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Development and Physico-Chemical Analysis of Vegan Lactose-Free Chocolate Milk extracted from Fagopyrumesculentum. (Buckwheat)

Rohit G Jagdale[1], Prof. Nisha Wagh[2], Sayali Pawar[3]
Department of Food Technology[1,2,3]
Parul University Applied Science, Parul University, Waghodia, Gujarat, India
rjagdale151@gmail.com

Abstract: *The study of Buckwheat flavoured vegan milk development was carried out at the Parul Institute of Applied Sciences, Parul University, Vadodara. The main goal of developing a flavoured Buckwheat milk is to provide a lactose-free and delightful product to people who are lactose intolerant i.e. indigestion of lactose as well as cow's milk allergy. Buckwheat milk does not contain natural sugar lactose, so it can be recommended for people with lactose intolerance. Proper and ideal packaging along with refrigeration storage makes the Buckwheat milk fit and in sound condition for up to 10 days. Buckwheat milk has tremendous nutritional value and can be an effective asset in eradicating lactose intolerance by supplementing young age groups, lactose-intolerant people, and vegans who do not prefer to consume regular milk. Flavoured buckwheat milk was produced by extracting milk from germinated and non-germinated soaked buckwheat and different compositions of milk were prepared. Three distinct dilutions were created using buckwheat milk and water ratios (1:2, 1:4, 1:8) for further milk formulations. The best formulation is used for further organoleptic evaluations such as taste, colour, mouth feel, and overall acceptability. Following that, proximal analysis and microbial parameters were evaluated. The ideal selected formulation contained 5.14% Protein, 63.5% Carbohydrate, 0.53% Ash, 2.42% Fat, and 293 Kcal per 100g. According to the findings, buckwheat milk is a vegan product with a high nutritional value which can be strongly suggested for people who are lactose intolerant.*

Keywords: Buckwheat, Lactose-free, Vegan, Chocolate, Sugar-free

I. INTRODUCTION

The market for drinks in India is estimated to be worth close to Rs. 1,95,000 crore and is expanding at a rate of 20–23%, according to research done by IMAGES Group, the publisher of Progressive Grocer India, for The India Food Report 2016. By 2020, this growth rate will have tripled the size of the category from where it is now. Given the enormous market potential for drinks and the population's increased knowledge of the health benefits of naturally occurring, minimally processed foods and beverages (Nakade et al., 2020).

The consumption of milk and milk products is rising quickly all across the world, and it is predicted that this trend will continue in the same direction going forward. The majority of people often drink cow's milk, which is regarded as a healthy, complete diet that contains important elements including fat, protein, and carbs (Plant Based Foods Association 2016). In addition to these macronutrients, milk includes a variety of other nutrients (micro and macro), including calcium, selenium, riboflavin, vitamin B12, and pantothenic acid (vitamin B5), all of which are crucial for the general development and upkeep of the body system (M€akinen et al. 2016). Even though milk is regarded as a complete diet, its recommendation as a complete food for babies older than 12 months is restricted by the low availability or almost complete lack of key minerals, like iron, and vitamins, like folate (Vanga, Singh, and Raghavan 2015). Additionally, lactose intolerance is a condition that is constantly spreading throughout the industrialized world, particularly in the elderly population groups, which limits the use of milk and milk products, especially by the elderly population. Additionally, there are a few drawbacks to milk, including its high cost, restricted availability in some countries (particularly desert ones), and the presence of several strong bacteria (Salmonella spp. and Escherichia coli O157:H7) that can lead to disease outbreaks and other health issues (Vanga and Raghavan 2018). The creation of milk alternatives, milk substitutes, and milk analogs may have been inspired by several factors, including cholesterol, cow's milk allergy, which is primarily observed in newborns and young children, antibiotic residues, vegetarianism, and vegan diets. However, recent research has clearly demonstrated the critical role that these plant-based beverages play in enhancing or managing the immune system, having potential antimicrobial effects, lowering the risk of cardiovascular and gastrointestinal diseases through improved physiological functions, lowering the risk of having low bone mass, and having extremely high antioxidant levels with free radical scavenging properties. (Paul et al., 2020).

Therefore, an attempt has been made to provide vegan flavor buckwheat milk as a replacement for regular animal-based milk in light of the situation. The development of Buckwheat Milk involved the use of two formulations, T□ and T□, where T□ is made using non-germinated Buckwheat and T is made with germinated Buckwheat. To enhance the flavor and palatability of vegan buckwheat milk, additional ingredients such as cocoa powder and dark chocolate were used. While lowering blood sugar levels, aspartame is used to provide sweetness. It benefits as it has an absence of lactose and contains all the critical elements needed for a balanced diet, making it appropriate for consumption by people with lactose intolerance and people with a vegan diet.

1.1 Benefits of Vegan Chocolate Flavoured Buckwheat Milk

- Recommended for lactose intolerant peoples
- Sugar-free
- High calorific value
- Cost Efficient
- Lactose-free

II. MATERIALS AND METHODOLOGY

The principal raw materials required for the formulation of vegan Flavoured Buckwheat Milk are Buckwheat, Cocoa powder, Aspartame, and Dark Chocolate, packaging material.

2.1 Processing Equipment

Processing equipment is anything needed to treat or process a raw material or finished good via mechanical, thermal, or chemical techniques. It has a refrigerator, a mixer/processor, a digital thermometer, and a weighing scale. The utensils required for the formulation of Buckwheat milk are vessels, spoons, Plates, and muslin cloth.

2.2 Analyzing Equipment

For the formulation and preparation of Flavoured Buckwheat milk, there is a requirement of Soxhlet apparatus for fat estimation, Kjeldhal's apparatus for protein estimation, a Muffle furnace for ash estimation, a Digital ph meter for measuring the ph of the sample, Laminar Air Flow for the determination of T.P.C. count, T.P.C. plates are incubated in an incubator, and crude fibre is estimated using a water bath.

Glassware includes Petri plates, glass bottles, burettes, bakers, volumetric flasks, glass rods, pipettes, silica crucibles, and measuring cylinders. This glassware is used during the analysis of the product.

2.3 Product Manufacturing Process

2.3.1 Preparation of Flavoured Buckwheat Milk

Buckwheat, cocoa powder, aspartame, and dark chocolate were procured as having sound and uniform quality. All raw materials were accurately measured in accordance with the milk ratio formulated. Two different methods were employed for the milk extraction. 1. Extraction of milk from Germinated buckwheat. 2. Extraction of milk from non-germinated soaked buckwheat. The Buckwheat is soaked in mildly hot water for 12 hours, followed by the germination/sprouting of the soaked buckwheat. This procedure leads to a further decrease in starch content. In both techniques, further procedures were followed in a similar process. Then buckwheat was ground with different ratios of water followed by filtration by using a muslin cloth to extract milk. The extracted milk was then heated at 70˚ C; and Aspartame, Cocoa powder, and Dark chocolate were added accordingly. The developed vegan milk was further cooled and filled in a Glass bottle and was stored in the refrigerator.

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2.3.2 Cleaning
After taking raw material, cleaning was done to remove the undesired particles, stones, dirt, immature beans, broken beans, etc.

2.3.3 Weighing
Weighing of the necessary amount of cleaned buckwheat for soaking was done.

2.3.4 Soaking
For 12 to 16 hours, buckwheat was soaked in gentle, lukewarm water. Buckwheat losses all of its antinutritional properties after soaking in somewhat lukewarm water.

2.3.5 Germination
The soaked buckwheat was then tied in a cotton cloth for 2 days for germination as it decreases the starch content of the cereal and milk was extracted by further grinding.

2.3.5.1 Non-Germinated Buckwheat
In non-germinated buckwheat milk, the buckwheat was soaked for 12hr and milk was extracted by further grinding with the addition of water in different proportions.

2.3.6 Filtration
Muslin cloth is used to physically filter and extract the milk from the processed buckwheat.

2.3.7 Heating
After filtration, heating was carried out and the temperature was checked with a glass tube thermometer till it reached 70°C.

2.3.8 Addition of Cocoa Powder
Cocoa powder was added according to the ratio and continuous stirring of milk was carried out to avoid lump formation.

2.3.9 Addition of Aspartame
Aspartame was added according to the ratio and continuous stirring of the milk was done.

2.3.10 Addition of Dark Chocolate
Dark chocolate was added according to the ratio and then continuous stirring was carried out for mixing the content evenly.

2.3.11 Bottling
Then milk was cooled down and packaged in a glass bottle and the sealing of the bottles was done.

2.3.12 Storage
After bottling milk was stored in the refrigerator at 4˚C temperature.

III. METHODS

3.1 Physio-Chemical Analysis
Buckwheat, cocoa powder, dark chocolate, and aspartame were used and prepared vegan flavoured buckwheat milk was analysed for proximate composition including moisture, ash, protein, fat, carbohydrate, and calories content according to the standard operating procedure (AOAC 2005).

3.1.1 Moisture Content

The moisture content was determined by drying the empty dish and weighing and grinding 5 g of the sample in the dish. The dish was then subjected to an oven for drying at 105˚C for 4hrs. It was again weighed after cooling in a desiccator until constant weight. The resulting weight loss was estimated as moisture content.

Moisture % = Initial weight (W1)-final weight(W2) / Initial weight (W1) × 100

3.1.2 Ash Content

Ash content was determined using the (AOAC 1980) procedure. 5g of sample was weighed into the pre-weighed crucible and it was heated at low flame till all the material was completely charred (smokeless) and cooled. The sample was then kept in the muffle furnace for about 4hrs, at 5500C. It was again cooled in a desiccator and weighed. The procedure was repeated until two consecutive weights were constant. The percent ash was calculated by knowing the difference between the initial and final weight.

Ash %=Weight before heating – Weight after heating/ weight of sample × 100

3.1.3 Determination of Protein content (By Micro-Kjeldhal Method)

% N = (Sample – blank N of HCL vol. of digest 0.014) / Aliquot taken Wt. of sample

3.1.4 Determination of Carbohydrates

The carbohydrate content was calculated by deducting the sum of the moisture, fat, protein, total ash, and crude fiber values. The NFE was determined using the formula below.

NFE % = 100 – (CP% + CF% + CF% + TOTAL ASH%)

CP = crude protein.
CF= crude fat.
CF= crude fiber.

3.2 Microbial Parameter

Microbial inspection is the ideal quality evaluation procedure used in food product quality analysis. The developed vegan milk's microbiological quality was assessed. In the current investigation, several microbiological characteristics such as total plate count, yeast, mold, and coliform were evaluated, as well as the samples' preservation at room temperature. Microbial tests were performed in accordance with APHA guidelines (1992).

3.2.1 Determination of Total Plate Count

The nutrient agar medium was made by adding 28 g of nutrient agar to 1000 ml of distilled water and heating the mixture until the agar was thoroughly dissolved. Its mouth was covered with cotton, and it was sterilized for 20 minutes at 120°C and 15 lbs of pressure in an autoclave.

The sample solution (serial dilution) was made by taking nine sterile test tubes and numbering them. 9 ml of distilled water was placed in each tube. The test tubes were sterilized in an autoclave at 121 °C for 15 minutes using cotton plugs under 15 lbs of pressure. A sterile test tube containing 9 ml of distilled water received 1 ml of sample serially.

Pipettes and Petri dishes were sterilized using an autoclave (moist heat treatment) or a hot air oven (dry heat treatment). A sterile petri dish was placed in the laminar airflow cabinet, and ultraviolet light was turned on for 30 minutes. After 30 minutes, the UV light was turned off, the fan was turned on, and 70% ethanol was used to clean the work surface. Plates were appropriately labelled before 1 ml of samples were put onto them, and 1 ml of samples were added to each plate. Each plate received 15–20 ml of molten medium. This was carried out close to a flame to avoid microbial contamination of the plate. The plates were vigorously stirred and kept there to solidify. After 48 hours at 37°C in the incubator, the plates were taken out to be checked for colonies.

3.2.2 Determination of Yeast and Mould count

Potato dextrose agar medium preparation: In 1000ml of distilled water, 39g of Potato dextrose agar medium was added and boiled to dissolve adequately. The mouth was closed with a cotton plug and sterilized in an autoclave at 121˚C for 15 minutes at 15 lbs pressure.

Sample solution preparation (serial dilution): 9 sterile test tubes were obtained and numbered correspondingly. Each tube received 9ml of distilled water. The test tubes were sealed with cotton plugs and sterilized in an autoclave at 121°C for 15 minutes under 15 lbs of pressure. 1 ml of the sample was serially added to 9 ml of distilled water in a sterile test tube.

Plate preparation: Petri plates and pipettes were sterilized in a hot air oven (dry heat treatment) or in an autoclave (moist heat treatment). Sterilized Petri dishes were placed in a laminar airflow cabinet and exposed to ultraviolet light for 30 minutes. After 30 minutes, the UV light was turned off, the blower was turned on, and the working surface was cleaned with 70% alcohol. Plates were appropriately labeled before 1 ml of samples were put onto them. Each plate received 15- 20ml of the molten medium. This was done near a flame to prevent microbial contamination of the plate. The plates were vigorously spun and left to solidify.

3.2.3 Enumeration of Coliforms (AOAC, 2005)

The second, third, and fourth dilutions of buckwheat milk samples that were prepared have been used for the enumeration of coliforms. The 1 ml from the 10^2, 10^3, and 10□ dilutions were taken in duplicate into Petri plates, and the violet-red bile agar was added and mixed well. The plates were allowed to solidify. These plates were incubated at 37 °C for 1–2 days.

3.3 Quality Control

When determining the degree to which a product's individual unit is acceptable to the consumer, the quality of the product may be defined as the sum of its differentiating qualities.

3.3.1 Raw Material Quality Control

Before purchasing raw materials in bulk, food manufacturers usually purchase samples to ensure that they meet the factory's criteria. Raw materials were tested for many characteristics, which vary according to the nature and kind of substances.

3.3.2 Process Control

All treatments used during processing were standardized, materials were utilized in the exact proportions, accurate techniques of preparation and mixing were used, and the containers used were checked to ensure that they were in good condition. During processing, satisfactory sanitary conditions are also maintained.

3.3.3 Inspection of the Finished Product

It is used to determine how well the intended quality requirements have been met. Some tests were performed to evaluate specific properties related to the product's palatability and acceptance.

3.4 Sensory Evaluation

Sensory evaluation of vegan flavoured buckwheat milk was carried out using a 9-point Hedonic scale by a panel of 10 semi-trained judges. Different milk samples were evaluated for their colour, mouthfeel, taste, flavor, and overall acceptability.

IV. RESULTS AND DISCUSSION

4.1 Optimization of Buckwheat Milk Formulation (Seeds: Water Ratio)

For optimization of the formulation of buckwheat milk, three different ratios of seed: water were evaluated i.e.1:2; 1:4, and 1:8. The best two combinations obtained therefore were analyzed for both proximate composition and sensory attributes. As buckwheat contains 75% starch, it is necessary to dilute it. The presence of starch directly affects the sensory parameter as well as the product's shelf life. So, to decrease the starch content buckwheat seeds were soaked and further kept for germination. During further processing, it was observed that the germination of buckwheat showed decreased or almost 0% starch content while the non-germinated showed the formation of starchy lumps. The dilutions were prepared by using a 1:2 buckwheat and water ratio.

Figure 1: Starch Formation

In the T1 formulation soaked-non germinated buckwheat was formulated, the water ratio used was 1:4, and for the T2 formulation germinated buckwheat was formulated, the water ratio used was 1:8. The T1 formulation contained 50 g of non-germinated buckwheat with 200 ml of water, whereas the T2 formulation, containing 50 g of germinated buckwheat with 400 ml of water, was used for further processing.

4.2 Proximate Composition

Table 1 shows the findings of the approximate composition of milk samples. All of the characteristics that were assessed, with the exception of crude fat concentrations, showed significant differences across the samples. T□ formulations show significantly higher values of protein, carbohydrates, and fat. Moisture levels obtained in this study for both samples were similar. The protein value obtained from the T□ formulation was significantly higher than that of the T□ formulation. The protein value obtained for T□ formulation was 5.14%. However, it is noteworthy that the protein content of buckwheat milk (5.14%) obtained in this study was higher than in market vegan milk. Generally, the composition and balance of amino acid in milk samples is of greater importance than the quantity of protein. The amount of fat in the T□ formulation was 2.42 %, similar to the value obtained for the T□ formulation in this study. The total ash content of the T□ formulation was higher than the value of the T□ formulation. The higher ash content of T□ in this study in comparison with T□ is suggestive of the richness of the sample as macronutrient sources. Noteworthy is the crude fiber content of the T□ formulation which was significantly higher than the value obtained for T□. The carbohydrate content of the T□ formulation in this study was lower than the value for the T□ formulation. The amount of carbohydrates in the T□ formulation was 63%. The energy value obtained for the T□ formulation in this study was higher than the values of the T□ formulation.

Table 1: Proximate composition of non-germinated buckwheat and Germinated buckwheat (Shreej et., 2021)

Parameters	T1 Formulation (%)	T2 Formulation (%)
Moisture	80.43	81.43
Protein	3.5	5.14
Ash	1.5	0.53
Fat	3.5	2.42
Fibre	0.42	0.69
Carbohydrate	67	63
Energy value (Kcal)	301	293
Starch	40.2	0
Lactose	NIL	NIL

Table 2: Proximate composition of developed chocolate-flavored vegan buckwheat milk samples (g/100)

Parameter	Non-germinated buckwheat (g/100)	Germinated buckwheat (g/100)
Protein	10	12
Carbohydrates	72.63	69.4
Fat	3.1	2.05
Ash	2.05	1.5
Moisture	11	12.7
Fibre	0.92	1.4

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Energy	359.6	345
Starch	42.8	39.2

Table 3: Effect of storage on the pH values of Buckwheat Milk:

Parameter	0 Day	3 Day	6 Day	9 Day
pH Values	6.5	6.5	6.5	6.3

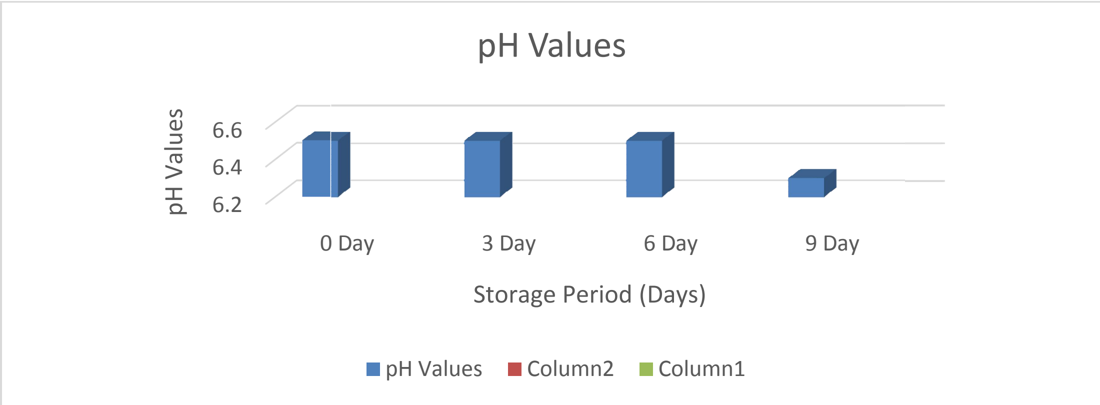
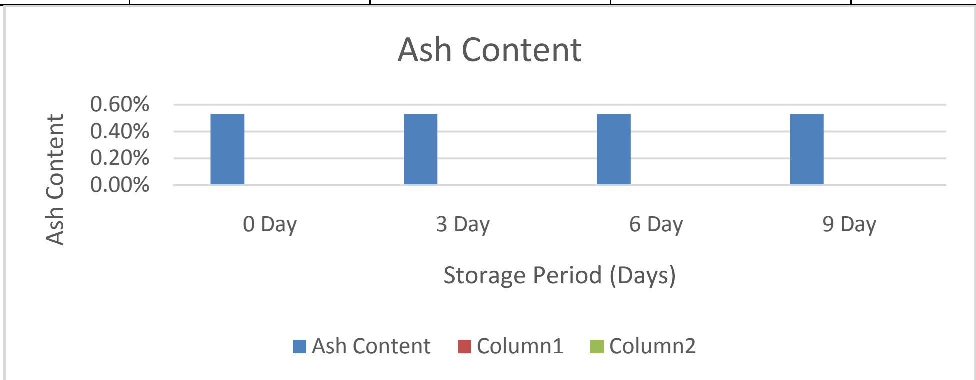


Table 4: Effect of storage on the Moisture content of Buckwheat Milk:

Parameter	0 Day	3 Day	6 Day	9 Day
Moisture Content	81.4%	81%	81%	81%

Table 5: Effect of storage on the Ash Content of Buckwheat Milk:

Parameters	0 Day	3 Day	6 Day	9 Day
Ash Content	0.53%	0.53%	0.53%	0.53%



4.3. Sensory Evaluation

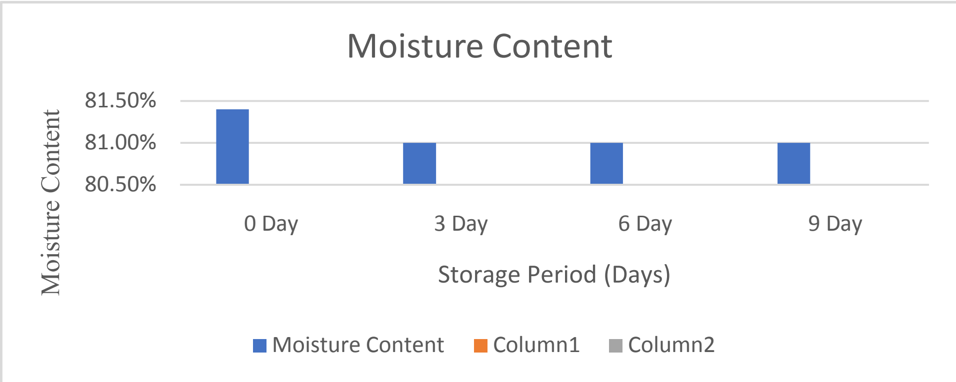
Figure 2: Formulation of vegan choco-flavored buckwheat milk.

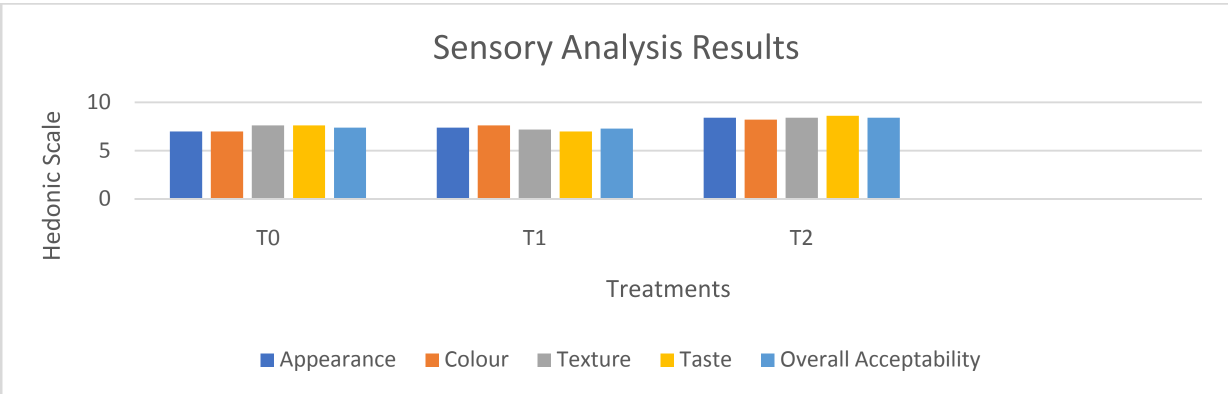
The sensory scores of vegan-flavored buckwheat milk are shown in Table No. 1. According to the results presented, there was a significant increase in taste, colour, flavor, and overall acceptability (Buckwheat) composition of vegan-flavored milk. No significant change was observed in all parameters of sensory evaluation in vegan-flavored milk prepared from buckwheat. The overall acceptability score indicated that the vegan-flavored milk prepared with up to

75-80% buckwheat has the most acceptable sensory attributes. Formulation T□ had the highest score of 8.4, while sample T□ had the lowest score of 7.3 for overall acceptability. The germination and the non-germination process were effective on vegan-flavored milk because the sensory parameters of germinated buckwheat were better than non-germinated buckwheat. This contradicted the result, which revealed that the panelists found the vegan-flavored milk containing buckwheat to be most acceptable.

Treatments	Appearance	Color	Texture	Taste	Overall Acceptability
T0 (Control)	7	7	7.6	7.6	7.4
T1	7.4	7.6	7.2	7	7.3
T2	8.4	8.2	8.4	8.6	8.4

Table 6: Sensory Evaluation of Vegan-flavoured buckwheat milk.





The vegan-flavored milk from Formulation (T□) buckwheat resulted in a better appearance, color, texture, taste, and overall acceptability. Sample (T□) was the highest value in appearance (8.4), color (8.2), texture (8.4), taste (8.6), and overall acceptability (8.4) as compared to sample (T□) sample was the lowest value in appearance (7.4), color (7.6), texture (7.2), taste (7), and overall acceptability (7.3).

The T□ formulation which contains non-germinated buckwheat milk found to have s a floury taste, whereas in the T□ formulation which contains germinated buckwheat, the floury taste was found to be NIL. The germination process affected all parameters of sensory evaluation. The presence of starch also affected the taste, viscosity, aftertaste, and thickness of vegan-flavored milk.

4.4 Packaging

Packaging may be viewed as a socio-scientific discipline that functions in society to guarantee that things are delivered to their final consumers in the best condition possible. Plastics, paper boards, metals, glass, and wood are the materials used in packaging most frequently. The cellulose, or wood fiber, used to make cardboard boxes for sale is also used. Providing physical protection, barrier protection, information transfer, and convenience are the goals of packaging (Nakade et al., 2020).

In this research, we have selected glass bottles for the packaging of buckwheat milk.

4.5 Cost Estimation

Table 7: Cost estimation of vegan-flavored buckwheat milk

	Cost (Rs)
Raw material	82
Electricity	0.8
Gas	13
Packaging	20
Total (400gm)	116

V. CONCLUSION

The present research comprises the development of "vegan lactose-free chocolate milk from buckwheat". According to a study carried out germinated buckwheat milk was found to be more suitable for further processing as it eliminated the starch content by up to 0%. As mentioned earlier buckwheat milk is Lactose-free, vegan, contains a high calorific value, and is cost-friendly. Through the research carried out it can be concluded that the developed chocolate-flavored vegan buckwheat milk can be served to lactose intolerant people as it does not contain lactose, additionally, the absence of starch was noted and therefore is easy to digest. As the developed milk was prepared from cereal therefore it can be consumed by people following a vegan diet. The Buckwheat Milk has a Shelf life of up to 15 days at refrigeration. The nutritional significance of buckwheat milk is great as it contains 5.14 g protein, 63.5 g carbohydrates, 2.42 g fat, 0.53 g ash, 0.69 g dietary fiber, and an energy content of 293 Kcal per 100 g. Along with these essential vital nutrients, buckwheat milk contains some other micronutrients like potassium, magnesium, phosphorous, and iron in considerable amounts. The production cost of the developed milk was around 116 Rs for 400ml therefore the product is cost-friendly.

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