biology and life sciences forum

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Proceeding Paper

Obtaining a Functional Food from Andean Grains through Lactic Acid Fermentation †

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† Presented at the IV Conference Ia ValSe-Food CYTED and VII Symposium Chia-Link, La Plata and Jujuy, Argentina, 14–18 November 2022.

Abstract: Quinoa and amaranth have excellent nutritional compositions. Lactic fermentation is capable of transforming the functional, structural, organoleptic and nutritional properties of raw materials. The objective of this study was to develop a product analogous to yogurt, which would be suitable for special diets. The product was formulated with quinoa and amaranth flours, water, sugar and strawberries, and it was fermented with an exopolysaccharide-producing strain of Lactobacillus Plantarum. Chemical parameters and BAL growth were monitored. Sensory analysis determined the best formulation and fermentation time. In the final product, the proximal composition, microbial count, pH, antioxidant activity, color, viscosity and content of exopolysaccharides (EPS) were determined. The formulation of the selected product was 15 g quinoa/amaranth (50:50) flour, 12 g sugar, 25 g strawberry pulp and 85 g water, fermented for 8 h. The composition of the functional product was 19.60 g carbohydrates and 1.74 g protein/100 g of the puree. The viable cell count was 7.60×10^8 CFU/g; the pH was 3.86; and IC50 = 10.3 mg/mL. The color parameters L*, a* and b* were 43.85, 15.24 and 11.72, respectively, with a reddish-brown color. During fermentation, the viscosity increased to 5029 mPa*s at 10 rpm due to the production of EPS (6.78 g EPS/L fermented pure). However, EPS production was not enough to significantly modify the viscosity, probably due to the amylolytic capacity of BAL. Fermented pure was described as having a rich, fruity and acidic flavor, with a mild and pleasant smell and a viscous texture. The food obtained was analogous to yogurt with acceptable sensory characteristics and was suitable for vegetarians, coeliacs and lactose intolerants.

Keywords: amaranth; fermentation; functional food; Lactobacillus Plantarum; quinoa

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Citation: Salinas Alcon, C.E.; Jiménez, M.D.; Lobo, M.O.; Sammán, N.C. Obtaining a Functional Food from Andean Grains through Lactic Acid Fermentation. *Biol. Life Sci. Forum* **2022**, *17*, 11. https://doi.org/10.3390/blsf2022017011

Academic Editors: Loreto Muñoz and Claudia M. Haros

Published: 25 October 2022

1. Introduction

In recent years, the demand for functional foods and beverages has increased globally due to consumer trends seeking functional, organic, nutraceutical and allergen-free foods, among other characteristics. Consequently, the food and beverage industry has researched new sources of raw materials, new technologies and new food processes in order to develop innovative products that meet these needs, for example, plant-based dairy analogues [1]. The health problems associated with dairy consumption, the growing trend of veganism and the limited availability of these products in many countries have led to the development of dairy-free probiotic foods. Andean grains are excellent substrates to produce fermented functional foods since they are complete matrices with adequate profiles of macro and micronutrients, which allow the growth of nutritionally demanding lactic acid bacteria [2]. Quinoa (*Chenopodium quinoa*) and amaranth (*Amaranthus* spp.) have a high nutritional value without lactose, gluten and cholesterol. The aim of this work was to develop a product analogous to yogurt through the lactic fermentation of quinoa and amaranth.

2. Materials and Methods

2.1. Raw Materials

Quinoa (variety INTA Hornillos) and amaranth (variety Cica) were obtained from the *Centro de Investigación y Desarrollo Tecnológico para la Agricultura Familiar* IPAF NOA (Research and Technological Development Center for Family Agriculture INTA Hornillos, Jujuy, Argentina). The saponin of the quinoa was removed with successive washes. Both grains were washed and dried in a forced convection oven at 40 °C and then milled to obtain flours.

Lactic bacteria (Lactobacillus Plantarum), producer of exopolysaccharides, was used. The starter culture was prepared with activation in MRS broth and was incubated at 36 °C for 24 h. Then it was replicated twice in MRS broth at concentration of 1% and incubated for 12 and 6 h, respectively, to reach the exponential phase.

2.2. Sample Preparation

Different amounts of quinoa and amaranth flours (50:50 ratio) were studied to select the optimal ratio of flour–water (between 5 and 30% *w/v* of flour) to obtain the product. The addition of sugar (12 and 15 g/100 g puree) and strawberry pulp (25 and 30 g/112 g puree) were also studied to choose the formulations with the best physicochemical and sensory characteristics.

According to the previous results, the puree was made by mixing 15 g of quinoa/amaranth flour (50:50), 12 g of sugar and 85 g of water. Sugar was used to promote exopolysaccharide (EPS) synthesis [3] and to improve flavor. The puree was cooked in a water bath for 10 min to cook and gelatinize the starch to obtain a suitable viscous texture and to avoid phase separation. Separately, the strawberry was processed to obtain a pulp and added to the cooked puree. The puree was packed in glass flasks with screwed-on metal caps and autoclaved (P = 0.9 kg/cm^2, 119 °C, 15 min). The sterilized purees were inoculated with a lactic acid bacterium L. plantarum (10^6 CFU/g) and fermented at 36 °C for 0, 6, 8 and 10 h. The fermented product was stored at 4 °C.

2.3. Sensory Analysis of the Fermented Products

The fermented products, fermented at different times, were analyzed by 21 semi-trained judges from the Faculty of Engineering of the National University of Jujuy. Acceptability and purchase interest were studied by 9-point hedonic scale, classified from 1 to 4, meaning dislike/do not buy; 5, meaning indifferent/maybe buy; and from 6 to 9, meaning like/buy. In addition, a check all that applied the analysis (CATA) [4] was performed; attributes of flavor, aroma, texture and appearance were evaluated.

2.4. Characterization of the Final Product

The following analyses were carried out on the fermented puree: proximal composition determined in triplicate by official AOAC methods [5]; lactic acid bacteria count by serial dilution technique; plate count on MRS agar (incubation at 36 °C, 48 h) [6]; pH by direct measurement (UltraBasic digital pH meter, Denver Instrument) of the dilution of the product in water (1:10 ratio); antioxidant activity (IC50) of the methanolic extracts of the samples by scavenging the free radical DPPH [7]; color by determining the coordinates L*, a* and b* in the CIELAB system with a colorimeter (MSEZ117 HunterLab, Reston, Virginia, USA); viscosity measured with a VISCO STAR plus rotational viscometer (Fungilab Expert Series, Barcelona, Spain) at 10 rpm and 22–23 °C; EPS quantification performed by adding trichloroacetic acid (20%), refrigerated incubation, recovery with ethanol (96%) and drying [8]; and the total titratable acidity determined by AOAC 981.12 technique.

2.5. Statistical Analysis

Physicochemical results were expressed as mean value ± standard deviation. An analysis of variance (ANOVA) was carried out with a significance level of 0.05. Means were compared using Tukey's test.

CATA analysis was studied by Principal Component Analysis (PCA) to describe the samples and select the best product.

Physicochemical and sensory results were analyzed using Microsoft XLStat software 2009 (Addinsoft, Paris, France).

3. Results and Discussion

3.1. Sensory Analysis of Fermented Purees

Sensory analysis was essential for the selection of the final product since it allowed the characterization and identification of the attributes responsible for the acceptance or rejection of the judges. Acceptability of the fermented purees was greater than 50% for those at up to 8 h of fermentation; on the other hand, at 10 h of fermentation, the product presented a strong acidity due to the development of lactic acid bacteria, causing sensory rejection. The purchase intention showed the same behavior as acceptability: the product with 6 h of fermentation had the highest score, while the rest of the samples did not exceed 30% (Table 1).

Table 1. Acceptability and purchase interest of the purees.

Fermentation Time (h)	Acceptability			Purchase Interest		
	Like (%)	Dislike (%)	Indifferent (%)	Buy (%)	Do Not Buy (%)	Maybe Buy (%)
0	62	19	19	29	43	29
6	67	14	19	43	29	29
8	52	19	29	24	38	38
10	38	52	10	29	57	14

The CATA analysis was studied by Principal Component Analysis (Figure 1), and it explained 84.71% of the total variability. The samples were associated in three different groups. There was the unfermented puree with attributes such as a sweet, vinegary, strange flavor as well as a viscous texture and a nutritional appearance. There were purees at 6 and 8 h of fermentation with positive sensory attributes such as an acidic, rich, fruity flavor; a fluid texture; a pleasant, nice smell; and a good appearance. Finally, there was the puree with the longest fermentation time with nonacceptance by the judges due to the development of the bacteria, which provided undesirable characteristics such as an ugly, bitter and very acidic taste; a strange, unpleasant and intense odor; and a bad appearance. Therefore, the puree with 8 h of fermentation was selected as the best option.

3.2. Formulation of the Food and Its Characterization

The functional product obtained with the 15% *w/v* of the flours (50:50 quinoa flour: amaranth flour) with water was inoculated with 10^6 CFU/g of the bacterial strain, which is the minimum concentration required by the Argentine Food Code (2021) [9], to be considered a probiotic. After 8 h of fermentation at 36 °C, a puree with 7.60×10^8 CFU/g was obtained. The bacterial growth produced a decrease in pH and an increase in total titratable acidity. The initial pH was 4.97, a slightly acidic value, due to the presence of the strawberry pulp, reaching a final pH of 3.86; furthermore, 0.408 g of lactic acid was produced in 100 g of the sample. Table 2 shows the proximal composition of the functional product, with 100 g of the product having 19.60 g of carbohydrates, 1.74 g of proteins, 0.39 g of lipids and 89 kcal.

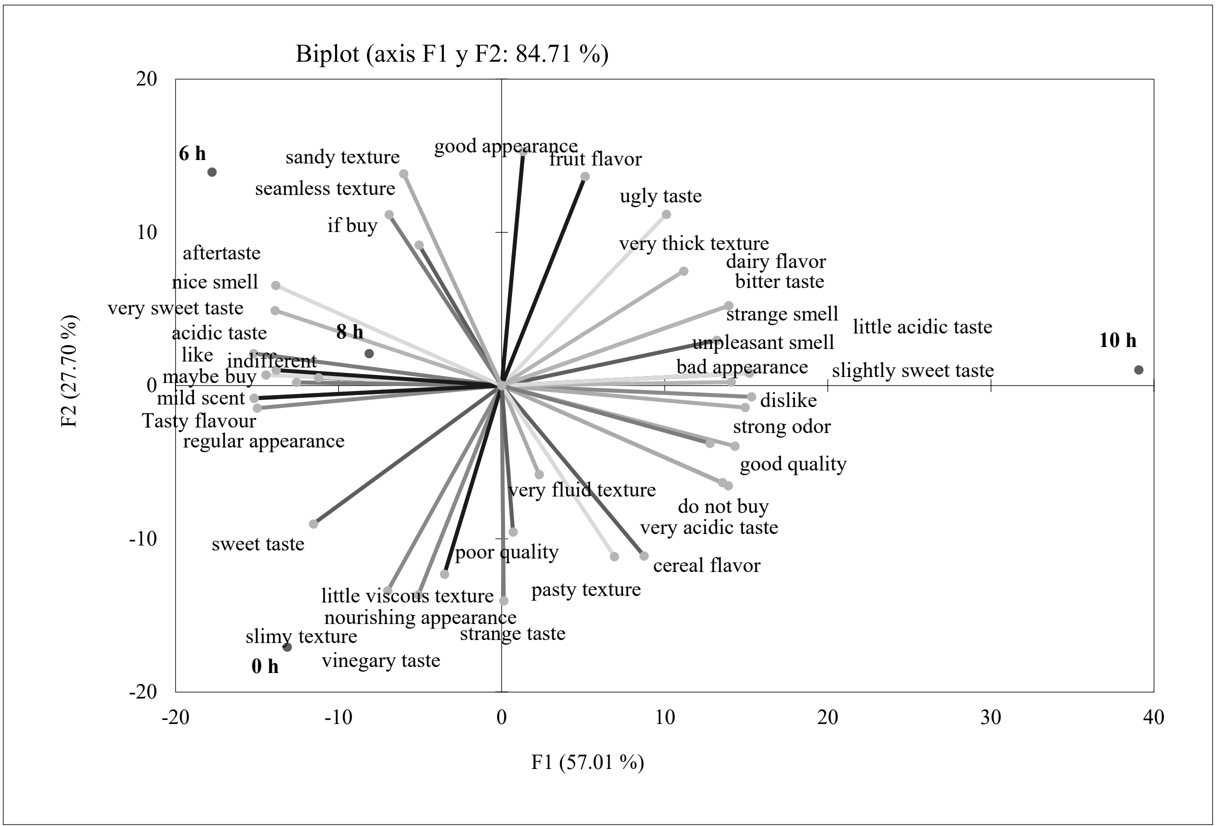

Figure 1. Principal Component Analysis of sensory attributes of purees.

Table 2. Proximal composition of the fermented puree.

	Humidity	Proteins	Lipids	Ash	Carbohydrates
Fermented puree	78.21 ± 0.10	1.74 ± 0.17	0.39 ± 0.04	0.06 ± 0.03	19.60

X ± DS = Mean ± standard deviation; n = 3 determinations.

The fermented puree had an IC50 of 10.3 mg/mL, which represents high antioxidant activity, since quinoa, amaranth and strawberry pulp provide significant amounts of compounds with antioxidant activity [10]. The fermented puree presented the following chromaticity coordinates for L*, a* and b*: 43.85 ± 1.34, 15.24 ± 0.34 and 11.72 ± 0.23, respectively. The reddish-brown color was due to the addition of strawberry pulp and the subsequent sterilization at high temperatures, which could produce non-enzymatic browning by the Maillard reaction, obtaining brown melanoidin pigments.

The viscosity of the fermented puree was 5029 mPa*s at 10 rpm, which had increased from 4126 mPa*s of the unfermented product. As the BAL used was characterized by generating EPS in the fermentation media [11], 6.78 g of EPS per liter of the fermented puree was developed during the fermentation. The production of EPS, in general, increased the viscosity and water-retention capacity, improving the rheological properties (in addition to improving the biofunctional contribution due to the soluble fiber content), but, due to the amylolytic capacity of the bacteria, it was not enough to significantly enhance the viscosity during the fermentation of the puree.

4. Conclusions

Quinoa and amaranth were excellent raw materials for the production of fermented foods, which constitutes an alternative for the revaluation of Andean crops and adds value to regional production chains. A fermented functional food based on Andean grains was elaborated with a concentration of Lactobacillus Plantarum of 10^8 CFU/g, so it could be defined as a possible probiotic food. In addition, the antioxidant characteristics of the fermented product could have a potential role in reducing the risk of contracting type 2 diabetes, cardiovascular diseases and hypertension. In this way, this food will allow for the diversification of healthy food options in the market, especially for populations with health problems associated with lactose intolerance, celiac disease and/or gluten-restricted diets as well as for the growing trend of veganism.

Author Contributions: Conceptualization, C.E.S.A.; validation, M.D.J., M.O.L. and N.C.S.; formal analysis, C.E.S.A. and M.D.J.; investigation, C.E.S.A.; resources, M.O.L. and N.C.S.; writing—original draft preparation, C.E.S.A.; writing—review and editing, M.D.J. and M.O.L.; visualization and supervision, M.O.L. and N.C.S.; project administration, M.D.J. and M.O.L.; funding acquisition, M.O.L. and N.C.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the grant Ia ValSe-Food-CYTED (119RT0567) and SECTER–Universidad Nacional de Jujuy—CONICET.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The works consulted are detailed in the bibliography.

Conflicts of Interest: The authors declare no conflict of interest.

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