Development and investigating parameters of fermented milk products based on various types of vegetable milk

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Abstract. Plant-based milks and fermented plant-based milk products are gaining popularity but may not possess the same physicochemical characteristics as the traditional fermented dairy products. The different chemical composition of plant-based milk affects the physicochemical and consumer acceptability of fermented plant-based milk. To compare their characteristics to the traditional dairy yoghurt and consumer acceptability, different fermented plant-based milk products were prepared as well as their two-component mixtures and their physicochemical and consumer acceptability evaluated. The single component fermented plant-based milk sample (soy milk) did not vary in terms of physicochemical parameters with respect to the traditional fermented dairy product, yoghurt. The physicochemical parameters including pH, water holding capacity, syneresis improved when the milk samples were composited and are comparable to the traditional fermented dairy product (yogurt). The results indicated that the use of two-component plant-based milk for fermentation provides the best nutritional and energy values for consumers rather than using just one component. Also, the high consumer acceptability for fermented plant-based milk was demonstrated from the findings.

1 Introduction

Globally, the demand for an alternative food protein source that could potentially replace animal proteins has been on the rise in recent years. This dietary shift has been because of sustainability, health, and ethical considerations (vegetarians, cultural values). To ensure food security, the threat of climate change must not be overlooked and as a result the world's growing population moving towards plant-based diet is laudable and inevitable [1, 2].
Other factors that might influence this increment in the demand for plant-based milk might be the increase in lactose intolerance particularly in old age population, limited availability of milk and milk products, high price and presence of some pathogenic microorganisms

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(*Salmonella* spp. and *Escherichia coli* O157:H7) that may lead to disease outbreaks and affect human health [3].

Present consumers expect more healthy and palatable food products which has made the dairy industry switched their attention to the production of non-conventional milk with health benefits beyond the conventional milk products [4]. Studies have revealed that plant-based milk improve the immune system, exhibit antimicrobial effects, reduce the risk of cardiovascular and gastro-intestinal diseases with improved physiological activities, reduced the risk of low bone mass and antioxidant activities. Plant milk can undergo fermentation either naturally or controlled, this improves the bioavailability of bioactive compounds they contain. Again, plant-based milk can be fortified to improve its nutritional bioavailability and components [5]. Generally, legumes, cereals and oilseeds are the major targets for plant-based milk production due to their high nutritional profile. Usually, these seeds contain specific bioactive compounds that make them comparable to animal milk. Soybean, oat, coconut, rice, almond just to mention few, are used to produce milk and flavored with either vanilla or straw berry or chocolate and many others. Sweeteners are also added to improve their organoleptic properties.

The global market of the dairy industry, due to high demand for the dairy products, has always been in the top position. However, dairy products involve the use of lots of resources and present a lot of environmental hazards. This makes the process very stressful and time-consuming. Most of the feed by the livestock is converted into other metabolic processes such as bones, muscles etc. formation, and only a few portions of the feed consumed are converted to milk. This leads to high input to produce less products as compared to plant-based milk. Also, the rearing of livestock such as cattle production has contributed to the release of methane gas to the environment leading to global warming. The production of plant-based milk in this case is more advantageous to the conventional animal milk when compared. It is making its way into the global market, capturing the attention of consumers. Soy milk is the leading produced plant-based milk, about 58.0% global production in 2017 and almond, rice, coconut and oat milk also contribute significantly [6].

Different food products including beverages are prepared and preserved by fermentation from age till date [7]. During fermentation process, complex carbohydrates such as starch and sugars are converted into simple products like alcohols and acids with the production of carbon dioxide concurrently using microorganisms such as yeasts and or bacteria under anaerobic conditions. The process of fermentation and the product of the fermentation is determined by the microbial biomass that is used, ranging from small scale production thus experimental purposes to large scale production thus industrial production [8]. It is known that in the fermentation process of raw materials, simpler, easily digestible molecules and biologically active compounds are formed. In this regard, the study of vegetable milk fermentation is relevant. Thus, research has shown that the extraction of biomolecules and improvement of phytochemical profile of plant-based milk products are made possible through the fermentation process using *Lactobacillus acidophilus* [9].

The objective of this study is to obtain fermented products using different plant milk types, their composites and characterize the physicochemical and consumer acceptability compared with the traditional dairy yoghurt.

2 Materials and methods

2.1 Materials

The plant-based milk products were purchased from the supermarket in Yekaterinburg, Russia. These include soy milk (Alpro), coconut milk (Alpro), almond milk (Alpro), coconut milk (Aroy-D), and cow milk (Irbitskoe, 2.5% fat) as a control. The starter culture for the fermentation (Streptococcus thermophilus and Lactobacillus bulgaricus) was also purchased from Kapria, Moscow (www.skvaska.com).

2.2 Methods

2.2.1 Preparation of fermented products

About 120 ml of milk was poured into a glass, placed in a water bath, heated to 80 ^{O}C, and kept for 10 minutes to pasteurize the milk. The milk was then cooled to 40 ^{O}C and in a sterile conditions of a laminar flow cabinet (BAVp-01-"Laminar-S"-1.2) 100 ml of milk was poured into a jar, 0.1 g of starter culture and 1 g of sugar were added, the contents of all jars were mixed with a sterile wooden stirrer. The milk mixture was fermented in a Memmert UN55 thermostat at 38 ^{O}C for 6 hours. The samples were cooled in a refrigerator at 4 ^{O}C for further experiments.

2.2.2 Water Holding Capacity, WHC

WHC of the samples were determine by centrifugation of about 5 g of the samples at 4500 rpm at 4 ^{O}C for 30 minutes. The water suspended was removed and weighed. The WHC was calculated as a percentage using the equation:

$$WHC(\%) = 1 - \frac{Weight\ of\ whey}{Weight\ of\ sample} * 100 \quad (1)$$

2.2.3 Measurement of Syneresis

The syneresis was determined using the method described in the work of Raikos et al. [10]. About 5 g of the samples were weighed onto a 2-V folded filter paper (Whatman) and placed on the top of a funnel. Syneresis was determined by gravity by measuring the weight (g) of liquid collected in a measuring cylinder of known weight. The drainage time was about 120 minutes and was done at room temperature. The percentage of syneresis was calculated according to the equation:

$$Syneresis\ (\%) = \frac{Weight\ of\ whey}{Weight\ of\ sample} * 100 \quad (2)$$

2.2.4 Measurement of pH and Titratable Acidity

The pH of the samples was measured at room temperature using FiveEasy pH meter (Mettler Toledo Ltd., Victoria, Australia). The titratable acidity (TA) was obtained through colorimetric titration method 942.15 (AOAC, 2016) [11] using a 2% (w/v) phenolphthalein solution in ethanol as an indicator. A 1 mL sample was mixed with 9 mL of purified water followed by addition of a few drops of indicator and titrated using 0.5 N NaOH solution. The TA, expressed as percentage lactic acid, was calculated as follows:

$$TA\ (\%) = \frac{10 * V_{NaOH} * 0.009 * 0.5}{W} * 100 \quad (3)$$

Where *10* is the dilution factor, V_{NaOH} is the volume of NaOH used to neutralize the lactic acid (ml), *0.009* is the conversion factor, *0.5* is the normality of NaOH, and *W* is the weight of sample.

2.2.5 Measurement of Dry Matter Content

The dry matter (DM) contents of the samples were determined by drying about 3 g of samples at 105 ˚C for 60 minutes in hot air oven. The final weight of the dried samples was measure and the DM content determined as a percentage using the equation:

$$\text{DM}\ (\%) = \frac{Weight\ of\ dried\ sample}{Weight\ of\ sample} * 100 \quad (4)$$

2.2.6 Sensory evaluation

Sensory analysis was performed within 24 hours after production at a temperature of 4 ˚C by untrained panels (staff and graduate students at the Institute of Chemical Technology, Ural federal University, Yekaterinburg, Russia). Evaluating flavor, appearance, taste, and overall acceptability on a 5-point hedonic scale (1-dislike extremely, 2-dislike, 3-neither like nor dislike, 4-like, 5-like extremely) for each sample. Water was used as a palate cleanser in between samples by the panelists.

2.2.7 Calculation of nutritional value

The calculation of the nutritional value of samples of fermented milk products was carried out on the basis of the nutritional value of the components used in their preparation according to the formula:

$$X_i = \sum \frac{X_{ij} * m_j}{100} \quad (5)$$

where x_i is the content of the i-th nutrient in 100 g of fermented milk product, g or mg;
x_{ij} is the content of the i-th nutrient in 100 g of component j, g or mg;
m_j is the mass of component j, g.

The energy value of samples of fermented milk products, kcal, was calculated by the formula:

$$y = k1 * mb + k2 * mg + k3 * my \quad (6)$$

Where y is the energy value of samples of fermented milk products, kcal;
$k_{1\text{–}3}$ is the caloric coefficients of proteins, fats and carbohydrates,
m_b is the mass of protein in 100 g of fermented milk product, g;
m_g is the mass of fats in 100 g of fermented milk product, g;

m_y is the mass of carbohydrates in 100 g of fermented milk product, g.

2.2.8 Statistical analysis

All statistical analyses were executed using GraphPad Prism 8 statistical software package. Two trials were made in parallel, and the outcomes are expressed as means ±standard deviation. The water holding capacity, syneresis, pH, titratable acidity, and dry matter were analyzed using One-way Analysis of Variance (ANOVA) and compared Dunnett's multiple comparisons. The sensory data were analyzed using Two-way ANOVA with Dunnett's multiple comparisons.

3 Results and discussions

The first part of the work is devoted to obtaining fermented milk products on the based whole plant-based milk, such as soy milk (Alpro), almond milk (Alpro), coconut milk (Alpro), and coconut milk (Aroy-D) and compare them with cow milk yoghurt. The second part includes the use of mixtures of different plant-based milk for fermentation.

3.1 Nutritional composition of milk types

Table 1 shows the nutritional composition of the various milk types used in the experiment according to the manufacturer. It can be observed that the protein content in soy milk (Alpro) is similar to that of cow milk, 3.0 g. This provides the probability of replacement in the production of fermented products. However, other milk samples, almond milk (Alpro), coconut milk (Alpro), and coconut milk (Aroy-D) have lesser protein content, and this may have effect on the fermented product formation. Other compounds such fat and carbohydrates are necessary for a better curd formation and stability of the fermented products.

Table 1. Nutritional composition of milk types (100 g of raw material).

Milk types	Protein, g	Carbohydrates, g	Fats, g	Energy, kcal	Organic acid, g	Ca, mg	K, mg	P, mg
Soy milk (Alpro)	3.0	2.5	1.8	39.0	-	120.0	122.0	43.0
Coconut milk (Alpro)	0.1	2.7	0.9	19.0	-	16.0	100.0	263.0
Almond milk (Alpro)	0.4	2.4	1.1	21.0	-	120.0	-	-
Coconut milk (Aroy-D)	0.8	9.5	10.5	18.5	0.005	18.0	220.0	96.0
Cow milk (2.5% fat)	3.0	4.7	2.5	53.0	0.1	120	146	90

3.2 Fermented plant-based milk and cow milk (yoghurt)

3.2.1 pH of fermented milk samples

The pH values obtained for the fermented soy milk, coconut milk (Alpro), and almond milk (Alpro) did not vary significantly ($p > 0.05$) from the pH for the control sample cow milk (4.5 ±0.02) according to table 2. However, the Coconut (Aroy-D) varies significantly ($p < 0.05$) in pH compared with the control sample. This result agrees with the findings of Mattison et al., [12] who also observed similar pH when a plant-based milk, cashew nut was fermented. The pH of the fermented milk sample is very crucial for the activities of the probiotics present in the fermented samples.

Table 2. pH of fermented milk samples.

Sample	pH
Cow milk	4.5 ±0.02
Soy milk (Alpro)	4.9 ±0.34
Coconut milk (Alpro)	4.9 ±0.06
Coconut milk (Aroy-D)	5.4 ±0.01*
Almond milk (Alpro)	4.9 ±0.35

Values are mean ± SD; n = 2. Means in the same column that do not have * are not significantly different ($p > 0.05$) from the control sample.

3.2.2 Water holding capacity and syneresis

The water holding capacity, WHC, of the soy milk (Alpro) and coconut milk (Alpro) samples presented in fig. 1, did not vary significantly ($p > 0.05$) from the control sample. These results obtained from the fermented soy milk (Alpro) are similar to that obtained by Madjirebaye et al. [13] when soy milk was fermented. The high fat content in the coconut (Aroy-D) could be responsible for the least water holding capacity.

The syneresis obtained in the coconut milk (Alpro), coconut milk (Aroy-D), and almond milk (Alpro) samples varies significantly ($p < 0.05$) with respect to the control sample, as shown in figure 2. Higher syneresis was observed in the almond milk (Alpro) while the least was observed in the coconut milk (Aroy-D). The syneresis observed in the all the coconut milk samples (Alpro and Aroy-D) did not vary statistically. However, the result obtained from the soy milk (Alpro) sample did not vary significantly ($p > 0.05$) from in the control sample, cow milk. This makes soy milk (Alpro) a very good substituent for fermented cow milk.

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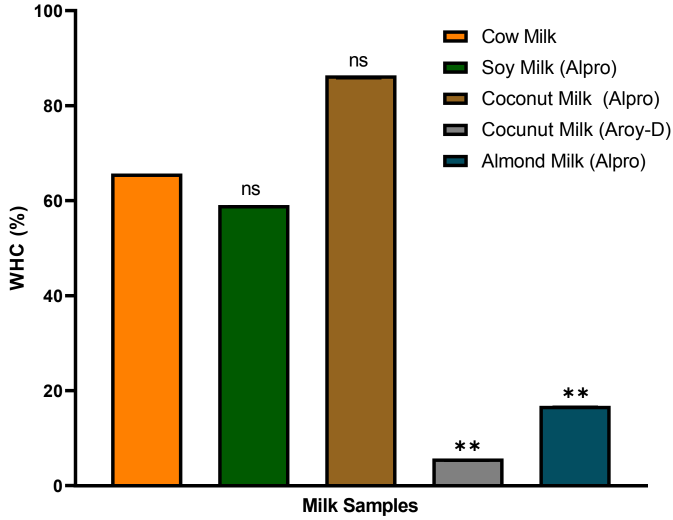

Fig. 1. Water holding capacity (%). Bars are means and Bar with ** differ significantly from the control sample (p < 0.05). Bar with *ns* did not differ significantly ($p > 0.05$).

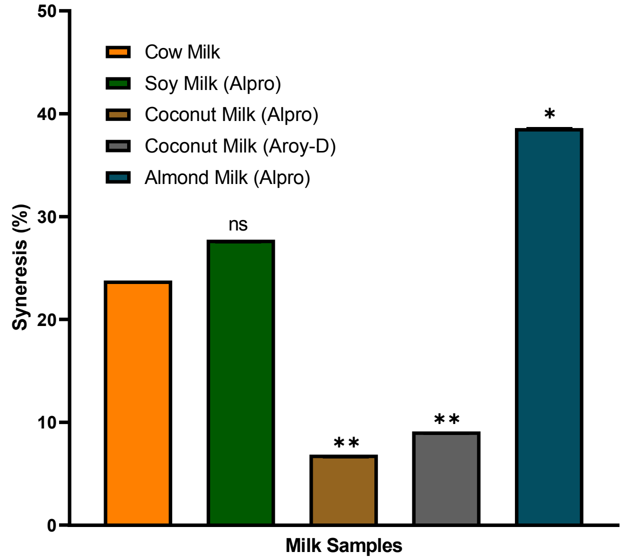

Fig. 2. Syneresis (%).Bars are means (n = 2). Bar with ** $p < 0.01$, * is $p < 0.05$ differ significantly from the control sample. Bar with *ns* did not differ significantly from the control.

3.2.3 Dry matter

The dry matter content of the fermented soy (Alpro) and coconut milk (Alpro) did not differ significantly ($p > 0.05$) from the control sample as shown in fig. 3. However, coconut milk (Aroy-D) and almond milk (Alpro) differs significantly from the control sample with the coconut milk (Aroy-D) having the highest dry matter content (25.68 ±0.96) whiles coconut milk (Alpro) has the least dry matter (3.95 ±0.40).

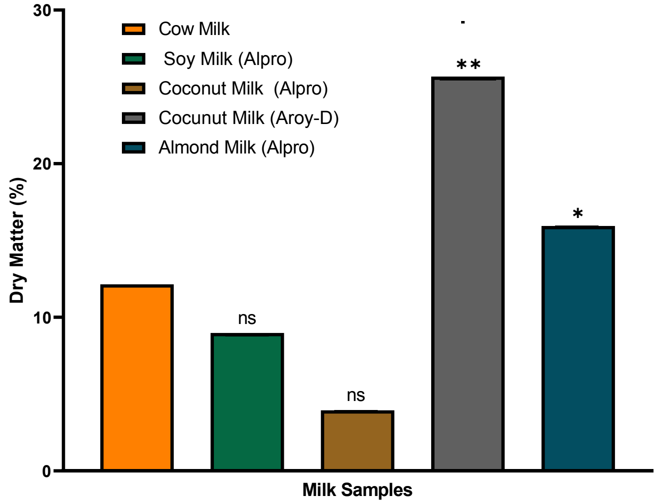

Fig. 3. Bars represent the mean values (n = 2) of the dry matter of the various milk samples. Significant difference ** $p < 0.01$, * is $p < 0.05$ and where *ns* means no significant difference.

3.2.4 Consumer acceptability

The results of the consumer acceptability as shown in table 3, indicates that, considering all the characteristics tested by the panelists (flavor, appearance, taste, consistency, and overall acceptability), soy milk (Alpro) and coconut milk (Aroy-D) did not differ significantly, ($p > 0.05$) from the control sample. These make them a perfect replacement for the traditional fermented dairy product (yoghurt). The low scores observed in the coconut milk (Alpro) and almond milk (Alpro) may be due to the inability of the product to conform physico-chemically with the control sample as shown in the earlier results.

Table 3. Consumer acceptability.

Samples	Organoleptic attributes				
	Flavor	**Appearance**	**Taste**	**Consistency**	**Overall acceptability**
Cow milk	3.88	4.38	4.25	4.25	4.25
Soy milk (Alpro)	4.13^{ns}	4.13^{ns}	4.25^{ns}	4.25^{ns}	4.38^{ns}
Coconut milk (Alpro)	3.25^{ns}	3.00^{***}	3.25^{*}	2.13^{****}	2.63^{****}
Coconut milk (Aroy-D)	4.38^{ns}	4.38^{ns}	4.25^{ns}	4.63^{ns}	4.25^{ns}
Almon milk (Alpro)	3.50^{ns}	3.13^{**}	3.38^{ns}	2.38^{****}	2.8^{***}

Values are mean, where ns = no significant difference ($p > 0.05$) and * = significant difference ($p < 0.05$). 1-dislike extremely, 2-dislike, 3-neither like nor dislike, 4-like, 5-like extremely.

3.3 Fermented products based on mixtures of different plant milk types

It was shown that soy and coconut fermented products have the best organoleptic and physicochemical parameters. For this reason, we used these milk types as two composite fermented products. This was done in the ratios (soy milk (Alpro): coconut (Aroy-D)) of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0. Thus, the best result was found in fermented soy (Alpro) and coconut (Aroy-D) milk in the proportion 80:20 and 90;10 respectively. This was selected based on the consumer preferences and the similarity of the physicochemical properties with the traditional fermented cow milk (yoghurt) as shown in table 4 as well as consumer preferences.

Table 4. Fermented milk (composite) parameters.

Parameters	**soy (Alpro) and coconut (Aroy-D) milk**		**Cow milk**
Compositions, %	80:20	90:10	
Dry matter, %	9.60	9.50	12.15
Titratable Acidity, ^{o}T	76.00	75.00	101.00
Syneresis, %	26.00	24.10	23.79
WHC, %	52.50	42.1	65.74
pH	4.92	4.82	4.58

To establish the degree of satisfaction of the daily human need for macro- and micronutrients, the nutritional value of the resulting product was calculated. Table 5 represents the results of calculating the energy and nutritional value of a fermented milk product from a composite of soy (Alpro) and coconut (Aroy-D) milk (80:20) and (90:10) respectively.

Table 5. Energy and nutritional value of fermented products from different plant milk types.

Nutrient	**Mass content in product, g/100 g**		**Energy value, kcal/100 g**		**Nutritional value**		
					Daily requirement, g	**% covering daily requirement**	
	80:20	**90:10**	**80:20**	**90:10**		**80:20**	**90:10**
Protein	2.56	2.78	10.50	11.40	75	3.41	3.71
Fats	3.54	2.67	32.92	24.83	83	4.27	3.22
Carbohydrates	3.90	3.20	15.99	13.12	65	6.00	4.92
Ca	0.1	0.30	–	–	1	10.00	30.00
K	0.14	2.00	–	–	3.5	4.00	57.14
P	0.23	0.94	–	–	1	23.00	94.00
Total		–	**59.41**	**49.35**	–		

The results in table 5 shows that the energy value (59.41 kcal / 100 g) of a two-component product from a mixture of soy (Alpro) and coconut (Aroy-D) milk is higher than that of a one-component product from soy milk (39.00 kcal / 100 g) due to the higher content of fats and carbohydrates in milk mixture. According to the results of the energy and nutritional value of the resulting product, it can be concluded that the fermented milk product from a mixture of soy and coconut milk (80: 20) is the best option considering the percentage covering daily requirement. In addition, the degree of satisfaction of the daily requirement of potassium and phosphorus has increased.

4 Conclusions

In this study, the use of composited plant-based milk products for fermented product formation is most preferred on nutritional and energy requirement bases. Physicochemical properties of fermented plant-based milk affect its consumer acceptability. Soy milk (Alpro) and coconut milk (Aroy-D) did not differ in many cases from the control sample and hence their high acceptability by consumers. However, their individual energy values are much lesser compared to cow milk and hence the need to composite them. The different plant-based fermented products have provided information about how to compose these products for fermentation based on their physicochemical characteristics. The results show that the change in the nutritional content of the samples due to fermentation and composition needs to be assessed in further studies and the optimal and best type of milk composition. The outcome of the work is beneficial to the plant-based milk industry for the production of composite plant-based fermented milk product for consumers. Overall, this work produced valuable insights into the potential benefits and drawbacks of fermenting different plant-based milks and could help inform future research in this area.

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