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ORIGINAL RESEARCH PAPER

PHENOLIC COMPOUND CONTENT OF DISTILLED QUINOA (CHENOPODIUM QUINOA WILLD.) BEVERAGES

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Abstract: The effect of processing of two Peruvian quinoa seed cultivars on the content of total phenolic compounds (TPC) was studied. The cultivars were INIA 420 "Negra Collana" (BNQ, black) and INIA 415 "Pasankalla" (RPQ, red). Seeds were washed to remove saponins and germinated at three different times at two temperatures. The total sugar content of the germinated seeds was measured, obtaining the highest value after 72 h at 25 °C (14.35 % for BNQ and 13.15 % for RPQ). These seeds were then roasted to get malts that were fermented to obtain washes of up to 5.5 % alcohol. After two distillations, distilled beverages of 45 and 42 % alcohol were obtained for BNQ and RPQ, respectively. TPC values increased after malting compared to ungerminated seeds, reaching 172.16 mg gallic acid equivalent (GAE)/100 g for BNQ. The wash of this cultivar also showed a higher TPC level compared to RPQ. There was no significant difference between the TPC levels of the distilled beverages of both cultivars. However, the one obtained from BNQ obtained a higher flavor score than RPQ in the sensory acceptability tests. These results are the first reported for a distilled beverage made from quinoa malts.

Keywords: *phenolic compounds, quinoa, total sugars*

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is an Andean pseudocereal whose cultivation extends from 43 °S to 5 °N on the South American continent, including parts of Argentina, Chile, Bolivia, Peru, Ecuador and Colombia from sea level to 4000 m altitude [1]. Its genetic variety allows the selection, adaptation and cultivation of different cultivars for different climates and conditions [2]. Furthermore, the plant is frost-resistant and can grow on poor soils with low annual rainfall (300 - 400 mm) [3]. Improved quinoa cultivars grown under irrigation have a shorter cultivation cycle (sowing in March-May and harvesting after July) than the native ecotypes grown in the highlands [4].

Because of its nutritional characteristics, it has aroused interest not only in South America but also worldwide. Starch is the main carbohydrate in quinoa, representing up to 32.6 % of the seeds [5]. Lipids represent 4.97 to 6.46 % of the seed, mainly linoleic and oleic acids [6]. The soluble protein has a high proportion of lysine, methionine and tryptophan [5]. However, quinoa is important not only because of its amino acid content but also because it is a source of bioactive compounds that have beneficial health effects. The quinoa cultivars “Pasankalla” and “Negra Collana” contain some bioactive compounds such as phenolic compounds [7]. Other authors have pointed out that for red quinoa varieties, the redder ones would have a higher antioxidant activity and a higher content of phenolic compounds [8]. Quinoa is used in food in a variety of ways, the main uses being soups and sweets, and a coarse bread called “kispina” [3]. It is also used to make flour, breakfast cereals and alcohol (the alcoholic drink “chicha” made by fermenting quinoa seeds). Cakes and biscuits can also be produced by mixing up to 60 % quinoa flour with wheat flour. Moreover, in certain regions where quinoa is grown and vegetables are scarce, quinoa leaves can be eaten in salads [3].

Different genotypes and cultivars of quinoa can be used to produce different products, depending on their composition and properties. When cultivars are used indifferently, products of reduced quality are usually obtained [2]. Ludena Urquizo *et al.* [9] investigated the development of a fermented beverage from quinoa seeds of a white and a red cultivar, finding that differences between cultivars have an effect on the processes and the final product. Their results showed that not only do the quinoa varieties have significantly different nutritional contents, but the flours behave differently when mixed with water. The effect of germination (without malting) and fermentation of quinoa seeds on their content of antioxidant compounds was studied by Carciochi *et al.* [10]. After 72 h of germination, the amount of antioxidant compounds increased significantly, while in fermented quinoa seeds the amount of antioxidant compounds decreased.

Since the fermentation or development of fermented beverages from malted black quinoa seeds or the production of a distilled beverage from different colored quinoa seeds has not been studied, this work aims to develop a distilled alcoholic beverage based on quinoa seeds of two cultivars of different color and to observe the effect of the process on the total phenolic compounds (TPC). The sensory acceptability of the beverages was also evaluated.

MATERIALS AND METHODS

Reagents and solvents for the analysis of total sugar content and total phenol content

Folin-Ciocalteu reagent (Merck KGaA), standard glucose solution, concentrated hydrochloric acid (EMSURE), sodium carbonate anhydrous analytical grade reagent (EMSURE), ferric sulfate (EMSURE), gallic acid (anhydrous) for synthesis (Merck KGaA), pure sodium hydroxide (EMPLURA), and potassium permanganate (0.01 N) (Crist. Puris) were purchased from Merck Millipore (Lima, Peru). Likewise, Fehling solution A (HANNA), Fehling solution B (HANNA), ethyl alcohol (95 %) (ALKOFARMA), distilled water (ALKOFARMA) were purchased from DELTA Quimica S.R.L. (Arequipa, Peru).

Quinoa seeds and conditioning

Seeds of quinoa cultivars INIA 415 “Pasankalla” (RPQ) and INIA 420 “Negra Collana” (BNQ) were used. The episperm of RPQ seeds is red, while that of BNQ is black [1]. Quinoa seeds were obtained from an experimental station of the Instituto Nacional de Innovacion Agraria (INIA) in Puno, Peru, where they were stored at 10 °C after harvest to maintain germination potential greater than 90 % (determined by INIA).
Seven kilograms of seeds of each cultivar were manually washed with water at 37 °C in a 1:2 (v/v, volume/volume) ratio to remove saponins. The process was repeated until the wash water was clear and without foam. The residual saponin content in the washed seeds was determined using the method described by Mujica *et al*. [11] with slide modifications. 0.5 g of quinoa and 5.0 mL of distilled water were vigorously mixed for 30 seconds. After that, let it rest for 10 seconds for the formation of the foam, the height of the foam is measured and later the percentage of saponin is calculated with the following formula: % Saponina = 0.441 x (height of the foam)/5. Disinfection was performed with 0.3 % (w/v, weight/volume) NaClO for 10 seconds and then rinsed six times with distilled water.

Germination and roasting

The disinfected seeds were soaked with sterilized water in a 1:1.5 ratio (seeds : water) for 3.5 h at room temperature. The final moisture content of the seeds was approximately 42 %. That was determined by differences between its initial weight and the weight once dried in the oven (gravimetric method), obtaining the percentage of moisture with the following formula: % Moisture = (weight of the sample without drying - weight of the dried sample)/ weight of the sample portion x 100. Then, they were then placed in containers in layers of 0.5 cm high, which were covered with moistened cloth. Germination was carried out in an Incucell incubation chamber (MMM Medcenter Einrichtungen GmbH, Germany) at two temperature levels (20 and 25 °C) and three time levels (24, 48 and 72 h). Drying was carried out in a Binder oven model A16-16930 (Binder GmbH, Germany) at 60 °C for 10 h to the humidity of approximately 12 %. Roasting was carried out by placing 500 g of germinated seeds in a

domestic stove at 160 °C for 120 s. The roasted seeds (malt) were then cooled to room temperature.

Mashing

The roasted seeds were ground to 1.4 mm using a grain disc mill (MIAG Braunschweig, Schorch- Werke A.G. Rheydt). Twelve litres of water were heated to 75 °C and 6 kg of ground seeds were added for each cultivar. The mixture was macerated for 1 h, maintaining a temperature above 65 °C. The mash was recirculated using a vessel with a false bottom made of a sieve at the bottom and a tap underneath the false bottom. The sieve of the false bottom is used to form a bed of unhydrolysed malt residue, which helps to filter out large impurities. After recirculation, the ground seedbed was washed by adding 12 L of water to the vessel in 3 equal volume fractions at 75 °C and allowed to filter.

Fermentation and distillation

The yeast (SafAle US-05, *Saccharomyces cerevisiae*) was activated according to the instructions of the manufacturer (S. I. Lesaffre, France). Then it was added to the wort to the concentration of 0.08 % w/v. The wort was placed in a closed vessel at room temperature and the top of the fermenter was secured with an airlock for CO_2 venting. The Brix degrees were measured in a refractometer with a measurement range of 0 to 32° (ATC, TR-032ATC) while the *p*H in an electronic potentiometer (*p*H 211, HANNA). Both were measured every day. The fermentation process was ended when the Brix degrees became constant.
The distillation was carried out twice in a copper still (pot still). The boiler of the still was filled with the fermented wort. The vapor rises to the cap, travels through a swan-neck shaped pipe and is directed to the cooling coil immersed in cold water, where it condenses. The low wine was collected when the still boiler was heated from 82 to 93 °C. The first fraction (heating up to 82 °C) and the last fraction (heating above 93 °C) were discarded. This process lasted 3 h and was repeated to obtain a new spirit ("new pot" or "new make"), using the same ranges and temperature separation of the still boiler. The spirits were packaged in 300 mL glass bottles and stored at room temperature in a dark place.

Total sugars content

The percentage of total sugars in the germinated samples was determined using the Bertrand method described by Fernández Segovia *et al.* [12]. Samples were ground and passed through a 50 mesh sieve (0.297 mm) to obtain flour. 1.5 g of flour was hydrolyzed by heating with 100 mL of water and 7 mL of concentrated HCl. Then 5 mL of alumina cream and 11 mL of 6 N NaOH were added. The solution obtained was placed in a 250 mL volumetric flask, made up to volume with distilled water and filtered. Five mL of the filtrate was taken into another flask and excess Fehling's liquor (10 mL Fehling's A and 10 mL Fehling's B) was added. The mixture was heated to boiling for 3 min to reduce the Cu which precipitated as Cu_2O. The precipitate was washed with distilled water and dissolved with hot $Fe_2(SO_4)_3$. The $FeSO_4$ formed was

titrated volumetrically using 0.01 N $KMnO_4$. The results were obtained by interpolation with a Bertrand table.

Total phenolic content

The content of total phenolic compounds (TPC) was determined by the Folin Ciocalteu spectrophotometric method using gallic acid as a standard. Hydroalcoholic extracts were obtained according to the methodology described by Repo de Carrasco and Zelada [13], with modifications. Five grams of sample were crushed with 20 mL of 95 % ethanol. Samples were then collected into a flask and left to stand for 24 h at 4 °C. They were then centrifuged for 15 min at 3000 × g. Using a micropipette, 0.5 mL of clear supernatant and 8 mL of ultrapure water were taken and mixed. At the same time, a blank was prepared with 0.5 mL of 95 % ethanol. Then 0.5 mL of Folin-Ciocalteu reagent (diluted with water 1:8 (v/v)) was added, mixed and allowed to react for three minutes before 1 mL of 20 % Na_2CO_3 was added. The samples were protected from light and stored for 30 minutes. Subsequently, absorbance was measured at 725 nm. The results were expressed as mg gallic acid equivalents (GAE) per 100 g sample on a dry basis (db), which is derived from the gallic acid reference calibration curve. Samples of disinfected seeds, malt (after germination for 72 h at 25 °C), wash and distilled beverages were analyzed in duplicate.

Physicochemical analysis and sensory acceptability

Moisture, ash content, protein and total fat content of the disinfected quinoa seeds were determined according to the methods of the Association of Official Analytical Chemists [14]. Alcohol content was estimated as alcohol by volume measured with a Gay Lussac alcoholmeter at 20 °C. The total acidity of the distillates was determined according to method NTP 210.017 [15], while the concentration of esters was determined by method NTP 211.003[16] and the concentration of aldehydes by method NTP 210.020 (gas chromatography, GC) [17]. The content of higher alcohols was determined by method NTP 210.021 (GC) [18]. Methanol and furfural were determined by Association of Official Analytical Chemists methods 9.089 and 9.081 [14], respectively. The acceptability of color, odor, flavor and overall appearance of the beverages were evaluated using a 5-point hedonic scale. The samples were evaluated by 15 judges.

Statistical analysis

Experimental data were evaluated by analysis of variance (ANOVA) and Tukey's HSD 157 test, considering a significance level of $p < 0.05$. Analyses were performed with Statgraphics XVII 17.2.07 (Madrid, MD., USA).

RESULTS AND DISCUSSION

Nutritional characteristics and total sugar content of the seeds

The moisture content of disinfected BNQ seeds was 9.29 ± 0.00 %, while RPQ seeds registered moisture of 9.49 ± 0.04 %. Ash content was the same for both quinoa

cultivars (2.58 ± 0.00 %, db). Likewise, the protein content of BNQ and RPQ seeds was 18.77 and 19.54 % (db), respectively. Regarding the content of fat and fiber, RPQ presented contents of 4.24 ± 0.01 and 1.62 ± 0.02 %, respectively; while for BNQ, 4.22 ± 0.01 and 1.58 ± 0.02 % (db), respectively. There was no significant difference ($p<0.05$) in fat and fiber content of the two quinoa cultivars. The residual content of saponins in the washed seeds of RPQ and BNQ was the same (0.02 ± 0.01 %).

Ash results for both cultivars are in the range reported by Diaz-Valencia *et al*. [19] (2.51 - 2.96 %). Concerning to the protein content, Vidaurre-Ruiz *et al*. [7] and Diaz-Valencia *et al*. [19] showed lower protein concentrations for both cultivars (between 9.4 and 14 %); likewise, in the analysis of different samples of black and red quinoa seeds, Encina-Zelada *et al*. [20] reported mean protein values of 14.6 and 15.6 %, respectively. The fat content of both cultivars is lower than the reported by Vidaurre-Ruiz *et al*. [7] (fat content range of 4.64 and 5.25 %) but closer to those measured by Díaz-Valencia et al. [19] (7.33 and 6.67 % for RPQ and BNQ, respectively). Regarding to the fiber content, Vidaurre-Ruiz *et al.* [7] reported higher crude fiber contents for RPQ and BNQ (1.93 and 2.59 %). Lastly, Díaz-Valencia *et al*. [19] reported a lower content of residual saponin (0 %) for both cultivars, determined by a similar method, as well as Castañeda *et al*. [21] obtained a saponin concentration of 0.01 % after washing quinoa grains for beer production.

Table 1 shows that the initial total sugar content of RPQ and BNQ seeds was 4.32 and 4.53 %, respectively. Moreover, the total sugar content increased with the time for the roasted germinated seeds of the two cultivars. At a temperature of 20 °C and time intervals of 24, 48, and 72 h of germination, the total sugar content of RPQ was 5.42, 6.71, and 8.58 %. However, at a temperature of 25 °C, the values were higher for the same cultivar at the same periods (5.68, 8.58, and 13.15 %). On the other hand, the germination of the BNQ cultivar at a higher temperature (25 °C) produced lower values of total sugars than the registered at 20°C temperature, after 24 and 48 h. But, after 72 h the value (14.35 %) was higher than that obtained with the lower germination temperature (13.65 %). In both cases, the best germination conditions for sugar production were 72 h and 25 °C. Authors like González *et al.* [5] determined the content of glucose (4.55 %), fructose (2.41 %) and sucrose (2.39 %) in Sajama quinoa seeds from Argentina.

Table 1. *Total sugar content (%) of both cultivars*

Germination time	**Quinoa cultivars**			
	RPQ		**BNQ**	
	20°C	25°C	20°C	25°C
24h	5.42	5.68	-	-
48h	6.71	8.58	-	-
72h	8.58	13.15	13.65	14.35

Mujica *et al.* [11] reported a significant increase in the concentration of reducing sugars after germination of quinoa seeds of different varieties even after 24 h of germination. Amylases hydrolyze starch into simple sugars, such as glucose and maltose, which are reducing sugars, and to a lesser extent, sucrose, which is a non-reducing sugar [22]. Temperature also affects the activity of α-amylase, being lower at lower temperatures [23]. The latter authors observed a higher a-amylase activity in quinoa seeds after 36 h, decreasing markedly up to 48 h and increasing slightly at 72 h.

Effect of the process on the TPC

Table 2 shows the TPC content of disinfected seeds, roasted seeds (malt), wash and distilled beverages for each cultivar. The TPC values of quinoa seeds were slightly above the range reported by Repo de Carrasco and Zelada [13] for different quinoa samples from Peru (37.15 - 139.94 mg GAE/100 g). Abderrahim *et al.* [8] reported a higher range for 13 quinoa cultivars from the Peruvian Altiplano (250 - 792 mg GAE/100 g, db). Vidaurre-Ruiz *et al.* [7] and Diaz-Valencia *et al.* [19] reported lower TPC levels for RPQ (61.1 - 108.9 mg GAE/100 g), while Abderrahim *et al.* [8] obtained a much higher value (707 mg GAE/100 g). The TPC level of BNQ obtained in the present work was very similar to that reported by Vidaurre-Ruiz *et al.* [7] for the same cultivar (142.3 mg GAE/100 g, db). In contrast, Diaz-Valencia *et al.* [19] obtained a lower value (95.9 mg GAE/100 g).

Table 2. *Total phenolic content of samples of both cultivars throughout the process*

Cultivar	**Stage**			
	Raw seeds [mg GAE/100 g]	**Malt [mg GAE/100 g]**	**Wash [mg GAE·L^{-1}]**	**Distilled beverage [mg GAE·L^{-1}]**
BNQ	143.67±0.92^{a}	172.16±0.03^{a}	62.98±0.79^{a}	1.61±0.22^{a}
RPQ	133.61±1.04^{b}	160.16±1.24^{b}	45.26±1.31^{b}	1.07±0.05^{a}

Values followed by the same letter in the column do not differ (Tukey's HSD, $p < 0.05$).
Values for raw seeds and malt are expressed in dry basis (db).

Malting increased the TPC value of BNQ and RPQ. However, these were lower than the values reported by Pilco Quesada *et al.* [24] (375.9 mg GAE/100 g) for quinoa seeds (variety "Chulpi") germinated for 72 h at 22 °C. This is because this variety had a high level of TPC before germination (231.9 mg GAE/100 g), which increased with time. Aguilar Izquierdo [25] reported lower TPC levels for BNQ and RPQ malts (118.7 y 145.9 mg GAE/100 g, respectively) after germination for 48 h at 25 °C. The seeds used by this author had a lower TPC content than those of the present study before germination. After germination for 48 h at room temperature, Leguía Damiano [26] determined even lower TPC levels for BNQ and RQP malts (40.33 y 41.77 mg GAE/100 g, respectively). Paucar-Menacho *et al.* [27] showed a high TPC level in germinated RPQ (unroasted) seeds after 72 h at 20 °C (376.61 mg GAE/100 g), although the highest TPC level they determined was obtained after 42 h of germination at the same temperature (499.24 mg GAE/100 g). The increase in TPC levels after germination was attributed to the release of these compounds by hydrolytic enzymes activated by germination [28] and also the synthesis of new phenolic compounds.

The BNQ samples also had a higher TPC level than RPQ in the wash and the distilled beverage. González *et al.* [29] analyzed 80 commercial beers in Spain and determined that the TPC level of pale and dark lager beers was 482.2 and 610.1 mg GAE·L^{-1}, respectively. Bogdan and Kordialik-Bogacka [30] determined the total polyphenol content of beers produced with 30 % substitution of barley malt by unmalted quinoa seeds (87 mg GAE·L^{-1}) and flakes (116 mg GAE·L^{-1}). These values were higher than those of the present study. The fermentation process can decrease the phenolic content of the wash due to absorption on the yeasts and the formation of phenol-protein polymers that precipitate [29]. The TPC level of the distilled beverages was lower than the total polyphenol content determined by Goldberg *et al.* [31] in several samples of

blended Scotch whisky (8.33 $mg \cdot L^{-1}$), rum (4.52 $mg \cdot L^{-1}$) and regular brandy (4.36 $mg \cdot L^{-1}$). These authors also pointed out that wood ageing was the most likely source of these phenolic compounds in the analyzed beverages.

Physicochemical characteristics of the distilled beverages

After the first distillation, the alcohol content increased from 5.5 and 4.4 (wash) to 21 and 20 % (v/v) for the BNQ and RPQ distilled beverages, respectively. After the second distillation, the alcohol content increased to 45 (BNQ) and 42 % (v/v) (RPQ). These values are above the minimum (40 %, v/v) established by the Peruvian standard for whiskies INACAL [32]. The content of methanol (0.010 and 0.016 mg/100 mL of pure ethanol (PE) for BNQ and RPQ, respectively) and furfural (0.07 and 1.01 mg/100 mL PE for BNQ and RPQ, respectively) was lower than the maximum values (30 and 6 mg/100 mL PE for methanol and furfural, respectively) also established by this standard. Regarding the level of aldehydes, esters and higher alcohols, the distilled beverage obtained from RPQ seeds presented values of 0.45, 4.92 and 0.82 mg/100 mL PE, respectively. In the case of the beverage obtained from BNQ seeds, the content of aldehydes was slightly higher (0.54 mg/100 mL PE), but the content of esters (4.22 mg/100 mL PE) and higher alcohols (0.57 mg/100 mL PE) was lower. These compounds would be removed after distillation, together with the heads or foreshots [33]. However, the higher alcohols also contribute to aromas and flavors characteristic of the raw material or generated during the fermentation process. The volatile acidity was higher in the distilled beverage obtained from RPQ (1.53 mg/100 mL PE) than that obtained from BNQ (1.31 mg/100 mL PE). The main responsible for volatile acidity is an undesirable organic acid (acetic acid) that imparts an unpleasant vinegar odor. Nóbrega *et al.* [34] determined that the volatile acidity of seven blended whiskies produced in Brazil is in the range of 16.5 to 58.8 mg/100 mL PE. These values were much higher than those obtained in the present work.

Sensory acceptability

Table 3 shows the sensory acceptability results of the distilled beverages. The scores obtained indicated good acceptability. The two samples presented similar values in the aspects of color, odor, and general appearance but differed in flavor. In this aspect, the beverage obtained from BNQ seeds scored significantly higher ($p < 0.05$). It is possible that the distillation process removed more of the compounds responsible for color and odor (such as higher alcohols) and thus reduced the sensory differences between the two beverages [30]. For flavor, the difference could be explained by colorless and odorless compounds in different amounts in each beverage, which may have originated in the wash obtained from the fermentation of each cultivar and have been carried over after distillation. Castañeda *et al.* [21] found sensory differences between beers brewed from malted and unmalted quinoa seeds that replaced different percentages of barley malt.

Table 3. *Sensory acceptability of distilled spirits*

Attribute	BNQ	RPQ
Color	4.07±0.46[a]	3.87±0.35[a]
Odor	4.33±0.49[a]	4.13±0.52[a]
Flavor	4.40±0.51[a]	4.00±0.38[b]
Overall appearance	4.33±0.49[a]	4.07±0.26[a]

Values followed by the same letter in the row do not differ (Tukey's HSD, $p < 0.05$).

CONCLUSIONS

Seeds of both quinoa cultivars showed good initial nutritional value concerning protein and TPC content. Germination for 72 h at 25 °C produced the highest total sugar contents in both seeds, which would be suitable for malting. After malting, the TPC content increased, probably due to the roasting process and release of phenolic compounds. The BNQ wash had a higher TPC content, but there was no difference between the distilled beverages. The content of methanol, furfural, aldehydes, esters, higher alcohols and volatile acidity of the beverages was lower than the maximum established by the Peruvian standard for whisky. The BNQ beverage could have odorless and colorless compounds in higher concentration or different from those present in the RPQ seed beverage, which allowed it to obtain a higher flavor score. Further identification of which compounds would be responsible would be needed at a later stage. Ageing the distilled beverages in wooden barrels to increase the TPC content could also be considered.

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