

Development of newly enriched bread with quinoa flour and whey

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Abstract. Ecuador, Bolivia, and Peru are countries with the highest amount of quinoa production in the world due to the proximity to the Andes. Further, Ecuador has a high production of dairy products, particularly fresh cheese of which production gives a high volume of whey, without further use, with the consequent loss of their nutritional value. The present study was performed to develop a new fortified bread through the incorporation of quinoa flour and whey at three different concentrations. The use of quinoa and whey improved the texture, shelf life and sensory characteristics of bread, compared to those prepared with wheat flour. This study shows the potential of quinoa flour and whey as ingredients in the development of baked products.

1. Introduction

Bread has been regarded for centuries as one of the most popular staple food products [1]. Epidemiological studies suggest that people diet significantly prevents many chronic diseases [2]. Since bread is a staple food in developing countries, it is considered to be the best way for fortification [3]. However, from 1999 to 2003, the consumption of bread declined by 13.8%, due to such factors as changing nutritional habits and increasing use of substitutes e.g. breakfast cereals and fast foods [4]. At this time, consumers are demanding healthy diets and nutritional foods, these requirements have allowed a continuous growth on sales of functional foods and in the process establishing an enormous commercial market [1]. Functional foods are defined as innovative, physiologically-active foods, which can provide additional health benefits beyond basic nutrition. Food fortification is one of the major techniques used to create functional food products [5].

New varieties of baked goods are becoming increasingly popular worldwide. Generally, from a consumer's point of view, bread should have a low glycemic index, be a source of protein and dietary fiber, vitamins, magnesium, calcium, microelements, and antioxidants [6]. Necessary components for bread production are cereal flour, water, yeast, and salt, but some optional ingredients can be added to improve processing or to produce specialty and novelty bread which often have an increased nutritive value. Quinoa is a pseudocereal and is a native plant in the Andean region. Quinoa grains are considered as potentially gluten-free with an excellent nutrient profile [2, 7]. They contain considerable amounts of fiber and minerals, such as calcium and iron [2, 8]. It is also rich in



antioxidants such as polyphenols [3, 9, 10]. Dairy ingredients are widely used in the baking industry because of their beneficial effect on the nutritional, organoleptic and some functional properties [11, 12]. Whey is defined as the fraction of milk obtained by rennet action or by acids during cheese making. It constitutes 90% of milk and contains water-soluble compounds. In this solution, it is possible to find soluble proteins, lactose, vitamins, and minerals [13]. Whey proteins represent about 20% of milk proteins and constitute a rich source of complete and bio-available amino acids. Lysine (9.1 g/100 g of whey protein) is a crucial amino acid that is lacking in a diet limited to direct consumption of cereal grains (1.6 g/100 g of wheat protein) [14,15]. The aim of this research was to establish the effect of the inclusion of quinoa flour and whey in bread.

2. Materials and methods

2.1. Materials

Whey was obtained from fresh cheese processing in a local factory. Pasteurization was performed at 63 °C for 30 minutes. Fresh yeast (Levapan S.A, Ecuador) (4 g/100 g of flour), salt (Ecuasal S.A. 2 g/100 g of flour). Butter (10 g/100 g of flour), sugar (9 g/100 g of flour), eggs (9 g/100 g of flour), quinoa and commercial wheat flours were purchased from a local supermarket.

2.2. Breadmaking process

The quinoa flour and whey were added at three substitution levels: 10%, 15% and 20% (w/w) and 50%, 75% and 100% (w/w), respectively. The control sample was prepared with wheat flour and water. Whey was poured directly to the dough. Fresh yeast and salt were diluted in water before being added to the flour. The amount of added water for the control samples was 60 g per 100 g of flour. Water for the experimental bread was calculated according to the levels of substitution (table 1). All ingredients were mixed for 3 min., in a mixer (Ferton, USA). The dough was divided into portions of 180 g and proofed at 37 °C and 80% relative humidity (RH) for 60 min. The dough was then baked in a rotatory oven (Zucchelli Mini Fanton, USA) at 210 °C for 20 min. For each formulation, the bread samples were analyzed in triplicates obtained from different bread loaves.

Table 1. Formulation for bread with substitution of quinoa flour and whey.

<i>Samples</i>	Wheat flour (g)	Quinoa flour (g)	Water (g)	Whey (g)
Control	100	-	60	-
BQW501	90	10	30	30
BQW502	90	10	15	45
BQW503	90	10	-	60
BQW751	85	15	30	30
BQW752	85	15	15	45
BQW753	85	15	-	60
BQW101	80	20	30	30
BQW102	80	20	15	45
BQW103	80	20	-	60

BQW501: bread from quinoa flour 10% and whey 50%, BQW 502: bread from quinoa flour 10% and whey 75%, BQW503: bread from quinoa flour 10% and whey 100%, BQW751: bread from quinoa flour 15% and whey 50%, BQW752: bread from quinoa flour 15% and whey 75%, BQW753: bread from quinoa flour 15% and whey 100%, BQW101: bread from quinoa flour 20% and whey 50%, BQW102: bread from quinoa flour 20% and whey 75%, BQW103: bread from quinoa flour 20% and whey 100%.

2.3. Technological characteristics

Height measurements were performed from the bottom to the highest point of the top of the bread. The texture analysis was carried out using a texturometer (CT3 Brookfield texture analyzer, USA). Probe speed was set to 2 mm/s to compress the center of the bread. Hardness values, corresponding to the maximum peak force of the first compression were recorded.

The slicing process was performed using an automatic cutter until obtain 10 mm thickness slices. The slicing was evaluated by weight loss before and after cutting and applying the equation (1).

$$\text{Slicing (\%)} = \frac{100 \times (\text{initial weight} - \text{final weight})}{\text{initial weight}} \quad (1)$$

2.4. Physicochemical properties

The pH was determined using a potentiometer (HI 9126 HANNA, Rhode Island, USA). The acidity was determined by titration with 0.1 N NaOH, using phenolphthalein as indicator (AOAC 947.05). Total solids analysis was performed following the standard method NTE INEN 95. The moisture of bread was measured according to AOAC standard method (AOAC, 2005).

2.5. Colour determination

After the bread was made, the colour parameters were measured, L* (Brightness), a* (red/green) b* (yellow / blue), yellow index (YI) and brightness (B) with a Hunter Lab Colorimeter (mini Scan 4500L EZ, Hunter Associates Laboratory INC, Reston, Virginia, USA) calibrated with an illuminator D65 (natural light) and standard observer D10. The chroma polar coordinate or saturation C* was calculated from the expression $C^* = \sqrt{(a^*)^2 + (b^*)^2}$. At least 15 measurements were performed in different areas of the sample, and the average recorded as the reported value.

2.6. Microbiological analysis and shelf life

Total aerobic mesophilic bacteria were evaluated following the official method AOAC 990.12. Molds and yeasts analyses were performed following the AOAC official method 997.02. The shelf life of baking products was determined based on total aerobic growth after a week period and the calculation of cfu/g of bread. The results were linearized to obtain the regression equation.

2.7. Proximal analysis

Fat was evaluated following the official method AOAC Ed.19 2012 2003.06; moisture following the official method AOAC Ed.19 2012 925.10; protein following the official method AOAC Ed.19 2001.11 and ash following the official method AOAC Ed.19 2012 923.03. This analysis was performed only for the best treatment.

2.8. Sensory evaluation

The sensory characteristics of the best treatment were evaluated using an incomplete block design with 15 semi-trained panelists. Attributes as colour, flavour, texture, appearance, and acceptability were considered. Three tastings sessions with a hedonic scale based on 5 points were developed.

2.9. 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 $P \leq 0.05$.

3. Results and discussion

3.1. Technological characteristics

Technological parameters such as height, slicing, and hardness are shown in table 2. In concordance to technological parameters, the greatest height value was observed in BQW501 (50% v/v whey and 10%

w/w of quinoa). Quinoa flour has a marked effect on height bread than wheat flour and was related with the percentages of quinoa incorporated to the formula when more quinoa was added the height was lower [16].

The sliced process showed that the best slicing sample was BQW503 treatment, which had 91.67%. The effect of quinoa in the hardness of the bread is highly significant. In contrast, the effect of whey was not significant. The interaction between quinoa and whey showed that increasing Quinoa flour produces an increase in the bread hardness (table 2).

Table 2. Technological parameters of bread.

Treatment	Height (cm)	Slicing (%)	Hardness (g)
BQW501	12.55±0.15 ^a	73.33±0.58 ^{a, b}	344.33±1.00 ^a
BQW502	12.35±0.19 ^a	76.67±1.00 ^b	434.83±1.00 ^b
BQW503	10.75±0.17 ^b	91.67±0.58 ^c	633.00±1.00 ^c
BQW751	11.70±0.15 ^c	80.00±1.15 ^d	388.67±0.75 ^d
BQW752	11.40±0.15 ^{c, d}	71.67±0.58 ^a	575.50±0.75 ^e
BQW753	11.30±0.20 ^{c, d}	75.00±0.58 ^b	690.00±0.75 ^f
BQW101	11.35±0.15 ^{c, d}	91.67±1.15 ^c	526.33±0.75 ^g
BQW102	11.10±0.18 ^{b, d}	83.33±1.15 ^e	603.17±1.00 ^h
BQW103	10.65±0.15 ^b	73.33±1.15 ^{a, b}	639.17±0.75 ⁱ

Results are the mean ± standard deviation. One-way ANOVA: different letters (a,b) in the same column indicate significant differences between samples ($P \leq 0.05$).

3.2. Physicochemical properties

The analysis of the different pH values (table 3) showed that there is a significant influence of whey in the production of bread. However, such result was not found with the use of Quinoa. Despite the effect of whey on the pH, all treatments are within acceptable values according to standard NTE INEN 95 (INEN, 1979) which states that the pH of any bread should be between 5.5 and 6.

Total solids are significantly affected not only by the amount of quinoa but also the amount of whey when they are added to each treatment. The greater influence factor is whey since it was added in higher amounts (50% to 100%, w/w) compared to the quantity of quinoa (10% to 20% w/w). The moisture content in the different treatments was within the values established by the INEN (<35%).

Table 3. Physicochemical properties of bread.

Treatments	pH	Total solids %	Moisture %
BQW501	5.81±0.03 ^a	81.37±0.56 ^a	18.63±0.03 ^a
BQW502	5.86±0.03 ^a	83.27±0.57 ^b	16.73±0.13 ^{b, c}
BQW503	5.86±0.05 ^a	83.58±0.13 ^b	16.42±0.13 ^{b, c}
BQW751	5.85±0.10 ^a	82.24±0.12 ^{a, b}	17.76±0.68 ^b
BQW752	5.93±0.09 ^a	86.05±0.68 ^{c, d}	13.95±0.13 ^b
BQW753	5.94±0.03 ^a	87.61±0.82 ^c	12.39±0.13 ^b
BQW101	5.93±0.03 ^a	85.22±0.74 ^d	14.78±0.03 ^b
BQW102	5.91±0.03 ^a	86.78±0.13 ^{c, d}	13.22±0.13 ^b
BQW103	5.92±0.03 ^a	86.60±0.56 ^{c, d}	17.03±0.13 ^c

Results are the mean ± standard deviation. One-way ANOVA: different letters (a,b) in the same column indicate significant differences between samples ($P \leq 0.05$).

3.3. Colour

The colour is one of the most important factors to determine consumer acceptance when working with partial or total substitutions of wheat flour. The effects of quinoa flour and whey on bread colour are shown in table 4. The colour parameters of a commercial bread were measured, with luminosity values (L) = +54; Axis green - red (a) = +16; Axis blue - yellow (b) = +35. L, a, and b values of quinoa bread were statistically different ($p < 0.05$), and the amount of quinoa flour created a noticeable difference in all color parameters. The treatments were found in the circle of the first quadrant of the a + and b + scale, which allows establishing that the different treatments are between brown and yellow earth colours. The yellow colour of bread could be enhanced to lactose levels, which also resulted in Maillard reactions at a higher degree. Likewise, the amount of quinoa flour created a noticeable difference in the colour of bread, especially, the yellowness [17].

Table 4. Colour of bread.

<i>Samples</i>	L	a	b	C
BQW501	47.1 \pm 0.3 ^a	17.3 \pm 0.5 ^{a,b,c,d,e}	32.2 \pm 0.5 ^{a,c,f}	36.5 \pm 0.3 ^{a,b,c,d}
BQW502	50.8 \pm 0.8 ^b	17.2 \pm 0.5 ^{a,b,c,d,e}	32.8 \pm 0.5 ^{a,f}	37.0 \pm 0.5 ^{b,c,d}
BQW503	49.2 \pm 0.3 ^c	17.6 \pm 0.1 ^{b,c,d,e}	32.9 \pm 0.5 ^{b,d,f}	36.8 \pm 0.2 ^{a,b,c,d}
BQW751	52.3 \pm 0.7 ^d	16.7 \pm 0.5 ^c	34.7 \pm 0.5 ^{a,b}	38.4 \pm 0.5 ^c
BQW752	48.0 \pm 0.8 ^{a,c}	17.9 \pm 0.6 ^{a,b,c,d}	32.7 \pm 0.6 ^c	37.0 \pm 0.4 ^{a,b}
BQW753	50.8 \pm 0.2 ^{b,e}	17.9 \pm 0.2 ^{a,b,c,d}	34.6 \pm 0.5 ^{a,d,e}	38.9 \pm 0.3 ^c
BQW101	48.8 \pm 0.2 ^c	17.8 \pm 0.4 ^{a,b,c}	33.4 \pm 0.3 ^{d,e}	37.8 \pm 0.3 ^d
BQW102	50.9 \pm 0.2 ^e	18.2 \pm 0.1 ^d	35.7 \pm 0.1 ^{b,f}	39.5 \pm 0.2 ^c
BQW103	53.2 \pm 0.1 ^f	16.7 \pm 0.6 ^{a,b,c,d}	36.2 \pm 0.2 ^{d,f}	39.8 \pm 0.2 ^c
Control	54.7 \pm 0.1 ^g	16.1 \pm 0.2 ^e	35.2 \pm 0.1 ^{b,c,f}	38.7 \pm 0.3 ^c

^{a-g} Different letters in the same column indicate significant differences between means ($p < 0.05$).

3.4. Proximal analysis

Nutritional characteristics of bread are shown in table 5. No significant differences were observed in the ash content ($p > 0.05$). On the other hand, protein, humidity and lipids had significant differences ($p < 0.05$). The control sample which includes only wheat flour and water, had the highest moisture content, while the sample BQW101 had the lowest value. Bread with quinoa flour had lower moisture content than other type of breads [18]. These differences could be attributed to the variation in soluble dietary fiber contents of quinoa flour. Presence of whey solids such as proteins (α -lactalbumine and β -lactoglobuline) and lipids, could explain higher protein and lipid values found in the sample BQW101 compared to the control. The replacement of water with pasteurized whey could have also increased the macro mineral concentrations and lipids of the bread [19].

Table 5. Nutritional properties of bread.

Sample	Ash (%)	Protein (%) (Nx6.25)	Humidity (%)	Lipids (%)
BQW101	2.40 \pm 0.04 ^a	10.7 \pm 0.7 ^a	29.9 \pm 0.2 ^a	4.61 \pm 0.1 ^a
Control	2.44 \pm 0.04 ^a	9.94 \pm 0.7 ^b	31.6 \pm 0.2 ^b	3.30 \pm 0.1 ^b

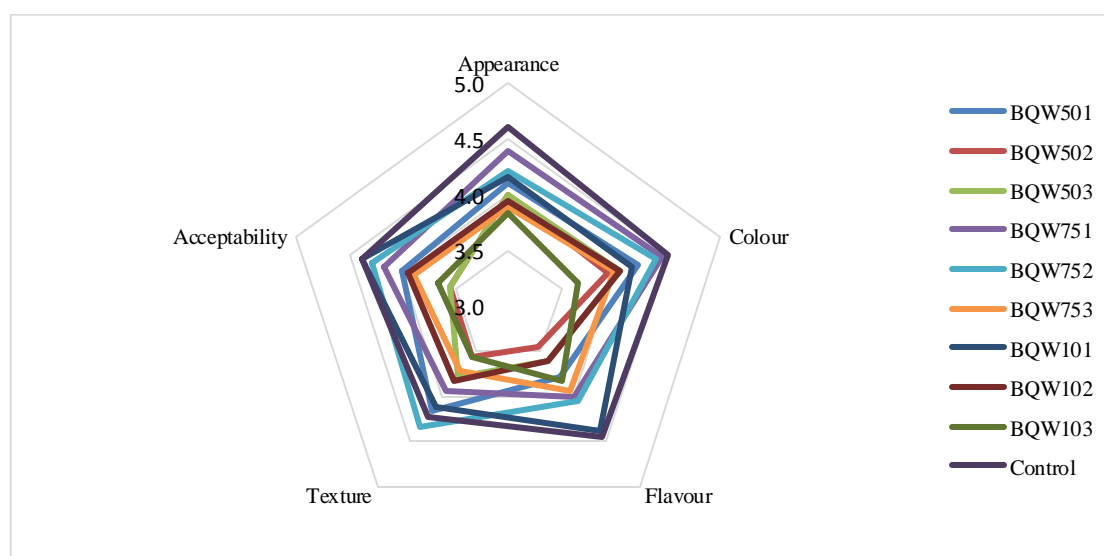
Results are the mean \pm standard deviation. One-way ANOVA: different letters (a,b) in the same column indicate significant differences between samples ($P \leq 0.05$).

3.5. Sensory evaluation

From the sensory evaluation of bread, it was possible to establish that at a 95% level of confidence there are significant differences between treatments. The sensory profile of treatments for the five

characteristics showed that the highest acceptability was obtained by the treatment BQW101 (figure 1) which had a significant amount of whey and the highest amount of quinoa. On the other hand, the control and treatment BQW101 had the highest evaluations in texture. It can be noted that this is not affecting the acceptability of treatments. However, appearance is significantly affected by quinoa and whey.

Figure 1. Sensorial profile of bread.



3.6. Microbiological analysis

The microbiological analysis establishes that the processed bread complies with the provisions of the INEN 2945; all the results are within the normative what indicate the absence of harmful microorganisms to public health. The shelf life of the best treatment was obtained by linearization of the concentration of total aerobic organisms ($\ln(\text{ufc/g}) = 0.5685 \cdot \text{time} + 5.973$; $R^2 = 0.9649$). The shelf life was calculated by the equation and was 5.69 days, which is an excellent value for bread with no preservatives added in its formulation.

4. Conclusion

The effect of quinoa flour and whey in bread showed that the maximum value of quinoa flour that can be added is 15%. Higher percentages cause loss of acceptability due to a slight bitterness of the bread. However, it helps the process of leavened as the sugar content in the flour provides sugars to the yeasts. In contrast, whey could completely replace the amount of water that is used to make bread. Whey does not affect the pH, height, texture, and generate greater acceptability in the sensory analysis because it hides unwanted flavours that could be attributed to the use of quinoa.

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