



Influence of Substitution of Wheat and Broad Bean Flour for Hydrolyzed Quinoa Flour on Cookie Properties [†]

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Abstract: Quinoa (*Chenopodium quinoa*) is an important pseudocereal for its high nutritional value, versatility in cooking, gluten-free nature, and potential contribution to food security and sustainable agriculture. The aim of this work was to evaluate the effect of different levels of substitution (10, 20, and 30%) of hydrolyzed quinoa flour (HQF) on the nutritional, physical, and antioxidant characteristics and protein digestibility of cookies elaborated with wheat and broad bean flours. Cookies without HQF were the control (C0). The addition of HQF increased the protein content by between 12 and 68% compared to C0. The increase in HQF improved the cookies' quality according to the spread ratio. Adding HQF resulted in more compact cookies, decreasing their specific volume (1.30 to 1.15 cm³/g) and increasing their hardness (2791 to 6515 g). The total polyphenols increased by 2 to 3 times, and the antioxidant activity increased by more than three times with a 30% addition of HQF with respect to C0. The oxygen radical absorbance capacity with fluoresceine (ORAC-FL) index (stoichiometry or amount of antioxidants) revealed that up to a 20% and 30% addition of HQF increased the antioxidant compounds by up to ~1.5 times. On the other hand, the antioxidant reactivity, according to the oxygen radical absorbance capacity with pyrogallol red (ORAC-PGR) index, increased by 2.4 times with a 30% addition of HQF. Finally, the cookies' digestibility improved with a 10% addition of HQF. Therefore, HQF represents a viable option in the development of cookies with highly reactive antioxidant compounds that are nutritionally improved. This application could be extended to other baked products. However, a 30% addition of HQF affects its textural properties and decreases its digestibility.

Keywords: *Chenopodium quinoa*; antioxidant; cookies; flour; hydrolyzed flour; properties; protein



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1. Introduction

In recent years, the interest of the food industry in the development of functional food products and the attraction of consumers to these have increased. Protein-enriched bakery products, especially cookies, are widely accepted due to their adequate physicochemical, rheological, textural, antioxidant, and sensory properties and long shelf life [1]. Furthermore, a higher protein intake may provide benefits to people on special diets, such as the elderly and athletes.

Quinoa (*Chenopodium quinoa*) is an important pseudocereal for its high nutritional value, versatility in cooking, gluten-free nature, and potential contribution to food security and sustainable agriculture. There are several investigations on the effect of protein

hydrolysates in bakery products [2]. However, studies on the incorporation of hydrolyzed quinoa to enrich these products is scarce. In our previous works, we studied the nutritional and functional properties of a hydrolyzed protein product from quinoa flour, which could be useful for the development of enriched baked products [3]. Therefore, the aim of this work was to evaluate the effect of different addition levels (10, 20, and 30%) of hydrolyzed quinoa flour (HQF), on the nutritional physical, antioxidant, and protein digestibility characteristics of cookies elaborated with wheat and broad bean flours.

2. Materials and Methods

2.1. Materials

Broad bean flour (BF) (*Vicia faba* L.) was provided by small producers from the Quebrada de Humahuaca, Jujuy, Argentina. Hydrolyzed quinoa flour (HQF), obtained in previous work (54.7% protein, 14.11% ash and 23.18% dietary fiber), was used [3]. Refined wheat flour (WF), baking powder, sugar, margarine, bitter cocoa, and vanilla essence was purchased from a local market.

2.2. Protein Cookies: Formulation

Protein cookies were formulated with a mixture of wheat flour (WF) and broad bean flour (BF) and the addition of different levels (10, 20, and 30%) of HQF (CQ10, CQ20, and CQ30). A control cookie (C0) was formulated with a mixture of WF/BF in a ratio of 70:30, respectively. Through preliminary tests, the amount of baking powder, sugar, margarine, bitter cocoa, and water to be incorporated was determined.

Cookies' preparation: The process began by creaming the margarine, sugar, and vanilla essence. When this step was completed, the flour mixture, baking powder, bitter cocoa, and water were added according to the ratios shown in Table 1. The dough formed was stretched and cut circularly with an approximate thickness of 8 mm. The dough circles were baked at 150 ± 2 °C for 30 min. Finally, the cookies were cooled at room temperature and then analyzed.

Table 1. Formulation of cookies (g/100 g).

Ingredients	C0 (g)	CQ10 (g)	CQ20 (g)	CQ30 (g)
WF	70	63	56	49
BF	30	27	24	21
HQF	0	10	20	30
Sugar	30	30	30	30
Margarine	30	30	30	30
Baking powder	3.2	3.2	3.2	3.2
Bitter cocoa	1	1	1	1
Water	20	18	18	22

WF: refined wheat flour; BF: broad bean flour; HQF: hydrolyzed quinoa flour; C0: control cookie; CQ10, CQ20, and CQ30: protein cookies elaborated with addition of 10, 20, and 30% of HQF.

2.3. Chemical Composition

AOAC (2017) methods were used to determine the moisture, protein, lipids, ashes, and total dietary fiber (TDF) of baked cookies. Digestible carbohydrate (DHC) content was calculated by difference.

2.4. Physical Properties

2.4.1. Diameter, Thickness, and Spread Ratio

The diameter and thickness of baked cookies were measured using a digital vernier caliper. These parameters were measured 4 times, rotating the cookie 90° after each measurement. An average of six baked cookies was determined. The spread ratio was

determined from the ratio between the average values of the diameter and thickness of the cookies using the following equation:

$$\text{Spread ratio} = \text{Diameter} / \text{Thickness}$$

2.4.2. Specific Volume (SV)

The volume was determined according to Encina-Zelanda et al. [4] using a modified standard displacement method with quinoa seeds. The specific volume was calculated using the following equation:

$$\text{SV (cm}^3/\text{g)} = \text{Cookie volume} / \text{Cookie weight}$$

2.4.3. Hardness

Hardness was determined using a Texture Analyzer (TA-XT Plus, Stable Micro Systems Godalming, UK). The bending test was performed using a platform and 3-point bending ring (HDP/3 PB) with a 5 kg load cell. The hardness, peak of maximum force, was measured in six baked cookies for each formulation. The maximum force to break the cookies is reported as the hardness in N.

2.5. Antioxidant Properties

Antioxidant compounds were extracted using ultrasound-assisted extraction (UAE). Total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay, ORAC-FL (stoichiometry or amount of antioxidants), and ORAC-PGR (antioxidant reactivity) indexes were determined according to Zuñiga-López et al. [5].

2.6. In Vitro Protein Digestibility (IVPD)

The IVPD was determined using the standardized static in vitro digestion protocol, suggested by international consensus within the framework of INFOGEST COST Action [6].

2.7. Statistical Analysis

The data were statistically treated by analysis of variance, while the means were compared through the LSD Fisher's test at a significance level of 0.05 using, in both cases, the statistical software INFOSTAT—Version 2017 p (UNC, Cordoba, Argentina). All experiments were performed at least in triplicate, and mean values $\pm$ standard deviation were reported.

3. Results and Discussion

3.1. Chemical composition

The proximal composition on a wet basis (Table 2) shows that the protein content of the cookies increased from 9.66 to 16.23 g/100 g sample. This increase was significant and due to the 10% substitution of HQF. The ash content and total dietary fiber of the cookies increased with the addition of HQF. This could be due to the higher ash and fiber contents of HQF [3], so it could serve as an alternative source of dietary fiber in bakery products.

Table 2. Chemical composition of cookies (g/100 g sample).

Parameters *	C0	CQ10	CQ20	CQ30
Moisture	12.84 $\pm$ 0.17 ^a	11.45 $\pm$ 0.48 ^b	9.49 $\pm$ 0.21 ^c	7.18 $\pm$ 0.65 ^d
Protein	9.66 $\pm$ 0.17 ^d	10.86 $\pm$ 0.07 ^c	14.97 $\pm$ 0.47 ^b	16.23 $\pm$ 0.07 ^a
Lipids	12.46 $\pm$ 0.53 ^a	12.07 $\pm$ 0.07 ^a	11.95 $\pm$ 0.14 ^a	11.83 $\pm$ 0.13 ^a
Ash	1.33 $\pm$ 0.24 ^d	2.39 $\pm$ 0.06 ^c	3.09 $\pm$ 0.08 ^b	3.55 $\pm$ 0.19 ^a
TDF	7.12 $\pm$ 0.13 ^d	9.28 $\pm$ 0.24 ^c	10.72 $\pm$ 0.34 ^b	12.15 $\pm$ 0.14 ^a
DHC	56.95	53.95	49.78	49.06

* Means $\pm$ standard deviations (n = 3). Values in each row followed by different superscript letters are significantly different ($p < 0.05$). TDF: total dietary fiber; DHC: digestible carbohydrates calculated by difference. C0: control cookie; CQ10, CQ20, and CQ30: protein cookies elaborated with addition levels of 10, 20, and 30% of hydrolyzed quinoa flour (HQF).

3.2. Physical Properties

Table 3 shows the physical properties of the cookies. Compared to C0, the diameter of the cookies increased due to the 20% substitution of HQF, while the thickness decreased significantly. Therefore, the spread ratio of the cookies increased significantly due to the 20% substitution of HQF. The decrease in total gluten content and the increase in protein content as HQF was added could be responsible for the increase in spread ratio. In this system, the water could interact with the proteins, peptides, and amino acids of the hydrolyzed flour and, consequently, be less available to interact with the gluten. The spread ratio is an important quality index in cookies, as those with higher spread ratio values are more desirable. Therefore, CQ30 would be considered the best quality with respect to this property.

Table 3. Physics properties of cookies.

Parameters *	C0	CQ10	CQ20	CQ30
Diameter (mm)	54.25 ± 0.81 ^a	55.06 ± 1.03 ^a	57.32 ± 1.51 ^b	57.73 ± 1.30 ^b
Thickness (mm)	11.84 ± 0.50 ^b	11.01 ± 0.78 ^{ab}	10.24 ± 0.20 ^a	10.08 ± 0.58 ^a
Spread Ratio	4.59 ± 0.23 ^{ab}	5.02 ± 0.40 ^b	5.60 ± 0.16 ^c	5.74 ± 0.20 ^c
SV (cm ³ /g)	1.30 ± 0.02 ^c	1.20 ± 0.06 ^b	1.15 ± 0.12 ^a	1.15 ± 0.18 ^a
Hardness (g)	2791 ± 497 ^a	3056 ± 449 ^b	3788 ± 221 ^c	6515 ± 304 ^d

* Means ± standard deviations (n = 3). Values in each row followed by different superscript letters are significantly different (*p* < 0.05). SV: specific volume. C0: control cookie; CQ10, CQ20, and CQ30: protein cookies elaborated with addition levels of 10, 20, and 30% of hydrolyzed quinoa flour (HQF).

The specific volume (SV) decreased with the increasing HQF levels, indicating a more compact structure. This behavior could be explained by a strong interaction between free water, limited in the dough, and the proteins and fiber of the HQF. On the other hand, the decrease in SV could also be attributed to the dilution of the gluten content due to the addition of HQF. Furthermore, the fiber content provided by the HQF would weaken the dough, decreasing the SV [7].

In general, the cookies' hardness increased after the 10% addition of HQF. The increase in hardness could be related to the more compact structure due to the increase in protein and fiber content [8]. When comparing the hardness values with those of a commercial cookie (CC), which contained whey concentrate in its formulation (hardness: 9795.55 g), it was found that they were significantly lower.

3.3. Antioxidant Properties

Figure 1a,b show that the gradual addition of HQF significantly increases the TPC and antioxidant activity measured via the DPPH radical scavenging of the cookies compared to G0. On the other hand, the stoichiometry, or amount of antioxidants measured through the ORAC-FL index, increased by 1.5 times with the 20 and 30% substitutions HQF with respect to C0. Furthermore, the antioxidant reactivity against radicals determined with the ORAC-PGR index increased by 2.5 times with the 30% addition of HQF (Figure 1c).

The substitution with HQF caused an effective supplementation of phenolic compounds in cookies. According to Batista et al. [9], there is a high correlation between DPPH radical scavenging and total phenolic content. The increase in antioxidant activity would help to reduce the effect of free radicals and peroxides and would increase the potency of anti-oxidative enzymes in the body [10]. In addition, the Maillard reactions produced during baking led to an increase in molecules that reduce free radicals. These molecules can react with Folin Ciocalteu reagent, contributing to the overall antioxidant activity [11]. Espinosa-Páez et al. [12] reported that the bitter cocoa used as an ingredient in the formulation of cookies contains phenols and melanoidins that, when exposed to temperatures between 130 and 150 °C, increase the content of bound polyphenols and the antioxidant activity. Therefore, processing can increase the availability of antioxidant compounds and their antioxidant activity.

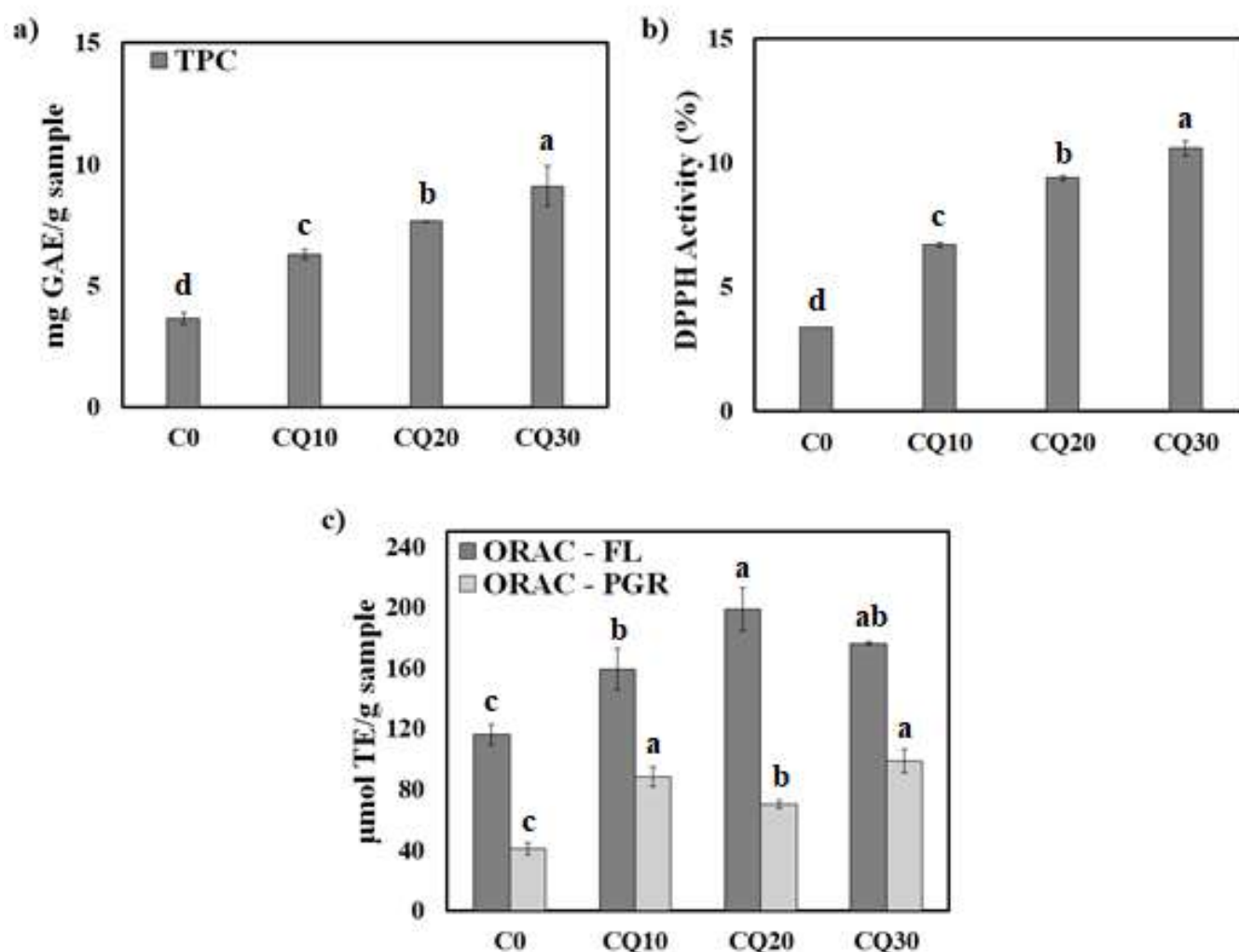


Figure 1. (a) Total phenolic content (TPC) of cookies. (b) Antioxidant activity of cookies measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay. (c) Antioxidant capacity measured by the oxygen radical absorbance capacity—Fluoresceine (ORAC-FL) and reactivity measured by the oxygen radical absorbance capacity—Pyrogallol red (ORAC-PGR) of cookies. Result are expressed as average $\pm$ standard deviation ($n = 3$). Bars with different lower-case letters are significantly different ($p < 0.05$). C0: control cookie; CQ10, CQ20, and CQ30: protein cookies elaborated with addition levels 10, 20, and 30% of hydrolyzed quinoa flour (HQF).

3.4. In Vitro Protein Digestibility (IVPD)

Figure 2 shows the IVPD of cookies as a function of time. Regarding G0, the IVPD increased significantly by 26% with the 10% addition of HQF. However, the addition of higher levels of HQF caused a decrease in IVPD, so no significant differences were observed between CQ20, CQ30, and G0. Bas and El [13] reported that the decrease in the IVPD is due to the Maillard reaction that occurred during baking and the methodology used to determine this property. Therefore, it is recommended to study other properties, such as the degree of denaturation and the microstructure of the food, which are factors that can affect protein digestibility.

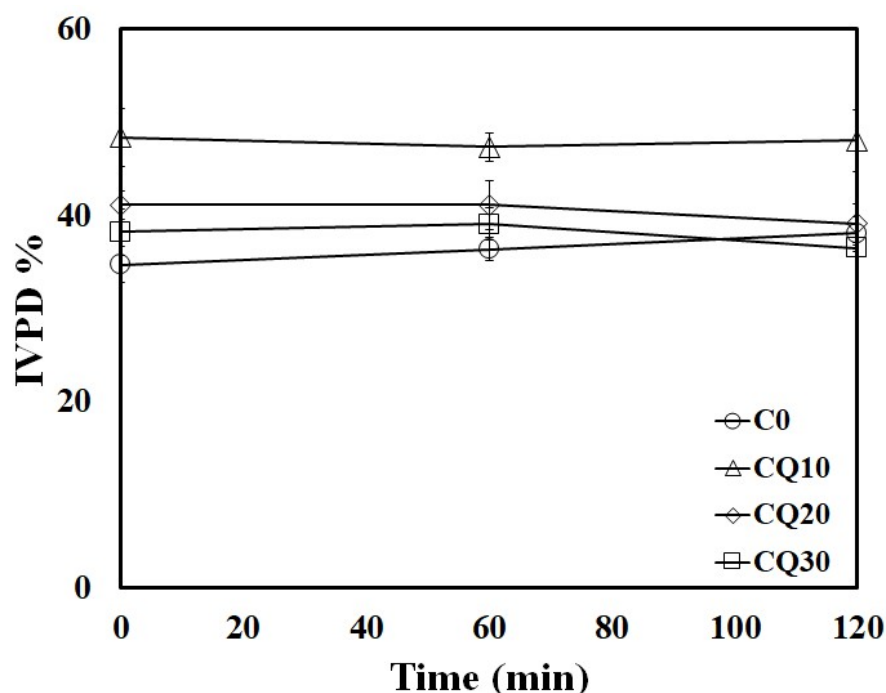


Figure 2. In vitro protein digestibility (IVPD) of C0 (o), CQ10 (Δ), CQ20 ($\diamond$), and CQ30 ($\square$) as a function of time. Result are expressed as average $\pm$ standard deviation (number of repetition, $n = 3$). C0: control cookie; CQ10, CQ20, and CQ30: protein cookies elaborated with 10, 20, and 30% addition levels of hydrolyzed quinoa flour (HQF).

4. Conclusions

The results showed that the substitution with HQF notably increases the protein and fiber content of the cookies. The 30% addition of HQF increases the hardness of the cookies; however, this hardness value is lower than those of some commercial cookies. Furthermore, the addition of HQF increases the spreading ratio of the cookies, improving the cookies' quality. Cookies could be an alternative to functional food, and their consumption could be beneficial to people's health. On the other hand, the protein digestibility of cookies decreases with high levels of HQF. Therefore, the use of HQF represents a viable and advantageous option in the development of nutritionally enhanced bakery products.

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