ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 22 No. 10
December 2022
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2022; 22(10):22041-22058 https://doi.org/10.18697/ajfand.115.20870

FORTIFICATION OF LOW-FAT YOGURT WITH MELLOCO FLOUR *(ULLUCUS TUBEROSUS):* PHYSICOCHEMICAL AND RHEOLOGICAL EFFECTS

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ajfand SCHOLARLY, PEER REVIEWED
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Volume 22 No. 10
December 2022
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ABSTRACT

Yogurt is a fermented milk product containing Lactic Acid Bacteria (BAL) (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). Although the application of additives is not mandatory, the use of gums or dairy extenders have been studied to establish the effect on the product. Among the additives most used in yogurt, production is focalized on natural or artificial sweeteners, stabilizing agents, flavors and colorants. Nowadays, consumers are looking for products that seem less artificial and more natural. In this sense, melloco, an Andean crop that, thanks to its mucilaginous content, could become an excellent additive that provides stability and viscosity. This study aimed to evaluate the effect of melloco flour (*Ullucus tuberorus*) yellow variety (INIAP-Quillu) on low-fat yogurt's quality properties. In this study, semi-skimmed milk (2% fat) was supplemented with 0.9% (w / v) of melloco flour, fermented and stored at 4°C for 20 days. Sensory attributes such as color, texture, taste, smell and overall acceptability were evaluated; the proximal analysis was performed using official methods of the Association of Official Analytical Chemists (AOAC). The physical and rheological properties and microbiological analysis were evaluated during 20 days of refrigerated storage. The use of melloco flour to enrich the reduced-fat yogurt improved acid production during fermentation. The average pH of the samples decreased from 4.8 to 4.18 after 20 days of storage. The color parameters did not differ significantly in all samples and remained constant during storage. The viscosity showed a rheological behavior of the pseudoplastic fluid. This study evidenced that the acid production in the yogurt enriched with melloco flour was higher during fermentation than the control sample, the typical end-point of pH (4.5) was reached by enriched yogurt in 5.5 hours compared to 7 hours for control. Sensory attributes also allowed establishing that the sample with 0.9% of flour presents the best overall acceptability. In general, the results suggest that melloco flour can be considered as good gelling and nutritional agent for the production of yogurt.

Key words: enriched, fermentation, Melloco, microbial growth, yogurt, physicochemical properties, rheology, sensorial properties

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INTRODUCTION

Yogurt is fermented milk obtained by the multiplication of lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgariccus*, which can ferment lactose, providing the characteristic lactic flavor of yogurt [1, 2]. Yogurt consumption has increased by 3.8% worldwide in the last ten years; currently, consumption is 14.5 liters per person per year [3]. Yogurt has been the subject of studies that focus on production technology and the use of ingredients other than those customarily used. In this sense, reducing the fat component in order to provide the consumer with a nutritionally acceptable product has become one of the most promising approaches [4, 5]. Yogurt's viscosity is affected by different factors such as the milk composition, the combination of lactic acid bacteria and the acidification time, the total solids content, and the fat content. The structural characteristics of yogurt can be altered by the factors mentioned above, causing poor sensory characteristics and high syneresis [6, 7]. To improve the attributes that could be affected, the use of gums, dairy extenders, among others, have been studied to establish the effect on the physicochemical, rheological, and sensory attributes, which are considered essential for consumer acceptance [8, 9]. In the food industry, additives have been used to provide physicochemical stability to food, improving its sensory characteristics and lengthening the shelf life of the product [6, 10]. The most common and used additives are sweeteners (glucose, sucrose), stabilizing agents (vegetable gums, gelatin, pectin and starches), natural or artificial flavorings, colorants and fruits [1, 11]. In this sense, it is essential to note that the thickeners used in the dairy industry are known as stabilizers, which are used to modify the stability, consistency and texture of yogurt, avoiding syneresis and improving its palatability [12, 13].

The melloco is an ancestral Andean tuber cultivated in South America, its distribution is extensive from Venezuela to the north of Argentina, and it is the second most consumed tuber in Ecuador after the potato [14]. Due to its nutritional value composed of carbohydrates 70.50%, proteins 10.01%, minerals such as potassium 59.44 ppm and iron 2.48%, it provides an important quality that could be used as an ingredient in processed products. Likewise, it could be used as flour because it has a high percentage of starch and mucilage [15]. Melloco starch could act as a gelling agent in gelatins, jellies and jams; this property could also be used to improve the viscosity of fermented dairy products [14, 16-18]. Notwithstanding the technological value that melloco could provide, processes that take advantage of the nutritional and techno-functional properties in the food industry have not been developed. The application of unconventional raw materials for the production of more natural and healthy foods aims to recover and contribute to new production opportunities for an underutilized traditional crop [19]. The objective of this research was to evaluate the effect of the addition of melloco flour on the physicochemical and rheological properties of low-fat yogurt.

MATERIALS AND METHODS

Melloco (*Ullucus tuberosus*) obtained from a market in Ambato (Ecuador) were thoroughly washed in water, cut into slices of approximately 0.3 cm thick and dried in a convective dryer (Gander MTN) at 60 °C for 24 hours. Once dry, they were ground in an industrial mill (Inox Equip, Ecuador) and hermetically packed at room temperature until later use. Whole milk with ~3% fat was collected from a local producer of Pillaro (Ecuador), skimmed and adjusted to 2% fat. The freeze-dried form of the yogurt culture containing *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* (CHR HANSEN, YFL-812, Denmark) was used. All these were stored at 4 °C until use.

Proximal analysis of melloco flour

The following AACC methods were used to determine the proximate composition of melloco flours: drying in a stove at 130 ºC ± 2 º C for 1 h for moisture (AACC, 2001- method 14-15 A), incineration in a muffle at 550 °C for ash (AACC, 1981- method 08-01.01), Soxhlet method with petroleum ether for fat; acid and alkaline hydrolysis for crude fiber (AOAC method 962.09) and Kjeldahl method for protein content estimated by the nitrogen portion using a 6.25 factor. Carbohydrate contents were estimated by difference. All determinations were performed in triplicate.

Preparation of yogurt

Dry melloco flour was mixed with liquid milk at 0.9% (w/v) level for 2 min. This mix was heated to 89 °C for 10 min, then cooled down to the incubation temperature of 42 °C. The stock yogurt culture was made according to CHR HANSEN's instructions by dissolving freeze-dried yogurt starter culture containing *S. thermophilus* and *L. delbrueckii ssp. bulgaricus* (YFL-812) in water. The milk (control contained milk only) and the mixtures of milk and melloco flour were inoculated with stock yogurt culture (20 mLL^{-1}) at 42 °C and were incubated at 42 °C. The mixtures' pH was monitored starting from the third hour of incubation (at 30 min intervals) until the value reached pH 4.5, then, they were chilled and stored at 4 °C. Control yogurt samples without melloco flour were prepared following the same manner. The process of sample preparation is outlined in Figure 1.

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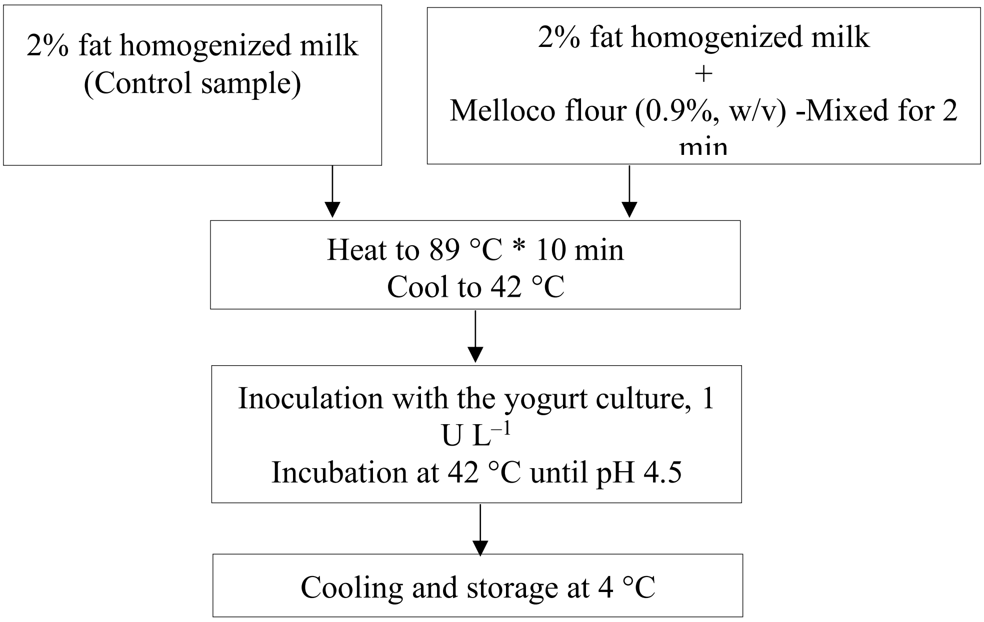

Figure 1: Procedure for production of yogurt (with and without melloco flour)

Proximal analysis of yogurt samples

The following AOAC (1990) methods were used to determine the proximate composition of yogurt: drying in a stove at 105 ºC ± 2 º C for 24 h for moisture (method 925.10), incineration in a muffle at 550 °C for ash (method 930.30), Gerber method for total fat and Kjeldahl method for protein content estimated by the nitrogen portion using a 6.25 factor. Carbohydrate contents were estimated by difference. All determinations were performed in triplicate.

pH

The pH of samples was measured according to the AOAC method (2011) using a digital pH-meter (HANNA HI 9126, Rhode Island, USA), and measurements were taken at room temperature (21 °C).

Titratable acidity

The acidity was determined using the AOAC standard TTA method (AOAC, 2011) and expressed as lactic acid %. The acidity of a mixture of yogurt in distilled water (1:9, v/v) was titrated with 0.1 M NaOH (Fisher Scientific, Chicago, IL, USA) and 0.1% phenolphthalein (Ricca Chemical Co, Arlington, TX, USA). The volume of NaOH required to produce color change was recorded; all tests were carried out in triplicate. The acidity was calculated with the following equation:

$$Titratable\ acidity = \frac{\text{Titer value} * \text{M} * 90 * 100}{\text{Volume of sample} * 1000}$$

Where: M is the molar concentration of sodium hydroxide, 90 is the equivalent weight of lactic acid, and the titer value and sample volume are in milliliters.

Microbial counts

The bacterial counts were taken immediately after culture inoculation and after 1, 5, 10, 15 and 20 days of storage. Every procedure was carried out under sterile conditions. For the microbiological tests, 10g of sample were taken, transferred to sterile bags (Sterilin, Stone, Staffordshire, United Kingdom) with 90 ml of buffered 0.1% peptone water (Difco, Le Pont de Claix, France) and vigorously shaken for 1 min in a Stomacher homogenizer (400C, Seward, London, UK). Next, appropriate dilutions were prepared for the determination of Mold and yeast count on PDA agar plates (Difco, Le Pont de Claix, France), incubated at 25 °C. Escherichia coli in Eosin Blue Methylene Agar for plate count, EAM (Acumedia, Neogen, Michigan, USA), which were incubated at 37 °C for 72 h, Enterobacteriaceae on a double layer of violet red bile agar (Acumedia, Michigan, USA), incubated at 37 °C for 48 h and Lactic bacteria on de Man- Rogosa- Sharpe (MRS) agar (Acumedia, Michigan, (USA) which were incubated at 37 °C for 48 h. All counts were expressed as the logarithm of colony-forming units per gram of sample (log CFU / g). The test was carried out for 20 days, and all analyses were performed in duplicate.

Viscosity and Color

The viscosity was determined in an MCR 302 Rheometer (Anton Paar, Austria), with a plate-plate geometry, 40 mm diameter, and a gap of 1 mm. About 800 µl of the sample was placed on the plate, and measurement proceeded. A strain scan was performed from 1 to 100 1 / s to characterize the yogurt samples' linear viscoelastic region. All tests were carried out at 4 ° C ± 0.1 ° C in triplicate. Color of yogurt was measured by a colorimeter (ColorFlex EZ, HunterLab, USA) using CIELAB® color scale with the parameters L * (lightness), a* (red / green), b* (yellow / blue). The polar coordinate or saturation Chroma C* was calculated using the expression $C^* = \sqrt{(a^{*2} + b^{*2})}$, Hue angle (°H) with the equation (°H) = arctg (b*/a*), and whiteness index (WI) with the equation WI= L- 3b + 3a. The yogurt samples were placed in small petri dishes at a depth of 0.5 cm to obtain a uniform distribution. Measurements were made in 5 sections of the box. At least 25 measurements were made in different parts of the sample.

Sensory evaluation

The color, texture, taste, smell and overall acceptability of yogurt were evaluated by 20 semi-trained panelists. Subjects conducted the evaluation individually using a 5-point hedonic scale (5 - liked very much, 4 - like moderately, 3 - neither liked

https://doi.org/10.18697/ajfand.115.20870

nor disliked, 2– disliked moderately, 1 - disliked very much). About 50 mL of each sample was presented coded with three digits in random order. Samples were served to each panelist accompanied by water and crackers to cleanse the palate while tasting.

Statistical Analysis

Statistical analysis was performed with the GraphPad Prism 5.0 program (GraphPad Software, San Diego, California, USA) with a bidirectional analysis of variance. The test of comparisons was carried out with the Tukey test with a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Proximal composition of melloco flour (Table 1) showed moisture content of 10%; this value is considered acceptable for commercial flours because a low content allows it to be stable for storage at room temperature since it would prevent deterioration due to the presence of microorganisms. Drying was effective in reducing moisture, ensuring a long shelf life. The fat content was around 1.3%, while previous studies of melloco showed a fat content of 2.1%; the difference could be attributable to the variety of tuber [20]. The proportion of fat in the flour was low; this low content is essential because it does not contribute to increasing calories when added to finished foods. They do not increase the total fat value substantially; also, it is essential to note that the fat of melloco is an unsaturated vegetable type [21]. The protein content in melloco flour was 9%; this value is higher than that of other Andean crops such as cassava, sweet potato and oca [22-24]. Due to its high protein content, possibly due to its legume nature (high content of lysine and tryptophan), it can be combined with cereal flours to increase the nutritional value of new products. The fiber content was 3.02%, similar to those reported in melloco tuber [15]. Fiber content measures the cellulose, hemicellulose and lignin contents in food. Fiber also helps maintain good health.

On the other hand, low-fat yogurt has 4.7% protein, 3.10% fat and 1.18% ash (Table 1). The proximal composition varied from the control sample due to the incorporation of melloco flour in the yogurt, and the fat content increased due to the fat that melloco provides. Likewise, the content of protein, ash and total solids increased when melloco flour was incorporated, as was expected. The incorporation of melloco flour in the yogurt formulation changes the original structure of the gel both physically and chemically. Also, the solids content in yogurt helps to prevent the separation of whey. An increase in the protein content could result in a firmer texture with less separation of whey [25]. In terms of caloric

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content, the energy observed in yogurt in which melloco was added, flour was higher ($p<0.05$) in a proportion of ~12% in contrast with control sample. Even so, the differences in energy content were not very big, this difference in caloric content could be attributed to the composition of proteins, lipids and carbohydrates that melloco flour presents.

The fermentation rate was related to the increase in acidity of yogurt due to the conversion of lactose to lactic acid by bacteria during incubation at 42 °C (Figure 2). As the control and LF-MY09 samples reached the typical end-point pH of 4.5 (~ 0.6 % lactic acid), it can be considered that the addition of melloco flour does not prevent the formation of the characteristic yogurt gel. Enriched yogurt with 0.9% reached the end-point of pH 4.5 in 5.5 hours compared to 7 hours for control. By reaching this end-point pH in a shorter incubation time than control, no apparent inhibition of microbial activity due to the addition of melloco flour was indicated. However, in the yogurt in which melloco flour was added, an increase in the fermentation rate was observed, which leads to a reduction in the processing time to produce yogurt, in this sense an increase in the acidification rates was also observed, similar to that reported in other yogurts fortified with chickpea and lentil flour [8, 26].

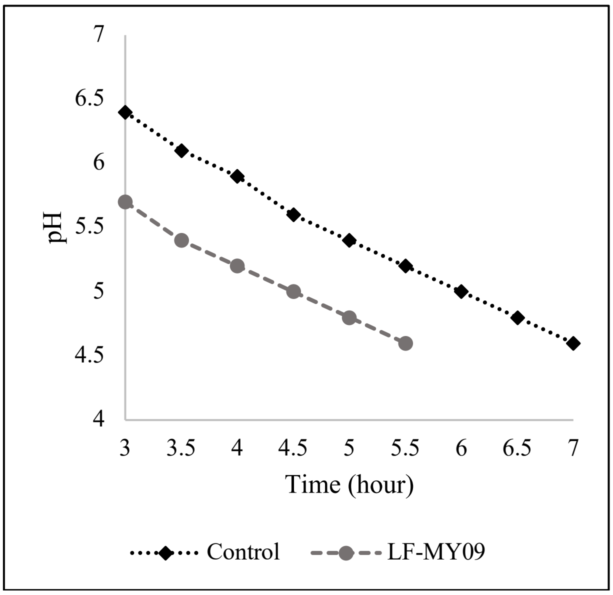
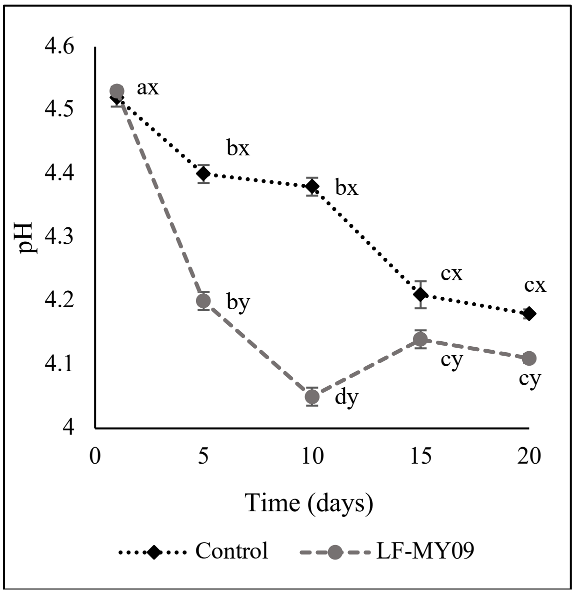


Figure 2: Changes in pH of yogurt of Control (Low-fat yogurt without melloco flour) and LF-MY09 (Low-fat yogurt with 0.9% of melloco flour), during incubation at 42 °C

During the storage of 20 days at 4 °C, the acidity continued increasing, and the yogurt with melloco flour had a higher acidity value compared to control starting from day 1 (Figure 3). The carbohydrates in the added melloco flour possibly affected the bacterial activity, even though the amount of flour was minimal. Likewise, the content of fiber and non-starch polysaccharides in the flour may have promoted the activity of the organisms in the yogurt. As a result, the production of hydrolytic lactose and the lactic acid fermentation enzyme may also have increased [27]. Similar results were reported in low-fat yogurt supplemented with *Pleurotus ostreatus* aqueous extract [28]. The acidity values of samples showed a general increase across the 20-day storage time. The acidity of yogurt can be affected by the organisms' metabolic activity in yogurt during storage; the high acidity value indicates a high metabolic activity of microorganisms.



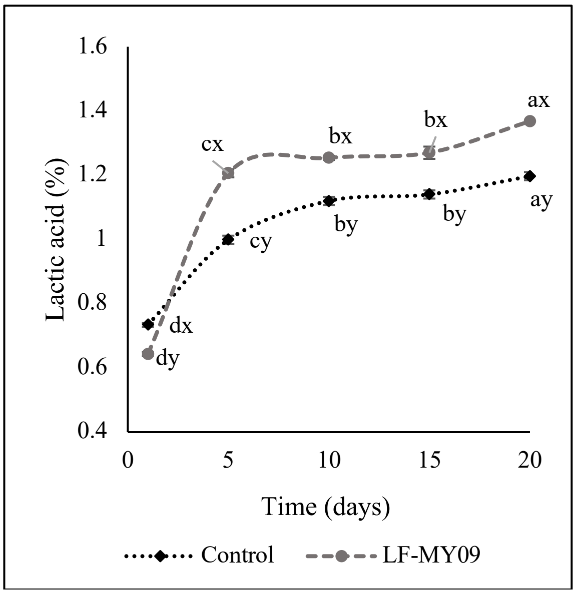


Figure 3: Changes in pH and acidity (Lactic acid) of yogurt: Control (Low-fat yogurt without melloco flour); LF-MY09 (Low-fat yogurt with 0.9% of melloco flour), during chilling storage at 4 °C for 20 days following. Different letters (a, b, c) in the same line indicate significant differences among days for each sample (P < 0.05). Different letters (x,y,z,.) in the same days indicate significant differences among samples for each day (P < 0.05)

Effects of melloco flour on viscosity

Viscosity is a measure of the fluid's resistance to deformation; in yogurt, the viscosity is affected by the structure, number and strength of the bonds between the casein micelles [29]. The samples presented a typical pseudoplastic behavior where a gradual decrease in viscosity was evidenced after the days of storage, which may result from the decrease in pH and the increase in acidity, causing a syneresis in yogurt (Figure 4). The yogurt samples' rheological properties showed

https://doi.org/10.18697/ajfand.115.20870

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a characteristic behavior of non-Newtonian fluids, showing a dependence on time. Similar behavior was reported in the production of fiber-fortified drink yogurt with the inclusion of dietary fiber from defatted rice bran [30]. Also, the reduction in yogurt viscosity values over storage was reported in other studies, and it was due to the low ability of the yogurt matrix to entrap all the serum phase resulting in instability and weakening the gel network [8, 31]. The viscosity in yogurt with 0.9% of melloco flour had the higher viscosity. The incorporation of melloco flour in the yogurt increased the viscosity since the rheological properties depend on the content of total solids and proteins present in the food. This effect is possible because the starch found in this tuber is a granular polysaccharide composed of amylose and amylopectin, and when it is subjected to heating with the presence of water, it swells and generates an increase in viscosity [32]. In general, a decrease during the 20 days of storage was observed for both samples. The yogurt samples showed a pseudoelastic behavior similar to that reported in a yogurt developed with guava and enriched with cereals [29]. The decrease in viscosity of yogurt with 0.9% melloco flour samples during storage could be related to the activity of microorganisms, which affect the protein-network interaction and consequently, product viscosity [33].

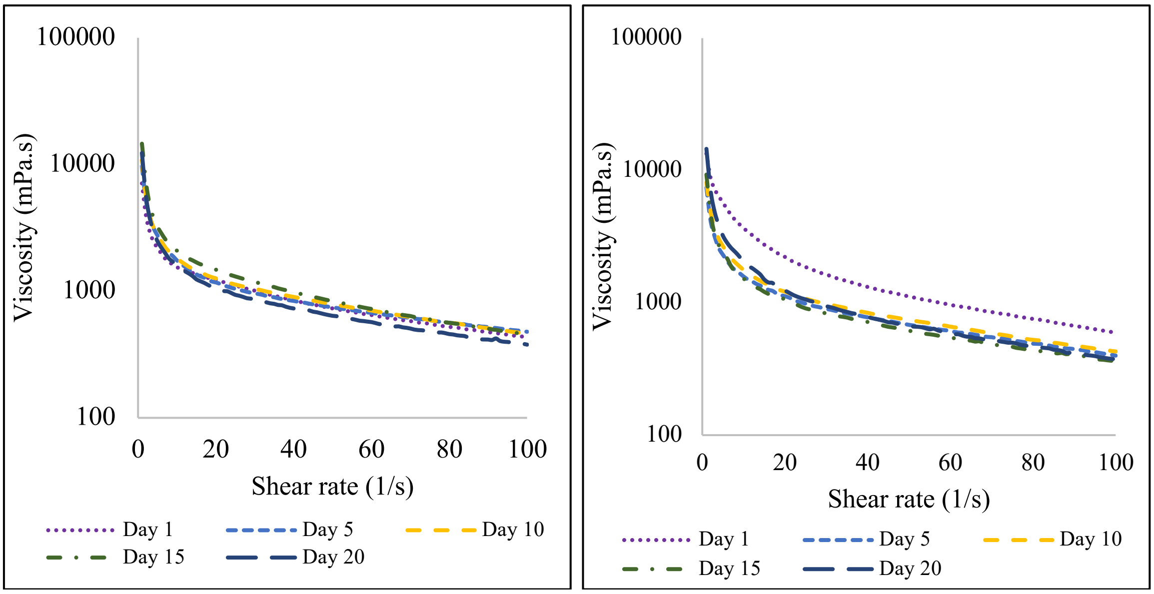


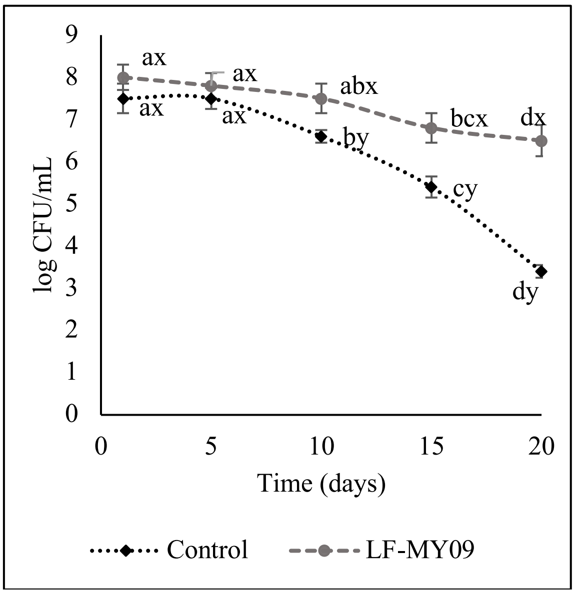
Figure 4: The viscosity of yogurt: A) Control (Low-fat yogurt without melloco flour), B) LF-MY09 (Low-fat yogurt with 0.9% of melloco flour) during chilling storage at 4 °C for 20 days

Effects of melloco flour on color

One of the most critical parameters that customers consider in new or alternative products is the color and appearance. In natural yogurt, clean white color with a pleasant sheen seems to be the most often demanded [34]. Therefore, the color of products developed with unconventional materials or additives should remain unchanged after production and during storage. Yogurt produced with melloco flour was white and lighter in color as indicated by a high L* value and low a* and b* values (Table 2). Thus, it is vital for a functional yogurt to provide nutritional benefits and to remain appealing to consumers. Table 1 shows differences (P<0.05) in the control and enriched yogurt's color parameters at days 1, 5, 10, 15 and 20 after production. On the first day of production, LF-MY09 had significantly less "L" values and higher "a" and "b" values in comparison with control samples. After 20 days, the "L" value in the LF-MY09 sample was significantly higher than skim milk control yogurt; also, the highest "a" and "b" values were found in this sample. The chromaticity and hue angle showed similar behavior between control and LF-MY09 samples. The whiteness index showed that the control sample has higher values, similar behavior as the luminosity. Multiple results have been obtained on the color properties of enriched yogurt by various authors [8, 35, 36].

Effects of melloco flour on microbial growth

Microbiological growth is shown in Figure 5. Counts of lactic acid bacteria (A), molds, and yeasts (B) during the storage at 4 °C for 20 days are presented. The number of lactic bacteria *(L. delbrueckii ssp. bulgaricus)* of the products ranged from 7.9 log CFU/ ml (control) to 8.15 log CFU/ ml (0.9% melloco flour added) with a significant difference between samples at the final day of storage. During the 20-days of yogurt storage, the viability of the lactic bacteria (LB) slightly reduced at day 10, but during the next ten days, the most significant reduction was observed in the control sample in contrast to the samples in which melloco flour was added. Compared to the control throughout the overall storage period, the growth of LB in yogurt with melloco flour maintained a stable microbial count and was significantly greater ($P < 0.05$) than the control. These results could be attributed to the properties of melloco flour in stimulating the growth of lactic bacteria. Similar behavior was observed in a study with yogurt with lentil flour (4%); the results showed that the probiotic bacteria improved the growth specifically during storage of 7 days [26]. Other studies with lentil, chickpea and soy flour added to yogurt enhanced Lactobacilli species' growth [8, 37, 38]. The count of molds and yeasts was within the regulation parameters (INEN 2395, 2011 based on the CODEX STAN 243-2003). Concerning *E. coli and Enterobacteriaceae*, no counts were obtained during chilled storage in any lots. Heat treatment inhibits the growth of these microorganisms due to a halt in active transport, oxygen absorption and oxidative phosphorylation in this type of bacteria. This behavior is interesting for public health because melloco is a tuber that could be used as new ingredient and could somehow generate some type of unconventional growth.



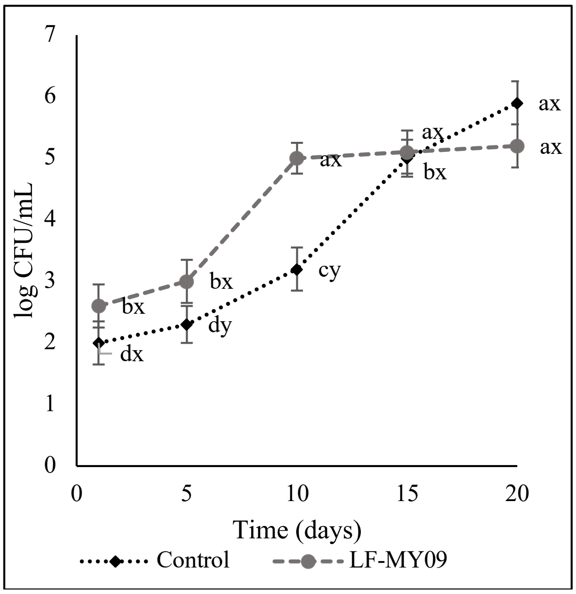


Figure 5: (A) Viable count of lactic acid bacteria and (B) mold and yeast during chilled storage at 4 °C for 20 days. Results are the mean ± standard deviation. Different letters (a, b, c) in the same line indicate significant differences among days for each sample (P < 0.05). Different letters (x,y,z,.) in the same days indicate significant differences among samples for each day (P < 0.05)

Effects of melloco flour on sensory evaluation

Sensorial assessment is an invaluable tool to assess the eating quality of yogurt. The sensory results between fortified yogurt and the control sample did not show a significant difference (P > 0.05) for scores on flavor, odour, color, viscosity and overall acceptability. However, the results showed that yogurt fortified with 0.9% melloco flour showed the highest values, except on flavor. A study on yogurt enriched with rubas *(Ullucus tuberosus)* extract reported higher acceptability in the color and flavor, and the concentrate added to the yogurt did not give it any unpleasant flavors or any undesirable characteristics [16]. The sensory analysis results in this study establish that it is possible to enrich a yogurt with melloco flour without the sensory parameters being affected and even present results superior to the control sample in odour, color, viscosity and overall acceptability scores.

CONCLUSION

In this study, the melloco flour was produced and characterized as a raw material for yogurt fortification. Melloco flour can be considered as an excellent thickening or gelling agent for the production of yogurt and other types of foods in which it could be intended to improve the viscosity parameters;also, it could be an excellent source for developing foods with high nutritional values. The results indicated that melloco flour's addition affected the fermentation process of yogurt, thus, the increase in acidity reduced the fermentation time from 7 in control to 5.5 hours in yogurt with 0.9% of melloco flour. The lowest pH and the highest acidity value were recorded for yogurt with 0.9% (w/v) of melloco flour after 20-day refrigerated storage. The viscosity values showed a general decrease for the low speed (1s-1) in the control sample during the 20 days of storage. The addition of melloco flour at 0.9% (w/v) did not affect the sensory quality parameters, and also, in color values, there were no significant differences. Higher UCF/g of Lactic Acid Bacteria in yogurts fortified with melloco flour were noted during the 20-day storage period, and no presence of E. Coli was detected. Finally, melloco flour has the potential as a raw material to provide nutritional benefits and improve the quality of yogurt-based on its physicochemical, rheological, microbial, and sensory properties.

ACKNOWLEDGEMENTS

The authors are grateful to Dirección de Investigación y Desarrollo (DIDE) and the Technical University of Ambato-Ecuador for financial support provided through the project “Development of a prototype of a gluten-free farinaceous mixture for pastry, using underused traditional Andean crops” approved by resolution 0193-CU-P-2018, PROJECT PFCIAL-029-DS.

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 22 No. 10 December 2022 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Table 1: Proximate composition and energy values of Melloco flours, Control yogurt (Low-fat yogurt without melloco flour), and LF-MY09 (Low-fat yogurt with 0.9% of melloco flour)

Parameters	Harina	Control	LF-MY09
Moisture	10.00±0.12^{c}	80.18±0.14^{a}	79.55±0.14^{b}
Protein	9.00±0.01^{a}	4.54±0.02^{c}	4.70±0.01^{b}
Fat	1.30±0.01^{c}	2.01±0.05^{b}	3.10±0.01^{a}
Fiber	3.02±0.01	0	0
Ash	4.70±0.12^{a}	0.94±0.07^{c}	1.18±0.17^{b}
Carbohidrates	75.00±0.07^{a}	12.31±0.07^{c}	12.65±0.03^{b}
Calories (Kcal/100g)	353.74±0.40^{a}	85.55±0.58^{c}	97.30±0.26^{b}
Fat Calories (%)	11.70±0.05^{c}	18.12±0.31^{a}	27.90±0.10^{a}
CH &F Calories (%)	306.04±0.30^{a}	49.25±0.20^{c}	50.60±0.12^{b}
Protein Calories (%)	36.00±0.05^{a}	18.17±0.05^{c}	18.80±0.04^{b}

Results are the mean ± standard deviation. Two-way ANOVA: Different letters (a, b) show significant differences between the samples in each parameter (P< 0.05)

Table 2: Influence of Melloco flour on the color parameters of yogurt. Control (Low-fat yogurt without melloco flour), LF-MY09 (Low-fat yogurt with 0.9% of melloco flour)

Color Parameters	Sample	Measurement Days				
		Day 1	Day 5	Day 10	Day 15	Day 20
L	Control	96,00± 0.89$^{a/x}$	95,33±0.52$^{a/x}$	95,67± 0.52$^{a/x}$	93,67± 1.03$^{a/y}$	96,00± 0.52$^{a/x}$
	LF-MY09	69,67± 0.52$^{b/x}$	69,00±0.63$^{b/x}$	70,00± 0.63$^{b/x}$	70,17± 1.17$^{b/x}$	69,00± 0.63$^{b/x}$
a*	Control	0,33± 0.52$^{a/x}$	0,33±0.52$^{a/x}$	0,67± 0.52$^{b/x}$	0,50± 0.55$^{a/x}$	0,33± 0.52$^{a/x}$
	LF-MY09	0,32± 0.52$^{a/y}$	1,00±0.63$^{a/xy}$	1,50± 0.55$^{a/x}$	0,67± 0.52$^{a/y}$	0,50± 0.55$^{a/xy}$
b*	Control	5,33± 1.03$^{b/x}$	5,67±0.52$^{b/x}$	5,33± 0.52$^{b/x}$	6,33± 0.82$^{b/x}$	6,00± 1.26$^{b/x}$
	LF-MY09	11,67± 0.52$^{a/x}$	11,17±0.98$^{a/xy}$	9,33± 0.52$^{a/y}$	9,83± 0.75$^{a/y}$	11,50± 0.55$^{a/x}$
C	Control	5,37± 0.96$^{b/x}$	5,68± 0.47$^{b/x}$	5,37± 0.54$^{b/x}$	6,35± 0,77$^{b/x}$	6,01± 1.23$^{b/x}$
	LF-MY09	11,68± 0.52$^{a/x}$	11,21± 1.03$^{a/xy}$	9,45± 0.57$^{a/xy}$	9,86± 0.77$^{a/xy}$	11,51± 0.56$^{a/x}$
H	Control	75.96± 0.01$^{b/x}$	78.69± 0.01$^{b/x}$	79.61±1.07$^{a/x}$	79.92± 1.06$^{b/x}$	78.25± 3.23$^{b/x}$
	LF-MY09	85.24± 0.01$^{a/x}$	84,10±1.58$^{a/x}$	80,97±2.97$^{a/x}$	84.42± 0.26$^{a/x}$	85.09± 0.24$^{a/x}$
IB	Control	93,33± 1.27$^{a/x}$	92,65± 0.53$^{a/xy}$	93,10± 0.59$^{a/x}$	91,03± 3.23$^{a/y}$	92,78± 0.95$^{a/x}$
	LF-MY09	67,50± 0.60$^{b/xy}$	67,03±0.0.78$^{b/xy}$	68,55± 0.73$^{b/x}$	68,58± 0.24$^{b/xy}$	66,93± 0.62$^{b/y}$

Results are the mean ± standard deviation. Two-way ANOVA: Different letters (a, b) show significant differences between the same samples in each parameter at the same time (P< 0.05). Different letters (x, y) show significant differences between samples in each parameter at a different time (P< 0.05)

https://doi.org/10.18697/ajfand.115.20870

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