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ORIGNAL ARTICLE

Utilization of Probiotics for the Development of Non-Dairy (Oat and Barley Based) Milk Products Targeting Lactose Intolerant Individuals

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ABSTRACT

Background: Lactose intolerance is a condition in people with inability to digest and breakdown the lactose sugar for metabolism. This disaccharide sugar is present in milk, and its products can be broken down into monosaccharide units by lactase enzyme, but its absence genetically or environmentally can make it difficult to consume the lactose containing products.

Objectives: The objective of this study is to develop and evaluate non-dairy products for consumer acceptance as milk alternate. Oats and barley milk can be the alternatives with addition of soluble and in-soluble fibers. Moreover, introduction of probiotics helps to improve nutritional characteristics and formulation of the product.

Methodology: The present study was designed to develop non-dairy milk from oats and barley, further fermented by using probiotics, namely *Lactobacillus acidophilus* and *Streptococcus thermophilus* to develop the drinking type yogurt product with better acceptability. Moreover, the milk products were examined through physical and chemical characteristics and the statistical analysis including pH, acidity, total soluble solids, specific gravity, and proximate analysis was conducted. Likewise, the fermented products were investigated for proximate, syneresis, color, texture, and sensory assessment to obtain best fit for milk substitute.

Results: The statistics for the prepared products showed that the color (using color meter) among the product range was L = 65.033-79.16; a* = -3.916 to -6.556 and b* = 13.847 to 23.0, while moisture was 87.233% to 97.713%. Furthermore, fat was 0.0143% to 2.3533%, protein 0.5433% to 3.286%, ash 0.095% to 0.4233%, texture 0.065 to 0.408, and syneresis range was 54.33 to 82.67 among the products.

Conclusion: After sensory evaluation and the analysis, the best treatment T_1 (100% oat milk) is considered fit-to-substitute cow milk and its products, with higher consumer acceptability.

Keywords	*Address of Correspondence	Article info.
Non-dairy product, *Lactose intolerance, Probiotics, Syneresis, Substitute.*	*saniyaramzan8@gmail.com*	*Received: January 24, 2021* *Accepted: February 28, 2021*

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INTRODUCTION

Lactose is a sugar (carbohydrate) that is present in dairy products. For its proper digestion and absorption in the body, lactose is hydrolyzed in the intestine by an enzyme β-galactosidase, which is usually called as lactase. Due to some reasons, there is a lack of lactase in humans and without it, there is difficulty in lactose digestion inherently. Even in normal conditions, lactose approaches the distal small intestine without being absorbed. Up to 8% of lactose

enters the ileum area of intestine without being processed. Fermentation of unabsorbed lactose occurs by the colonic bacterial flora in the colon area of the intestine, which results in the symptoms of lactose intolerance[1]. Lactase deficiency is present in 15% Northern Europeans approximately, while 100% of American Indians & Asians and up to 80% of blacks and Latinos[2]. Lactose malabsorption is prevalent in about 75% of the global population[3]. Primary adult lactose malabsorption in Pakistan was found to be 60%[4].

Oat (*Avena sativa*) belongs to family poaceae and genus Avena. It has many unique properties that makes it diverse from other cereal grains, such as oat hull is separate from endosperm and contains more fat content in comparison with other cereal grains. It is also known for its high percentage of soluble dietary fibres i.e., β-glucan. Moreover, phenolics and niacin are also present in appreciable quantities[5]. Oat's excellent lipid contents exist as lipid bodies, similar to emulsion droplets, surrounded by proteins and phospholipids which makes it a uniquely nutritious food. Oat milk is extracted from oat by adding water and salt, which extract these lipid bodies along with proteins[6].

Barley (*Hordeum vulgare*) is also a member of family poaceae and genus Hordeum. The most recently, barley based products are gaining popularity due to presence of β-glucan which has many physiological benefits. Additionally, it is also high in phenolic compounds such as phenolic acids, tannins, pro-anthocyanidins, flavonols, flavones, flavanones, chalcones and amino phenolic compounds. It has nutritional benefits as well such as high amount of dietary fibres, minerals (molybdenum, manganese, chromium, phosphorus, selenium, copper and magnesium) and vitamins (vitamin B1, E, and niacin)[7].

Plant-based milk alternatives are the extracts obtained from legumes, cereals, seed oil or pseudo-cereals that look like milk. These replacements are commonly prepared by the extraction of grains material in water. Then the elimination of solid particles and product formulation is done. Now-a-days, their trend is increasing due to peoples' awareness about their diet, as a lifestyle choice or for medical reasons (e.g. cow's milk allergy, lactose intolerance etc.)[8]. The cereal and grain milk are cholesterol and lactose free in comparison to bovine milk. So, for people, they are an attractive substitute of bovine milk[9]. Plant-based milk alternatives are a growing trend and can serve as an economical alternate to low income group of developing countries and places with insufficient cow's milk supply. Majority of these milk alternatives lack nutritional balance when compared to bovine milk, but they contain functionally active components with health promoting properties which fascinate health conscious consumers[3]. Also, in recent years, plant sources are accepted as functional food and nutraceuticals due to presence of health promoting components such as dietary fibres, minerals, vitamins and antioxidants[10]. For proper labelling, the legislation and labelling requirements allow names in accordance with the composition and the law and custom of the country, so that the product is sold and in a manner not to mislead the consumer. National legislation on food labelling varies from country to country, principle terminology in categorizing these plant based milk alternatives has been under debate at international level. In United States, the FDA covers these plant based milk alternatives under the definition of imitation milk and imitation milk products[3]. Interestingly, oat milk contains 1.033g/100g protein, 33.51g/100g carbohydrates, and 8.2mg/100g calcium[11].

Probiotics are living microbes that impart beneficial effect on the host which helps to maintain the health and have preventive and curative effects on host. Multiple researches have illustrated their health benefits on gastrointestinal tract infections, betterment in lactose absorption, antimicrobial activity, anti-mutagenic properties, decrease in blood cholesterol level, immune system stimulus, anti-cancer & anti-diarrheal properties, betterment in inflammatory bowel disease and many more[12]. Usually, members of the Lactobacillus and Bifidobacterium genus are recognized as probiotics. Now-a-days, probiotics are increasingly used in food products and development of new functional foods by the food industries[13]. Furthermore, in terms of acceptability of any new product in Pakistan, mothers generally will not feed their infants/children with foods to which they are not familiar, or which are not acceptable to them. So, there is a need to familiarize them with newly introduced products and their usage[14]. The present project has been designed to achieve the objectives including: Development and evaluation of cereal-based fermented yogurt-like product and accessing consumer acceptability of the developed

products through physico-chemical analysis and sensory evaluation.

MATERIAL AND METHODS

Preparation of oat and barley milk

Oat (*Avena sativa*) and barley (*Hordeum vulgar*) were procured from Ayub Agriculture Research Institute, Faisalabad. Oat and barley grains were soaked in water for 12hours before extraction of milk following the modified method described by Kljusric *et al.*[15]. The respective milk concentrates were diluted to get the oat and barley milk.

Analysis of Cow, Oat and Barley Milk

For raw milk obtained from oat and barley cereals, they were tested for different proximate analysis as for moisture, ash content, protein, fat, and nitrogen free extract and other physical analysis for acidity, pH, specific gravity, total soluble solids and lactose content as described by Association of Official Agricultural Chemists (AOAC)[16]. American Association of Cereal Chemists (AACC)[17] method was used to determine crude fiber. The color was measured by using colorimeter according to the modified method of Rocha and Morais[18] by putting the sample against the colorimeter through which light waves of different wavelengths passes.

Introduction of Probiotics for Fermented Product Preparation

Oat milk and barley milk were used in various proportions (Table **1**) to formulate fermented products by utilization of probiotics *Lactobacillus acidophilus* and *Streptococcus thermophilus* as stated by Bernat and colleagues[19]. Inoculum strains were activated from their frozen forms (stored in 40g/100ml glycerol at -80°C) by transferring each one to its selective broth until optimal bacterial growth is assured. Fermentation process was carried out by adding the corresponding amount of starter culture (prepared by mixing in a 1:1 volume ratio) to the formulated and sterilized oat milks. Further, incubation at 40°C was carried out because it is the optimal growth temperature of the mixed culture. Fermentation process was stopped when pH of samples reached 4.4-4.6 and cool the samples at 4°C (storage temperature) until the analyses were done.

Analysis of Fermented Product

For moisture, total ash, fat, crude protein, and color determination; previously described methods were used. Furthermore, texture of the treatments was determined by using TA-XT plus texture analyzer[20]. Another physical test namely syneresis of all the developed products was accomplished by utilizing the method of Li and Guo[21].

Sensory Evaluation

All the developed products were analyzed by the panel consisting staff, students, and faculty members of NIFSAT, UAF for sensory characteristics like appearance, texture, flavor, mouthfeel, consistency and overall acceptability by panel of judges following 9-point Hedonic Score System[22].

Statistical Analysis

Significant difference among obtained data for each parameter was analyzed statistically using analysis of variance technique $p < 0.05$. Completely randomized design ANOVA was further utilized to evaluate the level of significance for the data[23].

Table 1. Treatments Prepared by Different Proportions of Oat Milk and Barley Milk.

Treatments	Cow milk (%)	Oat milk (%)	Barley milk (%)
T_0	100	-	-
T_1	-	100	-
T_2	-	80	20
T_3	-	60	40
T_4	-	40	60
T_5	-	20	80
T_6	-	-	100

T_0 = 100% cow milk as control.

RESULTS AND DISCUSSION

Cow, Oat and Barley Milk Analysis

The grain milk was extracted from oat and barley, and then further subjected to the analysis that lead to the data obtained as mean square results of physical analysis of oat milk, barley milk, and cow milk ranges for different parameters, and shows a highly significant results for acidity, pH, Total soluble solids (TSS), lactose content, and color, while significant result was obtained for specific gravity. Furthermore, for the average of the samples, minimum pH 6.38 was found in oat milk, while maximum pH 6.86 was observed in barley milk. Furthermore, maximum acidity 0.13% was identified in cow milk while minimum acidity 0.03% in barley milk. Specific gravity had highly significant results among the samples that contain minimum value 0.97 for barley milk and maximum result 1.02 for cow milk. Total soluble solids among all the samples vary in extent and have the values (2.22 < 7.25 < 10.23ºBrix) in the manner having barley milk (TBM) least value and cow milk highest value[15]. Lactose content was found to be 0% in oat milk and barley milk as compared to cow milk with 3.8% lactose. Similarly, Pereira *et al.*[24] reported that cow milk contains 4.7% lactose sugar. The mean squares of the treatments for various parameters of chromaticity includes L, a* and b* that also showed highly significant results. a* and b* values are the four unique colors for human vision including red, green, blue and yellow. The values for the Lightness denoted by L have the highest value 79.09 for cow milk while least score was found in barley milk that is 65.267. In terms of a-value the observed results for cow milk have maximum value with minimum value for barley milk -3.8967. Furthermore, in terms of b value the highest score 22.753 was observed in oat milk (TOM) while, lowest score 13.813 was of cow milk (TM). The results of means of all the analysis are present in the Table **2**.

While, in case of the mean of the values (Table **3**), the highest percentage 97.55% in terms of moisture among all dairy and non-dairy milk samples was in TBM that is milk prepared from barley grain. The least value 85.67% was obtained by TM that is cow milk. Results in terms of crude fat have highest value 3.40% that is presented by TM treatment referred to cow milk, while the least result 0.024% was obtained from TBM that is barley milk. Results among the values for samples provided for protein indicates the highest value 3.27% for the cow milk. Furthermore, the oat milk TOM has the value for protein content 0.63%. The highest value among the samples for fiber is 0.22% that is represented by TOM prepared from oat milk. The lowest value is for milk that is 0% as milk does not contain any fiber in it. In case for ash content, the maximum ash percentage 0.70% was found in TM treatment that is 100% cow milk with the least score 0.22% obtained from TBM treatment that is barley milk. The highest value for Nitrogen free extract (NFE) is 6.97% represented by cow milk and the least value observed was 1.07% obtained by barley milk. These results are similar to studies done by Butt[25] and Makinen[26]. The results of means of the values are prominent in Table **3**.

Table 2. Average of Physical Analysis of Cow, Oat, and Barley Milk.

Samples		TM	TOM	TBM
Acidity %		0.13±0.0	0.11±0.0	0.03±0.0
pH		6.63±0.0	6.38±0.0	6.86±0.0
Specific gravity		1.02±0.0	1.01±0.0	0.97±0.1
Lactose %		3.8±0.0	0.00±0.0	0.00±0.0
TSS (ºBrix)		10.23±0.3	7.25±0.1	2.22±0.0
Color	L value	79.090±0.17	72.593±0.75	65.267±0.45
	a* value	-6.533±0.05	-5.25±0.24	-3.8967±0.02
	b* value	13.813±0.02	22.753±0.05	19.127±0.11

*Values are Mean + SD for samples analyzed in triplicate, TM = Cow milk, TOM = Oat milk, TBM = Barley milk.
L value = lightness, a* value = red/green coordinate, b* value = yellow/blue coordinate.

Table 3. Average for the Chemical Analysis of the Dairy and Non-Dairy Milk.

Treatments	TM	TOM	TBM
Moisture %	85.67±1.5	97.45±0.0	97.55±0.1
Fat %	3.40±0.1	0.09±0.0	0.024±0.0
Protein %	3.27±0.3	0.63±0.0	0.77±0.0
Fiber %	0.00±0.0	0.22±0.0	0.14±0.0
Ash %	0.70±0.0	0.32±0.0	0.23±0.0
NFE %	6.97±1.8	1.28±0.0	1.07±0.1

Values are Mean + SD for samples analyzed in triplicate, TM = Cow milk, TOM = Oat milk, TBM = Barley milk.

Table 4. Means for the Chemical Analysis of the Dairy and Non-Dairy Milk Fermented Products.

Treatments	Moisture %	Ash %	Fat %	Protein %
T_0	87.233±0.92	0.4233±0.02	2.3533±0.09	3.2867±0.24
T_1	97.067±0.20	0.1893±0.01	0.0717±0.00	0.5433±0.02
T_2	97.143±0.22	0.1680±0.00	0.0653±0.00	0.5857±0.00
T_3	96.90±0.45	0.1483±0.00	0.5233±0.03	0.6233±0.02
T_4	97.510±0.05	0.1377±0.00	0.3567±0.02	0.6617±0.00
T_5	97.713±0.17	0.1147±0.01	0.0227±0.00	0.6733±0.02
T_6	97.533±0.35	0.0957±0.00	0.0143±0.00	0.6477±0.01

Values are Mean + SD for samples analyzed in triplicate.

Prepared Products Analysis

Products prepared from different concentrations of oat milk, barley milk and their blend along with the control treatment cow milk were subjected to moisture, fat, protein, ash, color, texture and syneresis analysis. The means of the results were observed (Table **4**) then it showed that most of the treatments for moisture content fall into similar category and have only a little deviation from each other. As the highest result 97.713% was obtained by T_5 treatment prepared from 80% barley and 20% oat milk. While, the highest value for ash 0.4233% was found in T_0 (100% cow milk product) followed by T_1 with result 0.1893% that is 100% oat milk product. The least amount of ash 0.095% was found in 100% barley milk. The highest percentage for fat was presented 2.353% by cow milk product T_0 and the least value 0.0143% was obtained by T_6 (100% barley milk product). For protein the highest value 3.2867% was found in T_0 treatment with 100% cow milk followed by the 0.6733% of T_5 80% barley and 20% oat milk composition. The lowest percentage 0.5433% was obtained by T_1 (100% oat milk). The results are similar to the findings of Amanze and Amanze[27]. Such findings show that there is increased effect of the physical as well as chemical parameters on the products that may affect its quality based on time duration or the storage facilities.

By the results of color, texture and syneresis of the products and statistics applied to them showed the highly significant results. The mean results for the analysis performed on the treatments for color, texture and syneresis are stated in Table **5.** By the studies of these parameters, it was found that treatment T_1 prepared from 100% oat milk (Table **1**) is the best suited to substitute cow milk, followed by T_2 prepared from 80% oat milk and 20% barley milk as second-best option. For syneresis, the least score 54.33 was recorded by T_1 100% oat milk composition that means it has best water holding ability followed by T_2 80% oat milk and 20% barley milk having value of 61.83 even at high centrifugation rate rather than other

treatments and even the control treatment T_0 (100% cow milk) with value 82.67. So, it is better at maintaining its shape for more time rather than the other treatments. Furthermore, T_3 (60% oat milk and 40% barley milk) have shown the least stability during centrifugation with value 63.13. A study by Amanze and Amanze[27] on the oat flakes, non-dairy beverages showed the syneresis ranged from 48-67. For texture, the highest value 0.4087 was obtained by T_3 (60% oat milk and 40% barley milk). This is followed by treatments T_1 (100% oat milk) with value 0.3867 and T0 100% cow milk with 0.0813. Least score 0.0650 was obtained by T_6 (100% barley milk). The different parameters for color analysis are referred by L, a* and b* values. In case of L value, there seem to be a regular pattern among the treatments. The highest value 79.16 was for T_0 (100% cow milk) value followed by 71.53 for T_1 treatment (100% oat milk). The lowest value 65.033 was obtained by T_6 having 100% barley milk. As for a-value, the treatments got the values as: -6.5567 for T_0,-5.0100 for T_1 while least value was obtained -3.9167 for T_6. For the b-values of the fermented products, the highest result 23.0 was obtained by T_1 followed by T2 with value 19.79. While, the least value 13.847 was presented by T_0 (100% cow milk). Such findings are in agreement with the previous studies done by Kljusric[15] and Luana[28].

Sensory Evaluation

These treatments were subjected to sensory evaluation by sensory panel to check its acceptability in the market by consumers. These six treatments along with control treatment were analyzed for appearance, texture, flavor, mouthfeel, consistency and overall acceptability by the sensory panel based on 9-point hedonic scale (Table **6**). The analysis of variance of the results shows highly significant results for all the parameters. The mean values for overall appearance including color, shape of the treatments, the T_1 treatment (100% oat milk) was found to have the highest value 6.7 after T_0 7.7 that is the control treatment prepared from cow milk. Furthermore, T_2 (80% oat milk: 20% barley milk) is next best option after T_1 having score about 6.5. The lowest value for the appearance is T_6 that is 3.9 due to its color deviation from milky white to pinkish white. Moreover, it was not in such a good shape as the standard had. T_1 and T_2 was more near to the standard in appearance other than any treatment. The mean values for the texture of different treatments showed the highest score 7.5 for the T_1 treatment (100% oat milk) that was prepared with 100% oat milk. The score for T_0 treatment is 6.9 that is at 2nd place followed by T_2 with 6.4. The least score 4.5 is for treatment T_6 having 100% barley milk. These results showed that the texture in terms of hardness, firmness and shape stability is best suited to the treatments having higher content of oat milk that also exceeded to the control treatment. While there is a decreasing trend of texture maintenance among the treatments having higher ratio of barley milk. The mean values for flavor showed the results for the treatments among them the highest value is for T_1 (100% oat milk) that is 7.5 followed by the 7.1 for T_2 treatment. These two values gave better consumer acceptability than the control treatment that has score on 3rd place as T_0 with score 6.8. The lowest value for the flavor is 4.4 for T_6 prepared with 100% barley milk. The mean values for mouthfeel of the treatments showed highest score 7 was found in product prepared from 100% oat milk after the score of control treatment with score 7.8 prepared with 100% cow milk while lowest value was 4.1 showed by T_6. The mean values for consistency of treatments showed highest score 7.2 was found in treatment T_1 that was prepared from 100% oat milk. This value even exceeded the score of control treatment T_0 that is 6.8. T_2 treatment score is equal to the value of T_0. T_6 is the treatment with 100% barley milk showed the least value 4.4 for consistency. As in case of overall acceptability, T_1 (100% oat milk) got highest the score 7.6 followed by T_0 that has value 7.3 with least acceptability of T_6 that is 4.6. The results showed that T_1 prepared from 100% oat milk is the best suited to substitute cow milk followed by T_2 prepared from 80% oat milk and 20% barley milk as second best option. Both these treatments were not only found to be best among the treatments but they also outcast the preference of control treatment that is conventional yogurt prepared from cow milk. The mean values for the sensory parameters are available in Table **6**.

Table 5. Means for Texture, Syneresis and Color of Dairy and Non-Dairy Milk Fermented Products.

Treatments	Texture	Syneresis	Color		
			L value	a* value	b* value
To	0.0813±0.00	82.67±2.5	79.160±0.07	-6.5567±0.04	13.847±0.02
T_1	0.3867±0.01	54.33±2.1	71.530±0.11	-5.0100±0.2	23.0±0.22
T_2	0.2053±0.00	61.83±1.6	70.470±0.01	-4.7867±0.08	19.790±0.17
T_3	0.4087±0.01	63.13±2.7	69.517±0.04	-4.4067±0.11	18.997±0.04
T_4	0.3253±0.01	66.83±1.6	68.627±0.02	-4.4867±0.08	19.090±0.21
T_5	0.1253±0.01	66.83±2.0	68.940±0.12	-4.3100±0.09	19.067±0.28
T_6	0.0650±0.00	68.83±1.3	65.033±2.25	-3.9167±0.01	19.190±0.06

*Values are Mean + SD for samples analyzed in triplicate.

Table 6. Means for Sensory Attributes of Dairy and Non-Dairy Milk Fermented Products.

Treatments	Appearance	Texture	Flavor	Mouthfeel	Consistency	Overall Acceptability
T_o	7.7±0.7	6.9±0.7	6.8 ±0.8	7.8±0.8	6.8±0.8	7.3±0.7
T_1	6.7±0.7	7.5±0.7	7.5 ±0.7	7±0.5	7.2±0.6	7.6±0.7
T_2	6.5±0.5	6.4±0.5	7.1 ±0.7	6.9±0.9	6.8±0.9	6.9±0.3
T_3	5.8±0.6	6.2±0.6	6.6 ±0.5	6.3±0.8	6.4±0.7	6.3±0.7
T_4	5.2±1.1	5.8±1.1	6.2 ±0.6	6.1±0.6	5.8±0.8	6±0.5
T_5	4.7±0.9	4.9±0.9	5.6 ±0.7	4.8±0.8	5.3±0.5	5.3±0.5
T_6	3.9±1.3	4.5±1.3	4.4 ±0.7	4.1±1.1	4.4±1.0	4.7±0.7

Values are Mean + SD for samples analyzed in triplicate.

CONCLUSION

For the purpose of combating lactose intolerance, oats and barley milk was prepared and their products were utilized as substitute for the cow milk and its products. Among all the treatments T_1 and T_2 were best suited to substitute conventional cow milk. Both these treatments were not only found to be best among the treatments but they also outcast the preference of control treatment that is conventional yogurt prepared from cow milk. As cereal grains are major food constituents of daily food in Pakistan, it is staple food for the people, so people tend to like the flavor and unique properties of fermented product prepared from oat milk. They are more comfortable with the product prepared rather than the conventional dairy product. As oat and barley have no lactose sugar, so there are no chances of any lactose intolerance prevalence among the consumers. Study about non-milk derivatives or plant-based milk products is very vast and will be of main concern for the scientist and researchers in the near future. Not only its compositional analysis but more ways will be open in R & D section for innovative products development.

CONFLICTS OF INTEREST

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LIST OF ABBREVIATIONS

AOAC	Association of Official Agricultural Chemists
AACC	American Association of Cereal Chemists
R&D	Research and Development
TSS	Total Soluble Solids
NIFSAT	The National Institute of Food Science and Technology
UAF	University of Agriculture

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