Development of milk substitute from peas, sesame seeds and flaxseeds

Dissertation II Report

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CERTIFICATE

This is to that **Kiranpreet Kaur** (Registration No. 11707056) has personally completed M.Sc. dissertation II entitled "**Development of milk substitute from peas, sesame seeds and flaxseeds"** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of dissertation has ever been submitted for any other purpose at any University.

The project report is appropriate for the submission and the partial fulfillment of the conditions for the evaluation leading to the award of Master of Nutrition and Dietetics.

Date: 14 May 2018

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Chapter 1. Introduction

Milk is defined as the whole, fresh, lacteal secretion obtained by the complete milking of one or more healthy milch animals, excluding that obtained within 15 days before or 5 days after calving or such periods as may denecassory to render the milk practically colostrum free, and containing the minimum prescribed percentages of milk fat and milk solid not fat (Sukumar de, 2012). Cow milk is considered to be the wholesome food because of presence of all obligatory nutrients to human body expect vitamin C. The reason being milk so important for human health in the presence of bioactive compounds majoraly β- lactoglobulin, α-lactalbumin immunoglobulins, casein and whey protein, medium and shortchain fatty acids, mono-unsaturated and poly-unsaturated fatty acids, triglycerides, minerals, vitamins and nucleotides. Milk is functional for healthy growth and development of human body . Due to the significant amount of bioactive compounds present in milk , it devises to be an antihypertensive, anti-diabetic, anti-cholesterol, anti-carciogenic ,anti-obesity agent. It is also being evidence practically to be probiotic and prebiotic in nature. (FAO 2013).

Despite of these useful properties lactose intolerance and cow milk allergy have been spotted in recent past. Due to the deficiency of lactase enzyme in the digestive tract, people can lose their ability to digest lactose , this condition is known as lactose intolerance. It is been symphonized as abdominal pain, bloating and diarrhea(Sousa and Bolanz 2017).).The prevelance of lactose intolerance in South America 80-90% , and in parts of northen America around 40%. and in New delhi up to 27.4% (Deora and Deswal 2018).

Cow milk allergy is a type of food allergy , in this body's immune system shows adverse effect against one or more constitutes of milk i.e α-lactalbumin and β-lactalbumin (whey protein) responsible for cow milk allergy. The incidence of cow milk allergy among infants up to 2 to 6% and in adults about 0.1 to 0.5% along with respiratory (asthma, rhinoconjuctivitis), gastrointestinal (nausea, vomiting, diarrhea) and cutaneous (urticaria, eczema) symptoms (Crittenden et.al 2013).

To overcome these problems i.e lactose intolerance and cow milk allergy plant milk alternatives are originated. The popularity of plant milk alternatives as a functional drinks is increasing now days and these are also a suitable option in countries where mammal milk is scarce and expensive (Deora and Deswal 2018) .

According to the survey report of United States Department of Agriculture (USDA) , there is per capita decrease in the consumption of cow milk due to the growing interest of consumers among non dairy beverages (Singhal et.al 2016). Plant milk alternatives are fluids formed by extracting water from plant material such as cereals, legumes and oil seeds that mimic as a mammal milk

in consistency and appearance (Yadav et.al 2017). For making the final product it is necessary to improve microbial stability and suspensions by doing thermal treatments and homogenization (Makinen et.al 2015). Plant milk alternatives are fortified with same amount of calcium and vitamin D as resemble to cow's milk and it provide 30-40% of RDA of calcium and 25-35% RDA of vitamin D to toddlers and young children (singhal et.al 2017).

The trend of milk based beverage is nowadays shifting to plant based milk substitute because of the indispensable significant of the functional properties procure by them. These properties are proved to be handy in treating certain diseases, and have nutraceutical and therapeutic benefits too.

Chapter 2: Problem background

Milk serves as a major nutritional source in the diet of vegan people. It is consumed fresh, in the form of curd, butter, butter oil and as an ingredient of many sweets. Milk constituents like casein and lactose can act as allergens; can cause casein allergy and lactose intolerance and hence restricts its use in the diet of allergic people. A diet devoid of milk can leads to nutritional deficiency. Hence, there is need for the milk substitutes which can mimic the sensorial and nutritional value of milk.

Chapter 3. Review of literature

Plant milk alternatives are colloidal emulsions of plant materials and formed by grinding tha raw material into slurry. It can act as a cheap source for low economic group of developing countries and places, where supply of cow milk is insufficient. These milk alternatives are of various type including cereal based (Oat milk, rice milk), legume based (soy milk, peanut milk) , nut based (almond milk, coconut milk walnut milk) and seed based (sesame milk, flax milk sunflower milk) (Sethi et.al 2016).

3.1 Sesame seeds-

Sesame plant belonging to the order tubiflorae, family Pedaliaceae, is considered to be present since 1600 B.C. (Prasad et al, 2012 and Pathak et al, 2014). Popular as 'queen of oilseed' is also known with certain other names in different parts of world such as gingelly (India), benniseed (Africa), benne (Southern United States), gengelin (Brazil), and tila (Sanskrit) (Hassan, 2012). Origin of this plant is conjectured to be central Africa, unfurling to the areas of Egypt, India, and Middle-East, China and all over the globe. Sesame plant is majorly produced for its invaluable oil as it procures number of health and therapeutic properties (Namiki, 2007). The presence of natural antioxidants and certain bioactive compounds like sesamin, sesamolin, and tocopherols homologues makes it highly resistive to oxidation and rancidity, thus elevating keeping quality of the oil (Pathak et al, 2014). It is also been evidenced to procure anti-oxidative, anti-cancer, anti- hypersensitive, anti-immunoregulatory actions (Prasad et al, 2012). Beside this it also act to escalate taste and flavor of various food products (Namiki, 2007). On examining the chemical composition of the plant it is observed to have oil content ranging from 50-60% followed by 18-25% protein, 13-14% carbohydrate and 5% of the ash content (Prasad et al, 2012). This composition itself confirms that it is rich source of oils and fats. Not only rich in major nutrients it is also significant in trace materials like phosphorus, iron, magnesium, calcium, manganese, copper and zinc (Pathak, 2014).

3.1.1 Proximate Composition of Sesame Seeds

Constituent	**Composition in 100g**
Energy(Kcal)	570-580
Moisture (g)	5-7
Fat(g)	45.6- 50
Protein (g)	20-30
Carbohydrates(g)	18.6-21
Fiber(g)	7.17-11.8
Ash(g)	6.18-7.35
Calcium (mg)	1200- 1225
Magnesium (mg)	350-370.6

Potassium (mg)	540-550
Vitamin $B_{1\ (mg)}$	0.41.0.95
Vitamin $B_{2\ (mg)}$	0.35-0.58
Linoleic acid	40-47
Oleic acid	40-43
Palmitic acid	10-11
Stearic acid	8-10

3.2 Flaxseed-

Flaxseed, popular as Linseed is also named as Alsi, Jawas, Aksebija, in several parts on India belongs to the family of Linaceae (Ganorkar and Jain, 2013). The blue flowering Rabi crop is believed to be native of Eastern Mediterranean, extending itself to Western Asia, and the Middle East to India (Bernacchia et al, 2014). Flaxseed is majorly used for industrial purpose for extraction of its edible oil that is being utilized in production of natural health products (Shim et al, 2014). Traditionally, flaxseed is being reported to be used in paints and coatings, printing inks, soaps, core oils, etc but the presence of significant bioactive compound like linolenic acid, linoleic acid, and lignans make it useful for medicinal and therapeutic purpose (Bekhit et al, 2018 and Shim et al, 2014). In recent researches it is being proved to procure anti-tumoral and anti-inflammatory properties and also effective in treatment of cardiovascular diseases, various cancers and diabetes (Bekhit et al, 2018 and Rabetafika et al, 2010). On examining the chemical composition of flaxseed it was observed to be very rich source of fat accounting upto 41% followed by total dietary fiber content constituting about 28%, with 20% protein content and 3.4% of the ash constituents. Utilization of the flaxseed is new to food prospect because of increasing awareness among consumer towards functional food. Because of presence of effective bioactive component it is now being used for the preparation of certain salad dressings, food additive, and substitute for milk (Rabetafika et al, 2010).

3.2.1 Proximate Composition

Constituents	**Concentration in 100 g**
Energy	420-450
Protein	26.9- 31.9
Carbohydrates	18-29
Fat	35-41
Dietary fibre	25-28
Moisture	7.7-10
Oil	35-45
Linoleic acid	48.5 -51.2
Oliec acid	21.27- 23.45
Linoleic acid	15.62- 17.70

Calcium	220-236
Magnesium	420- 431
Phosphorus	600-622
Potassium	821-831
Sodium	25-27
Zinc	2.6-4
Copper	1
Iron	3.4-5
Manganese	3

3.3.1 Peas

Peas*(Pisum sativum L)* are available as yellow and green dried out cotyledon. They have for many years been a vital component of diet and are nutritionally rich in protein and starch content. Peas is mainly composed of phytochemicals ,proteins , starch , fiber, vitamins and vitamins. The starch digestibility is reduced because of the glycemic index which is low .On hydrolyzing pea protein the presence of peptides bioactive antioxidants and angiotensin-I converting enzyme activity was observed. The deficiencies diseases related to selenium and folate were tackled by the consumption of peas in diet. The consumption of peas in diet can helps in weight management and preventing cardiovascular diseases (Dahl et.al 2012).

3..3.2 Proximate composition

Constituents	**Concentration in 100g**
Protein	24.2-32.8
Carbohydrates	56.6-76
Saturated fat	8.6-19.5
Unsaturated fat	15.2-23.3
Starch	33.4-49.0
Amylose	21.7-33.7
Total dietary fibres	18-26
Total lipids	1.2-2.4
Ash	2.3-3.4
Calcium	67.9-75.5
Copper	0.4-0.7
Iron	3.4-5.5
Potassium	1100-1126.3
Magnesium	1.2-1.5
Phosphorus	380.9-401.9
Zinc	2..5-3.5

3.4 Bioactive compounds in different plant milks

Type of milk	Bioactive compound	Health benefits	References
Soy	Isoflavones (daidzein, genistein)	• Effective in preventing cardiovascular disease and breast, prostate cancer and relief of menopausal symptoms.	Chung et al 2014
		• Reduce the risk of uterine cancer, cure inflammatory diseases of airways and diminish oxidative damage.	Ziegler et.al 2017
		• Helps in improvement of bone health.	Rangel et. al 2017
	Diadzein	• Having anti-diabetic , anti-aging (helps to fight against skin aging and photodamaging), Neuroprotective properties (treating neurodegenerative disesases such as stroke , apoptosis i.e death of cells)	Sun et.al 2016

	Genistein	• Recover post-menopausal women from hot flashes • Hinder cell proliferation , cell adhesion , action of smooth muscle cell replication and enhance arterial elasticity.	Suthar et.al 2001
Almond	• α- tocopherol • Arabinose	• Antioxidant property inhibiting free radical activity,helps in lowering cholesterol level and also effective in reducing risk of cardiovascular, inflammation and oxidative stress. • Lowering lipid peroxidation and intracellular signaling. • Helps in treatment of metabolic syndrome, suppress obesity and improve insulin resistance , inflammation	Kamil and Chen 2012 Ryan 2011 Hao 2015

		and dyslipidemia.	
Rice	Phytosterols (β-sitosterol, γ- oryzanol)	Helps in reducing cholesterol level, having antioxidant , anti-inflammatory, chemo-preventive and lipid lowering properties.	Ryan 2011
	Polyphenols (ferulic acid ,α- lipoic acid)	Improving food quality by blocking oxidative degradation of lipids and also having antioxidant properties that helps in reducing oxidative damage.	Goffman and Bergman (2004)
		Play a protective role in suppressing breast and colon cancer cells.	Hundson et. al (2000)
	Carotenoids (α-carotene, β- carotene, lycopene, lutein and zeazanthin)	Inhibit neurological, light sensitive or eye related problems and also diminished risk of lung cancer.	Fiedor and Burda (2014)
Coconut	Sterols (campesterol 7.20%, stimgasterol 12.30%, β- sitosterol 38.97%)	Decreasing total cholesterol , LDL level and supress platelet aggregation	Schwartz et.al 2007

Flax seed	Phenolic compounds (lignans, flavonoids and phenolic acid)		Kasote and DM 2013
	Lignans (seciosolariciresinol diglucoside SDG)	Having antioxidant , anticancerous properties, helps in treating hypertension	R Bernacchia et.al 2014
	Flavonoids (kaempferol)	Having anti-oxidant , anti-diabetic properties and protective action against various types of cancers.	Zuk et.al 2011
	Phenolic acids (ferulic acid, chlorogenic acid, gallic acid)		
	Ferulic acid	Act as a growth enhancer, anti-oxidant and having anti-thrombotic, hepatoprotective anti-allergic anti-viral,anti-aging ,anti-diabetic prorerties and also hinder apoptosis.	Kumar and Pruthi 2014
	Chlorogenic acid	Having neuroprotective effect in neurodegenerative diseases (such as Parkinson's disease and Alzheimer 's disease)	Nabavi et.al 2017
Sesame	Sesamol, sesaminol, sesamolinol and sesamin	Helps in lowering cholesterol level and high blood pressure	Pathak et.al 2014

3.5 Antinutritional Factors in Different plant milks

Type of milk	Anti- nutritional factors	Adverse affects	References
Sesame	Tannins	Decreases feed intake, growth intake, and lowers effectiveness in converting absorbed nutrients by the body.	(El-Himed and El-Bramawy 2011)
Soy	Soybean Agglutinin(N-acetylgalactosamine)	Intake of high concentration (0.1-0.2%) will lead to increased permeability of intestine.	(Zhao, 2011)
	Proteinase inhibitors (Trypsin and chymotrypsin)	Increase in output of pancreatic enzymes.	(Brandon, 2003)
Peas	A-Galactosides	Creates intrusion in maceration of other nutrients. High intake will lead to decrease in amount of dietary net energy contribution.	(Villaluenga et al,2008)
	Phytates	Lowers availability of minerals as they chelate multivalent metal ions.	(Omoruyi et al,2013)
	Trypsin Inhibitor	Creates reluctance in protein digestion and enlargement of pancreas.	(Wang et al,1998)
Rice	Oxalates	High amount lead to stone formation in urinary tract, toxic effect may lead to death, also documented to lead to corrosion of mouth, and gastrointestinal tract.	(Kaur et al, 2011 and Noonan)
	Saponins	Some Saponins are responsible for reducing feed intake, growth rate, and responsible	(Kaur et al, 2011 and Desai et al,2007)

		for weight loss, diarrhea and extreme conditions may lead to death.	
Flaxseed	Caynogenic compounds (diglucoside linustatin, neolinustatin, and linamarin) Cadmium Trypsin inhibitors	Small amount leads to headache, tachycardia and dose of more than 100mg is lethal for human health. Responsible for renal dysfunction, aminoaciduria, glucosuria, pulmonary emphysema Slows down the absorption of proteins by hinder the action of proteases	(Imran et al,2013) Bernacchia et.al 2014 Bernacchia et.al 2014
Almonds	Phytic acid	Effect the bioavailability of minerals, especially cause zinc deficiency	Oatway 2007

3.6Pros and Cons of Different plant milks

Type of milk	PROS	CONS	References
Cow's milk	Rich in protein , calcium and vitamin D	Not suitable for those with allergic or lactose intolerance, higher in saturated fat	Crittenden and Bennett 2016
Soy milk	Highest protein and least processed among plant-based milk	Higher fat than other plant-based milks, sweeten varieties can contain up to 19g sugar	Meghwal and Sahu 2015
Almond milk	Less calories , rich in vitamin E	Low in protein	Chen et al. 2006
Rice milk	Best option for those with multiple allergies	Lesser amount of protein	Walter and Marchesan 2011 Ryan 2011

Oat milk	Contains fibre, iron and a moderate amount of protein	Presence of potential allergens	Sethi et. al 2016
Coconut milk	Good source of potassium, contain iron and fibre	Low in protein, highest fat content than other plant based milk	Mikolajczak 2017
Cashew milk	Creamy taste and texture	Very low in protein	Sethi et .al 2016
Flax milk	Low in calorie, high in essential fatty acids	Very low-protein	Ganorkar et.al 2013 Rabetafika et al. 2011
Pea milk	Higher amount of protein ,low in saturated fat, cholesterol and sodium	Not widely avialable	Dahl et.al 2012
Sesame milk	Rich in protein and having unique balance of amino acids	Sesame proteins are not soluble in water because they are salt soluble and susceptible to heat denaturation	Sethi et.al 2016

Chapter 4:PROPOSED RESEARCH OBJECTIVE

1. Quality evaluation of the raw materials.

2. Removal of anti-nutrients from peas and sesame seeds by different methods.

3. Development of milk substitute from peas ,flax seeds,sesame seeds.

4. To study the Quality evaluation and shelf life of the developed milk substitute.

Chapter 5: PROPOSED RESEARCH METHODOLOGY

Detailed plan work

5.1. Experiment 1: Quality evaluation of the raw materials.

1. Raw Materials to be used

a. Peas

b. Sesame seeds

c. Flax seeds

2. Tests to be performed

5.2 Experiment 2:Removal of antinutrients from peas and sesame seeds and flax seeds by different methods.

The effect of different processing techniques on the antinutritional factors of peas will be investigated for the following processing techniques:

Proximate composition

a. Moisture (olawuni et al. 2013)

b. Carbohydrates AOAC 2000 (Mundi et al.2012)

c. Protein AOAC 2000 (Mundi et al.2012)

d. Fats (soxhlet extraction method)

e. Ash (Elizabeth and shubramniyam 2013)

f .Crude fibre (AOAC 2000)

g. Dietary fibre (Van Soest and Wine 1967, AOAC 2000)

Antinutrient Factors

a. Total Phenols (Sakakibara et al. 2013)

b. Phytates (AOAC 98611-1988)

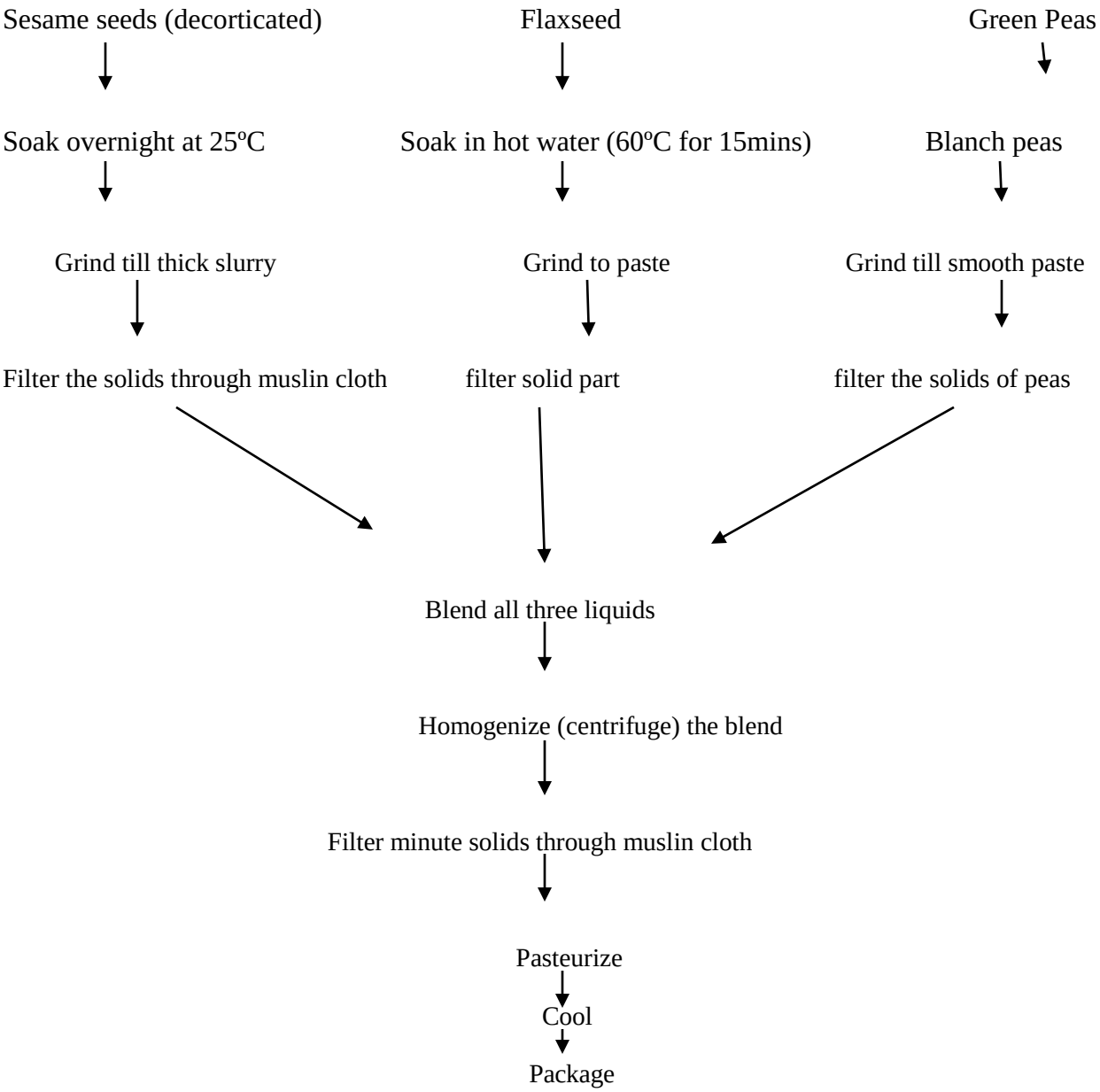
c. Oxalates (Moureau & Savage 2009)

d. Tannins (Ricci et al. 2015)

e. Anthocyanins (Elizabeth and shubramniyam 2013)

f. Flavonoids (Elizabeth and shubramniyam 2013

Materials and Methodology –



5.4 Experiment 4:To Study quality Evaluation and shelf life of Developed Milk Substitute.

Proximate composition

a.Moisture (olawuni et al. 2013)

b.Carbohydrates (Lakhvir et al. 2011)

c.Protein AOAC 2000 (Mundi et al.2012)

d. Fats (soxhlet extraction method)

e. Ash (Elizabeth and shubramniyam 2013)

f.Dietary fibres (Van Soest and Wine (1967), AOAC 991.43

Antinutrient Factors

a.Phytates (AOAC 98611-1988)

b.Oxalates (Moureau & Savage 2009)

c.Tannins (Ricci et al. 2015)

d. Anthocyanins (Kalpana et al. 20140

e. Total Phenols (Sakakibara et al . 2003)

Sensory Evaluation

Final Product Will be evaluated for the sensory characteristics on the basis of 9 point hedonic scale and composite sensory scale.

A.Sensory analysis

A 9 point hedonic scale will be used to evaluate the experimental samples using 100 semi trained panelist. Healthy male and female will be selected for the evaluation.

a. Color and appearance

b. Flavor and sweetness

c. Body texture

d. Mouth feel

Overall acceptability

Expression	Points to be assigned
Liked extremely	9
Liked very much	8
Liked moderately	7
Liked slightly	6
Neither liked or dislike	5
Dislike slightly	4
Disliked moderately	3
Disliked very much	2
Disliked extremely	1

Sample code	Color and apperance	Flavour and sweetness	Body and texture	Mouthfeel	Overall acceptibility	Remaks (if any)

Optimized product will be obtained using the above experimental setup.

B. Mean Sensory Score on Composite Scale for Milk substitute:

Sensory attributes	Possible Score	Mean Score±SD	Number of Judges
Apperance			
Consistency			
Flavour			
Absence of Defects			
Total Score			

Experiment 4: Microbiological Study

a. TPC

b .Yeast Count

c. Mould count

Chapter 6: EXPECTED RESEARCH OUTCOME

1. Potential anti-nutrient reduction for peas and sesame seeds will be achieved.

2. An energy rich, nutrient dense milk substitute acceptable on sensory physical and chemical properties will be developed.

3. The obtained optimized product will be evaluated for shelf life and an optimum shelf life of Milk Substitute will be predicted.

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