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ORIGINAL ARTICLE

Chemical and sensory evaluation of dark chocolate with addition of quinoa (*Chenopodium quinoa* Willd.)

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Abstract Quinoa (*Chenopodium quinoa* Willd) is a good source of vitamin E containing high quality protein. A dark chocolate with the addition of 12, 16 or 20% quinoa was developed. The protein concentration of the products increased as the percentage of quinoa increased. The product containing 20% quinoa showed only 9% increase in vitamin E, while the quantity of polyphenols decreased from 23.5 to 18 μmol pirocatechin/g. The amount of essential amino acids was improved in samples containing quinoa. Cysteine, tyrosine and methionine increased by 104, 72, 70%, respectively in chocolate containing 20% quinoa. The amino acid pattern was as per WHO standards, which was adequate to human needs. The chocolate with quinoa was approved by 92% of the sensory panel. All the samples showed an index of acceptance above 70%. Quinoa could be used at the levels evaluated in this study adding its potential health benefit to the dark chocolate.

Keywords Cocoa · *Chenopodium quinoa* · Protein · Amino acids · Vitamin E · Polyphenol

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Introduction

Development of new food products with improved quality and health benefits have been gaining increased attention in recent years. In this regard, the consumption of chocolate with high levels of cocoa has been studied because this food is a good source of Mg and polyphenols, which are beneficial to human health (Nebesny et al. 2007). It has been reported that cocoa and dark chocolate contain high amounts of polyphenols (epicatechin, catechin, anthocyanins, flavonols), which are important to reduce cardiovascular disease risk (Keogh et al. 2007). In the same way, quinoa (*Chenopodium quinoa* Willd.), an Andean grain crop belonging to *chenopodiaceae* family, has gained worldwide attention (Bhargava et al. 2006a). This grain is a good source of minerals, vitamins and natural antioxidants like vitamin E. The most important and appreciated characteristic of this pseudocereal is the high amount and quality of its protein (Bhargava et al. 2007). Studies have been conducted to investigate the use of quinoa as a food ingredient to increase the amount of protein and for taste improvement (Ng et al. 2007).

Schoenlechner et al. (2007) reported comparative study about pseudocereals and cereals. They verified that quinoa and amaranth had higher amounts of dietary and crude fiber and contained about 4 times more folic acid than the studied cereals (5 varieties of wheat, barley, and buckwheat). They also reported that an alternative to improve the nutritional quality of some food, for example the increase of folic acid, may be achieved by blending cereal flours with pseudocereals. Quinoa has revealed to be a good alternative when compared with traditional cereal products mainly as an ingredient collaborating in diets with good sources of functional substances (Berti et al. 2005).

In this work a dark chocolate with addition of quinoa was developed. The aim of this addition was to verify the improvement of protein content and quality of chocolate, to evaluate the amount of polyphenols and vitamin E of the

samples, and to verify the acceptance of this by a sensory panel.

Materials and methods

Preparation of quinoa: Quinoa obtained from a local supermarket was toasted in a stove (Central Scientific Co., Chicago, IL, USA) at 100°C for 3 h to reduce moisture. The quinoa seeds were placed in a metallic plate and during the drying process was mixed manually at 20 min intervals to get uniform drying and colour. The roasting process also reduced the saponin residue (Brady et al. 2007).

Dark chocolate samples with quinoa: Dark chocolate (40% cocoa mass) was provided by Chocolates Neugebauer (Florestal Alimentos S/A, Porto Alegre, Brazil). Three formulae were developed with different percentage (12, 16 or 20%) of quinoa. The chocolate was cut, melted in a static bath at 100°C, tempered manually in a cool surface (Cohen et al. 2004) and then toasted quinoa was added at the indicated percentages. Immediately after that, the mixture was distributed in forms and crystallized in refrigerator at 3°C until the samples were untied of the molds.

Composition analyses: The analyses were carried out in duplicate for 2 different samples, and the values were averaged. Protein concentration was determined by semi micro-Kjeldahl method (AACC 2000) and nitrogen to vegetable origin protein conversion factor of 5.75 was used (Leite et al. 2008). The quantity of fat, fiber, ash and moisture were measured (AOAC 1995). Total carbohydrates were determined by difference.

The method described by Vinson et al. (1998) with minor modifications was used for preparation of samples for the determination of total polyphenols. Analyses were carried out in triplicates. The samples were weighed (100 mg), ground and defatted by 3 sequential extractions with hexane. Samples were dried and 50 mg each was mixed with 5 ml of 1.2 mol/l HCl in 50% (v/v) methanol and heated for 2 h at 90°C. The samples were homogenized every 30 min. An aliquot (100 µl) of chocolate extract was reacted with 1 ml of Folin Ciocalteau's reagent diluted 1:9 (v/v) with distilled water (Vinson et al. 2001). After 20 min the absorbance was measured at 750 nm using a spectrophotometer (Shimadzu, UV mini 1240, Tokyo, Japan). A standard curve was prepared with 1000 µmol/l stock solution of pirocatechin (Vetec, Rio de Janeiro, Brazil).

Determination of amino acid composition of quinoa and chocolate with more quinoa content (20%) was performed at Faculdade de Medicina de Ribeirão Preto (UNESP, Ribeirão Preto, Brazil). Amino acid analysis was carried out by ion-exchange chromatography. The amino acids were obtained by peptide hydrolysis with 6 mol/l HCl at 110 ± 1°C for 22 h and purified with Amberlite IR-120. Tryptophan was determined with the same method but the hydrolysis was carried separately using 4 mol/l LiOH at 110 ± 1°C for 24 h (Penke et al. 1974). Each amino acid was detected based on the retention time of external standards for the individual amino acids. Amino acids concentrations were calculated based on the area under the peak established for a given amino acid (Cohen et al. 1989). The results were expressed as mg/g of the sample.

Vitamin E quantification was performed by HPLC analysis at Centro de Pesquisa e Processamento de Alimentos (CEPPA, Curitiba, Brazil). Sample preparation and the parameters for vitamin E determination (α, β, γ, and δ- tocopherols) were in accordance with standard protocols (Manz and Philipp 1988). The results were expressed as mg of vitamin E/100 g of the sample.

Twenty five non-trained panelists evaluated the chocolate samples, using a 9-point Hedonic scale (Rosas-Nexticapa et al. 2005). One end corresponded to the qualification "dislike extremely," the center to "neither like nor dislike" and the other end to "like extremely". The index of acceptance (IA) was calculated using the following equation:

$$IA = M/9 \times 100$$

where M indicates the average of the evaluations carried out by sensory panel. For the evaluation of the preference, the panelists were questioned about their preferred sample and the percentage of preference was calculated for each one.

Statistical analysis: The experiment was carried out in 3 replicates. Statistical significance among samples was performed using one-way-ANOVA and comparisons between means were performed by Tukey's test (Da Silva et al. 2007). Differences were considered significant at $p<0.01$ level.

Results and discussion

Toasted quinoa showed about 10.5% crude protein (Table 1). Repo-Carrasco et al. (2003) reported that the seed of quinoa contains 14.4% protein, whereas wheat and rice have 10.5 and 9.1% protein, respectively. Bhargava et al. (2006b) reported that the quantity of protein in grains usually ranges from 7.5 to 22.1%. The maximum protein content was in sample with 20% of quinoa, which amounts to an increase of 36.5% in the protein content. This result was relevant because the seed of quinoa contains not only high levels of protein but also a balanced amino acid composition (Bhargava et al. 2006b). Also, dark chocolates have a smaller amount of protein when compared with milk and white chocolates (Afoakwa et al. 2007). Thus, the incorporation of quinoa to dark chocolate can contribute to the improvement of protein quality.

Ghosh et al. (2005) had described that all the solids in chocolate mass are covered by a continuous lipid phase. When the chocolate moisture content reaches higher than 1.5%, water is absorbed by sugar causing a white crystal migration to chocolate surface. Addition of toasted quinoa did not cause a significant increase in the moisture content (Table 1). The fat concentration of chocolate samples was not significantly modified by quinoa addition (Table 1). The major contribution of quinoa as ingredient is that it has

higher amount of unsaturated fatty acids (Wood et al. 1993). The amounts of fat, carbohydrate and ash after addition of quinoa were not correlated to percent addition, although the differences among the formulations were not significant. This discrepancy could be attributed to the random distribution of seeds in each chocolate sample.

It has been reported that the amount of fiber may be increased with the addition of quinoa (Repo-Carrasco et al. 2003, Schoenlechner et al. 2007). For CAC (2007), the claim "source of fiber" corresponds to a quantity of 30 g/kg, but this value was not reached in the toasted quinoa (Table 1).

The results observed for amino acid composition of toasted quinoa (Table 2) were similar to that described by Van Etten et al. (1963). An increase in the amount of amino acids was observed by the addition of quinoa, particularly for some essential amino acids. The essential amino acids cysteine, tyrosine and methionine increased 104, 72 and 70%, respectively in the chocolate containing 20% quinoa.

The amino acid results, when compared with the FAO/WHO (1991) pattern showed low values for 3 groups of essential amino acids (Met + Cys, Leu, and Phe + Tyr) (Table 3). For quinoa and dark chocolate when they were compared with scoring pattern, tryptophan had a relevant higher value. It could be observed that the chocolate was deficient in those amino acids as that observed in toasted quinoa amino acids and lysine. In general quinoa contained higher amount of protein and the relative amount of essential amino acids was also higher than chocolate without quinoa addition, and this increased amino acids value of sample with 20% of quinoa. It is possible to affirm, that quinoa addition (20%) contributed to increase of tryptophan, lysine, methionine+cystine and isoleucine. However, this did not change the limiting amino acids content of chocolate.

The maintenance of antioxidant potential is important when developing new products with cocoa. Lee et al. (2003) suggested that cocoa is more beneficial to health than teas and red wine in terms of its higher antioxidant capacity. A significant decrease in the amount of polyphenols was observed when quinoa was added at 16 and 20%, as compared with chocolate without quinoa (Fig. 1). The differences between both formulae (16 and 20%) were not statistically significant. Recent investigation on the antioxidant activity of extracts of *C. quinoa* seeds indicated a weak correlation between phenolics concentration and antioxidant potency, suggesting that non-phenolic compounds might play major free radicals scavenging activity (Nsimba et al. 2008).

Some studies showed 5.3 mg of α-tocopherol and 2.6 mg γ-tocopherol content in 100 g of quinoa seeds (Bhargava et al. 2006b). The vitamin E content in dark chocolate was 3.1 ± 0.06 mg/100 g and in chocolate containing 20% quinoa it was 3.5 ± 0.01 mg /100 g, indicating a 9% increase. Ng et al. (2007) affirmed that the quinoa oil is stable owing its high amount of vitamin E content, which helps to prevent lipid oxidation.

Table 1 Composition (%) of toasted quinoa and chocolate samples

	Toasted quinoa	Dark chocolate	Quinoa (%) in chocolate		
			12	16	20
Protein	10.5 ± 2.8	3.5 ± 0.13[a]	4.3 ± 0.19[b]	4.6 ± 0.3[c]	4.8 ± 0.1[d]
Fat	6.5 ± 2.4	26.1 ± 16.3[a]	23.3 ± 20.9[a]	21.7 ± 0.2[a]	22.6 ± 4.3[a]
Moisture	5.1 ± 0.2	0.78 ± 0.4[a]	1.1 ± 2.3[a]	1.0 ± 0.2[a]	1.2 ± 0.1[a]
Ash	2.1 ± 0.4	1.4 ± 1.5[a]	1.4 ± 2.3[a]	1.4 ± 0.8[a]	1.5 ± 2.1[a]
Fiber	1.5 ± 1.1	0[a]	0.18 ± 0.1[b]	0.23 ± 0.2[c]	0.29 ± 0.2[d]
Carbohydrate	74.3 ± 5.7	68.3 ± 16.3[a]	69.8 ± 25.6[a]	71.1 ± 1.1[a]	69.6 ± 0.2[a]

Values followed by superscripts within a row differ significantly ($p<0.01$), comparisons between chocolate samples only

Table 2 Essential amino acid composition (mg/g sample) of quinoa, chocolate and chocolate with quinoa

Amino acid	Toasted quinoa	Dark Chocolate	Chocolate 20% quinoa
Trp	2.7 ± 0.03	0.78 ± 0.01	1.2 ± 0.01
Lys	7.9 ± 0.13	1. 6 ± 0.01	2.6 ± 0.01
Thr	4.0 ± 0.01	1.4 ± 0.01	2.0 ± 0.01
Cys	1.2 ± 0.01	0.28 ± 0.01	0.57 ± 0.03
Val	4.8 ± 0.07	1.7 ± 0.01	2.5 ± 0.03
Met	2.1 ± 0.07	0.43 ± 0.01	0.73 ± 0.03
Ile	4.0 ± 0.01	1.2 ± 0.01	2.0 ± 0.01
Leu	6.6 ± 0.01	2.1 ± 0.05	3.2 ± 0.03
Tyr	2.5 ± 0.13	0.71 ± 0.01	1.2 ± 0.01
Phe	4.2 ± 0.01	1.4 ± 0.03	2.1 ± 0.01

Table 3 Essential amino acid composition (g/100 g protein) of the samples in comparison with the FAO/WHO pattern

Essential amino acids	Toasted quinoa	Dark chocolate	Chocolate 20%quinoa	FAO/WHO pattern*	Ratio experimental/FAO pattern		
					Toasted quinoa	Dark chocolate	Chocolate 20%quinoa
Trp	2.6	2.2	2.4	1.1	2.4	2.0	2.2
Lys	7.5	4.5	5.5	5.8	1.3	0.8	0.9
Thr	3.8	4.1	4.2	3.4	1.1	1.2	1.2
Val	4.6	4.9	5.2	3.5	1.3	1.4	1.5
Met +Cys	2.0	1.2	1.5	2.5	0.8	0.5	0.6
Ile	3.8	3.5	4.2	2.8	1.4	1.2	1.5
Leu	6.3	6.0	6.8	6.6	0.9	0.9	1.0
Phe +Tyr	4.0	4.0	4.5	6.3	0.6	0.6	0.7

*Values indicate the provisional amino acid scoring pattern by FAO/WHO (1991)

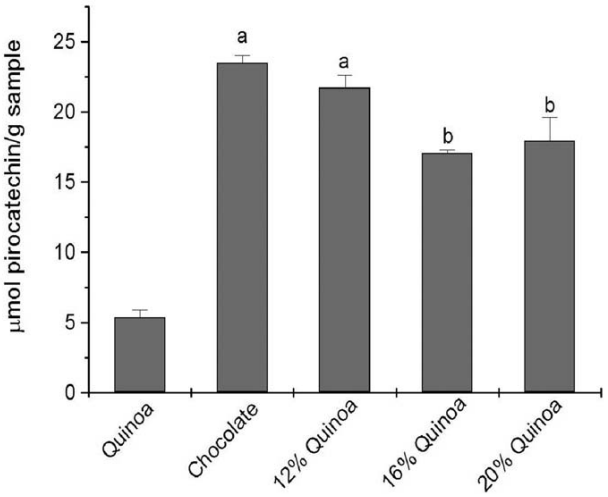

Fig. 1 Polyphenol concentration of toasted quinoa and chocolate samples. Different letters indicate significant differences ($p < 0.01$) (n=3)

Table 4 Sensory analysis of the chocolate samples with addition of quinoa

	Quinoa level, %		
	12	16	20
Index of acceptance	82.9	80.3	77.3
Preference, %	28	36	28
Mean	7.5 ±0.99[a]	7.2 ±1.58[a]	7.0 ±1.46[a]

Values followed by different superscripts differ significantly ($p < 0.01$) (n = 25 panelists)

Ninety two percent of sensory panelists liked chocolate with quinoa (Table 4). The preference of the panelists to the samples with different percentage of quinoa showed no significant differences. All the formulae showed an index of acceptance above 70%.

Toasted quinoa had increased protein and essential amino acids content. The total polyphenol content reduced when quinoa was added and the addition of 20% of quinoa caused 9% increase in vitamin E. All samples were acceptable by the taste panel, indicating that quinoa could be used in the chocolate at the levels evaluated in this study.

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