

# Quinoa malt and blueberry functional beverage: effect of malting and consumer sensory evaluation

Bebida funcional de malta de quinua y arándanos: efecto del malteado y evaluación sensorial del consumidor

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## ABSTRACT

**Background:** Consumers are looking for functional, natural products, valuing minimally processed, additive-free foods that include ancestral crops such as quinoa for their sustainability, nutritional quality and cultural connection. **Objective:** To evaluate the influence of processing on the antioxidant capacity and total phenolic compounds and consumer liking of a functional beverage based on quinoa malt (*Chenopodium quinoa* Willd.) and blueberry (*Vaccinium corymbosum*) pulp. **Methods:** Total phenolic compounds (TPC) were determined by ultraviolet-visible spectrophotometry (UV-Vis). The antioxidant capacity was determined by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods were applied. Consumer liking level was determined using a five-point verbal scale on 86 untrained evaluators. **Results:** The mixture design was employed with minimum-maximum constraints to obtain the optimized beverage formulation based on the blend of three types of quinoa malt [black quinoa malt (BQM), red quinoa malt (RQM), and white quinoa malt (WQM)]. The response variables were antioxidant activity using the DPPH and ABTS methods. Each of the response variables was maximized and fitted to the quadratic regression model. The optimum formula had 70% black quinoa malt (BQM), 20% red quinoa malt (RQM) and 10% white quinoa malt (WQM). Antioxidant capacity increased from stage to stage from  $45.11 \pm 0.26 \mu\text{g TE/g}$  in sprouted quinoa (SQ) to  $767.55 \pm 4.94 \mu\text{g TE/g}$  in quinoa malt-based beverage with the inclusion of blueberry pulp (QMB) using the DPPH method and from  $271.64 \pm 1.23 \mu\text{g TE/g}$  in SQ to  $834.32 \pm 2.14 \mu\text{g TE/g}$  in QMB using the ABTS method. The sensory evaluators rated QMB between "good" and "super good" according to the attributes of odor, color, taste and appearance, evaluated. **Conclusions:** Quinoa malting and fruit addition significantly influenced the antioxidant capacity and total phenolic compounds. Furthermore, the sensory characteristics of the beverage were described within the range of "good" to "super good" in the evaluated attributes.

**Keywords:** *Chenopodium quinoa*, malt, phenolic compounds, sensory evaluation, antioxidant activity, Mixture design.

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## RESUMEN

**Antecedentes:** Los consumidores buscan productos funcionales, naturales, valorando alimentos mínimamente procesados, libres de aditivos y que incluyan cultivos ancestrales como la quinua por su sostenibilidad, calidad nutricional y conexión cultural.

**Objetivo:** Evaluar la influencia del procesado sobre la capacidad antioxidante y los compuestos fenólicos totales y el agrado del consumidor de una bebida funcional a base de malta de quinua (*Chenopodium quinoa* Willd.) y pulpa de arándano (*Vaccinium corymbosum*).

**Métodos:** Los compuestos fenólicos totales (CFT) se determinaron mediante espectrofotometría ultravioleta-visible (UV-Vis). La capacidad antioxidante se determinó con los métodos del ácido 2,2'-azino-bis(3-etilbenzotiazolina-6-sulfónico) (ABTS) y del 2,2-difenil-1-picrilhidrazilo (DPPH). El nivel de agrado del consumidor se determinó utilizando una escala verbal de cinco puntos en 86 evaluadores no entrenados.

**Resultados:** El diseño de mezcla fue empleado con restricciones mínimo-máximo para obtener la formulación optimizada de la bebida basada en la mezcla de tres tipos de malta de quinua [malta de quinua negra (BQM), malta de quinua roja (RQM), y malta de quinua blanca (WQM)]. Las variables respuesta fueron la actividad antioxidante con los métodos DPPH y ABTS. Cada una de las variables de respuesta fue maximizada y se ajustó al modelo de regresión cuadrática. La fórmula óptima tenía 70% de BQM, 20% de RQM y 10% de WQM. La capacidad antioxidante aumentó de una etapa a otra, de  $45,11 \pm 0,26 \mu\text{g TE/g}$  en la quinua germinada (SQ) a  $767,55 \pm 4,94 \mu\text{g TE/g}$  en la bebida a base de malta de quinua con inclusión de pulpa de arándano (QMB), utilizando el método DPPH y de  $271,64 \pm 1,23 \mu\text{g TE/g}$  en SQ a  $834,32 \pm 2,14 \mu\text{g TE/g}$  en QMB con el método ABTS. Los evaluadores sensoriales calificaron QMB entre "buena" y "super buena" según los atributos olor, color, sabor y aspecto, evaluados. **Conclusiones:** El malteado de quinua y la adición de fruta influyeron significativamente en la capacidad antioxidante y los compuestos fenólicos totales. Además, las características sensoriales de la bebida se describieron dentro del rango de "bueno" a "súper bueno" en los atributos evaluados.

**Palabras clave:** *Chenopodium quinoa*, malta, compuestos fenólicos, evaluación sensorial, actividad antioxidante, diseño de mezcla.

## INTRODUCTION

Quinoa is an annual dicotyledonous plant native to the Andean region of northwestern South America. It was an important part of the diet of the people of the ancient Inca Empire. It has notable attributes in its nutritional composition that include proteins (13 % – 16 %) with an amino acid pattern close to the ideal protein balance (recommended by the FAO in 2011), micronutrients (minerals and vitamins), and polyphenols (1). Quinoa, compared to other grains such as barley, oats, rice, and corn, has a higher mineral (Mg, Zn, Fe, and Ca) and vitamin (A, B, and E) content (2, 3). Furthermore, it is classified as having a low glycemic index as it lacks gluten and contains many polyunsaturated fats and dietary fiber (4). It has beneficial effects on consumer health, such as antidiabetic, antioxidant, antiobesity, and heart-healthy effects (5).

Quinoa is a world-renowned food and has been grown in Europe, North America, Africa, and Asia since the 20th century (1, 2, 6). Quinoa grains can be used without processing or can be transformed into products by the food industry (flour, flakes, noodles, bread, cakes, cookies, chocolates, yogurt, beverages, beer, liqueurs, baby food, desserts, and gourmet dishes) (4, 6-8). Because of the increasing demand for healthy foods, food developers have been able to minimize the negative effects of technological processes on nutritional and/or sensory quality using the appropriate methodologies. Thus, the use of optimization methods has been shown to be effective for developing functional foods (8).

Malting is an effective way to enrich the antioxidants in quinoa grains to produce gluten-free functional foods and beverages (9). This process includes the following stages: soaking, germination, drying and toasting (10). Soaking the grains dissolves the hydrophilic saponins, thereby lowering the level of bitterness. Furthermore, it activates intercellular enzymes and hydrolyzes stored nutrients, which causes the grain to germinate. Germination, in turn, enhances the nutritional properties of quinoa-based beverages (8). Complex carbohydrates are degraded during germination to reduce sugars and low molecular weight compounds, which reduce antinutritional components, improve the digestibility of proteins and starch, and increase the levels of polyphenolic and antioxidant compounds. The grains are finally dried, leading to further enhancement of the flavor profile through the formation of Maillard reaction products (3, 11). A final stage may include toasting, which enhances the phenolic content of the grains (12).

In summary, malting can improve the nutritional and sensorial quality of the grain, by lowering the level of bitterness without abrasively removing the pericarp of the grain, thus preserving its dietary fiber, polyphenolic compounds, and other bioactive agents which are concentrated in its outer layers (3). Applications of quinoa malts include gluten-free beer (13, 14), baked goods, and nonalcoholic beverages (3). Each stage in the malting process is known to affect the bioactive components of quinoa. For example, the germination process improves the phenolic and nutritional content of

quinoa (15). Toasting can change the phenolic level, composition, and antioxidant activities of millet foods (12). In addition, malting mobilizes nutrient reserves through enzymatic activity and significantly increases polyphenols, antioxidant capacity (AC), and protein, and reduces sugars in quinoa (3).

The addition of fruit pulp improves the nutritional and organoleptic characteristics of beverages. Blueberries contain flavonoids-including flavan-3-ols (procyanidins, catechin, epicatechin etc.), flavonols (kaempferol, quercetin, myricetin etc.), and nonflavonoid polyphenolic compounds (hydroxycinnamic acid esters, such as chlorogenic, caffeic, gallic and ferulic acids, and stilbenes) (16, 17). Blueberries have health-promoting properties related to polyphenols and condensed tannins with a demonstrated influence on the activity of antioxidant enzymes (catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase) (17). Considering the above, this study aimed to (i) determine the optimal formulation of a quinoa malt-based clean label functional beverage, (ii) evaluate the effects of malting and the inclusion of blueberries in the optimized beverage formulation on the antioxidant capacity and total phenolic content (TPC), and (iii) evaluate the level of consumer liking of the beverage (optimal formulation).

## MATERIALS AND METHODS

### Plant material

Samples of quinoa were obtained from the Instituto Nacional de Investigación Agraria (INIA), Peru: INIA-420 Negra Collana (black quinoa), INIA 415-Pasankalla (red quinoa), and Salcedo INIA (white quinoa). Fresh blueberry fruits were purchased at the central market in Lima, Peru. Undamaged fruits were selected, disinfected with sodium hypochlorite solution (0.5 % w/v), rinsed with filtered water, drained, and crushed. The product obtained was immediately stored at  $-18 \pm 1$  °C until use.

### Malting process

The quinoa seeds were washed with 0.1 % sodium hypochlorite solution (1:5, w/v) for 30 min at room temperature. They were rinsed several times with distilled water until reaching a neutral pH. The seeds were subsequently soaked in distilled water (1:5, w/v) for an additional period of 6 h and shaken every 30 min (15). Subsequently, the hydrated seeds were placed on absorbent paper moistened with distilled water in trays covered with paper towels

and incubated at room temperature for 72 h. Water was sprayed for 15 min at 12-h intervals using a hand sprayer to maintain the relative humidity (8). The sprouts were harvested and toasted at 60 °C for 20 min to obtain the malted quinoa seeds (18).

### Preparation of the beverage

The malt of three varieties was combined according to the extreme vertex experimental design. Subsequently, the quinoa malt mixture was crushed using a blender, while water was added gradually. The ratio used between the quinoa malt mixture and water was 1:4 (w/v). This preparation was subjected to the mashing process, carried out in specific stages: first, the mixture was heated at a temperature of 45 °C for 30 min, then at 65 °C for 30 min, and finally at 74 °C for 30 min (19). The resulting wort was filtered and the filtrate obtained was brought to boiling. At this stage, blueberry pulp in a proportion of 15% w/v and sugar were added until a concentration of 10° Brix was reached. The mixture was pasteurized at 80 °C for 20 minutes. Finally, the beverage was packaged in 250 mL glass bottles and stored in refrigerated conditions ( $1.0 \pm 0.5$  °C) until further analysis.

### Antioxidant capacity by DPPH method

DPPH radical scavenging was conducted following the methods of Brand-Williams *et al.* (20). Using 100 µL of the sample and 2.9 mL of the DPPH reagent, absorbance was measured at a wavelength of 517 nm on the spectrophotometer (Shimadzu UV/Vis 2550, Shimadzu Scientific Instruments, MD, USA) in comparison with that of the reagent blank. This was done to identify antioxidant capacity through radical scavenging. The calibration plot was linear (Eq. 1) and  $R^2$  is the coefficient of determination.

$$A = 14.059c_x + 0.735 \quad (R^2 = 0.998) \quad (1)$$

where  $A$  represents absorbance,  $c_x$  is DPPH radical concentration expressed in micrograms per millilitre. Based on the obtained equation, the AC was calculated and expressed as micrograms of Trolox equivalents (TE) per gram of sample.

### Antioxidant capacity by ABTS method

AC using the ABTS assay was determined according to the procedure described by Re *et al.* (21). For the preparation of the ABTS cation radical solution (ABTS•+) 77.6 mg of the diammonium salt of ABTS

was dissolved in 20 mL of deionized water, then 13.2 mg of potassium persulfate was added, the solution was left to react at room temperature for 16 h in the dark. The ABTS•+ solution was then diluted with 80% ethanol to an absorbance of  $0.70 \pm 0.02$  at 734 nm. To prepare the reaction tubes (samples), 100  $\mu$ L of the solution was taken and 4.9 mL of the ABTS reagent was added to it. The preparation was allowed to stand for 7 min in the dark, and the absorbance was measured using a spectrophotometer at 734 nm against that of the reagent blank. The calibration plot was linear (Eq. 2) and  $R^2$  is the coefficient of determination.

$$A = 21.234c_x + 5.522 \quad (R^2 = 0.991) \quad (2)$$

where  $c_x$  is ABTS radical cation concentration expressed in micrograms per millilitre. Based on the obtained equation, the AC was calculated and expressed as micrograms of Trolox equivalents (TE) per gram of sample.

### Total phenolic content

TPC was estimated using the Folin–Ciocalteu assay as suggested by Singleton & Rossi (22), with some modifications. Folin–Ciocalteu reagent was used to determine total polyphenols based on its reducing capacity. The sample was prepared in a test tube, which was diluted with ethanol. Subsequently, 0.5 mL was extracted and 2.5 mL of Folin–Ciocalteu reagent was added. After 5 min of reaction, 2 mL of 7.5 % sodium carbonate was added, and the entire mixture was incubated at room temperature for 30 min in complete darkness. The blue color that developed was quantified using a spectrophotometer at 760 nm against the reagent blank. The TPC in the tested samples was determined by the equation of the calibration line (Eq. 3) and expressed as micrograms of gallic acid equivalents (GAE) per gram of sample.

$$A = 0.0774c_x + 0.0194 \quad (R^2 = 0.9948) \quad (3)$$

where  $A$  represents absorbance,  $c_x$  is GAE concentration and  $R^2$  is the coefficient of determination.

### Physicochemical analyses

pH was determined using a pH-25 digital pH meter (Leici, Shanghai, China), calibrated with pH 4.0 and 7.0 buffer solutions. Soluble solids were determined using a digital refractometer with

automatic temperature compensation SKU:MA871 (Milwaukee Instruments Inc., Rocky Mount, NC, USA). In addition, total flavonoids and tannins were determined in the fruit according to the method followed by Borowiec *et al.* (17). The total flavonoids concentration was expressed as milligrams of quercetin equivalent (QE) per milliliter. The tannins content was expressed as the micrograms of catechin equivalent (CE) per gram.

### Consumer sensory evaluation

The malt-based beverage made with three varieties of quinoa and blueberry was assessed in terms of its organoleptic characteristics of odor, color, flavor, and appearance, to know potential consumers' perception. Consumer perception was assessed through the responses of a total of 86 untrained tasters who agreed to participate in the evaluation after providing informed consent and indicating their voluntary participation, without which, their responses were not considered. The panelists included 61.6 % students, and the rest 38.4 % were students' relatives and professors and administrative staff of the School of Pharmacy and Biochemistry of the Universidad Nacional Mayor de San Marcos, in Peru. The sensory test was carried out at the Sensory Analysis Laboratory of de School of Pharmacy and Biochemistry of the Universidad Nacional Mayor de San Marcos. The beverages were kept at room temperature for 30 min prior to testing, and the final serving temperature was between 18 to 20 °C. Approximately 100 mL of sample was poured into clean glasses of 200 mL capacity and capped to prevent volatile compounds from escaping from the glass. All samples were numbered with three-digit random codes. Participants were instructed to rinse their mouths with water before starting the tasting, and they were also asked to hold a sip of the beverage in their mouth for 5 s before swallowing. The consumer liking test was carried out using a five-point Kroll verbal scale with friendly descriptors, where (5) super good, (4) good, (3) not very good, (2) bad, and (1) super bad (23).

### Experimental design and Optimization of quinoa malts blend ratio

An extreme vertex mixture design was used to determine the optimal malt ratio of the three quinoa varieties in a beverage and maximize its antioxidant capacity. The design was used because additional constraints were set as upper and lower limits for the proportions of components located within the design (24). The constraints (minimum/maximum



composition) included 60 % – 70 % *BQM*, 20 % – 30 % *RQM*, and 10 % – 20 % *WQM*. These restrictions were set in preliminary tests. The experimental design consisted of 20 run order with three independent variables (*BQM* or  $X_1$ , 0.60 – 0.70; *RQM* or  $X_2$ , 0.20 – 0.30; and *WQM* or  $X_3$ , 0.10 – 0.20), and the response variables were the *DPPH* and *ABTS*. The sum of proportions in the mixture is one (24). For optimization, the combination of factors that elicited the best response was determined based on the influence of each factor. Equation (4) was used to model the responses after data analysis, depending on the goodness of fit, predictive power, and robustness of the model for the three components. Quadratic model:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (4)$$

where  $Y$  represents the modeled response variable;  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  are coefficients of the linear terms of *BQM*, *RQM*, and *WQM*;  $\beta_{12}$ ,  $\beta_{13}$ , and  $\beta_{23}$  are the coefficients of the interaction term; and  $X_1$ ,  $X_2$ , and  $X_3$  are the independent variables of *BQM*, *RQM*, and *WQM*, respectively. Goodness of fit of the ensuing model was determined by calculating the coefficients of determination ( $R^2$ ), adjusted coefficient of determination (*adjusted*  $R^2$ ), *predicted*  $R^2$  and the regression coefficients of the adjusted polynomial equation for the response variable. The desirability function was used to optimize response variables. The Minitab (v.19, Minitab MLL, USA) software was used to assign a level of importance to each response variable and an objective (maximize).

The predictive performance of the model was validated using the accuracy factor (*AF*) (Eq. 5) and the bias factor (*BF*) (Eq. 6) (25):

$$AF = 10^{\frac{\sum \log \left| \frac{V_p}{V_e} \right|}{n_e}} \quad (5)$$

$$BF = 10^{\frac{\sum \log \left( \frac{V_p}{V_e} \right)}{n_e}} \quad (6)$$

where *AF* values close to 1.00 suggest a perfect model fit in which the predicted and actual response values are similar. The *BF* is generated based on the agreement between the model and the experimental data. Furthermore, the average mean deviation (*E*) defined as in Eq. 7 was used to determine data fitting efficiency.

$$E(\%) = \frac{1}{n_e} \sum_{i=1}^n \frac{v_e - v_p}{v_e} \times 100 \quad (7)$$

where *E* is the average mean deviation,  $n_e$  is the number of experimental data,  $v_e$  is the experimental value, and  $v_p$  is the predicted or calculated value.

## Statistical Analysis

Analysis of variance was performed to determine the effects of malting and the inclusion of blueberry pulp in the quinoa and blueberry malt beverage on the antioxidant capacity and phenolic compounds. The Tukey multiple comparison test was performed once the statistical assumptions of standardization and homogeneity of variance were verified. The sensorial acceptability was analyzed with Friedman's nonparametric test.

## RESULTS AND DISCUSSION

### Raw material characteristics

The water content of quinoa seeds ranged from 8.34 to 9.91 %, although no significant differences ( $P > 0.05$ ) were found among varieties. The means values of water content were for white quinoa  $8.86 \pm 0.16$  %; red quinoa  $9.18 \pm 0.44$  % and black quinoa  $9.28 \pm 0.83$  %. There is a very small difference in water content with other samples. Pellegrini *et al.* (26) reported a range of 5.27 to 8.24 % (white Spanish quinoa obtained from organic farming; two different brand of white Bolivian Real quinoa obtained from organic farming; white Peruvian quinoa; red Bolivian Real quinoa obtained from organic farming; black Bolivian Real quinoa obtained from organic farming). Regarding total phenolic content (*TPC*), the black and red quinoa samples stood out for its *TPC* ( $3327 \mu\text{g}$  of GAE/g db and  $2739 \mu\text{g}$  of GAE/g db, respectively), while the white quinoa sample showed the lowest *TPC* ( $2181 \mu\text{g}$  GAE/g db). Phenolic compounds are beneficial to health due to their AC, and can vary significantly depending on different factors such as soil composition, growing conditions, altitude and post-harvest conditions, among others (27).

The average value of soluble solids and pH of the blueberry were  $11.50 \pm 0.12$  °Brix and  $3.10 \pm 0.06$ , respectively. These values are consistent with the physicochemical characteristics of blueberries ( $9.0$  °Brix and pH of 3.4) (28), which are considered adequate for avoiding microbial growth, thus extending the pulp's useful life. Regarding the

antioxidant capacity of the blueberry, the results showed that it contained  $1\,929.99 \pm 1.53 \mu\text{g TE/g}$  and  $1\,347.30 \pm 2.03 \mu\text{g TE/g}$  as determined by the ABTS and DPPH methods, respectively. TPC was  $1\,077.33 \pm 4.83 \mu\text{g GAE/g}$ . The antioxidant capacity was superior to blueberries harvested in the Amazon Region of Peru ( $493.00 \pm 0.05 \mu\text{g TE/g}$  and  $848.00 \pm 0.06 \mu\text{g TE/g}$  by ABTS and DPPH methods, respectively) (29). The growing conditions were probably different from those of the fruits used in this investigation. However, TPC was lower than those ( $1\,910.00 \pm 0.02 \mu\text{g GAE/g}$ ,  $4\,620.00 \pm 0.13 \mu\text{g GAE/g}$  and  $1\,912.00 \pm 136.00 \mu\text{g GAE/g}$ ) reported by Rojas-Ocampo *et al.* (29), Borowiec *et al.* (17), and Busso Casati *et al.* (30), respectively. Therefore, the differences between the values obtained for antioxidant capacity and TPC were likely due to the different geographical origins, agronomic conditions, and different methods for obtaining the phenolic extracts (29). The tannin content was  $1\,704.64 \pm 6.27 \mu\text{g CE/g}$  of sample. This result was higher than that ( $1\,050.00 \pm 0.08 \mu\text{g CE/g}$ ) reported by Borowiec *et al.* (17). Tannins are considered polyphenolic antinutritional substances that lower the nutritional value of foods because of their ability to interact with proteins and large molecules depending on the pH of the foods (3, 5). Furthermore, they impart astringency and impair the absorption of vitamin B12, iron, and glucose

(3). However, in blueberry, condensed tannins (proanthocyanidins) are crucial for the sour taste of the fruit and stabilize anthocyanins by forming copolymers with them (17).

The total flavonoids concentration of blueberry was  $10.49 \pm 0.29 \text{ mg QE/mL}$  of sample. Quercetin and derivatives stand out among the flavonols of blueberries, and they also contain anthocyanins, flavan-3-ol, and proanthocyanidins (16). The value obtained was similar to that ( $9.73 \pm 0.24 \text{ mg QE/mL}$ ) reported by Borowiec *et al.* (17), but lower than those reported by Guevara-Terán *et al.* (27), who compared the flavonoid concentration of Andean blueberries that grew at two different altitudinal levels ( $58.45$  and  $31.48 \text{ mg QE/mL}$  at  $3\,641$  and  $2\,836 \text{ m}$  altitude, respectively) and demonstrated that the flavonoid concentration increased with the increase in the altitude of blueberry growth. Therefore, there are several factors that can impact its bioactive components.

### Proportions of the components of the quinoa malt mixture

The beverages made with different proportions of quinoa malts (BQM, RQM, and WQM) according to the mixture design (Table 1) were analyzed for antioxidant capacity using the DPPH and ABTS methods.

**Table 1.** Antioxidant capacity in beverage brewed with different proportions of BQM, RQM and WQM by extreme vertex mixture design.

Run order	BQM ( $X_1$ )	RQM ( $X_2$ )	WQM ( $X_3$ )	DPPH ( $\mu\text{g TE/g}$ )	ABTS ( $\mu\text{g TE/g}$ )
1	0.60	0.20	0.20	$787.69 \pm 0.69$	$826.88 \pm 0.85$
2	0.70	0.20	0.10	$805.60 \pm 0.28$	$845.83 \pm 0.42$
3	0.60	0.30	0.10	$800.65 \pm 0.35$	$840.96 \pm 0.44$
4	0.60	0.25	0.15	$796.75 \pm 0.35$	$841.54 \pm 0.23$
5	0.65	0.20	0.15	$796.70 \pm 0.28$	$835.36 \pm 0.06$
6	0.65	0.25	0.10	$799.35 \pm 0.35$	$838.54 \pm 0.57$
7	0.63	0.23	0.13	$797.25 \pm 0.92$	$837.12 \pm 0.31$
8	0.62	0.22	0.17	$791.15 \pm 0.21$	$830.88 \pm 0.16$
9	0.67	0.22	0.12	$803.12 \pm 0.29$	$842.18 \pm 0.28$
10	0.62	0.27	0.12	$802.55 \pm 0.64$	$842.43 \pm 0.18$
11	0.60	0.20	0.20	$787.75 \pm 0.21$	$824.86 \pm 0.14$
12	0.70	0.20	0.10	$804.45 \pm 0.35$	$843.84 \pm 0.33$
13	0.60	0.30	0.10	$801.75 \pm 0.21$	$841.92 \pm 0.31$
14	0.60	0.25	0.15	$795.95 \pm 0.07$	$835.52 \pm 0.39$
15	0.65	0.20	0.15	$795.75 \pm 0.49$	$835.34 \pm 0.24$
16	0.65	0.25	0.10	$802.66 \pm 0.47$	$839.04 \pm 0.09$

Run order	BQM ( $X_1$ )	RQM ( $X_2$ )	WQM ( $X_3$ )	DPPH ( $\mu\text{g TE/g}$ )	ABTS ( $\mu\text{g TE/g}$ )
17	0.63	0.23	0.13	797.45 $\pm$ 0.64	837.22 $\pm$ 0.25
18	0.62	0.22	0.17	795.75 $\pm$ 0.35	835.36 $\pm$ 0.23
19	0.67	0.22	0.12	801.90 $\pm$ 0.28	841.62 $\pm$ 0.86
20	0.62	0.27	0.12	800.75 $\pm$ 0.92	840.64 $\pm$ 0.49

Abbreviations: *BQM*: black quinoa malt, *RQM*: red quinoa malt, and *WQM*: white quinoa malt, *DPPH*: 2,2-diphenyl-1-picrylhydrazyl; *ABTS*: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

The mean values of the AC were within the range of 787.69 – 805.60  $\mu\text{g TE/g}$  and 824.86 – 845.83  $\mu\text{g TE/g}$  by DPPH and ABTS methods, respectively. A small positive effect was observed for DPPH and ABTS, which increased by 2.27 and 2.29 % when *BQM* increased from 60% to 70% and *WQM* decreased from 20% to 10% (runs #1 and #2), respectively. This is probably because black quinoa malts are characterized by a higher content of hydroxycinnamic acids, flavan-3-ols, magnesium, potassium and AC, while white quinoa malts are characterized by a lower content of saponin and phytic acid (3).

Each of the response variables was fitted to the quadratic regression model. The P values of the models were 0.040 and 0.006 for the DPPH and ABTS methods, respectively. Further, the coefficients of determination of the models were greater than 0.80;  $R^2 = 0.93$ , adjusted  $R^2 = 0.90$ , predicted  $R^2 = 0.89$  and  $R^2 = 0.92$ , adjusted  $R^2 = 0.89$ , predicted  $R^2 = 0.83$  for the DPPH and ABTS methods, respectively demonstrated the adequacy of the quadratic models. Furthermore, both regression models showed no *Lack of fit* ( $P = 0.075$  and  $P = 0.476$  for the DPPH and ABTS methods, respectively).

Once the quadratic model was selected as the best model. The coefficients were  $\beta_1 = 805.23$ ,  $\beta_2 = 801.56$ ,  $\beta_3 = 787.38$  and  $\beta_1 = 845.18$ ,  $\beta_2 = 841.71$ ,  $\beta_3 = 825.58$  for the DPPH and ABTS methods, respectively. The null hypothesis  $H_0: \beta_{12} = \beta_{13} = \beta_{23} = 0$  was tested against the alternative hypothesis  $H_a$ : at least one of the coefficients of the interaction term is nonzero. For the DPPH method, with significance level  $\alpha = 0.05$ ,  $\beta_{12}$  is -11.58 ( $P = 0.027$ ), indicating that there was a negative correlation in the mixture of *BQM* with *RQM*, which was significant;  $\beta_{13}$  is 1.47 ( $P = 0.758$ ), i.e., there was no significance in the interaction between *BQM* and *WQM* in the mixture; and  $\beta_{23}$  is 9.94 ( $P = 0.052$ ), i.e., there was a positive correlation between *RQM* and *WQM* with significant effect. While for ABTS method, with significance level  $\alpha = 0.05$ ,  $\beta_{12}$  is -15.29 ( $P = 0.019$ ), indicating that there was a negative correlation in the mixture

of *BQM* with *RQM*, which was significant;  $\beta_{13}$  is 0.95 ( $P = 0.872$ ), i.e., there was no significance in the interaction between *BQM* and *WQM* in the mixture; and  $\beta_{23}$  is 20.29 ( $P = 0.004$ ), means that, there was a positive correlation between *RQM* and *WQM* with significant effect. These estimates were provided by the software package. The sign and magnitude of the regression coefficients in the equation indicate the effect of each independent variable on the AC of quinoa malt made with the three varieties (*BQM*, *RQM*, and *WQM*). The negative sign implies a decrease in the response when the level of the variable increases—an effect observed only for the *BQM*\**RQM* interaction.

The most suitable conditions for maximizing the DPPH and ABTS variables corresponded to exactly *BQM* = 70 %, *RQM* = 20 % and *WQM* = 10 %. The composite desirability value under these conditions was 0.974, which was within the range between acceptable and excellent ( $0.8 \leq \text{desirability} \leq 1.0$ ) (31). Higher desirability levels (i.e., close to 1.0) are the most sought after (32), since the desirability value indicates the degree of accuracy of the optimal solution (33). The desirability function has been widely used in the manufacturing industry and is the most popular method for simultaneously analyzing various factors in optimizing product quality (31). In one study, the desirability function was 0.600 in optimizing the proportions of banana, strawberry, and juçara in a smoothie using a blend design, indicating that the optimal formula had acceptable accuracy (34). When applying the blend design method to the optimal formulation of a powdered tempe drink, the desirability was 0.943, a value that supported obtaining a formula within the acceptable and excellent range (33). The desirability level close to one suggested an adequate result, which reinforced the ratio of components for the preparation of the beverage. Subsequently, two contour plots were generated to better visualize the combined effect of the independent variables (*BQM*, *RQM* and *WQM*) on the antioxidant activity in the mixture by the DPPH and ABTS methods (Fig. 1).

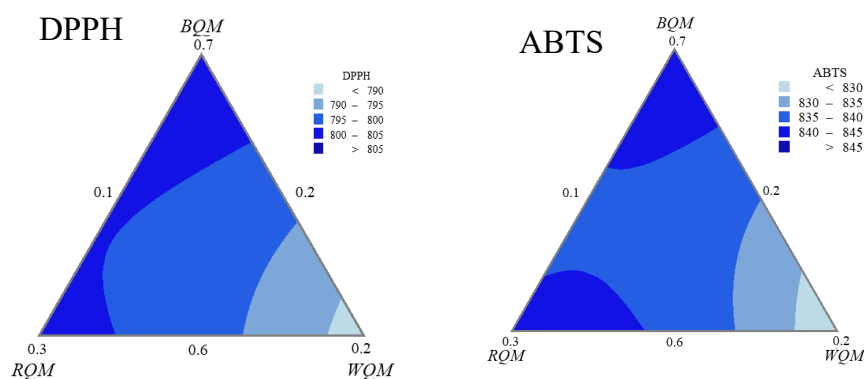


Fig. 1. Contour plots predicted by DPPH and ABTS methods

### Model validation

The model was validated to determine its accuracy, suitability, and the limits of its performance. Therefore, a predictive model can only be used reliably in decision making when it has been tested and validated (32). The predicted value for antioxidant capacity were 805.23 and 845.19  $\mu\text{g TE/g}$  by DPPH and ABTS methods, respectively. The experimental values were  $767.55 \pm 2.85$  and  $834.32 \pm 5.63$   $\mu\text{g TE/g}$  by DPPH and ABTS methods, respectively. Equations (5), (6) and (7) were used to determine the AF values ( $AF_{\text{DPPH}}=1.007$  and  $AF_{\text{ABTS}}=1.002$ ), the BF ( $BF_{\text{DPPH}}=1.155$  and  $BF_{\text{ABTS}}=1.039$ ) and  $E\%$  ( $E\%_{\text{DPPH}}=0.7$  and  $E\%_{\text{ABTS}}=0.3$ ) to quantitatively determine the applicability and accuracy of the models described above. The models show a good fit when AF and BF are close to 1.00 for the key responses (25). Both models showed good fit for the optimized formulation, as shown by AF, thus indicating an almost perfect fit of the model,

also the BF values showed a trend like that of the precision factor. The variations between predicted and obtained experimental values were within the acceptable error range, as indicated by the average mean deviation ( $E \leq 5\%$ ) (32). Thus, the predictive performance of the established models can be considered as acceptable, which demonstrates the high applicability of the models and the mixture design to develop the optimized formulation of the quinoa malt-based beverage.

### Effect of malting and fruit inclusion

Figure 2 shows the effect of the malting process from sprouting (SQ), drying (DSQ), and toasting (TSQ), as well as the inclusion of blueberry (QMB) in the beverage, on the antioxidant capacity and TPC. The results show a significant effect ( $P < 0.05$ ) between the malting stages and the addition of fruit regarding antioxidant capacity by DPPH and ABTS methods and TPC.

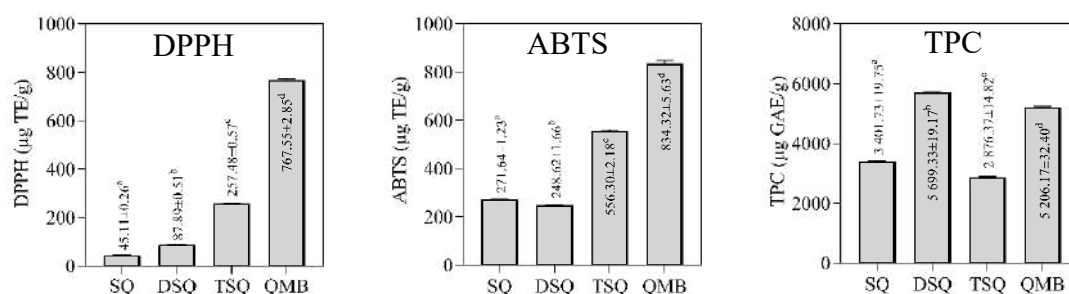


Fig. 2. Effects of beverage production stages on antioxidant capacity by DPPH and ABTS methods and total phenolic content (TPC). Lower case letters (a-d) indicate significant differences ( $P < 0.05$ ) between samples according to Tukey's multiple range test. Each column represents the mean of three replicates  $\pm$  standard deviation.



The *TPC* was  $3\,401.73 \pm 19.75 \mu\text{g GAE/g}$  in the SQ stage, and this value is within the range of *TPC* for germinated quinoa grains ( $1\,844.00 - 4\,992.40 \mu\text{g /g dw}$ ) reported by Paucar-Menacho *et al.* (15). Eight phenolic compounds were detected in quinoa (gallic acid, protocatechuic acid, vanillic acid, p-hydroxybenzoic acid, transferulic acid, sinapic acid, p-coumaric acid and caffeic acid) (3). Some are free, and others are bound, and are released during germination by hydrolysis of cell wall structures. Moreover, the amount of gamma-aminobutyric acid, antioxidants, amino acids, dietary fiber, vitamin E, and other bioactive compounds increases at this stage, whereas the content of antinutritional factors such as phytic acid, tannins, and saponins decreases, thus resulting in higher mineral bioavailability (35). Therefore, the nutritional value increases, and the enzymes are activated as an effect of germination, thus improving the grains' vitamin content and softness (2). The DSQ process yielded the highest *TPC* content ( $5\,699.33 \pm 19.17 \mu\text{g GAE/g}$ ) compared to the other malting processes (SQ and TSQ), a value that did not necessarily indicate a high AC. It is probably related to the degradation of proteins and carbohydrates within the cell wall due to drying, thus leaving the polyphenols free. Some bound phenolics are released during germination, and this improves their solubility and bioavailability. In the TSQ stage, the *TPC* value was  $2\,876.37 \pm 14.82 \mu\text{g GAE/g}$ , being the lowest value compared to that of the other malting stages (SQ and DSQ). Toasting benefits the flavor profile of malted quinoa through the formation of Maillard reaction products (3, 12). This result is consistent with the *TPC* ( $2\,900 \mu\text{g GAE/g}$ ) of the malted quinoa beverage developed by Kaur & Tanwar (18).

In general, the stages of the malting process (SQ, DSQ, and TSQ) had a positive effect on the antioxidant capacity, and the highest values were obtained in the TSQ stage, equal to  $257.48 \pm 0.99$  and  $556.30 \pm 3.78 \mu\text{g TE/g}$ , based on the DPPH and ABTS methods, respectively. The malting has a significant effect on total AC due to the formation of Maillard reaction products that have antioxidant properties, and the color of the grain also contributes to this variation (3).

The antioxidant capacity determined using the DPPH method increased from one stage to another, from  $45.11 \pm 0.26 \mu\text{g TE/g}$  in SQ to  $767.55 \pm 4.94 \mu\text{g TE/g}$  in the QMB. On the other hand, the antioxidant

capacity determined with the ABTS method decreased significantly ( $P < 0.05$ ) from the SQ stage ( $271.64 \pm 1.23 \mu\text{g TE/g}$ ) to DSQ ( $248.62 \pm 1.66 \mu\text{g TE/g}$ ), and then increased in the following stages (TSQ:  $556.30 \pm 2.18 \mu\text{g TE/g}$  and QMB:  $834.32 \pm 2.14 \mu\text{g TE/g}$ ). Malting with proper heat treatment is effective in increasing antioxidants in quinoa grains which could be used as an ingredient to make a gluten-free beverage (9). In conclusion, quinoa malting has a significant effect on antioxidant capacity in correlation with the *TPC* and other components, which are products of the Maillard reaction. Therefore, this beverage could have potential as an antidiabetic and antihypertensive preparation, within the classification of gluten-free beverages, similar to that obtained in the investigation by Kaur & Tanwar (18).

Adding blueberry pulping to the quinoa malt beverage significantly increased ( $P < 0.05$ ) the antioxidant capacity and *TPC* compared to the values obtained during the malting stages. Regarding *TPC*, the malted quinoa beverage with the inclusion of blueberry (QMB) presented  $5\,206.17 \pm 32.41 \mu\text{g GAE/g}$ , which meant an increase of 81.0 % from the previous stage. And AC increased to  $767.55 \pm 2.85 \mu\text{g TE/g}$  and  $834.32 \pm 5.63 \mu\text{g TE/g}$ , with the DPPH and ABTS tests, respectively. This represents an increase of 198.1 % and 49.9 %, respectively, compared with the previous stage (TSQ). This result was as expected, because of the presence of several anthocyanins, such as quercetin, 3-O- $\beta$ -glucoside, and delphinidin-3-O-galactoside, for blueberries of the species *Vaccinium corymbosum* (36), which provide a functional contribution to the beverage.

## Sensory evaluation

The demographic variables of the recruited participants obtained through the questionnaire were age, gender, and marital status. According to the data collected, the sensory evaluators comprised both women (53.5 %) and men (46.5 %). Most respondents were under 25 years old (41.9 %). Among all respondents, 64.0 % were single, 26.7 % were married and 9.3 % answered other. All agreed to participate in the evaluation. The quinoa malt beverage with blueberry pulp was evaluated using the sensory test of level of liking (Table 2). Consumer-centered sensory orientation is recommended for product development (23).

**Table 2.** Descriptive statistics of the sensory test (n = 86).

Attribute	Scale	Frequency	Percentage	Mean $\pm$ SD
Odour	(5) super good	63	72.1	4.66 $\pm$ 0.61
	(4) good	20	23.3	
	(3) not very good	3	3.5	
	(2) bad	1	1.2	
	(1) super bad	0	0.0	
Colour	(5) super good	66	76.7	4.72 $\pm$ 0.55
	(4) good	16	18.6	
	(3) not very good	4	4.7	
	(2) bad	0	0.0	
	(1) super bad	0	0.0	
Flavor	(5) super good	69	80.2	4.81 $\pm$ 0.40
	(4) good	17	19.8	
	(3) not very good	0	0.0	
	(2) bad	0	0.0	
	(1) super bad	0	0.0	
Appearance	(5) super good	63	73.3	4.71 $\pm$ 0.51
	(4) good	21	24.4	
	(3) not very good	2	2.3	
	(2) bad	0	0.0	
	(1) super bad	0	0.0	

Most respondents (72.1 %) rated the beverage's odor as "super good," followed by 23.3 % who rated it as "good," and only a minority (1.2 %) of those surveyed found the odor "bad." The mean rating for odor was  $4.66 \pm 0.61$ , thus indicating that consumers rated the beverage's odor between "super good" and "good". For the majority of respondents (76.7 %), the color was perceived "super good," and the mean color rating was  $4.72 \pm 0.55$ , thus indicating that consumers rated the beverage's color between "super good" and "good". Regarding the flavor evaluation, 80.2 % of respondents rated it as "super good" and 19.8 % rated it as "good," with an average color rating of  $4.81 \pm 0.40$ , thus suggesting that the beverage's flavor had high acceptability, between "super good" and "good". The beverage's appearance was rated by the majority of respondents (73.3 %) as "super good," followed by 24.4 % who rated it as "good," and only 2.3% rated it as "not very good". The mean appearance rating was  $4.71 \pm 0.51$ , thus

indicating the beverage's high acceptability in terms of appearance.

In general, for the odor, color, flavor, and appearance attributes of the beverage, the results showed scores between  $4.66 \pm 0.61$  and  $4.81 \pm 0.40$ , which place the beverage within the range of "good" to "super good" in the attributes evaluated. The malting process is probably responsible for the beverage's high flavor scores by improving its flavor profile through the formation of Maillard reaction products (3). The color of the beverage was due to the blueberry, which has high anthocyanin content. (17).

The growing demand for clean label foods reflects consumers' desire for healthier foods. A clean label food is a product that is presented as "natural" and/or free of artificial ingredients/additives (37). The results of this investigation can serve as a basis for future research regarding the development of functional beverages with a clean label and high consumer acceptability.

## CONCLUSIONS

The mixture design methodology using an extreme vertex design turned out to be an effective technique for determining the ideal proportion of quinoa malts in a beverage formulation. The optimal formula for BQM, RQM, and WQM was 70 %, 20 %, and 10 %, respectively. Malting of the quinoa and the addition of fruit significantly influenced the beverage's AC and TPC. However, AC does not come exclusively from the TPC. Maillard reaction products are formed in the drying and toasting stages, which are responsible for the beverage's flavor scores. Furthermore, the addition of blueberry affected its sensory characteristics, which was described within the range of "good" to "super good" in the evaluated attributes. This investigation serves as a basis for the development of functional beverages with a clean label using the malting process and including fruits in their formulation. This work serves as a basis for developing functional beverages that meet the demands of modern consumers for clean-label products. By utilizing the malting process and integrating fruits into the formulation, the study opens pathways to create beverages with enhanced nutritional, sensory and health benefits while maintaining a natural and minimally processed profile. This research not only highlights the potential of ancient crops, like quinoa, in innovative food applications, but also bridges the gap between traditional practices and contemporary trends, promoting sustainable and culturally relevant food systems.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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## AUTHOR CONTRIBUTIONS

Conceptualization was devised by E.C.-L., T.R.-A. and E.C.-F.; methodology and analysis by E. Ch.-M. and A. A.-Ch.; research, resourcing, writing-preparation of the original draft and writing-revising and editing, data visualization was done by E.C.-L., T.R.-A. and E.F.-C.; fundraising was done by A.M.M.

All authors have read and accepted the published version of the manuscript.

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