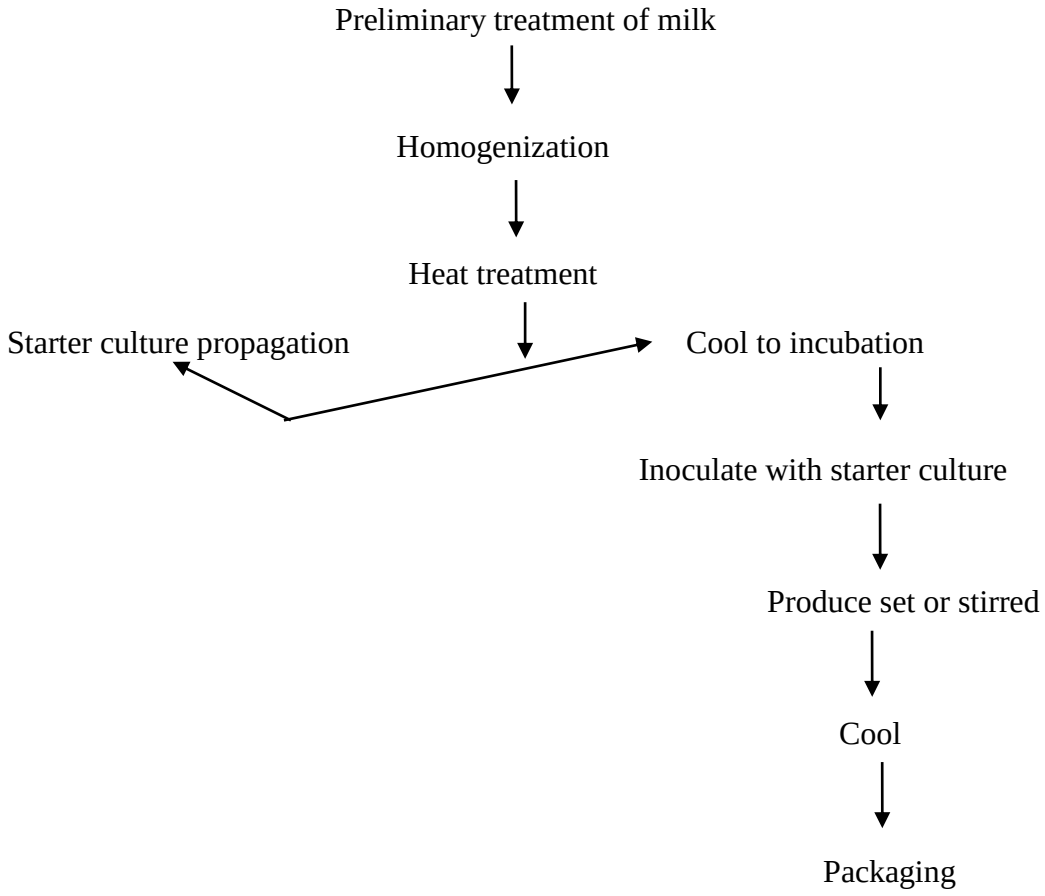


Figure 1. Generalized scheme illustrating the modern method for the production of yogurt. (Devcich, 2007)

2.3. Milk imitation and analogue products

Cow is a major source of milk-consumed worldwide. In view of the scarcity of cow's milk in developing countries, an over increasing gap between this requirement and population growth, there is a trend of shifting from cholesterol to phytosterol (Elizabeth, 2002). The short supplies of milk in various counties and the need for alternatives have motivated the development of beverages from locally available raw materials of plant origin. Efforts have been made over the years to develop alternative milk-like products from vegetable sources popularly called imitation milk (milk analogue). The development of imitation milk extracted from legumes is an alternative way of producing an acceptable nutritious food. Such milks could be used to replace or extend animal milk (Harkins and Sarett, 1967).

Many developing countries today suffer from shortages of fluid milk and other dairy products due to climatic conditions, breeding practices and prevalent diseases from parasites. These shortages have to a great extent affected the protein intake of both infants and adults (Fashakin and Unokiwedi, 1992). A remedy could be sought through extending fresh cow milk with soy milk in the production of certain products such as traditional cheese like product "warankasi" (Igyor et al, 2006).

2.4 Probiotic, prebiotic and synbiotics

Probiotics are food supplements composed of living bacteria, whose oral uptake results in health advantages for the consumer above the normal diet. Probiotics, literally meaning 'for life', are micro-organisms proven to exert health-promoting influences in humans and animals (Marteau et al, 2001). The role(s) of probiotic bacteria in dairy fermentations is to assist in:

(i) The preservation of the milk and developing cereals, legumes-based yoghurt by the generation of lactic acid and possibly antimicrobial compounds

(ii) The production of flavor compounds (e.g. acetaldehyde in yoghurt and cheese) and other metabolites (e.g. extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer

(iii) To improve the nutritional value of food, for example, the release of free amino acids or the synthesis of vitamins

(iv) The provision of special therapeutic or prophylactic properties as cancer (Stein et al, 1992) and control of serum cholesterol levels (Lin et al 1989) (Table 3).

Prebiotics are non-digestible food ingredients that improve the health status of the consumer through selective stimulation of growth and metabolic activity of a small number of bacteria in the colon, in particular *Bifidobacteria* (Table 3).

Synbiotics are food products combining probiotic and prebiotics (Glenn and Roberfroid, 1995) (Table 3).

Table 3. Important characteristics of pro-, pre- and synbiotics products

Probiotic	+	Prebiotic =	Synbiotics
Stability problems of probiotic bacteria during storage of the product		Good stability of oligo-saccharides during food processing and storage	Stability problems of probiotic bacteria during storage of the product partly corrected by the prebiotic
Stability problems of bacteria during passage of the upper gastrointestinal tract		Good stability of oligo-saccharides during passage of the upper gastrointestinal tract (exceptions possible)	Stability problems of bacteria during passage of upper gastrointestinal tract
Surviving bacteria exert			Surviving bacteria exert
beneficial effects		Oligosaccharides reach the colon and stimulate beneficial resident micro-organism	beneficial effects, in addition to stimulation of resident beneficial microflora
no side effects			possible side effects

In this study, *Lactobacillus plantarum* P2R3FA isolated from traditional cereal (tef)-based fermented food (Injera) by the Center for Food Science and Nutrition researcher in Addis Ababa University were used to develop the non-dairy yoghurt analogue product. It is well known this lactic acid bacteria produce a high concentration of folate (Aynadis Tamene, 2018). Moreover, sweet lupin like other legumes is a good source of fiber materials. Non-starch polysaccharides are well known to have a prebiotic activity. Therefore, the formulated fermented product in this study is sort of synbiotics.

2.5 Dairy alternatives

Nondairy probiotic products have a big worldwide importance due to the ongoing trend of vegetarianism and high prevalence of lactose intolerance in many populations around the world also the scarcity of milk in developing countries. However, still the dairy sector is strongly linked to probiotics, accounting for nearly 33% of the broad market, while cereal products have just

over 22%. A total of 78% of current probiotic sales in the world today are delivered through yogurt. Fruit juices, desserts, and cereal-based products featuring probiotics may be other suitable media for delivering probiotics (Cargill, 2009). Traditions and economic reasons that limit the use of dairy products in countries, such as Japan, China, and some African countries, promote the idea of reducing milk components as vehicles for the probiotic agents or even replacing milk with other media, such as cereals, fruits, and vegetables. Lactose intolerance, cholesterol content, and allergenic milk proteins are the major drawbacks related to the intake of dairy products, which makes the development of new nondairy probiotic foods essential. For instance, the potential of producing a probiotic fermented gruel based on a cereal and legume blend combined with a fresh tomato has been investigated. However, over the long years of research and testing of various nuts and seeds, milk analogue mainly had been prepared from soybean and other cereals (Adenekan et al, 2009).

Among which, the development of milk imitation extracted from legumes is an alternative way of producing an acceptable nutritious food. Such milks could be used to replace or extend animal milk (Harkins and Sarett, 1967). Thus, the present study evaluated the potential of sweet lupin to produce yoghurt analogue product. This would alleviate the intermittent use of existing dairy facilities in Ethiopia; it also improves the utilization of the underutilized crop, provides a synbiotics effect that is used to increase the health benefit for the consumer. As to our knowledge no study has been conducted on the sweet lupin-based yoghurt analogue.

2.6 Lupin seeds

Lupins have an ancient history in agriculture that traces back more than 4000 years (Kurlovich and Kartuzova, 2002). Domestication occurred first in the Mediterranean region and the American continent, but the real breakthrough that made lupin a modern agricultural crop occurred in Europe and Australia, it

serves as a fodder and food crop, as well as an ornamental plant. Lupin seeds are known for their high crude protein content. For instance, different cultivars of *Lupinus albus* (bitter species) from Ethiopia had average crude protein content between ranges of 33% to 39% (Tizazu et al, 2010), (Paulos et al, 2009), (EHNRI,1997). In some sweet lupin varieties, the amount reaches up to 50% (Petterson et al, 1998).

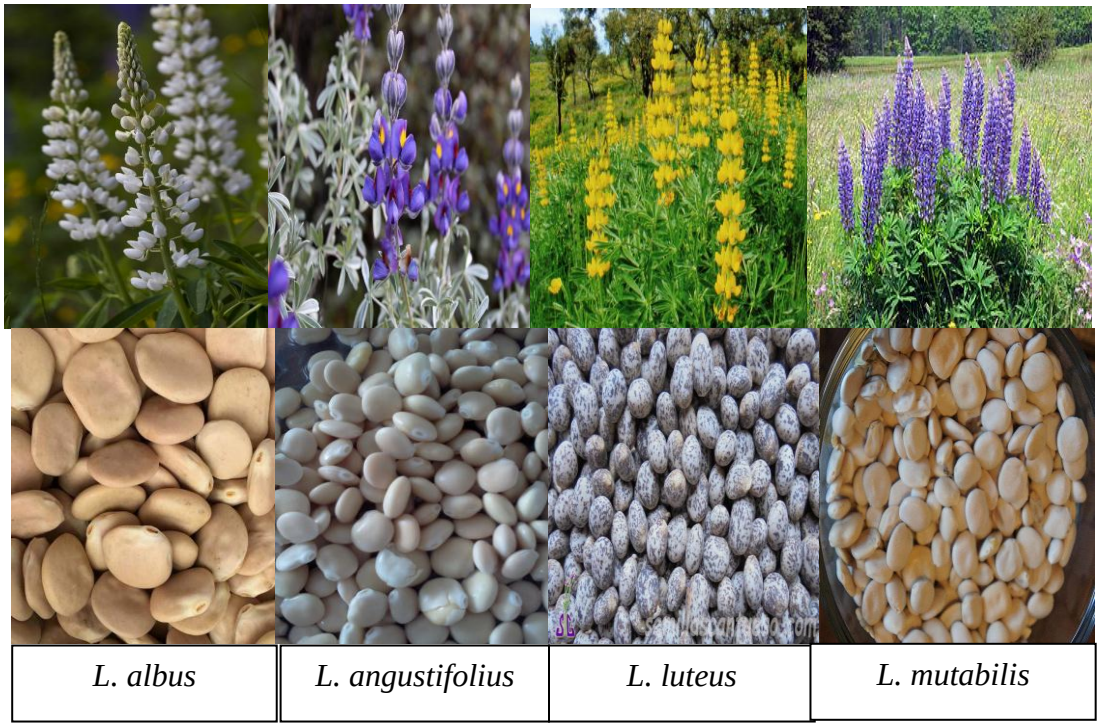


Figure 2. Worldwide existing lupin species

In their wild state, lupins have 'hard' (water impermeable) seeds, shattering pods and high level of alkaloids that makes lupin seeds toxic for human and animal consumption. The breakthrough in selecting natural mutants in *L. luteus* with low alkaloids (sweet type) by Von Sengbusch in Germany in 1927/1928, after the development of a quick method for detecting alkaloids, opened a new era in modern lupin breeding (Hondelmann, 1984).

Modern lupin breeding has focused on developing lupin species/varieties which produce seeds that are sweet and water-permeable, and non-shattering pods to facilitate mechanical harvest. Nutrient components of the lupin seed, like other grain legumes, it is a source of high-quality protein, essential amino acids, oil and other nutritive substances (Glencross, 2009) (Table 4). Australia, Europe and Japan use sweet lupins in dairy production. Lupins also have a long history of being consumed by humans in the Mediterranean and Andean regions (Petterson, 1998). However, less than 4% of global lupin production is used as human food (Lawrance, 2007).

Among the four commercially important large seeded sweet annual lupin species, narrow-leafed lupin (*Lupinus angustifolius* L.), white lupin (*Lupinus albus* L.), and yellow lupin (*Lupinus luteus* L.) are widely grown species in the world. The common cultivation areas of these species are Europe, Australia, South Africa and North and South America. According to (Erbas et al, 2005), a total of 1,387,660 t of lupin seed was produced worldwide in 2001. In most parts of the world the vast majority of lupin seed produced each year is used for livestock feeding. In addition to its use for livestock, it is also used in human nutrition.

Table 4. Nutritional composition of different lupin species

Nutrients	*L. angustifolius*		*L. albus*		*L. luteus*		*L. mutabilis*	
	Whole seed (%)	kernel (%)	Whole seed (%)	kernel (%)	Whole seed (%)	kernel (%)	Whole seed (%)	kernel (%)
Moisture	9	12	9	11	9	12	8	10
Protein	32	41	36	44	38	52	44	52
Fat	6	7	9	11	5	7	14	17
Ash	3	3	3	4	3	4	3	4
Fiber	15	9	10	2	13	2	7	10
Lignin	<1	<1	<1	<1	<1	<1	<1	<1
NSP	22	29	17	21	8	11	9	10
Oligosaccharides	4	6	7	8	9	12	5	6
Starch	ND	-	ND	-	-	ND	-	-

Sources: (Petterson, 1998). NSP: non-starch polysaccharides; ND: not detectable.

In Ethiopia white lupin is a traditional crop, grown in the north-western part of the country (Francis, 1999). (Yeheyis et al, 2010) reported that under traditional management systems the average grain yield potential of the crop is 1.2 t/ha. However, the use of the crop as human food and as livestock feed is limited. Lupin seeds possess many nutritional and food processing qualities, making them an attractive alternative to dry beans and soybeans. Foods derived from lupins are commercially manufactured in Europe, North America and Australia. These include lupin kernel flour-based products such as bread, pasta, milk, tofu,

tempe, miso, soy sauce and snack. Also, lupin hull is used as dietary fibre products or fibre additive to bread (Petterson, 1998). In Ethiopia there are legumes, cereals products such as flour, bread so as conducting researches to address sweet lupin-based product.

2.7 Folate

Folate, an important vitamin B9, participate in many metabolic pathway activities such as DNA and RNA biosynthesis and amino acid inter-conversions (Duthie, 2007). Folate deficiency has been implicated in a wide variety of disorders from Alzheimer's to coronary heart diseases osteoporosis increased risk of breast and colorectal cancer poor cognitive performance hearing loss and of course, neural tube defects Due to the occurrence of problems associated with current folic acid fortification programs, researchers have been looking for novel methods to increase concentrations of naturally occurring folates in foods (Durga et al, 2007).

Folate has more available in green leafy vegetables foods. Total folate content in selected cereals has wheat products (10-87 µg/100g, rice has 10-16µg/100g, maize and products 10-26µg/100g, sorghum and products 30-38µg/100g, millet and products 8-50µg/100g and sweet lupin has 98 µg/serving.) (Souci et al, 2000).

Cereal, vegetable and milk-based food products were investigated to increase the folate level although most of the studies focused on dairy products (Saubade et al, 2017). When compared to other food groups like offal's, green leafy vegetables and cereals, milk is not a rich source of dietary folate (Kneifel, 2000).

2.8 Functional and physico-chemical properties

Functional properties are the fundamental physicochemical properties that reflect the complex interaction between the composition, structure, molecular conformation and physico-chemical properties of food components together

with the nature of environment in which these are associated and measured (Kinsella, 1976); (Kaur and Singh, 2006); (Siddiq et al, 2009). Functional characteristics are required to evaluate and possibly help to predict how new proteins, fat, fibre and carbohydrates may behave in specific systems as well as demonstrate whether or not such protein can be used to stimulate or replace conventional protein (Mattil, 1971); (Kaur and Singh, 2006); (Siddiq et al, 2009).

The food property is characterized of the structure, quality, nutritional value and /or acceptability of a food product. A functional property of food is determined by physical, chemical, and/or organoleptic properties of a food. Example of functional properties may include solubility, absorption, water retention, frothing ability, elasticity and absorptive capacity for fat and foreign particulars. Typical functional properties include emulsification, hydration (water binding), viscosity, foaming, solubility, gelation, cohesion and adhesion. For a new food product to penetrate into the market its color, appearance, aroma, and flavor should be acceptable by the consumer (Zenthenbaur and Groh, 1998); (Purlis and Salvadori, 2007).

As a commercial product, it is important that the yoghurt has curd with sufficient hardness to stand up to the impact caused by shaking during transportation (Horiuchi et al, 2009). (Nielsen, 1975) suggested that the texture of yogurt should be firm enough to remove it from the container with a spoon. According to (Lewis and Dale, 1994), yoghurt should have a glossy surface appearance without excessive whey. Whey and Syneresis is a major defect of yoghurt (Lucey, 2001). The formulation of yoghurt products with optimum consistency and stability to whey syneresis is of primary concern to the dairy industry (Biliaderis et al, 1992). Factors influencing yoghurt texture and whey syneresis include total solids (TS) content especially proteins, homogenization, type of culture, acidity resulting from growth of bacterial cultures and heat treatment of milk (Harwalkar and Kalab, 1986).

Therefore, the main objective of this study was to formulate sweet-lupin based yoghurt like product using high folate producing LAB.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

Five kilograms of sweet lupin (*Lupinus luteus*) was procured from Holeta Agricultural Institute, Holeta, Ethiopia. *Lactobacillus plantarum* was obtained from Center for Food Science and Nutrition of Addis Ababa University, Addis Ababa, Ethiopia (Appendix 1). For dairy-free yoghurt alternative high folate producing *L. plantarum* strain was used, the most efficient folate producing strain (L. plantarum P2R3FA). The isolation of potential folate producing LAB was performed from tef dough. In order to have high chance of isolating folate-producing strains, the LAB was isolated and collected using a selection pressure on folic acid casei medium (FACM, Difco, France) which was a culture medium without folate but containing folate synthesis precursors (PABA and guanosine triphosphate). The physiological properties of the LAB were gram positive, non-spore former, catalase-negative characterized as fastidious, acid tolerant and fermentative microorganisms. (Aynadis et al, 2018).

Most glassware's, reagents, purity (sodium ascorbate, mercaptoethanol, hydrochloric acid, potassium hydroxide, ammonium hydroxide, petroleum ether, sodium hydroxide, lanthanum chloride, acetic acid, ethanol, methanol) and equipment were provided by the Center of Food Science and Nutrition, Addis Ababa University.

3.2. Methods

3.2.1. Yoghurt like product development method

In this study prior to the product development, according to (Agosin et al, 1989), a pretrial- was done at home following backslope fermentation. But the product from the pretrial did not possess the desired characteristics of yoghurt like

products. Hence, the processing method was further modified following the method by (Jimenez-Martinez et al, 2003). The parameters modified to obtain the desired characteristics were water to lupin seed ratio, soaking time, after blending the slurry to water ratio and the percentage of LAB (inoculum) used for fermentation.

3.2.2. Production of sweet lupin-based yoghurt-like product

Sweet lupin yoghurt was produced using the method described by (Agosin et al, 1989) and (Jimenez-Martinez et al, 2003) after some modifications based on the pre-trial processing. Briefly, 150g of the dry sweet lupin was cleaned to remove foreign, damaged and undesirable matter, and dust particles. The cleaned lupin was soaked in (1:9) water to seed ratio for 12hrs, then the water was removed and the seeds were dehulled discarding the hull. The dehulled lupin seeds were grinded with (1:1) water to slurry ratio with Laboratory Miller (Model: polymix LABE-7B, California, 2005).

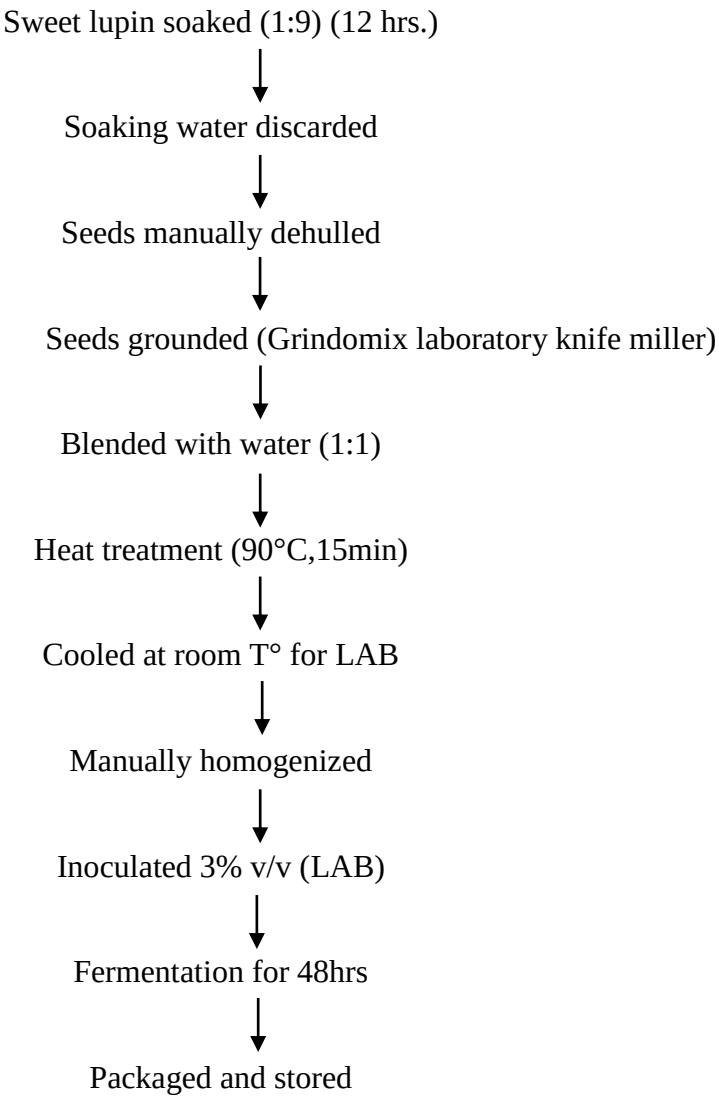


Figure 3. Sweet lupin-based yoghurt-like fermented product processing flow chart Modified from (Agosin et al, 1989) and (Jimenez-Martinez, 2003).

The slurry was pasteurized at about 90ºC with continuous stirring for 2 minutes and a holding period of 15 minutes. The pasteurized slurry was cooled at room temperature to create optimum temperature for the bacterial growth. Three percent of *Lactobacillus plantarum* folate producing strain of actively growing microbial starter culture was inoculated and incubated at a temperature of 30 ± 3ºC for two days until desired degree of acidity was achieved (Figure 3).

3.3. Analytical methods

Proximate composition

3.3.0 Preparation of flour

Flour was first prepared by heating the bean at 78ºC for 25min (Agosin et al.1989) then manually dehulled. The raw sweet lupin was grinded with Laboratory Miller (Model: polymix LABE-7B, California, 2005) for 10 to 15 minutes, followed by sieving through 500mm mesh size sieve (Model: AS200 digit, Germany, 2001).

3.3.1 Determination of moisture content

Moisture determination was carried out by the air oven method described by AOAC (2000) 9 25.09. About 5g of the sample (in triplicate) were measured into petri dishes (moisture dishes) of known weight. The sample was dried at 130^0C for 6 hours (U 10, DIN 12880-KI, Memmert, 854 Schwabach, West Germany) after which the dried sample was placed in a desiccator. Then, it was cooled to room temperature and weighed accurately. The percentage moisture content of the sample was calculated using the following formulae:

$$\text{Moisture (\%)} = \frac{(W2-W3)}{W1} * 100 \qquad \text{Equation (1)}$$

Where,
W_1 = weight of sample
W_2 = final weight of crucible + fresh sample
W_3 = weight of crucible + weight of sample after oven dried

3.3.2 Determination of crude protein

Crude protein was determined by microkjeldahl method as described by AOAC (2000) 979.09. About 0.5 g of sample was digested using Kjeldahl digester (Kjeltec 2300 Analyzer unit, FOSS, Sweden) with a concentrated sulfuric acid in a kjeldahl flask until the digest became clear. The digest was made up to 100ML and transferred quantitatively into a volumetric flask and 5ML aliquot was taken for distillation. The ammonia distilled was trapped into a mixture of

boric acid and titrated using 0.1N hydrochloric acid to pink colour end point. The crude protein was obtained by multiplying the nitrogen value by a conversion factor of 6.38 (for yoghurt). The total nitrogen content was calculated using equation 2.

$$\%\text{of Nitrogen} = \frac{(V_2 - V_1) * N * 14.01 * 100}{\text{Sample wt. (mg)}} \qquad \text{Equation (2)}$$

Where,

N = normality of titrant (standard hydrochloric acid) (0.1N)

V_1 = volume (ml) of hydrochloric acid solution used in blank sample titration

V_2 = volume (ml) of hydrochloric acid solution used in the titration for the sample

14.01 = molecular weight of nitrogen

The % of nitrogen is converted to % of protein by using a conversion factor of 6.38.

Crude protein content (%, w/w) = %Nitrogen* conversion factor (%N*6.38)

3.3.3 Determination of crude fiber

The crude fibre content was determined by AOAC (2000) method. About 2g of the sample was defatted using petroleum ether and boiled under reflux for 30 minutes with 200ML of a solution containing 1.25g of sulfuric acid per 100ML of diluted water solution. The solution was filtered through linen on a fluted funnel and washed with boiling water until the washings were no longer acidic. The residue was transferred to a beaker and boiled for 30 minutes with 200ML of a solution containing 1.25g of carbonate-free NaOH per 100ML of diluted water solution. The final residue was filtered through a thin but closed pad of washed and ignited asbestos in a Gooch crucible. The residue was dried in an electric oven and weighed. The residue was incinerated, cooled and weighed. The loss in weight after incineration multiplied by 100 percent is the percentage of crude fibre as shown below (Equation 3):

Crude fiber content (%)$= \frac{(M2-M3)}{M1}$*100 Equation (3)

Where,

M_1 = weight of sample

M_2 = weight of crucible and sample after drying

M_3 = weight of crucible and sample after ashing

3.3.4 Determination of crude fat

This was carried out in accordance with the Soxhlet extraction method described by AOAC (2000) 24.5.01 using soxhlet machine (2055 SOXTEC extraction unit, FOSS extractor, Sweden). Two grams of the sample was weighed into a thimble. The thimble and contents were transferred to the extraction apparatus and the beaker was rinsed several times with petroleum ether (40:60). The sample contained in the thimble was extracted with petroleum ether in a soxhlet extraction apparatus for 4-5 hours at a condensation rate of 3-6 drops per second. Upon completion of the extraction, the fat extract in the extraction flask was dried in a (U 10, DIN 12880-KI, Memmert, 854 Schwabach, West Germany) for 30 minutes at 100^0C until odour of petroleum ether was not detected. Then, the extract was collected in a desiccator and weighed. The crude fat content was calculated using equation 4.

Crude fat content (%) = $\frac{W_3-W_2}{W_1}$*100 Equation (4)

Where,
W_1= weight of sample (g)
W_2= weight of extraction flask (g)
W_3= weight of extraction flask with the dried crude fat (g)

3.3.5 Determination of total ash

The total ash content of the sample was determined by following the analytical method of AOAC (2000) 923.03. Porcelain dishes used for the analysis were washed using dilute hydrochloric acid on boiling followed by washing with distilled and de-mineralized water respectively. Then, the dishes were dried at

120°C in an oven (U 10, DIN 12880-KI, Memmert, 854 Schwabach, West Germany) and ignited at 550 ^{o}C in muffle furnace (Carbolite, Aston Lane, Hope, Sheffield s30 2RR, England) for 3hrs. Then, the dishes were removed from the furnace and cooled in a desiccator. The mass of the dried dish was measured as W2 and 2.5 gm of sample was being weighed into it and recorded as W1. Then, the sample was charred at 120°C for 30 minutes in a hot plate (Wagtech, hot plate SH3, UK), until the whole content became carbonized. The charred sample was placed in a furnace at 550°C until free from carbon and the residue appears grayish white after 5 hrs. The sample was removed from the furnace and placed in a desiccator. Finally, the mass was weighed as W3, and the total ash content was calculated with the following formula (Equation 5): -

Crude ash content (%) = $\frac{(W3-W2)}{W1}$ *100 Equation (5)

Where,

W_1= weight of sample

W_2= weight of empty crucible

W_3= weight of crucible and sample after ashing

3.3.6 Determination of total carbohydrate

The total carbohydrate content was estimated as the difference between 100 and the total sum of moisture, fat, protein, and ash

Total carbohydrate (%) =100 – (Moisture + Ash + Protein + Fat) % Equation (6)

Utilizable carbohydrate content (%) = (Total carbohydrate – crude fiber) %

3.3.7 Determination of total energy

The caloric value of the samples was estimated by calculation using the quantification factors of 4, 9 and 4Kcal/100g respectively for protein, fat and carbohydrate.

Gross caloric value (Kcal/100g) = (P*4) + (F*9) + (C*4) Equation (7)

Where,

P = protein content (%)

F = fat content (%)

C = total carbohydrate (%)

3.4 Physicochemical analysis

3.4.1 Determination of Titrable acidity

Titrable acidity was measured according to AOAC (1990) protocol. Briefly, 6 gm of sample was placed in 250 ML conical flask. Then, 50 ml of distilled water was added followed by 2 drops of 1% phenolphthalein indicator. The mixture was titrated with 0.1N NaOH. The result was recorded as soon as the first appearance of a pink color. Titration continued until the color persisted. The result obtained was calculated as follows (Equation 8):

$$\text{TTA}\ (\%) = \frac{\text{Volume of NAOH used} \times 0.1\text{N NAOH} \times 0.09 \times 100}{\text{Sample wt. (gm)}} \qquad \text{Equation (8)}$$

3.4.2 Determination of pH

pH of was measured with a Townson pH meter as described by (Danbaba and Oyeleke et al, 2014). The sample homogenate was prepared by blending 10ml sample in 100ml of deionized water. The mixture was filtered and the pH of the filtrate was measured. The average of triplicate readings was recorded for each sample, the pH was calibrated using pH 7 and pH 2 buffer solutions.

3.4.3 Determination of Water Absorption Capacity (WAC %)

The water absorption capacity of the flour was determined by the method of (Sosulski et al, 1976). Briefly, 1gm of sample was mixed with 10 ML distilled water and allowed to stand at ambient temperature (30 ± 2°C) for 30 minutes. Then, centrifuged (DYNAC II centrifuge, Clay Adams, division of Becton and

Dikinson Company, USA) for 30 minutes at 3000 rpm or 2000 × g. Then, the volume of the supernatant was measured. Similarly, WAC of the seed, 50gm of seed was soaked in 100ML of distilled water for 24hrs. Then, the seed was discarded and remaining water was measured.

$$\text{WAC (\%)} = \text{ml of water/ gram of sample} * 100 \qquad \text{Equation (9)}$$

3.4.4 Determination of foaming capacity and stability

The foaming capacity (FC) and foaming stability (FS) were determined using the method by (Narayana and Narasinga, 1982) with slight modifications. Briefly, 1g flour sample was added to 50 ML distilled water at 30 ± 2°C in a graduated cylinder. The suspension was mixed and shaken for 5 min to foam. The volume of foam was recorded one hour after whipping to determine foam stability as per percent of initial foam volume. The volume of the foam at 30 secs after whipping was expressed as foam capacity using the following formula:

$$\text{Foaming capacity (\%)} = \frac{\text{Volume of foam AW} - \text{Volume of foam BW}}{\text{Volume of foam BW}} \times 100 \qquad \text{Equation (10)}$$

Where,

AW = after manually whipping BW = before manually whipping

3.4.5 Determination of hull weight

This was carried out in accordance with the method by AOAC (2010). About 5 grams of dry seed was measured then soaked in distilled water (1:9) ratio for 24 hrs. Then, the seed was dehulled and the hull was dried at 150 ^{o}C in drying oven (U 10, DIN 12880-KI, Memmert, 854 Schwabach, West Germany) for 1 hr. until constant weight was obtained and calculated as followed: -

$$\text{Hull size (\%)} = \frac{M}{SW} \times 100 \qquad \text{Equation (11)}$$

Where:

M = dried weight of hull (gm), SW = sample weight (gm)

3.4.6 Determination of viscosity

The viscosity of the yoghurt like product was determined using Brookfield rotational viscometer. (Massachusetts, Dongguan China, KJ-LVDV-S) followed the (Sathe and Salunkhe, 1981) method. About 200 ML of sample was poured into a beaker at room temperature. Then, the spindle number #62 was immersed into the test fluid up to the notch cut in the shaft and the viscometer motor was off. Then, the motor was set at the lowest speed revolutions per minute 100 (rpm) with 42 centipoises. The power will be on. Once the digital display showed a stable reading for 1 minutes, the percentage of full scale torque reading was recorded. After the reading the motor was stopped and the spindle was slowly raised from the sample. Finally, the spindle was removed and cleaned with soap/water and dried. The reading was replicated three times and calculated as follow:

$$\eta = \text{display reading } \% \times \text{factor} \qquad \text{Equation (12)}$$

3.4.7 Determination of color

The colour of the yoghurt like fermented product was measured by Digital imaging system (Computer Vision) as described by (Saeed et al, 2012). Digital imaging system includes the capturing, processing and analyzing images, facilitating the objective and nondestructive assessment of visual quality characteristics in food products (Timmerman, 1998). The L*a*b color space was used for determining yoghurt colors because color of foods has been measured in with this method adopted by the Commission International ed‟Eclairage (CIE) in 1976. Where L* is the luminance or lightness component, which ranges from 0 to 100, and parameters a* (from green to red) and b* (from blue to yellow) are the two chromatic components, which range from -120 to 120 (Segnini et al, 1999); (Papadakis et al, 2000); (Yam and Papadakis, 2004). The L*a*b* color space gives uniformity in color distribution and closeness to

human perception (León et al, 2006). The detailed procedure for crust color determination was as follows:

Image capturing

The sample was selected and captured. To avoid light reflection in the space and preventing from fluctuation in imaging, a chamber having light bulb was prepared and a yoghurt sample was placed in the chamber for imaging (Figure7). The images were captured by a SONY Camera (Model: DSC-W610, 14.1 mega pixels) which was connected to computer via USB port. The camera was fixed parallel to and at a distance of 30 cm from yoghurt like product samples. The Imaging was performed using SIGMA Capture Pro 1.1.

Image Processing

500*500-pixel (Area = 250000 pixels) pieces are cut from the captured images and saved under BMP format. The captured image was in RGB color space, we have to convert it to L*a*b* color spaces by using ImageJ 1.46r software. By aid of the ImageJ package referred to as "Color_Space_Converter".

Analyzing color

The converted L*a*b* images were analyzed using ImageJ 1.4 software and the mean value and standard deviation of color intensity in the image pixels was obtained and saved in Microsoft Excel 2010. The total color change, ΔE of the yoghurt from the reference is:

$$\Delta E^{*} = [(L^{*})^{2} + (a_{*})^{2} + (b_{*})^{2}]^{1/2} \qquad \text{Equation (13)}$$

Where:

$L_0=100$, $a_0=0$ and $b_0=0$

3.4.8 Determination of bulk density

The bulk density of both the raw and processed samples was determined by the method of AOAC (2010). Briefly, the mass and volume of the samples were measured and the respective ratio was considered as bulk density (Equation 14).

$$\text{Bulk Density} = \frac{\text{Mass of the sample}}{\text{Volume of the sample}} \qquad \text{Equation (14)}$$

3.5 Minerals analysis

The mineral analysis was conducted according to (Rustom et al, 1998). The ash was dissolved by 5 ML of 6 M HCl at low temperature on hotplate (Wagtech, hot plate SH3, UK) for about 2 hrs. Then, 7 ML of 3 M HCl was added and heated on a hot plate until the solution boiled. The digest was cooled and filtered through a filter paper (42 nm, whatmann) into a 50 ML volumetric flask. Then, 5 ML 3 M HCl was added to the dishes and heated to dissolve the residue in the dishes and then transferred to the volumetric flask. Then, the filter paper was washed thoroughly and the washing was collected in the 50 ML flask made to the mark. Afterwards the minerals concentration was determined by Atomic Absorption Spectrophotometry (AAS) (BECKMAN, Du-64 Japan). For calcium determination 2.5 ML of 10 % Lanthanum chloride solution was added to the flask. Then diluted to 50 ML mark with de-ionized water. The blank was prepared by the same procedure without the sample.

The instrument was set based on the instruction given in the manual. The calibration solutions and the reagent blank solutions were measured first. Then, the samples were run following the calibration values. The calibration curve was prepared for the required metal by plotting the absorption values against the metal concentration in ppm. The mineral contents of each sample were calculated using the following equation: -

$$\text{Mineral content}\left(\frac{\text{mg}}{100\text{gm}}\right) = \frac{\text{conci} \times \text{v} \times \text{df}}{\text{s}} \times 100$$ Equation (15)

Where: - $conc_i$ = initial concentration

V = volume (liter)

df = dilution factor, S = sample weight (gm)

3.6. Analysis of antinutritional factors

3.6.1 Phytic Acid

The phytate content in the sample was determined accordingly to modified by (Vaintraub and Lapteva, 1988). About 50mg of dried sample was extracted with 10 ML, 2.4 % HCl in methanol for 1 hr. at ambient temperature and centrifuged (DYNAC II centrifuge, USA) (3000 rpm) for 30 minutes. The clear supernatant was used for the phytate estimation. One ml of wade reagent (0.03 % solution of $FeCl_3$:$6H_2O$ containing 0.3 % sulfosalicylic acid in water) was added to 3ML of the sample solution and the mixture was centrifuged. The absorbance at 500 nm was measured using UV-spectrometer (BECKMAN, Du-64 Japan). The phytate content was calculated from the difference between the absorbance of the control (3 ML of water + 1 ML wade reagent) and that of the assayed sample. The concentration of phytate was calculated using phytic acid standard curve and the results were expressed as of phytic acids in mg per 100 gm dry weight.

$$\text{phytate}\left(\frac{\text{mg}}{100\text{gm}}\right) = (\text{As} - \text{Ab}) - \frac{\text{int}}{(\text{Slope} \times \text{W})}$$ Equation (16)

Where: -

As = sample absorbance

Ab= blank absorbance

Int= intercept, w= weight of sample

To construct the phytic acid standard curve, a series of standard solution were prepared containing 5-40 mg/ML phytic acid in water. 3 ml of the standard were

pipetted into 15 ml centrifuge tubes with 3 ML of water used as zero level. To each tube was added 1 ML of the wade reagent, and the solution was mixed on vortex mixer (Maxi mix II M 37610-26 Thrmolyne Dubaque lowa, USA) for 5s. The mixture was further centrifuged for 10 minutes and the absorbance of the supernatant was read at 500 nm by using water as a blank.

3.6.1.1. Bioavailability of minerals

The use of phytate: zinc molar ratios of the diet and recommend the use of dietary phytate: iron molar ratios to estimate the negative effect of phytate on zinc and iron bioavailability, respectively (Hurrell and Egli, 2010). Calculated as follow:

$$\frac{\text{Phytate (mg)}}{\frac{\text{660 (MW)}}{\text{Zn (mg)}}} \qquad \frac{\text{Phytate (mg)}}{\frac{\text{660 (MW)}}{\text{Fe (mg)}}}$$

65.38 (AtW) 55.845 (AtW)

Where: - AtW: Atomic weight
- MW: Molar weight

3.6.2. Determination of total alkaloid

The alkaloid content was determined gravimetrically by the method of (Adeniyi et al, 2009). Briefly, 5 gm of each sample was weighed and dispersed into 50 ML of 10 % acetic acid solution in ethanol. The mixture was well shaken and then allowed to stand for about 4 hrs. before filtration. The filtrate was then evaporated to one quarter of its original volume on a hot plate (Wagtech, hot plate SH3, UK) at 30ºC. Then, concentrated ammonium hydroxide was added drop wise in order to precipitate the alkaloids. A pre-weighed filter paper was used to filter off the precipitate and the precipitate was washed with 1 % ammonium hydroxide solution followed by drying in an oven at 60ºC for 30 minutes. Then it was transferred into a desiccator to cool and then reweighed until a constant weight was obtained. The weight of the alkaloid was determined

by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed. The experiment was repeated three times for each sample type and the reading recorded as the average of the triplicates (Adeniyi et al, 2009).

$$\text{Total alkaloid conten (\%)} = \frac{Wp - Wf}{Ws} \times 100 \qquad \text{Equation (17)}$$

Where;

Wp = weight of precipitate on the filter paper

Wf = weight of filtrate

Ws = weight of sample

3.6.3. Determination of condensed tannin

Tannin content was determined by the method of (Burns, 1971). Briefly, 2 g of the raw flour and new product was weighed in a screw cap test tube. The samples were extracted with 10 ML of 1% HCl in methanol for 24 h at room temperature with mechanical shaking (Tecator, CYLOTEC 1093, Swden) at 1000rpm. Then, the solution was centrifuged (DYNAC II centrifuge, USA) at 1000 rpm for 5 minutes. One ML of the supernatant was taken and mixed with 5 ML of vanillin-HCl reagent (prepared by combining equal volume of 8% concentrated HCl in methanol and 4% Vanillin in methanol). D-catechin was used as standard for condensed tannin determination. Accordingly, 40 mg of D-catechin was weighed and dissolved in 1000 ML of 1% HCl in methanol, which was used as stock solution. Then, stock solutions of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml were taken in a test tube and the volume of each was adjusted to 1 ML with 1% HCl in methanol. Five ML of vanillin-HCl reagent was added into each test tube. After 20 minutes, the absorbance of sample and standard solutions was measured at 500 nm by using water as a blank using Uv- spectrophotometer (BECKMAN, Du-64 Japan).

$$\text{Tannin content } \left(\frac{\text{mg}}{\text{g}}\right) = \frac{((\text{A} - \text{B}) - \text{Intercept}) \times 10}{\text{Slope} \times \text{d} \times \text{Sw}} \qquad \text{Equation (18)}$$

Where;
A = sample absorbance
B = blank absorbance
d = density of solution

3.7. Determination of folate content

The total folate content of sweet lupin flour, slurry and fermented sweet lupin yoghurt analogue was determined using the reference microbiological method, after trienzyme extraction (Kariluoto et al, 2004). The steps of folate analysis are presented in Figure 4.

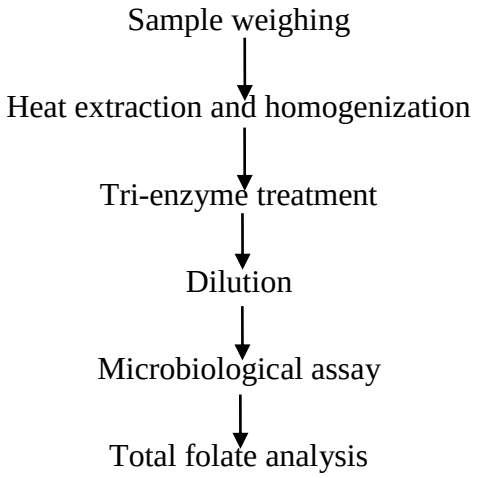


Figure 4. Flow chart of folate analysis

A. Extraction and tri-enzyme treatment

The first step in the folate analysis using microbiological assay was extraction followed by tri-enzyme treatments (conjugase, α-amylase and protease). Sample amount was 1.0-1.5 g depending on the estimated folate content. Sample was weighed into plastic tubes and 15ML extraction buffer (50 mM Ches, 50mM Hepes containing 2% sodium ascorbate and 10mM 2-mercaptoethanol, pH=7.85) was added. Samples were flushed with nitrogen, placed in a boiling water bath (DANI, model ALS100, 0305041069, Italy) for 10 min, cooled in ice

and homogenized. Samples were cooled and the pH was adjusted to 4.9 with HCl, Hog kidney conjugase (HK) was prepared from fresh pork kidneys according to (Gregory et al, 1984). Its activity was tested in every batch according to (Vahteristo et al, 1996).

Sample extracts were first incubated under a nitrogen atmosphere with α – amylase (EC 3.2.1.1, St. Louis, MO) and HK for 3 h at 37 ºC in a water bath. After that, the pH was adjusted to 7.0 with KOH and protease (EC 3.4.24.31; Sigma, St. Louis, MO) was added. Extracts were incubated under a nitrogen atmosphere for 1 h at 37 ºC, after which they were boiled for 5 minutes in a boiling water bath to inactivate the enzymes and cooled on ice. Samples were filled to an exact volume of 25 ML with 0.5 % (w/v) sodium ascorbate, pH 6.1 and then analyzed directly with the microbiological assay. A blank sample was analyzed in each set of samples and the results were corrected accordingly.

B. Microbiological assay

Total folate content was determined as previously described by (Kariluoto et al, 2004); (Piironen et al, 2008). The assay was carried out on 96-well microtiter plates and the total folate content was determined based on the growth of folate dependent strain, *Lactobacillus rhamnosus* ATCC 7469 as the test organism and [6S]-5-formyltetrahydrofolate.

(5-HCO-H4, folate) as the calibrant. Cryoprotection and preparation of working inoculum were performed according to (Molloy and Scott, 1997). Two dilutions were made from each sample extract using 0.5 % Sodium ascorbate solution and eight levels of calibrant (0–80 pg/well) were pipetted into 96-well microtiter plates (Tissue culture treated; Costar Corporation, Cambridge, MA), four wells for each level. Sodium ascorbate (0.5 %) was added to the calibrant-containing wells so that the final volume in each well before adding the inoculated medium was 100 µl. From each dilution, 100 µl was pipetted into four wells. Inoculated medium was then added into each well (200 µl). Plates were incubated for about

18 h at 37 ºC and turbidity was measured with a microplate reader (iEMS Reader MF; Labsystems, Helsinki, Finland) at 595 nm. All of the total folate results were given as the means of triplicates on a dry matter basis. Readings were adjusted using sample blank. Method performance was confirmed by analyzing a blank sample and as well as using certified reference material (CRM 121 wholemeal flour) in each set of samples.

3.8. Sensory analysis

The formulated probiotic yoghurt- analogue from sweet lupin was subjected to sensory test using 18 trained panelists according to the procedure described by (Wichchukit and Mahony, 2015). Rating acceptance sensory test was scored for attributes of colour, flavor, mouthfeel/consistency, taste and overall acceptability on a 9-point hedonic scale where 9 being extremely like and 1 being extremely dislike (Appendix 2). The panelist was arrived in sensory analysis room. The room have a box chair which protect them from disturbance. The sample was coded in different numbers (Appendix 2). For each sample they rinsed their mouth in the available sink using water. The new fermented product was also rated relative to commercial yoghurt product (farm fresh yoghurt). The acceptance rating was also done after a vanilla flavoring agent (to mask the beany flavor) was added to the new yoghurt like product. The order of product presentation to the panelists was randomized.

3.9. Data analysis

The collected data were analyzed performed using SPSS® (version 17). p-test was carried out to determine significance of difference at $p<0.05$. All analyses (except sensory analysis) were conducted in triplicate and results were expressed as mean ± standard error.

CHAPTER FOUR

RESULTS and DISCUSSION

4.1.1 Proximate composition of raw sweet lupin and yoghurt like product

In this study, the crude protein content of the sweet lupin (*L. luteus*) was founded 35.28% (Table 5). In other study finding shows value the sweet lupin had higher protein content than other legumes such as chickpea (22.9%) (Marconi et al, 2000); pea (23.9%) (Fernández-Quintela et al, 1997), soybean (30%) (Sosulski & McCurdy, 1987). The high protein content of sweet lupin plays important functional roles in food processing beyond nutrient source. These functional properties include emulsification, water absorption and adhesion, which are important for formulating yoghurt like products to make the product stable.

In this study, the crude fat content of the raw *L. luteus* seed was 9.36 %. Similar amount was reported in sweet lupin by (Petterson et al, 1998). However, in bitter *L. albus* collected from Ethiopia, higher fat content (9%) was reported by (Paulose et al, 2009). In other bitter lupin seeds also a higher (between 8-11) % fat content was reported (Uzun et al, 2007); (Sujak et al, 2006). From these studies one can assume a higher fat content in sweet lupin than the bitter species. The crude fiber content of the sweet lupin in this study was 3.61 %, which was slightly higher than the value reported by (Petterson et al, 1998). In bitter lupin species collected in Ethiopia, around 11% crude fiber content was reported by (Paulose et al, 2009), (Shimelis Emire et al, 2010) and (EHNRI, 1997). The total ash content of the raw sweet lupin was 3.03%. Whereas for the same species (Petterson et al, 1998) reported a total ash content of 4.0%. Same species for the bitter *L. albus*, average 3% total ash content was reported by (EHNRI, 1997) and (Sileshi, 1985). The raw *L. luteus* seed had total carbohydrate content of 44.23%. In general, the sweet lupin seed in this study had comparable and higher contents of macronutrients with other lupin species and legumes.

Moisture content in commercial dairy-based yoghurt products is (70-80) % (Sagdic et al, 2002). In this study, the yoghurt-like product had a moisture content of 19.87%. Hence, all the nutrient compositions were reported on dry basis taking into consideration the high moisture content. The crude protein content in the yoghurt-like product was 36.40 % (Table 5), which was a higher value than in the raw seed. Similarly, (Rao et al, 1988) reported higher crude protein levels in yoghurt-like product prepared from cowpea and mung bean than in the raw seeds. In contrast, the crude fat, total ash, crude fiber and gross energy contents of the new fermented product were lower compared with the raw seed (Table 5). (Obizoba and Egbuna, 1992). Though not investigated in this study, during fermentation of legumes a reduction in oligosaccharide levels is expected due to enzymatic activity. The reduction in the levels of these flatulence inducing sugars is significant in human nutrition (Nnam, 1997).

The average protein content in commercial yoghurt products is higher than that of milk because of the addition of non-fat dry milk (Buttriss, 1997). On average, the protein content of commercial yoghurt products is 5% (Dave and Shah, 1997), which is much lower than the amount in the sweet lupin-based yoghurt like product. In fact, the crude fat, crude ash, crude fiber, total carbohydrate and gross energy contents were also higher compared with commercial milk-based yoghurt product.

Table 5. Proximate composition of raw sweet lupin and yoghurt-like fermented product

Nutrient	Raw sweet lupin (g/100g)	Yoghurt-like product (g/100g)
Moisture	8.10 ± 0.53	19.87 ± 0.23
Crude protein	35.28 ± 0.99	36.40 ± 0.00
Crude fat	9.36 ± 0.76	8.96 ± 0.35
Total ash	3.03 ± 0.30	2.64 ± .001
Crude fiber	3.61 ± 0.00	2.07 ± 0.50
Total carbohydrate	44.23 ± 0.00	32.13 ± 0.00
Gross energy (kcal/100g)	402.28 ± 0.00	354.76 ± 0.00

Data are expressed as mean ± SE. (n=3) on dry basis.

4.1.2. Hull weight and functional property of raw sweet lupin

For new product development information on functional property of ingredients is important. As reported in Table 6, 21% of sweet lupin seed was the hull by weight. The WAC for the flour and seed were 310 and 460%, respectively. In fact, the WAC of the sweet lupin flour was higher than the reported value for wheat flour (140%) (Chandra 2013). Similarly, the bitter lupin, *L. albus* flour had a lower (276 %) WAC than the sweet lupin in this study. WAC is considered as an important functional property in viscous foods, such as sauces, doughs, yoghurt and baked products (Sosulski, 1976). In the yoghurt-like product processing, primarily the seed was soaked in water (1:9) then the grounded slurry was mixed with water (1:1) ratio. As water was major ingredient of the product, for its consistency the high WAC of the sweet lupin was important. Furthermore, (Sodini et al, 2004) mentioned that there is a

relationship between TA/pH and WAC and WHC (For instance, WHC of 67% and 65% was reported for yoghurt with pH 4.50 and 3.85, respectively).

The other functional properties of the sweet lupin flour determined in this study were foaming capacity (FC) and stability (FS). The FC and FS values of the sweet lupin flour were 12 and 10% respectively (Table 6). Foaming capacity and stability of legume flours depend on the type of protein, degree of denaturation, pH, temperature and whipping methods (Kinsella, 1976). The crude protein content of the sweet lupin in this study was 35.28% (Table 5). The high crude protein content might be linked with the foaming capacity reported. Since foaming capacity and stability appears to be due to solubilized protein, higher values will enhance its functionality in its uses for the production of cakes, whipping toppings etc. Therefore, the foaming capacity and stability of sweet lupin flour in this study were important to formulate scooped and stabilized creamy yoghurt like product (Figure 7). The other common legume pea had FC and FS values of 68% and 20% respectively (Oshodi & Ekperigin, 1989). Similarly, (Ghavidel & Prakash, 2006) reported a 22% FC for lentil flour.

The bulk density of the sweet lupin seed was 0.78 g/ml (Table 6). Similar bulk density values were reported on three bitter lupin cultivars by (Tizazu et al, 2010) (0.58, 0.61, 0.75) g/ml. The bulk density of milk yoghurt is 1.06g/ml, which is lower than the value in the new product. Bulk density is important in packaging and transportation of food products. Bulk density gives an indication of the relative volume of packaging material required and high bulk density is a good physical attribute when determining the mixing quality of a particulate matter (Aloys and Zhou, 2006).

Table 6. Hull weight and functional property of raw sweet lupin seed

Parameters tested	Content
Hull weight (g/100g)	20.60 ± 0.00
WACF (g/100g)	310.00 ± 1.41
WACS (g/100g)	460.00 ± 0.00
FC (g/100g)	12.00 ± 0.00
FS (g/100g)	10.00 ± 0.00
BDA (g/ml)	0.78 ± 0.00
BDB (g/ml)	1.25 ± 0.00

Data are expressed as mean ± SE. (n=3). WACF (Water Absorption Capacity of Flour), WACS (Water Absorption Capacity of Seed), FC (Foaming Capacity), FS (Foaming Stability), BDS (Bulk Density of the seed), BDN (Bulk Density of the new product)

4.1.3 Concentrations of macro-, micro-minerals and folate in raw sweet lupin seed and yoghurt like product

White lupin seeds are rich sources of macro- and micro-minerals (i.e. on average 30-40) mg/kg (Straková et al, 2006); (Sujak et al, 2006); (Saastamoinen et al, 2013). Among the macro elements (calcium, potassium, manganese, and magnesium) and microelement (iron) are dominant (Zelalem and Chandravanshi, 2014). In this study, the raw sweet lupin seed and yoghurt like product had concentrations of sodium, calcium, potassium (10.36, 3.11), (27.78, 0.46), (4.74, 1.76) mg/100gm respectively (Table 7). Previous reports on sodium levels of sweet lupin included (Carvalho et al, 2011) (30 mg/100g), (Fudiyansyah and Petterson et al, 1995) (0.5 mg/100g) and according to (Janusz, 2017) (0.2-1.2) mg per kg. Sodium and potassium are two critical minerals that have been identified as nutrients of concern by World Health Organization (WHO). The ratio of sodium to potassium in the diet is more important than the

amount of either one alone. Hence, it is important to examine not only individual mineral's intake but also their ratio in food products. Higher ratio is more strongly associated with an increased risk of hypertension and cardiovascular disease (Holmes et al, 2012). Accordingly, WHO recommends sodium intake of <2000mg/day and adequate potassium intake of ≥3510mg/day for healthy adults. Based on this, the Na: K ratio of the sweet lupin-based yoghurt like product was 1.7, which was a modest amount as per the recommendation. The calcium concentration in this study was lower than the report by (Fudiyansyah and Petterson et al, 1995) from African and Australian sweet lupin (0.502 and 1.2 g/kg) respectively. The amount of Na, Ca and K in commercial yoghurt is 0.35, 0.18, 0.24mg/100g (Buttriss, 1997). The Ca amount of commercial yoghurt has a higher value than sweet lupin yoghurt analogue. But the Na and K contents were higher in the new product. At the end of fermentation (48hrs), the levels of sodium, calcium and potassium decreased significantly (Table 7).

In this study, the concentrations of iron, zinc and manganese in the raw sweet lupin seed and yoghurt like product were (7.71, 0.21), (0.84, 0.46) and (4.40, 3.75) mg/100gm respectively, on dry basis (Table 7). (Trugo et al, 1992) reported the iron content of bitter raw *L. albus* of various cultivars in the range of 3.5-7.7 mg/100 gm. In this study, the iron content in the raw sweet lupin was within this range. Raw bitter lupin seed collected from open market in Ethiopia had zinc content of 2mg/100gm (Paulos et al, 2009), which was higher than the value in the present study. In previous study by (Paulose et al, 2009), manganese level beyond safety limit was reported in raw and processed white *L. albus* (59mg/100gm). Similarly, (El-Adawy et al, 2000) has reported 10.8 mg/100 gm manganese content in raw bitter lupin seed. The upper limit of safe intake of manganese for human consumption is 5 mg/day (Trugo et al, 1992). Thus, manganese was evaluated in the present study along with the other micro-minerals. Accordingly, the manganese content of raw sweet lupin and yoghurt-like product were 4.40 and 3.75 mg/100 gm dry sample (Table 7), which was a

lower level than the reports on the bitter species. Also, this amount was within the allowed safety limit. In commercial yoghurt the contents of iron, zinc and manganese are 0, 0.52 and 0.009 mg/100g (Buttriss, 1997) respectively. This implies higher iron amount in the new sweet lupin-based yoghurt-like product. However, upon fermentation for 48hrs the iron concentration decreased significantly (from 7.71 in raw seed to 0.21mg/100gm). In fact, the levels of zinc and manganese also decreased upon completion of the fermentation (Table 7).

Table 7. Macro, micro-minerals, and folate concentrations of raw sweet lupin seed and yoghurt-like product.

Minerals	Raw sweet lupin (mg/100gm)	Yoghurt-like product (mg/100gm)
Sodium	10.36 ± 1.41	3.11 ± 0.75
Calcium	27.78 ± 0.00	15.75 ± 1.31
Potassium	4.74 ± 0.99	1.76 ± 0.11
Iron	7.71 ± 0.01	0.21 ± 0.02
Zinc	0.84 ± 0.76	0.46 ± 0.01
Manganese	4.40 ± 0.00	3.75 ± 1.03
Folate (µg/100gm)	83.12 ± 0.01	36.02 ± 0.01

Data are expressed as mean ± SE on dry basis. All analyses were done in triplicate

As reported in Table 7, raw sweet lupin seeds had a folate content of 83 µg/100gm on dry basis. In other legumes reported folate contents were soybean (165 µg/100gm), pea (274 µg/100gm), chickpeas (557 µg/100m) and lentil (479 µg/100gm) (Singh et al., 2018). The sweet lupin flour had a lower folate content than the other legumes. Food composition tables and review papers based on microbiological assay reported total folate values for cow's milk in the range of 5–7µg/100gm. Similarly, folate contents of cultured dairy products (i.e. yogurt) were between (4 and 19) µg g/100gm. These values are very low compared with

the recommended daily allowance of dietary folate (400 µg per day) (Yates et al, 1998). During the last few years, most countries have established increased recommended intakes of folates (300–400µg per day for adults). Thus, providing the consumer with folate enriched food products is highly recommended. With this regard, the sweet lupin-based yoghurt analogue had higher folate content than dairy products. During the pasteurization of the slurry at 90 ^{o}C for 15 minutes, the folate concentration was reduced significantly from the raw seed. However, during the fermentation step the folate producing *L. plantarum* had increased the folate concentration significantly (19.45 µg/100gm to 36.02 µg/100 gm) (Figure 5).

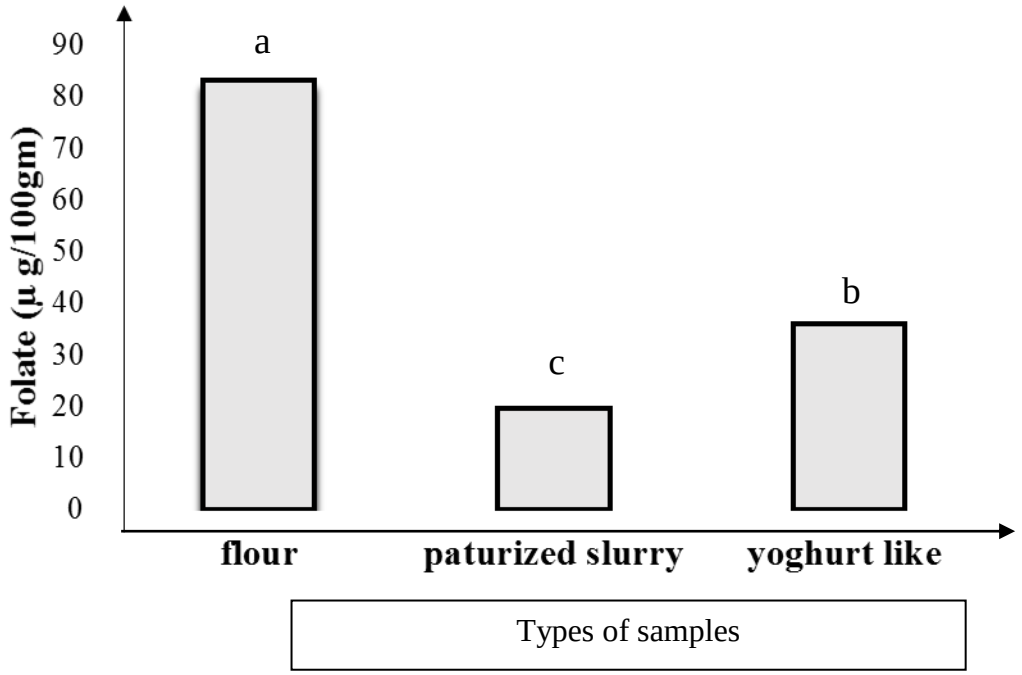


Figure 5. Total folate content of sweet lupin flour and yoghurt analogue in µg/100 g

Analysis were done in triplicate on dry basis. Different letters indicate a statistically significant difference ($p < 0.05$)

4.1.4 Concentrations of antinutritional factors in the raw sweet lupin seed and yoghurt like product

The composition of anti-nutritional factors in the raw sweet lupin seed and yoghurt like product was reported in Table 8. Accordingly, the raw seed had 0.60, 0.13 and 0.05 g/100gm phytate, total alkaloids and tannin concentrations respectively. Fermentation of the raw seed with folate producing LAB for 48 hrs. had reduced these levels significantly at ($p \leq 0.05$) to 0.16, 0.02 and 0.01 g/100gm (Table 8). Alkaloids are nitrogen-containing, water-soluble compounds produced in the chloroplasts of lupin (Nigussie, 2012). The major anti-nutritional factors in lupin are quinolizidine alkaloids which are responsible for the bitter taste (lower palatability) and human/animal toxicity (neurotoxins) maximum limit of 200mg/kg. The alkaloid levels in bitter and sweet cultivars of lupin are between (0.5 and 6.0%) and (<0.02%) respectively (Yeheyis et al, 2010). Apparently, the total alkaloid content in sweet white lupin cultivars has been significantly reduced in the process of domestication and breeding (Janusz, 2017). Similarly, in bitter lupin seeds from Ethiopia (Paulose et al, 2009) reported a total alkaloid content of 2.46%. The total alkaloid content in the sweet lupin in this study was significantly lower than the value in the aforementioned bitter seeds. Yet, this concentration is higher than the expected <0.02%. This may have occurred because of the species difference. However, fermentation had reduced the alkaloid levels to 0.02% significantly ($p<0.001$) (73% reduction) (Table 8). Similarly (Santana et al, 2002) and (Martinez et al, 2006) reported that the use of *Rhizopus oligosporus* for fermentation resulted in strong alkaloid degradation by 90%.

The other anti-nutritional factor determined was phytate, in which the sweet lupin in this study had 0.60 g/100gm (Table 8). Phytate belong to anti-nutritional substances because it affects phosphorus bioavailability, since they are permanently bound in the molecule and also limit the utilization of minerals by animals. Phytic acid is one of the undesirable compounds found in lupins (Trugo et al, 1992) reported phytic acid content of 0.4-1.2 g/100gm in bitter lupin

species. Upon folate producing LAB fermentation, the phytic acid concentration was significantly reduced to 0.16% ($p<0.006$). It is assumed that, for foods, the bioavailability of iron is affected by a molar ratio of phytate: iron, in which a ratio above 1, will hamper iron absorption (Hurrell et al, 2010). Also, International Zinc Nutrition Consultative Group (IZiNCG), suggests that phytate: zinc molar ratios > 18 are likely to adversely affect zinc bioavailability (Brown et al, 2004). In the new sweet-lupin yoghurt-like product both phytate to zinc and phytate to iron were 0.02 and 0.06 respectively, in which the bioavailability of the minerals was not affected.

Table 8. Anti-nutritional factors composition of raw sweet lupin seed and yoghurt like product

Anti-nutritional factors	**Raw sweet lupin (g/100gm)**	**Yoghurt-like product (g/100gm)**
Phytate	0.60 ± 0.01	0.16 ± 0.00***
Total alkaloid	0.13 ± 0.01	0.02 ± 0.00***
Tannin	0.05 ± 0.00	0.01 ± 0.00*

Data are expressed as mean ± SE, on dry basis. All the analyses were done in triplicate
*indicates mean values in the same row are significantly different at *$p<0.05$, *** 0.001

4.1.5 Parameters considered to develop sweet lupin-based yoghurt like product

After several trials at home and laboratory scale, the parameters for the new product formulation were optimized as shown in (Table 9).

Table 9. Optimized parameters to develop sweet lupin-based yoghurt-like product

Optimized parameters	Values
Seed to water ratio	1:9
Soaking time	12hrs
Slurry to water ratio	1:1
Inoculum (%)	3

The first step in the yoghurt-like product formulation was soaking the sweet lupin in water. Hence, the ratio of lupin to water was evaluated to obtain the maximum water retention in the seed so as to ease the de-hulling of the seed. The ratios tested were 1:1, 1:4, 1:6 and 1:9. Among which soaking the lupin seed (1:9) in water overnight (>16hrs) made the de-hulling easy, thus this ratio was selected for further processing (Table 9). After the lupin seeds were soaked in 1:9 (w/v/ ratio), the soaking time was optimized also. When, sweet lupin seeds were soaked for prolonged time the grinded slurry had an initial pH of 6 and titratable acidity of 0.75%. Hence, in this study the soaking time of the raw seed was optimized to get acceptable initial pH value (5.8) for as such products before fermentation. As shown in Figure 6, the acceptable soaking time was 12hrs with a pH value 5.8.

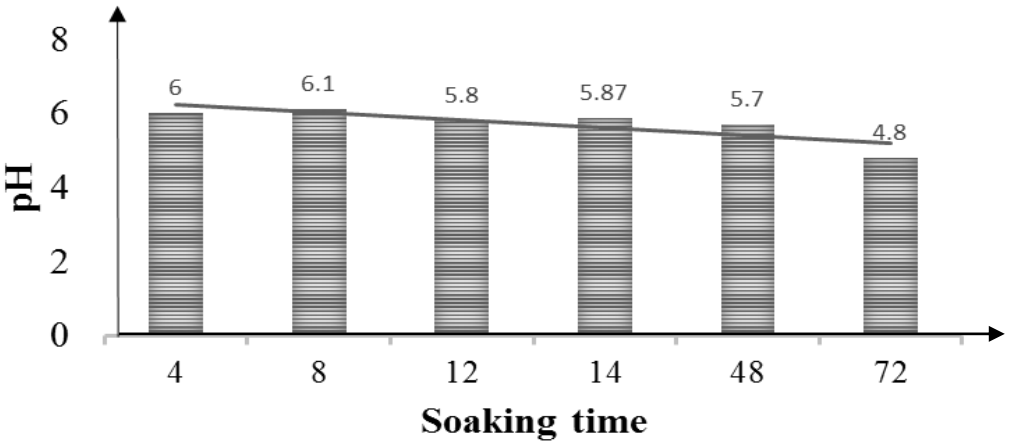


Figure 6. Optimization of raw sweet lupin soaking time to make yoghurt like product

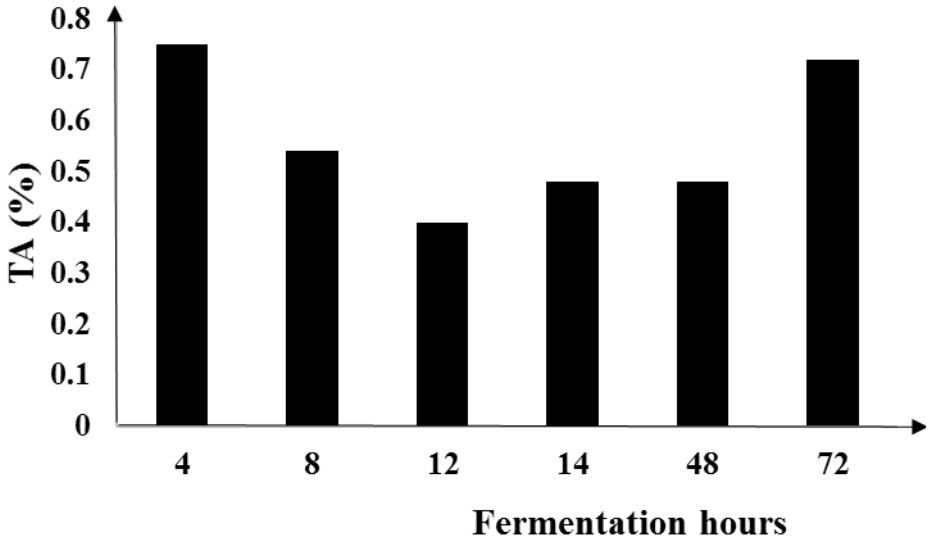
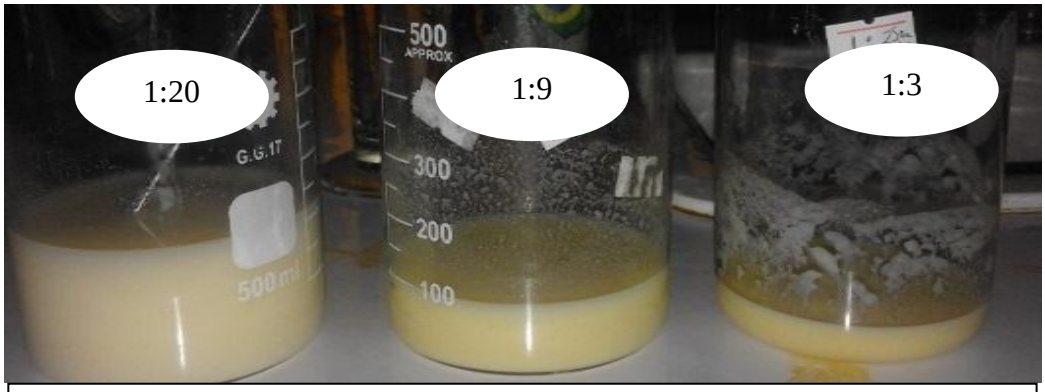


Figure 7. Optimization titratable acidity verses soaking time

After the sweet lupin was soaked for 12hrs and grinded, the slurry to water ratio was optimized before fermentation. Based on previous literature, the tested ratios were 1:20, 1:9, 1:3 and 1:1 (Figure 8A). The best ratio was selected based on viscosity and homogenization of the slurry with water. Thus, the 1:1 slurry to

water ratio was selected for further processing. This ratio provided a yellowish, creamy scooped yoghurt like product (Figure 8B).



(A). Different slurry ratio of yoghurt from sweet lupin

(B). Final thickness of (1:1) slurry to water ratio

Figure 8. Optimization of raw sweet lupin slurry to water ratio based on consistency and viscosity

In order to evaluate the possibility of making yoghurt-like product, two basic trials were made before the major experiment. As shown in Figure 9, the 1:1 slurry and water mixture was inoculated with 1% folate producing LAB bacteria according to (Agosin et al, 1989) and after 48 hrs of fermentation the pH of the slurry slightly dropped from 5.7 to 5.6 (Figure 9). This product had a very low pH change even after 48 hrs of fermentation. The slurry mixture was also mixed with 3 spoons of commercial yoghurt (i.e.as backslope). Instead of fermented product, the back sloping resulted in syneresis and decantation of the slurry. Thus, apparently the 1% inoculum gave a better fermented like product and increasing the inoculum percentage was anticipated. First, 1% inoculum was

added into the slurry. Then, following the method by (Martinez, 2006), 3% inoculum addition was tested for 48hrs fermentation. In this case, the pH dropped from 5.7 to 4.8 (Figure 9).

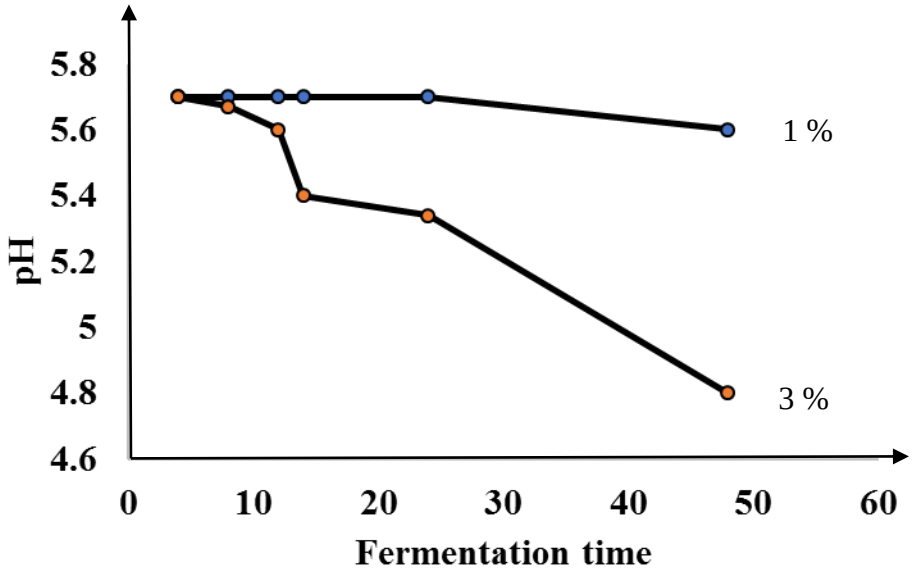


Figure 9. pH change of the sweet lupin slurry inoculated with 1% (A) and 3% (B) inoculum and fermented for 48 hrs.

4.2. Physico-chemical characteristics of sweet lupin slurry and sweet lupin-based yoghurt like product

4.2.1. pH and titratable acidity

In this study prior to the yoghurt like product formulation, the physico-chemical properties of the sweet lupin slurry were characterized. The raw sweet lupin was cleaned, soaked with (1:9) seed to water ratio for 12hrs. After discard dehulled and grinded with Laboratory Miller (Model: polymix LABE-7B, California, 2005) for 10 to 15 minutes with addition of (1:1, w/v) of distilled water to make a slurry. Accordingly, the pH and titratable acidity of the slurry were (5.80, 0.66) respectively (Table 10).

Table 10. pH and titratable acidity of raw sweet lupin slurry and yoghurt like product

Sample type	pH	Titratable acidity (%)
Raw sweet lupin slurry	5.80 ± 0.44	0.66 ± 0.08
Sweet lupin yoghurt like product	4.80 ± 0.06	0.99 ± 0.06

Data are expressed as mean ±SE. All analyses were done in triplicate

pH and titratable acidity are important parameters to evaluate the quality of fermented products. In this study, the pH and titratable acidity of the new product were 4.80 and 0.99 (%) respectively (Table 10). Apparently, pH decreased as titratable acidity increased in the sample. Lactic acid was used as an index of activity of the bacteria (*Lactobacillus plantarum*). The observed decrease in pH could be due to dominance by these lactic acid bacteria which degrade carbohydrates resulting in acidification. These observations are in conformity with earlier studies by (Singh and Bains et al, 1988). However, (Laye et al, 1993) had reported a lower pH value of (4.20-4.58) for probiotic yoghurt analogue product. This might be due to the difference of in the raw material. According to (Camacho and Sierra et al, 1991), LAB fermentation of *L. albus* resulted in a pH of 4.5 within 8 hrs. This difference with the findings of the present study might be also associated with the LAB strain used for fermentation. Different LAB strains have varied ability to grow and ferment different samples and carbohydrate sources (Ogbonna, et al, 2013).

Titratable acidity of milk-based yoghurt is 0.6% (codex standard FAO, 2008), which is lower that the value in the new yoghurt analogue. Furthermore, (Chang and Stone, 1990) reported pH range between 4.74 to 5.88 for fermented soymilk and titratable acidity values of between 0.06-0.33%. Also, for grain based similar products (barley, wheat and corn) pH range of 5.2-5.3. The lower pH values have vital roles in preservation from undesirable bacteria's, aiding in thickening and giving it the characteristics tartness.

4.2.2. Colour

Colour is one of the most important sensory attributes for the acceptance of new food products by the consumer. The colour values for the new sweet lupin-based yoghurt like product in this study were reported in Table 11. Accordingly, the lightness value (from black to white, L*) of 112.09 in the new product was similar with the value in cow's milk (114.47) and in yoghurt analogue made from *Lupinus campestris* (104.34) reported by (Jimenez-Martinez, 2003). A lower L* was reported for soybean-based milk (86.25) (Jimenez-Martinez, 2003). The a* (green to red) and b* (blue to yellow) values of the sweet lupin yoghurt like product were higher than the values in cow's milk and *L. campestris* based yoghurt analogue.

Table 11. Colour evaluation of sweet lupin-based yoghurt like product

Sample type	L*	a*	b*
Sweet lupin-based yoghurt-like product	112.09 $\pm$ 0.50	96.90 $\pm$ 0.45	34.81 $\pm$ 0.26

Data are expressed as mean $\pm$ SE. All the analyses were done in triplicate
L* - Lightness from black to white; a*= From green to red; b*= From blue to yellow

4.2.3. Viscosity

Determination and control of the flow properties of fluid foods is critical for optimizing processing conditions and obtaining the desired beneficial effects for the consumer. As shown in Table 12, the viscosity of the sweet lupin-based yoghurt-like product was 309 mPa^{-s}). Viscosity of milk-based yoghurt is 312 (mPa^{-s}). The viscosity of the product is valuable in order to get a spoony characteristics and a good syneresis stability.

Table 12. Viscosity of sweet lupin-based yoghurt like product

Spindle speed (rpm)	%Reading	Factor	Viscosity (mPa^{-s})
100	309.40	0.23	309.40 ± 0.01

Data are expressed as mean ±SE. The analysis was done in triplicate. mPa- megapascal

4.3. Sensory evaluation of the sweet lupin-based yoghurt like product

New food products to be accepted by the consumer and then to penetrate into the market, sensory acceptability is crucial. In this study, the new fermented product was rated as compared to commercial yoghurt product using a line scale (size of the scale/ruler, 6.0 cm). Panelists were asked to put a mark on the scale considering the overall acceptability of the new product compared with commercial yoghurt. The scale had an increased rate going from left to right (weak to strong). As reported in Table 13, the milk-based yoghurt being rated the highest scale of the ruler, the sweet lupin-based yoghurt had scored 4.2 based on overall acceptability test. Thus, one can assume a lower acceptability of the new product as compared with the commercial yoghurt product. The lower overall acceptability rating of the new product might be due to the beany flavor of the sweet lupin. Thus, for the acceptance rating sensory test, for comparison purpose vanilla was added in the new product to mask the beany flavor.

Table 13. Relative sensory overall acceptability score of sweet lupin-based yoghurt like product compared with milk-based yoghurt

Parameter tested	**Overall acceptability**
Similarity with milk-based yoghurt	4.20 $\pm$ 1.70

Also, acceptance rating sensory test was done using 9-point hedonic scale based on the sensory attributes of consistency, colour, flavor, taste, texture and overall acceptability. Accordingly, the vanilla added sweet lupin-based yoghurt like product had significantly higher acceptance rating scores for consistency and taste than the plain new product ($p \leq 0.05$). But, the plain new fermented product had higher scores for colour and flavor significantly ($p \leq 0.05$) (Table 14). Also, the overall acceptability of the sweet lupin-based yoghurt-like product was higher.

Table 14. Acceptance rating sensory scores of plain and vanilla added sweet lupin-based yoghurt like product

Sample type	Consistency	Colour	Flavor	Taste	Texture	Overall acceptability
PSYLP	6.89 ± 1.60^{b}	8.72 ± 1.27^{a}	6.80 ± 1.91^{a}	5.00 ± 1.94^{b}	7.30 ± 1.13	6.50 ± 2.00
VSYLP	7.63 ± 1.28^{a}	7.38 ± 1.54^{b}	6.00 ± 2.14^{b}	7.90 ± 2.04^{a}	7.40 ± 1.29	6.00 ± 1.80

Sensory scores are expressed as mean $\pm$ SE from 18 trained panelists. Mean values in the same column with different superscripts are significantly different at $p \leq 0.05$.
PSYLP: Plain Sweet lupin-based Yoghurt like Product; VSYLP: Vanilla added Sweet lupin-based Yoghurt like Product

Flavor is a strong determinant factor of beverage acceptability compared to other qualities (Gaffa and Ayo, 2003). (Jimenez-Martinez, 2003) reported acceptance rating scores of 6.4 and 5.3 for milk based and lupin-based yoghurt like product respectively based on flavor (i.e. based on a 7-point hedonic scale). Moreover, masking the beany flavor of the new product with 1% (1 spoon for 1 cup of the lupin-based yoghurt) vanilla had increased the flavor acceptability score to 6.8 ($p<0.05$) (Table 14).

The appearance of the yoghurt refers to the level of visual appeal obtained by fermenting the various milk substrates with relevant microbes (Sanful, 2009). Colour is an important quality of many foods including yoghurt and yoghurt analogue. It is a quality attribute that with flavor and texture play an important role in food acceptability. According to (Ihekoronye and Ngoddy, 1985), colour could be defined as a physiologic response by the eye and brain to the physical stimulus of light radiation at different wavelength. In this study, the colour of the sweet lupin-based yoghurt-like product was liked very much and had a significant higher score than the vanilla added product ($p \leq 0.05$) (Table 14). In fact, the colour acceptance score of the new product was higher than previous reports for milk-based-and lupin-based yoghurt like product (6.2 and 5.5 respectively) (Jimenez-Martinez, 2003). The colour of the scooped sweet lupin-

based yoghurt like product can be observed in Figure 8 of this document. Consistency is a measure of the attribute of the yoghurt to flow without forming lagging insoluble particles on the inner side of the containers (Sanful, 2009). It refers to the property of the yoghurt to exhibit smoothness and good flow properties. The vanilla containing new product had a higher consistency score than the plain product (Table 14). Consistency of the product also can be an evidence for the effectiveness of mixing (homogenization) of the ingredients.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDTIONS

5.1 Conclusions

In this study, the raw sweet lupin seed and flour had high values of Water Absorption Capacity, which was considered as an important functional property in viscous foods such as yoghurt. Similarly, due to the high crude protein content, the sweet lupin flour had given the required foaming capacity and stability for as such products. Apparently, the crude protein content of the raw sweet lupin was higher than other legumes. In general, the sweet lupin seed had comparable and higher contents of macronutrients with other lupin species and legumes. The new sweet lupin-based yoghurt like product had higher content of crude protein than in the raw seed, while the crude fat, crude ash and crude fiber contents were lower. The new fermented product had a significantly higher crude protein content than the value in commercial milk-based yoghurt product. In fact, the fat, ash, fiber, total carbohydrate and gross energy contents were also higher than in the new product.

The raw seeds had significant amounts of macro- and micro-minerals, in which during fermentation were decreased. However, the amount of all the tested minerals in the fermented product were in the level to satisfy the RDA of adult consumers. Also, the concentration of the minerals in the new product were higher compared with milk-based yoghurt product. Besides the sodium to potassium ratio in the new product was (1:7) healthy as per WHO recommendation (1:16).

At first, the yoghurt-like product processing was optimized. With the optimized conditions a scooped, homogenized (no phase separation) product with acceptable pH and titratable acidity was obtained. As one of the specific objectives of this study, the fermentation of sweet lupin using folate producing *L. plantarum* strain had significantly ($p \leq 0.05$) increased the folate content in the final product. In fact, the sweet lupin-based yoghurt analogue had higher folate content than milk-based products. Moreover, the

fermentation of the raw seed with folate producing LAB had reduced the levels of phytate, total alkaloids and tannin significantly at ($p \leq 0.05$).

The sweet lupin yoghurt-like product had a lower relative overall acceptability with commercial yoghurt based on a line scale marking. This might be due to the beany flavor of the sweet lupin. Hence, vanilla was incorporated in the new product for the acceptance rating sensory evaluation. According to the 9-point acceptance rating test vanilla added sweet lupin-based yoghurt like product had significantly higher ($p \leq 0.05$) acceptance rating scores for consistency and taste than the plain new product. Moreover, the overall acceptability of the sweet lupin-based yoghurt-like product with vanilla was higher. In contrast, the plain new fermented product had higher scores of colour and flavor significantly. The lightness value was similar with the value in cow's milk; and a* and b* values were higher than the values in cow's milk and other lupin-based yoghurt analogue.

Generally new sweet lupin was found to be a potential legume to make a new dairy analogue product. Also, the product had a comparable sensory acceptability with existing products. Besides, *L. plantarum* was found to increase the folate level in the new product.

5.2 Recommendations

The potential of sweet lupin to make dairy analogue products was demonstrated in this study. However, future investigations are highly recommended on the following points: -

-The fermentation should also be studied using normal LAB (not high folate producing)

-The optimization should be done in detail using software if any. These include, (soaking time, soaking seed to water ratio) (response can be pH and titratable acidity), (slurry to water ratio, fermentation time, inoculum percentage) (response can be pH, titratable acidity, folate content) etc.

-The crude protein content of both the raw sweet lupin seed and yoghurt-like product were high. But further study on the protein fractions, characteristics and amino acid composition is important.

-The shelf-life of the new product should be investigated (shelf stability, syneresis, microbial load) etc.

-The incorporation of vanilla into the new product was just based on previous literatures. Hence, detailed study on masking the beany lupin flavor using different flavoring agents should be carried out.

-In the long run the large-scale production of the folate producing LAB strain should be considered.

References

Adenekan, M. K, Akinlotan, J. V. and Odunmbaku, L.O. (2009). Milk extracted from Pigeon pea (*Cajanus cajan*) Seeds. Nigerian Institute of Food Science and Technology. University of Yola. Yola: 143-144.

Adeniyi, S. A. A, Orjiekwe.C, C. L and Ehiagbonare.J, J. E. (2009). Determination of alkaloids and oxalates in some selected food samples in Nigeria. African Journal of Biotechnology. 8(1):110-112.

Agerholm-Larsen, L., Raben, A., Haulrik, N., Hasen, A. S., Manders, M., and Astrup, A. (2000). Effect of 8-week intake of probiotic milk products on risk factors for cardiovascular diseases. European Journal of Clinical Nutrition. 54(4):288.

Agosin, E., Jarpa, S., Rojas, E., and Espejo, E. (1989). Solid-state fermentation of pine sawdust by selected brown-rot fungi. Enzyme and Microbial Technology. 11(8): 511-517.

AOAC. (2000). Official methods of analysis of AOAC International Gaitherburg. USA: AOAC International.

Aloys, N., and Zhou, H. M. (2006). Functional and chemical properties of lkivunde and lnyange, two traditionally processed Burundian cassava flours. Journal of Food Biochemistry, 30(4), 429-443

Aynadis Tamene. (2018). Screening of folate producing bacteria from injera: implication on folate production during fermentation and bioavailability. Ph.D. Thesis. Addis Ababa University. Addis Ababa, Ethiopia.76-132.

Biliaderis, C. G., Khan, M. M., and Blank, G. (1992). Rheological and sensory properties of yoghurt from skim milk and ultrafilter retentate. International Dairy Journal. (2): 311 323.

Brown, K. H., Rivera, J. A., Bhutta, Z., Gibson, R. S., King, J. C., Lönnerdal, B., Sandtröm B, Wasantwisut E, Hotz C. (2004). International Zinc Nutrition Consultative Group (IZiNCG) technical document# 1. Assessment of the risk of zinc deficiency in populations and options for its control. Food and Nutrition Bulletin, 25 (1 Suppl. 2).

Burns, R. E. (1971). Method for estimation of tannin in grain sorghum 1. Agronomy Journal, 63(3), 511-512.

Buttriss, J. (1997). Nutritional properties of fermented milk products. International Journal of Dairy Technology (50)**:** 21-27.

Camacho, L. A. V. I. N. I. A., Sierra, C. E. C. I. L. I. A., Marcus, D., Guzman, E., Campos, R. O. L. A. N. D. O., Von Baer, D., & Trugo, L. U. I. S. (1991). Nutritional quality of lupine (Lupinus albus cv. Multolupa) as affected by lactic acid fermentation. International Journal of Food Microbiology, 14(3-4), 277-286.

Cargill, (2009). Cargill beverage concepts will address consumer demands for health, taste and texture Available from: http://www.cargill.com/newscenter/news releases/ 2008/NA3007612.jsp. Accessed Jul 20, 2009.

Carvalho, A. F. U., Farias, D. F., da Rocha-Bezerra, L. C. B., de Sousa, N. M., Cavalheiro, M. G., Fernades, G. S., and Gouveia, S. T. (2011). Preliminary assessment of the nutritional composition of underexploited wild legumes from semi-arid caatinga and moist forest enviroments of northeastern Brazil. Journal of Food Composition and Analysis,24(4-5), 487-493

Chandra, S. Samsher. (2013). Assessment of functional properties of different flours. African Journal of Agricultural Research, 8(38), 4849-4852.

Chang, C., and Stone, M. B. (1990). Effect of total soymilk solids on acid production by selected *Lactobacilli*. Journal of Food Science. (55): 1643-1646.

Dave, R. I., and Shah, N. P. (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. International Dairy Journal. 7**:** 31-41.

Danbaba, N., Oyeleke, S.B., Maji, A.T., Kolo, I.N., Hauwawu, H., & Kolo, I.F. (2014). Chemical and microbiological characteristics of Soy-Kununzaki (a non-alcoholic beverage) produced from millet (Pennisetum typhodeum) and soybean (Glycine max). International Journal of Microbiological Applied Science,3(11), 649-656

Devcich, D. A., Pedersen, I. K., and Petrie, K. J. (2007). You are what you eat. Modern health worries and the acceptance of natural and synthetic additives in functional foods. Appetite. 48(3): 333-337.

Duthie, S. J. (2007). Berry phytochemicals, genomic stability and cancer. Evidence for chemoprotection at several stages in the carcinogenic process. Molecular Nutrition & Food Research. 51(6): 665-674.

Durga, J., van Boxtel, M. P., Schouten, E. G., Kok, F. J., Jolles, J., Katan, M. B., and Verhoef, P. (2007). Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial. A randomised, double blind, controlled trial. The Lancet. 369(9557): 208-216.

El-Adawy, T.A. (2000). Functional properties and nutritional quality of acetylated and succinylated mung bean protein isolate. Food Chemistry, 70(1), 83-91

Elizabeth, A. C. (2002). Over-the-counter products: non-prescription medications, Nutraceuticals and herbal agents. Clinical Obstetrics and Gynecology. 45 (1): 89-98.

Eapen, S. (2008). Advances in development of transgenic pulse crops. Biotechnology. (26): 162-168.

Erbas, M., Certel, M., Uslu, M. K. (2005). Some chemical properties of white lupin seeds (*Lupinus albus* L.). Food Chemistry. 89: 341–345

Fashakin, J. B., and Unokiwedi, C. C. (1992). Chemical analysis of warankasi prepared from cow milk partially substituted with melon milk. Nigerian Food Journal. 10: 103-110.

Fisberg, M., & Machado, R. (2015). History of yoghurt and current patterns of consumption. Nutrition reviews, 73(suppl_1),4-7.

Francis, C., (1999). New crops and oil seeds from Ethiopia. The Australia New Crop Newsletter (11).

Fernández, Quintela. A., Macarulla, M., del Barrio, A., Martínez, J. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. Plant Foods for Human Nutrition. 51(4): 331–341.

Founou, L. L., Founou, R. C., & Essack, S. Y. (2016). Antibiotic resistance in the food chain: a developing country-perspective. Frontiers in Microbioligy,7, 1881.

Fudiyansyah, N., Petterson, D. S., Bell, R. R., and Fairbrother, A. H. (1995). A nutritional, chemical and sensory evaluation of lupin (*L. angustifolius*) tempe. International Journal of Food science and Technology, 30(3), 297-305.

Gaffa, T. and Ayo, J. A. (2003). Physicochemical and sensory effects of cadaba farinose crude extract on cereal starches during kunun zaki production. Pakistan Journal of Nutrition: 1-32.

Ghavidel, R. A., & Prakash, J. (2006). Effect of germination and dehulling on functional properties of legume flours. Journal of the Science of Food and Agriculture, 86(8), 1189-1195.

Glenn, R. G. and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. Journal of Nutrition 125 (6)**:** 1401-1412.

Glencross, B. D. (2009). Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture. 1(2): 71-124.

Gomes, A. M. P., Malcata F. X. and Klaver, F. A. M. (1998). Growth enhancement of *Bifidobacterium* and *Lactobacillus acidophilus* by milk hydrolyzates. Journal of Dairy Science. (81): 2817–2825.

Gotcheva, V., Hristozova, E., Hristozova, T., Guo, M., Roshkova, Z and Angelov, A. (2002). Assessment of potential probiotic properties of lactic acid bacteria and yeast strain. Food Biotechnology. 16(3): 211-225.

Gregory III, J. F., Sartain, D. B., & Day, B. P. (1984). Fluorometric determination of folacin in biological materials using high performance liquid chromatography. The Journal of Nutrition, 114(2), 341-353.

Harkins, R. W. and Sarett, H. P. (1967). Method of comparing proteins quality of soybean infant formulas in the rat. Journal of Nutrition. 91**:** 213-216.

Harwalkar, V. R. and Kalab, M. (1986). Relationship between microstructure and susceptibility to syneresis in yoghurt made from reconstituted nonfat dry milk. Food Microstructure. (5): 287-294.

Hickisch, A., Beer, R., Vogel, R. F. and Toelstede, S. (2016). Influence of lupin-based milk alternative heat treatment and exopolysaccharide-producing lactic acid bacteria on the physical characteristics of lupin-based yogurt alternatives. Food Research International. (84):180–188.

Holmes, D. R., Mack, M. J., Kaul, S., Agnihotri, A., Alexander, K. P., Bailey, S. R., and Francis, G. S. (2012). Expert consensus document on transcatheter aortic valve replacement. Journal of the American College of Cardiology, 59(13), 1200-1254.

Hondelmann, W. (1984). The lupin-ancient and modern crop. Theory of Applied Genetics 68:1–8.

Horiuchi, H., Inoue, N., Liu, E., Fukui, M., Sasaki, Y. and Sasaki, T. (2009). A method for manufacturing superior set yoghurt under reduced oxygen conditions. Journal of Dairy Science. (92): 4112–4121.

Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. The American Journal of Clinical Nutrition, 91(5), 1461-1467.

Igyor, M. A., Igbian, E. K. and Torbo, C. I. (2006). Effects of soymilk supplement on the yield and quality of Warankasi‖ a Nigerian soft cheese made from a mixture of soymilk and cow milk. Nigerian Food Journal. 7**:** 50-56.

Ihekoronye, A. I. (1999). Cereal grains and oilseed processing technology. In: manual on small-scale food processing. The Academic Publishers. Nigeria. 11-50.

Ihekoronye, A. I. and Ngoddy, P. O. (1985). Integrated Food Science and Technology for the Tropics. Macmillan Education. 28-50: 236-265.

Janusz, P. (2017). White lupin (*Lupinus albus L.*) – nutritional and health values in human nutrition-a review. Czech Journal of Food Science, 35(2), 95-105.

Jimenez-Martinez, C., Hernandez-Sanchez, H. and Davila-Ortiz, G. (2003) Production of a yogurt-like product from *Lupinus campestris* seeds. Journal of the Science of Food and Agriculture. 83(6): 515-522

Kaur, M., Singh, N. (2006). Relationships between selected properties of seeds, flours, and starches from different chickpea cultivars. International Journal of Food Properties. (9): 597-608.

Kariluoto, S., Vahteristo L, Salovaara H, Katina K, Liukkonen K, Piironen V. (2004). Effect of baking method and fermentation on folate content of rye and wheat breads. Cereal Chemistry 81(1):134-139.

Khan, R., Stehli, D., Wei, L. S. and Steinberg, M. P. (1989). Activity and mobility of water in sweetened whole soy concentrates and their rheological properties. Journal of Food Science. 54**:** 931 935.

Kinsella, J. E., (1976). Functional properties of protein in food. Critical Review of Food Science and Nutrition. (5): 219.

Kneifel, W., (2000). Functional foods with lactic acid bacteria: probiotics-prebiotics-nutraceutical. In Progress in Biotechnology. 17: 101-107.

Kreisz, S., Arendt, E. K., Hubner, F and Zarnkov, M. (2008). Cereal-based gluten-free functional drinks. In gluten-free cereal products and beverages. 373-392.

Kurlovich, B. S., and Kartuzova, L. T., (2002). Lupin breeding. Geography, classification, genetic resources and breeding: 351-374

Lampart-Szczapa Korczak, J., Nogala-Kalucka, M. and Zawirska-woji. (2003). Antioxidant properties of lupin seed products. Food Chemistry. 83(2): 279-285.

Lawerance, L. (2007). Lupins-Australia's role in world markets. Australians Bureau of Agricultural and Resource Economics.

Laye, I., Karleskind, D. and Moor, C. V. (1993). Chemical, microbial and sensory properties of plain non-fat yoghurt. Journal of Food Science. (58): 991-995.

Leo´n, K., Mery, D., Pedreschi, F. and Leo´n, J. (2006). Colour measurement in L*a*b* units from RGB digital images. Food Research International. (39): 1084–1091.

Lewis, M. and Dale, R. H. (1994). Chilled yoghurt and other dairy desserts. In: Shelf life evaluation of foods. 127- 155.

Lin, S. Y., Ayres, J. W., Winkler, Jr. Wand Sandine, W. E. (1989). Lactobacillus effects on cholesterol in vitro and in vivo results. Journal of Dairy Science. 72(11): 2885-2899.

Lock, A. Land Bauman, D. E. (2004). Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids. 39(12): 1197-1206.

Luana, N., Rossana, C., Curiel, J. A., Kaisa, P., Marco, G. and Rizzello, C. G. (2014). Manufacture and characterization of yoghurt-like beverage made with oat flakes fermented by selected lactic acid bacteria. International Journal of Food Microbiology. 185(5): 17-26.

Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in milk gels. Food Hydrocollator. 15(4): 603–608.

Madsen, K., Cornish, A., Soper, P., Mckaigney, C., Jijon, H., Yachimec, C., Doyle, J., Jewell, L. and Desimone, C. (2001). Probiotic bacteria enhance murine and human intestinal epithehal barrier function. Gastroenterology. 121(3): 580-591.

Marconi, E., Ruggeri, S., Cappelloni, M., Leonardi, D and Carnovale, E. (2000). Physicochemical, nutritional, and microstructural characteristics of chickpeas (*Cicer arietinum* L.) and common beans (*Phaseolus vulgaris* L.) following microwave cooking. Journal of Agricultural and Food Chemistry. 48(12): 5986–5994.

Martensson, O., R. and Holst, O. (2002). The effect of yoghurt culture on the survival of probiotic bacteria in oat-based, non-dairy products. Food Reports International. 35:775–84.

Marteau, P. R., De ‘Vrese, M., Cellier, C. J. and Schrezenmeir, J. (2001). Protection from astrointestinal diseases with the use of probiotics. American Journal of Clinical Nutrition. 73: 430-436.

Martínez-Villaluenga, C., Frías, J. and Vidal-Valverde, C. (2006). Functional lupin seeds (*Lupinus albus* L. and *Lupinus luteus* L.) after extraction of α-galactosides. Food Chemistry. 98: 291-299.

Mattil, K. F. (1971). The functional requirement of protein in foods. Journal of America Oil Chemistry Society. (48): 477.

Molloy, A. M., Daly, S., Mills, J. L., Kirke, P. N., Whitehead, A. S., Ramsbottom, D., & Scott, J. M. (1997). Thermolabile variant of 5, 10-methylenetetrahydrofolate reductase associated with low red-cell folates: implications for folate intake recommendations. The Lancet, 349(9065), 1591-1593.

Narayana, K and Narasinga, R. M. S. (1982). Functional properties of raw and heat processed winged bean (*Psophocarpus tetragonolobus*) flour. Journal of Food Science. (42):534-538.

Nielsen, V. H. (1975). Factors which control the body and texture of commercial yoghurts. American Dairy Revolution. (37): 36–38.

Nigussie, Z. (2012). Contribution of white lupin (*Lupinus albus L.*) for food security in North-Western Ethiopia: a review. Asian Journal of Plant Science, 11(5), 200.

Nomoto, K. (2005). Prevention of infections by probiotics. Journal of Bioscience and Bioengineering. 100(6):583-592.

Nnam, N.M. (1997). Chemical and Sensory evaluation of vegetable milks from African yam bean (*Sphenostylis stenocarpa* (*Hochst ex A. Rich*) Harms and Maize. Plant Foods for Human Nutrition 51: 265-275.

Nsofor, L. M. (1996) Suitability of ultra-filtered soybean extract for developing evaporated cow milk analogue. Journal of Food Science and Technology. 29(5): 333-334.

Obizoba, I.C and Egbuna, H.I. (1992). Effects of germination and fermentation on the Nutritional quality of Bambara nut (*Voandzeia subterrranea L. Thouars*) and its product (Milk). Plant Foods for Human Nutrition 42: 13-23.

Ogbonna, J. C. and Ogbonna, C. N. (2013). Lecture notes on Industrial Biotechnology I Fundamentals of Microbial Cell Cultivation. Praise House Publishers. 16-38.

Oshoid, A. A., & Ekperigin, M. M. (1989). Functional properties of pigeon pea (Cajanus cajan) flour. Food Chemistry, 34(3), 187-191.

Papadakis, S. E., Abdul-Malek, S., Kamdem, R. E. and Yam, K. L. (2000). A versatile and inexpensive technique for measuring color of foods. Journal of Food Technology. 54(12): 48–51.

Paulose Getachew. (2009). Chemical composition and the effects of traditional processing on nutritional composition of gibito (*Lupinus albus* L.) grow in, Gojam area. M.Sc. Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Petterson, D. S. and Mackintosh, J. B. (1998). The chemical composition of lupin seed grown in Australia. 39-48.

Piironen, V., Edelmann, M., Kariluoto, S. and Bedő, Z. (2008). Folate in wheat genotypes in the health grain diversity screen. Journal of Agricultural and Food Chemistry. 56(21): 9726-9731.

Pilvi, T. K., Jauhiainen, T., Cheng, Z. J., Mervaala, E. M., Vapaatalo, H. and Korpela, R. (2006). Lupin protein attenuates the development of hypertension and normalises the vascular function of NaCl-loaded Goto-Kakizaki rats. Journal of Physiology and Pharmacology. (57): 167–176.

Potter, N. N. and Hotchkiss, J. H. (1996). Milk and milk products in Food Science. Chapman and Hall. (5): 279-316.

Purlis, E. and Salvadori, V.O. (2007). Bread browning kinetics during baking. Journal of Food Engineering. (80): 1107–1115.

Rao, D. R., Pulusani, S. R. and Chawan, C. B. (1988). Fermented soybean milk and other fermented legume milk products. In Legume-based Fermented Foods. 119-134.

Rustom, I. Y., Foda, M. I. and Lopez-Leiva, M. H. (1998). Formation of oligosaccharides from whey UF-permeate by enzymatic hydrolysis analysis of factors. Food Chemistry. 62(2): 141-147.

Saeed, A. N., Gale, S. M. A., Ranji, A. and Nekahi, A. (2012). Investigation of physical characteristics of bread by processing digital images (machine vision). Life Science Journal. 9(3): 1674-1678.

Sagdic, O., Arici, M., & Simsek, O. (2002). Selection of starters for a traditional Turkish yayik butter made from yoghurt. Food Microbiology, 19(4), 303-312

Sanful, R. E. (2009). The use of tiger nut (*Cyperus esculentus*), cowmilk and their composite as substrates for yoghurt production. Pakistan Journal of Nutrition. 8(6): 755-758.

Santana, F. M., Pinto, T., Fialho, A. M., Sa-correia, I., and Empis, J. M. (2002). Bacteria removal of quinolizidine alkaloids and other carbon sources from a Lupinus albus aqueous extract. Journal of Agricultural and Food Chemistry, 50(8), 2318-2323.

Sathe, S.K. and Salunkhe, D.K. (1981). Functional Properties of Great Northern bean (*Phaseolus vulgaris L.*) proteins Emulsion, Foaming, Viscosity and Gelation Properties. Journal of Food Science 46: 71-81.

Saubade, F., Humblot, C., Hemery, Y. M and Guyot, J. P. (2017). PCR screening of an African fermented pearl-millet porridge metagenome to investigate the nutritional potential of its microbiota. International Journal of Food Microbiology. (3)244: 103-110

Segnini, S., Dejmek, P. and O¨ste, R. (1999). A low-cost video technique for color measurement of potato chips. Food Science and Technology. 32(4): 216–222.

Siddiq, M., Nasir, M., Ravi, R., Dolan, K. D., Butt, Ms. (2009). Effect of defatted maize germ addition on the functional and textural properties of wheat flour. International. Journal of Food Properties. (12): 860-870.

Sileshi.Z, (1985), Protein Quality Evaluation of Dagussa (*Eleusin coracanal*) and gibto (*Lupinusalbus L.*) and the Supplementary Value of Gibto When Added to Dagussa, Master' s Thesis, Addis Ababa University

Singh, T. and Bains, G. S. (1988). Grain extract milk beverage-processing and physiochemical characteristics. Journal of Food Science. 53(5): 1387-1390.

Sodini, I., Remeuf, F., Haddad, S., & Corrieu, G. (2004). The relative effect of milk base, starter, and process on yoghurt texture: a review. Critical Review in Food Science and Nutrition, 44(2), 113-137.

Sosulski, F. W., Garatt, M. O and Slinkard, A. E. (1976). Functional properties of ten legume flours. International Journal of Food Science Technology. (9): 66-69.

Sosulski, F., McCurdy, A. (1987). Functionality of flours, protein fractions and isolates from field peas and faba bean. Journal of Food Science. 52(4): 1010–1014.

Souci, S., Fachmann, W., and Kraut, H. (2000). Food composition and nutrition tables. (6).

Saastamoinen, M., Eurola, M., and Hietaniemi, V. (2013). The chemical quality of some legumes, peas, fava beans, blue and white lupinues and soyabeans cultivated in Finland. Journal of Agricultural Science and Technology. B, 3(2B), 92.

Stein, M., O'sullivan, P., Wachtel, T., Fisher, A., Mikolich, D., Sepe, S. and Mayer, K, (1992). Causes of death in persons with human immunodeficiency virus infection. The American Journal of Medicine. 93(4): 387-390.

Strakova, E., Suchy, P., Vecerek, V., Serman, V., Mas, N., and Juzl, M. (2006). Nutritional composition of seeds of the genus Lupinus. Acta Veterinaria Brno, 75(4), 489-493

Sujak, A., Kotlarz, A. and Strobel, W. (2006). Compositional and nutritional evaluation of several lupin seeds. Food Chemistry. (2)98: 711-719.

Tamime, A.Y. and Robinson, R. K. (2007). Yogurt Science and Technology. Pergamon Press. 431.

Tefera, M. M. (2010). Cause of rural household food insecurity: A case from Kuyu district, Central Ethiopia. Journal of. Sustainable Development of Africa. (11): 286-304.

Timmermans, A. J. M. (1998). Computer vision system for online sorting of pot plants based on learning techniques. Acta Horticulture. (421): 91–98.

Tizazu, H. and Emire, S. A. (2010). Chemical composition, physicochemical and functional properties of lupin (*Lupinus albus*) seeds grown in Ethiopia. African Journal of Food, Agriculture, Nutrition and Development. 10(8).

Trinick, M. J., Dilworth, M. J. and Grounds, M. (1977). Factor affecting the reduction of acetylene by root nodules of Lupinus species. New Phytologic. (77): 359-370.

Trugo.L.C, Donangelo.C.M, Duarte.Y. A, And Tavares.C. L, (1992), Phytic Acid and Selected Mineral Composition of Seed from Wild Species and Cultivated Varieties of Lupine, (47):391-394.

Uzun, B., Arslan, C., Karhan, M. and Toker, C. (2007). Fat and fatty acids of white lupin (*Lupinus albus* L.) in comparison to sesame (*Sesamum indicum* L.). Food Chemistry. (102): 45-49.

Vahteristo, L., Ollilainen, V., Koivistoinen, P. and Varo, P. (1996). Improvements in the analysis of reduced folate monoglutamates and folic acid in food by high-performance liquid chromatography. Journal of Agricultural and Food Chemistry. (44): 477-482.

Vaintraub, I. A., & Lapteva, N. A. (1988). Colorimetric determination of phytate in unpurified extracts of seeds and the products of their processing. Analytical Biochemistry, 175(1), 227-230

Van Barneveld, R. J. (1999). Understanding the nutritional chemistry of lupin (*Lupinus* spp.) seed to improve livestock production efficiency. Nutrition Research Reviews. 12(2): 203-230.

Wichchukit, S., & O'Mahony, M. (2015). The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives. Journal of the Science of Food and Agriculture, 95(11), 2167-2178

Yam, K. L. and Papadakis, S. E. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. Journal of Food Engineering. (61): 137–142.

Yates, A. A., Schlicker, S. A., & Suitor, C. W. (1998). Dietary reference intakes: the new basis for recommendations for calcium and related nutrients, B vitamins, and choline. Journal of the American Dietetic Association, 98(6), 699-706.

Yeheyis, L., Kijora, C., Melaku, S., Girma, A and Peters, K. J. (2010). White lupin (*Lupinus albus* L.), the neglected multipurpose crop: Its production and utilization in the mixed crop-livestock farming system of Ethiopia. Livestock Research for Rural Development. (22):1-17.

Zelalem, K. A., and Chandravanshi, B. S. (2014). Levels of essential and non-essential elements in raw and processed *Lupinus albus L*. (White lupin, Gibto) cultivated in Ethiopia. African Journal of Food, Agriculture, Nutrition and Development, 14(5), 2015-2035

Zenthenbaur, G. and Grosh, W. (1998). Crust aroma of baguettes. Key odorants of baguettes prepared in two different ways. Journal of Cereal Science. (28): 81-92.

Appendix 1 Lactobacillus MRS Agar M641

Lactobacillus MRS Agar is recommended for cultivation of all *Lactobacillus* species. **Composition****

Ingredients	gms / Liter
Protease peptone	10.000
Beef extract	10.000
Yeast extract	5.000
Dextrose	20.000
Polysorbate 80	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium phosphate	2.000
Agar	12.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 67.15 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Appendix 2 Sensory evaluation consent form

Descriptive Analysis for sweet lupin yoghurt product Study

Dear panelist, you are invited to participate in a study involving sweet lupin-based yoghurt evaluation. The overall objective of this study is to develop a descriptive list of terms for the yoghurt to be tested. This product will be evaluated using a sensory evaluation method known as descriptive analysis. You will be oriented to identify, name and classify a range of **color**, **taste, flavor, mouthfeel(consistency), texture** and **overall acceptability** characteristics of these samples. You will be asked to taste and expectorate the samples, and to rate the samples for intensity of each characteristic. If you have prior experience of any allergic reactions to legume products, you should not participate in this study. If you experience allergic reactions any time during the study, you should discontinue the study. There is no direct benefit to you for participating in this study. You are free to withdraw from the study at any time and for any reason. We also reserve the rights to terminate your participation of the study at any time and for any reason.

Your performance and data in this research is confidential. Responses are coded to be confidential and any publications or presentation of the results of the research will only include information about group performance. Names or other identifiable information will not be disclosed or published. You are encouraged to ask any questions that you might have about this study whether before, during, or after your participation. Questions can be addressed to Ms. Betelhem shemelse (+251943447260, addiskidan786@gmail.com)

I understand the above information and voluntarily consent to participate in the study described above. I have been given a copy of this consent form.

Signature

Date

Code:_____537__

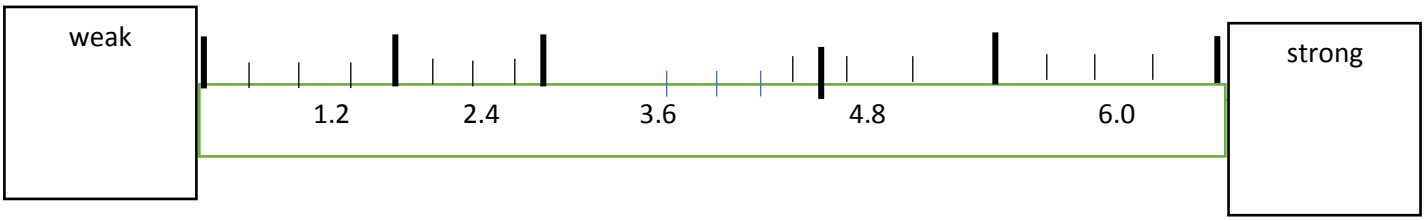
Dear panelist, you are invited to participate in a study involving sweet lupin-based yoghurt evaluation. Please taste the samples in the order presented, moving from top to bottom and rank them in level of score given in the table. (Put 'X' sign on the Box).

	Like extremely (1)	**Like very much (2)**	**Like moderately (3)**	**Like slightly (4)**	**Neither like nor dislike (5)**	**Dislike slightly (6)**	**Dislike moderately (7)**	**Dislike very much (8)**	**Dislike extremely (9)**
Color									
Taste									
Flavor									
Texture									
Overall acceptance									
Mouthfeel consistency									

Thank you!!!

Code: 394

How do you compare the overall similarity(Equivalence) of the sweet lupin-based yoghurt with the milk yoghurt?



Thank you!!!

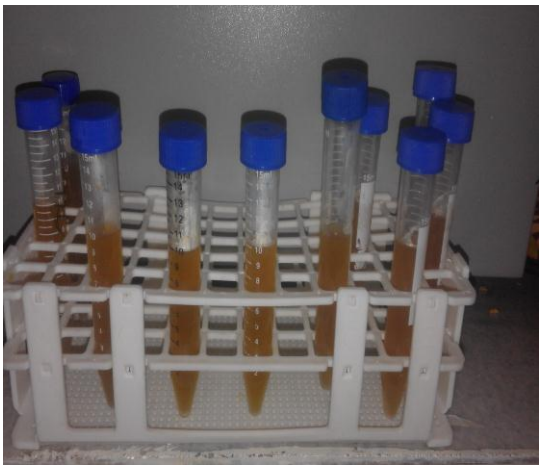
Code: 648

Dear panelist, you are invited to participate in a study involving sweet lupin-based yoghurt with vanilla evaluation. Please taste the samples in the order presented, moving from top to bottom and rank them in level of score given in the table. (Put 'X' sign on the Box).

	Like extremely (1)	**Like very much (2)**	**Like moderately (3)**	**Like slightly (4)**	**Neither like nor dislike (5)**	**Dislike slightly (6)**	**Dislike moderately (7)**	**Dislike very much (8)**	**Dislike extremely (9)**
Color									
Taste									
Flavor									
Texture									
Overall acceptance									
Mouthfeel **Consistency**									

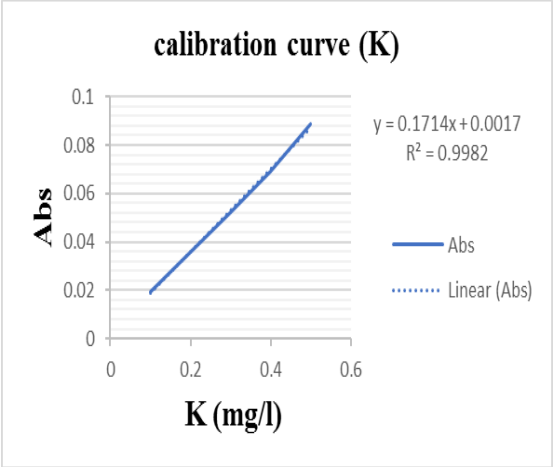
Thank you!!!

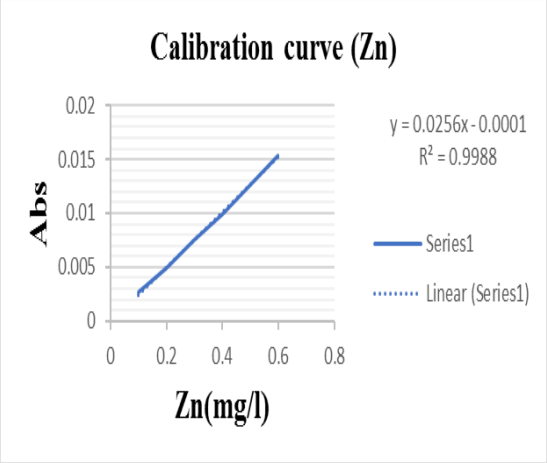
Appendix 3. Photos of Laboratory Equipment used during laboratory session



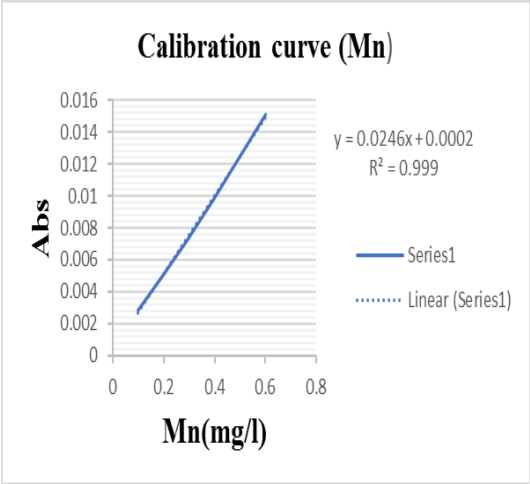
Appendix 4. Standards for minerals

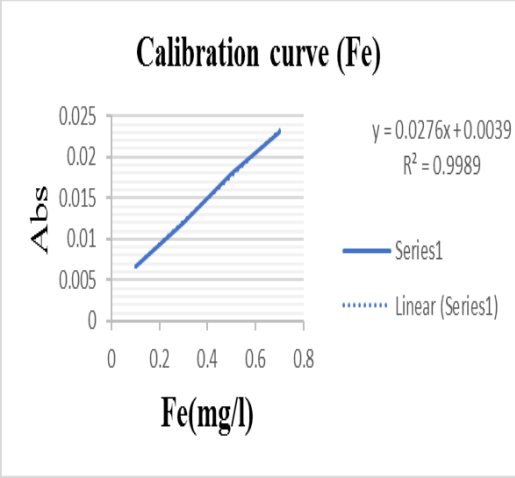
Potassium Concentration (mg/l) Absorbance	Absorbance	Zinc concentration (mg/l)
0.1 0.0026	0.0196	0.1
0.2 0.0049	0.0359	0.2
0.3 0.0075	0.052	0.3
0.4 0.0099	0,689	0.4
0.5 0.0154	0.0888	0.5





Manganese Concentration (mg/l) Absorbance	Absorbance	iron concentration (mg/l)
0.1 0.0066	0.0028	0.1
0.2 0.012	0.0051	0.2
0.3 0.018	0.0074	0.3
0.4 0.023	0.0099	0.4
0.6	0.0151	0.5





Sodium Concentration (mg/l) Absorbance	Absorbance	calcium concentration (mg/l)
0.25 0.0166	0.0111	0.5
0.5 0.0278	0.0292	1
0.75 0.0389	0.0456	1.5
1 0.0522	0.0624	2
1.25 0.0767	0.0799	3