Research Article**Enhancement of the rancidity stability in a marine-oil model by addition of a saponin-free quinoa (*Chenopodium quinoa* Willd.) ethanol extract[†]**

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ABSTRACT

This research focused on the antioxidant capacity of quinoa (*Chenopodium quinoa* Willd.), being its basic objective to analyze the retention of this property in the case that a previous saponins removal by water washing was undertaken. Chemical analyses related to saponin content and antioxidant behavior were carried out on ethanolic extracts of two quinoa ecotypes (Ancovinto and Cancosa) and compared to quinoa that was not subjected to saponins extraction; comparison to positive control (BHT-including condition) was also achieved. Lower contents on total polyphenols, total flavonoids and γ -tocopherol, and antioxidant activity (DPPH assay) were observed in saponin-free quinoa extracts from both ecotypes when compared with their saponin-including counterpart. Further, the effect of the different kinds of quinoa extracts was comparatively analyzed in a marine-oil heated model system. As a result, saponin-free quinoa extracts led to marked lipid oxidation inhibition assessed by the determination of conjugated dienes, peroxides, thiobarbituric acid reactive substances and polyenes, this effect being similar to the one produced by saponin-including quinoa extracts. Consequently, the water-washing process did not produce a detrimental effect over the inhibitory capacity of lipid oxidation of quinoa grain when evaluated in a lipid-food system with high degree of unsaturation (i.e., marine-oil model).

Practical application

Quinoa (*Chenopodium quinoa* Willd.) is a highly appreciated pseudo-cereal of Andean origin that has shown a wide range of profitable nutritional and functional properties. However, saponin compounds located in the pericarp of quinoa seed are bitter and interfere with its palatability and digestibility, making difficult to use this grain as a practical food source for commercial processing. In the present study, the antioxidant

behavior of a saponin-free quinoa was investigated and compared to its counterpart saponin-including quinoa. As a result, although the elimination of saponins of quinoa grain led to a loss of phenolic compounds and a consequent decrease in antioxidant activity (assessed by the method of DPPH), this process did not produce a detrimental effect over the inhibitory capacity of lipid oxidation when evaluated in a marine-oil model. Such pre-treatment would avoid the inconveniences of saponins presence while providing a source of potential food additives including antioxidant properties.

1. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a highly appreciated pseudo-cereal of Andean origin that has been used in South America for centuries in the same manner as cereals like wheat and rice [1]. Seeds are the main edible part of the plant showing a balanced composition on amino acids, lipids, trace elements, fiber and vitamins, high contents of proteins, and absence of gluten [2, 3]. Quinoa-derived products and their individual chemical constituents have demonstrated biological activities with various applications to benefit human health and nutrition. Thus, it has successfully been employed in the preparation of emulsion-type products and malted beverages, as a fat/cream substitute, to enhance the quality of baked foods, as well as for the preparation of quinoa protein concentrates [4-6]. Concerning food preservation, an antimicrobial activity has recently been proved [7], while its high content on vitamin E and phenolic compounds has been found responsible for its antioxidant properties [8, 9].

In spite of such great quinoa advantages, saponin compounds located in the pericarp (seed coats) of quinoa seeds avoid its utilization as a practical food source for commercial processing. Quinoa can be classified in accordance with the saponins concentration and its content would depend on the quinoa variety: “sweet” (free from or containing less than 0.11 % of free saponins) or “bitter” (containing more than 0.11 % of free saponins) [10]. Saponins are complex compounds composed of sugar and steroid or triterpenoid moieties which are widely distributed in plants, and have shown beneficial effects such as hypertension prevention [11] as well as anti-inflammatory [12] and antioxidant [13] activities. However, saponins of quinoa are bitter, interfere with quinoa’s palatability and digestibility representing the major drawback concerning commercialization of grain [3, 6]. Additionally, saponins have been reported as being toxic compounds because of their activity in lowering surface tension and hemolytic

activity, which has been attributed to the interaction with cholesterol in the erythrocyte membranes [14, 15].

The present research had as objective to evaluate the effect of the saponin removal pretreatment on the antioxidant capacity of quinoa seeds. With this purpose, saponins content and antioxidant capacity of ethanolic extracts of quinoa seeds, both with and without saponins, were comparatively studied.

2. MATERIALS AND METHODS

2.1. Starting quinoa and preparation of ethanolic extracts

Two different quinoa ecotypes (Ancovinto and Cancosa) cultivated in the Elqui Valley (Vicuña, Chile; 700 m a.s.l.; 30°1'0''S; 70°42'0''W) and harvested in 2011 were used. Homogeneous grains of both quinoa varieties were selected according to similar ripening conditions, color and size; visual inspection was carried out to discard contaminant particles or impurities. For both quinoa varieties, two different grain groups were considered. The first one was washed with distilled water (quinoa/water ratio of 1/10, w/w) at 20 °C with constant stirring for one hour, with water changes every 10 min [4, 16]. After draining the quinoa grains for 5 min, the drying process was carried out at 60 °C using a convective dryer with a constant air flow rate of $2.0 \pm 0.2 \text{ m s}^{-1}$. Samples were dried until they reached constant weight (equilibrium condition); as a result, quinoa grains from Ancovinto (AN batch) and Cancosa (CA batch) ecotypes without saponins were obtained. Moisture contents were 94.04 and 95.11 g kg⁻¹ for AN and CA batches, respectively.

The second group of Ancovinto and Cancosa grains to be tested was directly dried at 60 °C and further processed under the same conditions than for the first grain group [16]. As a result, dried quinoa grains from Ancovinto (ANS batch) and Cancosa

(CAS batch) including saponin compounds were obtained. Moisture contents were 91.75 and 93.23 g kg⁻¹ for ANS and CAS batches, respectively.

Quinoa samples were finely ground and subjected to a 500-micro sieve (Dual Manufacturing Co., U. S. Standard Sieve Series, Sieve N° 35, Chicago, IL, USA). Extracts from the four quinoa batches were obtained from a mixture of 100 g of ground and sieved samples and 1,000 mL of an 80 % aq. (v/v) ethanol solution (dried grains/alcoholic solution ratio of 1/10, w/v) [9]. Each mixture was homogenized and shaken for 24 h in an orbital shaker (BOECO, OS2, Hamburg, Germany), and thereafter centrifuged (Eppendorf, 5804 R, Hamburg, Germany) for 15 min at 5,000 rpm, being the supernatant filtered through a Whatman filter N° 1. Then, the filtrate was concentrated under reduced pressure at 40 °C on a rotary evaporator (Büchi RE 121, Flawil, Switzerland) and afterwards lyophilized in a freeze dryer (Virtis, Advantage Plus, Gardiner, NY, USA). The lyophilized quinoa extracts (ANS, AN, CAS and CA batches) were kept under refrigerated conditions (4 °C) to be employed throughout a 1-month period.

All chemical reagents and solvents were of reagent grade (Merck, Darmstadt, Germany). Otherwise, the kind of reagent is indicated.

2.2. Chemical analyses of lyophilized quinoa extracts

Determination of total saponins (TS) content was carried out based on a modified reversed-phase HPLC procedure described by Quispe-Fuentes, Vega-Gálvez, Miranda, Lemus-Mondaca, Lozano and Kong [17]. For it, an HPLC device (Agilent 1200 Series HPLC system, Santa Clara, CA, USA) including a diode array detector (DAD) set at 210 nm and an automatic injector was employed. The separation was carried out with a Kromasil C-18 RP column (250 × 4.8 mm, 5 µm) at 25 °C. The mobile phase consisted

of water acidified with formic acid (0.1 %, v/v) and acetonitrile. A gradient system was then applied starting with a ratio of 75:25 (water/acetonitrile, v/v) up to 65:35 (water/acetonitrile, v/v) for 15 min with a 0.7 mL min⁻¹ constant flow rate. Then, the ratio of the solvents was changed to 55:45 (water/acetonitrile, v/v) and the flow rate to 1.0 mL min⁻¹ for 20 min. To carry out the analysis, 20 mg of lyophilized quinoa ethanolic extract were dissolved in 1 mL of water, being 5 µL injected in the chromatographic system. For quantitative purposes, a purified extract of total saponins (70 %, w/w) was used as standard. This standard was obtained in our laboratory from quinoa seeds in agreement with the Madl, Sterk, Mittelbach and Rechberger [18] procedure; for it, water/ethanol (1:1, v/v) extraction, lyophilization, column chromatography, thin-layer chromatography and HPLC were employed. Actual results on total saponins content are expressed as g 100 g⁻¹ dry quinoa.

Total polyphenols (TP) content was determined colorimetrically by using the Folin-Ciocalteu (FC) reagent according to Chen, Fan, Yue, Wu and Li [19] with some modifications. A lyophilized quinoa extract of 0.5 g was dissolved in 25 mL of an 80 % aq. ethanol solution (v/v) and kept refrigerated at 4 °C. Then, a 0.5 mL aliquot was transferred to a glass tube; after 5 min, 0.5 mL of the FC reagent and 2 mL of a Na₂CO₃ aq. solution (200 mg mL⁻¹) were added. The mixture was allowed to react for 15 min at room temperature (20 °C). Ten mL of ultra-pure water (Heal Force, Super Series, Shanghai, China) were then added and the resulting precipitate was removed by centrifugation at 5,000 rpm for 5 min. Finally, absorbance was read at 725 nm in an UV-Visible spectrophotometer (PerkinElmer, UV-VIS Lambda 35, Waltham, MA, USA); quantification was achieved by means of a gallic acid (GA) standard calibration curve (0-500 µg GA mL⁻¹). Results were expressed as mg of GA equivalents (GAE) g⁻¹ dry quinoa.

Determination of total flavonoids (TF) content was carried out according to a previously described colorimetric protocol [20] with some modifications. For it, a lyophilized quinoa extract of 0.5 g was dissolved in 25 mL of an 80 % aq. ethanol solution (v/v) and kept refrigerated (4 °C). Then, a 0.5 mL aliquot was mixed with 2.0 mL distilled water in a 15 mL centrifugation tube; after addition of 150 μ L of a NaNO₂ aq. solution (0.5 g L⁻¹), the mixture was allowed to react for 5 min. Then, 150 μ L of a 10 % aq. AlCl₃ solution were added and the mixture was allowed to stand for further 5 min. Finally, 1 mL of a 1 M aq. NaOH solution and 1.2 mL of distilled water were added and the absorbance was read at 510 nm against a blank solution. TF was calculated from a calibration curve using quercetin as standard (0-500 μ g), being the concentration expressed as mg quercetin equivalents (QE) g⁻¹ dry quinoa.

The content of tocopherol compounds (α , β , γ and δ) was determined in the different kinds of quinoa samples according to the method of Cabrini, Landi, Stefanelli, Barzani and Sechi [21] with some modifications. For it, lyophilized quinoa extract (1 g) was homogenized with distilled water (3 mL) in an Ultra Turrax homogenizer (two times for 90 min). The homogenate was then mixed with 80 mM SDS (4 mL), absolute ethanol (8 mL) and n-hexane (8 mL), being the mixture vortexed for 2 min. The separated aqueous phase was again extracted with n-hexane, being the organic extracts pooled together and evaporated under nitrogen flux. The residue was dissolved in methanol and injected for HPLC analysis in agreement with previous research [21]. Tocopherols were identified and quantified using the corresponding external standards. Results were expressed as mg tocopherol kg⁻¹ dry quinoa.

2.3. Assessment of antioxidant activity of lyophilized quinoa extracts

The antioxidant capacity of lyophilized quinoa extracts was measured by means of the DPPH assay, according to the Turkmen, Sali and Velioglu [22] procedure with some modifications. Thus, different 25-mL water dilutions of the quinoa extracts were prepared and tested in order to fit into the trolox calibration curve. For it, an aliquot of 3.9 mL of a 0.5 mM DPPH radical solution in an 80 % aq. ethanol mixture (v/v) was added to 0.1 mL of the quinoa sample extract. The reaction mixture was vortex-mixed for 30 s and left to stand at room temperature in the dark for 30 min. The absorbance was read at 517 nm by means of an UV-Visible spectrophotometer, being an 80 % aq. ethanol mixture (v/v) used to calibrate the spectrophotometer. For quantitative purposes, a calibration curve was made using Trolox as standard (0-1 mM Trolox). The results were expressed as $\mu\text{moles of Trolox equivalents (TE) g}^{-1}$ dry quinoa.

2.4. Antioxidant behavior of quinoa extracts in a model system including a marine oil

Model system preparation and sampling procedure

The antioxidant effect of the different lyophilized quinoa extracts (ANS, AN, CAS and CA batches) was tested in a model system including a marine oil (cod liver oil, CLO; Merck, Darmstadt, Germany). CLO is considered an appropriate model system for studying oxidation mechanisms in food containing significant PUFA levels [23]; previous research has shown its usefulness in order to test the antioxidant behavior of citric acid [24] and jumbo squid (*Dosidicus gigas*) skin extract [25].

This part of the present research was carried out by means of two separated experiments. The first one with Ancovinto batches, while the second one included both Cancosa batches. For it, stock solutions of the different lyophilized quinoa extracts (0.5

g quinoa extract/25 mL aq. 80 % ethanol) were prepared. As a first step for the model system preparation, 0.5 g of CLO were dissolved in a mixture of 14 mL of chloroform and 5 mL of an aq. 80 % ethanol solution (v/v). Then, different amounts of the stock solution of each quinoa extract were added to the oil solution.

In the first experiment, 0.010, 0.050 and 0.250 mL from the ANS stock solution (a quinoa extract/marine oil ratio of 0.4 mg g⁻¹, 2.0 mg g⁻¹ and 10.0 mg g⁻¹, respectively) were added, being the total volume of the model system adjusted to 20 mL with aq. 80 % ethanol. The resulting reacting conditions obtained were named as ANS-1, ANS-2 and ANS-3, respectively. In the case of the AN batch, the same quantities of its stock solution were taken into account, so that AN-1, AN-2 and AN-3 reacting conditions were obtained. A control condition including CLO without quinoa extract was also prepared for the Ancovinto experiment (Control-ANC). Additionally, a BHT (butylated hydroxytoluene) positive control (1 mg BHT/20 mL marine-oil system; 0.005 %, w/v) was tested; this concentration was chosen in agreement with the recommended level of synthetic antioxidants to be employed for the preservation of lipid solutions [26].

The second experiment was carried out with both Cancosa extracts. In agreement with the first experiment, the same quantities of both stock solutions from the CAS and CA batches were taken into account, diluted similarly and named as CAS-1, CAS-2, CAS-3, CA-1, CA-2 and CA-3 reacting conditions, respectively. A control condition including CLO without quinoa extract was also prepared for the Cancosa experiment (Control-CAN). Additionally, a BHT positive control (1 mg BHT/20 mL marine-oil system; 0.005 %, w/v) was tested.

In both experiments, the resulting mixtures were vortexed for 30 s and then stored at 50 °C for up to 30 days. Sampling analyses were carried out after 2, 9, 16, 24 and 30 days, as well as in the initial oil (day 0). Triplicates (n = 3) of each reacting

condition were run. At each sampling time, different and complementary analyses were carried out in order to assess the lipid oxidation progress.

Assessment of the lipid oxidation development in marine oil

Conjugate diene (CD) formation was read at 233 nm following the Kim and Labella [27] procedure. Results obtained are expressed according to the formula $CD = B \times V \times 1000/w$, where B is the absorbance reading at 233 nm (Beckman Coulter DU 640, London, UK), V denotes the aliquot volume (mL) taken of the model-system and w is the aliquot mass (mg) taken of the model-system.

Peroxide value (PV) was determined in the lipid extract by peroxide reduction with ferric thiocyanate, according to the Chapman and McKay [28] method. Results are expressed as meq active oxygen kg^{-1} oil.

Thiobarbituric acid (TBA) value was determined according to Pokorný, Valentová and Davidek [29]. This method is based on the reaction between an aliquot of the model-system sample and thiobarbituric acid at 95 °C for 120 min. Content on thiobarbituric acid reactive substances (TBARS) was spectrophotometrically measured at 532 nm and results are expressed according to the following formula: $TBA \text{ value} = B \times V/w$, where B is the absorbance reading at 532 nm, V denotes the aliquot volume (ml) taken of the model-system and w is the aliquot mass (mg) taken of the model-system.

The fatty acid composition of the CLO was analyzed in samples corresponding to days 16 and 30 of storage at 50 °C. For it, oils were converted into FAME with acetyl chloride and analyzed by gas liquid chromatography (Perkin-Elmer 8700 chromatograph, Waltham, MA, USA), employing a fused silica capillary column SP-2330 (0.25 mm i.d. x 30 m, Supelco Inc., Bellefonte, PA, USA) [30]. The temperature program was as follows: (i) increased from 145 to 190 °C at 1.0 °C min^{-1} , (ii) from 190

°C to 210 °C at 5.0 °C min⁻¹, and (iii) held for 13.5 min at 210 °C. The carrier gas was nitrogen at 10 psi and detection was performed with a flame ionization detector (FID) at 250 °C. A programmed temperature vaporizer injector was employed in the split mode (150:1) and was heated from 45 to 275 °C at 15 °C min⁻¹. Peaks corresponding to FAME were identified by comparing their retention times with those of standard mixtures (Qualmix Fish, Larodan, Malmo, Sweden; FAME Mix, Supelco, Inc.). Peaks were automatically integrated; C 19:0 fatty acid was used as internal standard for quantitative purposes. Content of each fatty acid (FA) was calculated as g FA/100g total FA. Results obtained are expressed by means of the polyene index (PI), which was calculated as the following fatty acids concentrations ratio: (C 20:5 ω 3 + C 22:6 ω 3)/C 16:0.

2.5. Statistical analysis

Chemical composition analyses and antioxidant activity measurements were carried out in triplicate (three independent determinations; n = 3). Data obtained were subjected to one-way ANOVA method (p<0.05) to explore differences as a result of the removal of saponin compounds and the quinoa ecotype.

In the model-system experiment concerning the rancidity stability of the marine oil, each experimental condition (quinoa ecotype and quinoa extract content) considered was analysed throughout the storage time in triplicate (n = 3). Data obtained were subjected to one-way ANOVA method (p<0.05) to explore differences as a result of the following parameters: saponins removal, quinoa extract concentration and storage time (Statsoft, Statistica, version 6.0, 2001).

In all cases, comparison of means was performed using a least-squares difference (LSD) method.

3. RESULTS AND DISCUSSION

3.1. Saponin content and analyses related to antioxidant capacity

TS content was determined in the different kinds of quinoa extracts (Table 1). After the washing step, no saponins could be detected (detection level of 0.003 %) in both quinoa ecotypes extracts (AN and CA batches), this showing that the water washing process was effective. Table 1 also reveals that a higher level of this compounds group was obtained in quinoa from Ancovinto variety (ANS batch) than in its counterpart from Cancosa (CAS batch).

Saponin values obtained in Ancovinto and Cancosa quinoa extracts can be considered in the range of previous studies. As in the actual research, most information accounts for values included in the 0.9-1.4 % range (i.e., bitter quinoa) [31, 32]. With the aim of obtaining sweet quinoa seeds (i.e., lower content than 0.11 %), Gómez-Caravaca et al. [10] reduced the total saponins content from 2,443 (i.e., 0.24 %) to 508.8 (i.e., 0.06 %) mg kg⁻¹ d.w. after applying a 30 % pearling process.

Phenolic compounds present in vegetal samples may act as free radical scavengers, reducing agents, and potential chelators of metal ions, contributing to reduce the oxidative stress [33]. Polyphenols are divided into several subgroups, among which the flavonoids have been the most extensively studied [34]. Accordingly, TP and TF contents were analyzed in the present study.

TP content showed to be included in the 111-202 mg GAE g⁻¹ dry quinoa for all kinds of quinoa samples (Table 1). It can be observed that the process of saponins removal has led to a TP content decrease in both Ancovinto and Cancosa samples. Additionally, a higher content ($p < 0.05$) was observed in samples corresponding to the Ancovinto ecotype (ANS batch) when compared with its counterpart from the Cancosa variety (CAS batch).

Previous research accounts for information concerning the TP content of quinoa samples. Taking into account a fresh basis measurement, Tang et al. [35] studied separately white, red and black quinoa cultivars showing in all cases a TP content included in the 2-12 mg GAE g⁻¹ fresh seed range. Also on fresh basis, Abderrahim et al. [36] showed a 1.23-3.24 mg GAE g⁻¹ fresh seed range, while Gómez-Caravaca et al. [10] obtained 2,610.4±36.8 and 164.6±10.8 mg kg⁻¹ fresh seed for free and bound phenolic compounds, respectively. When the dry basis (d.w.) is employed, Carciochi et al. [9] obtained a 1.036 mg GAE g⁻¹ value, while Miranda et al. [8] showed that initial 0.284 score in raw seeds could decrease to 0.016 mg GAE g⁻¹ as a result of the drying process.

Results obtained for TF compounds are also shown in Table 1. Comparison among the different kinds of quinoa samples led to similar conclusions as for the TP assessment. Thus, a general reduction was implied as a result of the washing step during the saponins removal, while a higher content was obtained in samples from Ancovinto ecotype (comparison between ANS and CAS batches).

Previous studies account for the TF contents assessment in quinoa samples. When the dry basis is considered, Carciochi et al. [9] obtained a 0.25 mg QE g⁻¹ value. By considering the fresh basis, Abderrahim et al. [36] led to a 0.47-2.55 QE g⁻¹ range, while Tang et al. [35] studied white, red and black quinoa cultivars and found a 0.4-1.8 mg catechin equivalents g⁻¹ range.

Tocopherol compounds composition was also analyzed (Table 1). Thus, α - and γ -tocopherol showed to be the most abundant, being δ -tocopherol found in a lower level; no presence of β -tocopherol could be assessed in any of the quinoa samples tested. Lower mean values in all detected tocopherol compounds could be observed for saponin-free quinoa samples. As for total phenolic and flavonoid compounds, this

reduction can be explained on the basis of the water washing process; however, differences were only found significant in the case of γ -tocopherol. Taking into account the mean values obtained, the percentage loss in Ancovinto and Cancosa samples was 7.4 and 9.4 (α -tocopherol), 12.6 and 16.7 (γ -tocopherol) and 10.5 and 11.9 (δ -tocopherol), respectively. Accordingly, the lowest mean loss was obtained in the less polar tocopherol compound (i.e., α -tocopherol).

Previous studies have described the α -tocopherol content of quinoa seeds. Thus, Ruales and Nair [32] obtained a 26 mg kg⁻¹ (dry basis) value, while Graf et al. [6] indicated in their revision a 37-60 mg kg⁻¹ (dry basis) range on the basis of different studies. Current values obtained for both quinoa samples (ANS and CAS) can be considered in agreement with such previous results.

The antioxidant activity was measured by the DPPH assay, this showing values included in the 101-190 μ moles TE g⁻¹ dry quinoa range for all kinds of quinoa samples (Table 1). After the washing step, an antioxidant capacity decrease was implied, this decrease being bigger in the Ancovinto ecotype. Comparison between quinoa ecotypes showed a higher antioxidant activity in quinoa extracts from Ancovinto variety.

Previous research reports on the antioxidant activity assessment of quinoa samples by the DPPH assay. This accounts for 4.2-4.8 [37] and 5-14 [35] μ moles TE g⁻¹ fresh quinoa range and 28.6 % (expressed as percentage of DPPH radical scavenging) [9]. As a result of the drying process of quinoa (60 °C), Miranda et al. [8] showed that an initial value round 2,800 in raw seeds (expressed as IC₅₀-concentration, μ g mL⁻¹ sample) could decrease to values round 100.

According to the present results, the water-washing process has led to a marked loss of polyphenol (TP assessment) and flavonoid (TF assessment) compounds and consequently, to a significant decrease of the antioxidant capacity (i.e., DPPH assay) in

both quinoa ecotypes. In agreement with water extraction ability, hydrophilic polyphenol and flavonoid molecules would be likely to be extracted in a greater extent than lipophilic-type compounds. In this sense, previous research has demonstrated that the use of different extraction methods (i.e., water, methanol, ethanol, acetone, etc.) on different kinds of macroalgae can affect the extraction yield as well as the content on phenolics and other preserving compounds [38-40].

3.2. Antioxidant behavior of quinoa extracts in a marine-oil model system

According to values observed in control samples (Control-ANC and Control-CAN; Table 2), a progressive CD formation ($p < 0.05$) was obtained throughout the storage time, this indicating an increasing lipid oxidation development. Scarce differences could be observed as a result of saponin compounds removal; thus, a lower CD content was observed in some reacting conditions corresponding to saponin-free quinoa extracts (AN-1 at the 24-30-day period; CA-1 at day 24) when compared with their saponin-including counterparts. Ancovinto quinoa extracts showed a marked inhibition of CD formation at the 16-30-day period; additionally, an increasing preservative effect as a result of increasing their contents could be concluded. In the case of the experiment including the quinoa extracts from the Cancosa variety, an inhibitory effect on CD formation could only be detected in the 24-30-day period for reacting conditions corresponding to the two most concentrated quinoa extracts (CA-2, CA-3, CAS-2 and CAS-3). An inhibitory effect on the CD formation in the marine-oil system was also observed for the BHT presence (BHT-ANC and BHT-CAN conditions) in the Ancovinto (16-30-day period) and Cancosa (24-30-day period) experiments, respectively

A marked peroxide formation ($p < 0.05$) could be observed in control samples corresponding to the Ancovinto (Figure 1) and the Cancosa (Figure 2) experiments. Scarce differences could be observed as a result of the previous saponins removal in quinoa samples. In the case of the Ancovinto experiment, the most remarkable result was the lower peroxide content obtained in the AN-1 reacting condition for the 24-30-day period when compared with its counterpart (i.e., ANS-1); further, when the Cancosa experiment is taken into account, a lower PV was obtained in CA-2 and CA-3 reacting conditions at the end of the experiment when compared with their counterpart conditions (i.e., CAS-2 and CAS-3, respectively). In both experiments, quinoa extracts led to an inhibitory effect on peroxide formation in the marine oil for the 16-30-day period when conditions including the two most concentrated quinoa extracts are considered. An inhibitory effect on peroxides content in the marine-oil system was also obtained in both experiments for the positive control (i.e., BHT-including conditions) in the 24-30-day period.

Secondary lipid oxidation development was measured by means of the TBARS content. Both in the Ancovinto (Figure 3) as well as in the Cancosa (Figure 4) experiments, control samples (Control-ANC and Control-CAN) revealed a marked formation ($p < 0.05$) of such kinds of compounds throughout the storage time. Scarce differences could be observed as a result of previous saponins removal in quinoa extracts. Thus, in the Ancovinto experiment, the most remarkable effect was a lower TBARS content in samples corresponding to the AN-1 reacting condition for the 16-30-day period when compared with their saponin-including counterparts; contrary, some higher TBARS levels were obtained in samples belonging to the AN-3 reacting condition for the 16-30-day period. Concerning the Cancosa experiment, a clear tendency could not be implied as a result of previous saponins removal; thus, some

higher values were observed in samples corresponding to the CA-3 condition when compared with their saponin-including counterparts (16-24-day period), while lower values were found in saponin-free samples from CA-2 reacting condition at the end of the experiment. Both kinds of Ancovinto extracts led to an inhibitory effect at day 16, while in the 24-30-day period only reacting conditions belonging to the two most concentrated quinoa extracts (AN-2, AN-3, ANS-2 and ANS-3) maintained this inhibitory effect (Figure 3). Concerning the Cancosa experiment, an inhibitory effect on TBARS formation was obtained in the 9-24-day period for both kinds of quinoa extracts (Figure 4); at the end of the experiment, this inhibitory effect was only maintained in the reacting conditions including the two most concentrated quinoa extracts (CA-2, CA-3, CAS-2 and CAS-3). An inhibitory effect on the TBARS formation in the marine-oil system was also observed for the BHT presence in the Ancovinto (16-30-day period) and the Cancosa (9-30-day period) experiments.

Marine oil damage was also determined by the possible PUFA content loss (i.e., PI assessment). This quality index showed a slight decrease in the marine oil throughout the storage time (Table 3) in agreement with all the previously mentioned lipid oxidation indices. No effect ($p>0.05$) on the PI value was implied as a result of saponins removal in both quinoa varieties. Meantime, a general inhibitory effect of PUFA damage was obtained in the reacting conditions including Ancovinto extracts at both 16 and 30 days for all the quinoa concentrations tested; this effect was also observed in the BHT-including condition (namely, BHT-ANC). Concerning the Cancosa study, a general inhibitory effect of PUFA damage was obtained in all reacting conditions at day 16; however, at the end of the experiment, only the reacting conditions including the two most concentrated quinoa extracts (CA-2, CA-3, CAS-2 and CAS-3) showed higher

PI values than the Control-CAN condition; meantime, an inhibitory effect on PUFA loss was observed in the reacting conditions including BHT (i.e., BHT-CAN).

Lipid oxidation development has been recognized as a complex process in which different kinds of molecules are produced throughout the time course, most of them unstable and susceptible to breakdown that originate lower weight compounds or react with other molecules (nucleophilic-type, mostly) present in the reaction medium. So, the determination of individual compounds cannot always provide an accurate method for the quality loss assessment of a lipid and complementary analyses have to be carried out [41, 42]. In the present research (Ancovinto and Cancosa experiments), all lipid oxidation indices tested showed to be sensitive throughout the time course. Thus, the different indices tested revealed no lipid oxidation development throughout the 0-9-day period. Then (16-30-day period), a progressive lipid oxidation development could be observed in control conditions (Control-ANC and Control-CAN) for all indices tested, except for the CD assessment in Cancosa samples (24-30-day period). Consequently, antioxidant compounds present in the quinoa extracts (namely, the two most concentrated conditions) have shown an inhibitory effect on both primary (i.e., CD and peroxides) and secondary (TBARS) compounds formation in a heated marine-oil model system. Accordingly, quinoa ethanolic extracts have shown to be able to partially inhibit the stage of radical addition to the double bonds structure of lipids (i.e., formation of primary compounds), as well as have shown to be able to partially avoid the breakdown stage of primary compounds into secondary ones. Such preserving effect of quinoa extracts is in agreement with the above-mentioned presence of antioxidant compounds such as polyphenols, flavonoids and tocopherols, as well as the antioxidant capacity proved by means of the DPPH assay. Previous research has shown an important antioxidant activity in quinoa extracts, being this effect attributed to the presence of

polyphenolic compounds and vitamin E [8, 9]. In spite of the fact that a marked loss of TP and TF contents was produced in quinoa extracts as a result of the previous water-washing process intended for saponin removal, the results obtained from the marine oil model system have shown that such pretreatment did not produce a decrease of the antioxidant capacity of the ethanol extract in both quinoa ecotypes. As an explanation for this apparently contradictory result, it could be argued that the actual model system is a lipid-type medium, in which lipophilic-type antioxidants would be likely to be more active than hydrophilic ones. In agreement with the previous section results, since the water-washing process would mostly eliminate preservative hydrophilic molecules, a negative effect on the antioxidant capacity of the quinoa extract in the actual lipid-type model system would not be expected to occur.

The antioxidant activity has shown to depend on many factors, most of them related to the antioxidant structure, the food system to be stabilized and conditions of storage/processing [43]. As a result, the activity of such preservative compounds is often difficult to predict, because of the different mechanisms involved in the antioxidant effectiveness [44, 45]. The distribution of antioxidants in multiphase systems is thus an important parameter necessary to clarify the antioxidant mechanism and its relative effectiveness; thus, an important role of the more or less polar molecular structure has been observed in the partitioning coefficients between oil and water phases [46]. When a lipid-type medium is concerned as in the actual case, the effectiveness of oxidation inhibition has seemed to be more dependent on the physico-chemical properties (i.e., lipophilic/hydrophilic balance), rather than on the intrinsic redox capacity of the antioxidant (i.e., number and type of hydroxy groups, ability to delocalize radicals, and so on) [47].

A wide number of studies account for the antioxidant effect in lipid-type systems (i.e., marine oils) of lipophilic-type extracts of different natural sources. Concerning marine-origin extracts, an acetic acid-ethanol extract of jumbo squid (*Dosidicus gigas*) skin showed an antioxidant behavior in a marine-oil system [25], a methanolic extract of red alga (*Grateloupia filicina*) led to a lipid peroxidation inhibition in a fish-oil model [48], and an ethanol extract of different kinds of Danish algae led to a higher oxidative stability of fish oil during an accelerated test (Oxipress) [45]. Related to extracts from plant sources, pomegranate peel (*Punica granatum*) ethanolic extract inhibited lipid oxidation development in anchovy-fish-oil system [49], oregano and rosemary methanol extracts proved their capabilities in retarding oxidation of menhaden (*Brevoortia tyrannus*) oil [50] and extra virgin olive oil proved a preservative effect on lipid composition of canned albacore (*Thunnus alalunga*) when employed as coating medium [51].

4. CONCLUSIONS

Total saponin content and antioxidant properties and effectiveness of saponin-free quinoa were comparatively studied with quinoa including saponin compounds, taking into account two different quinoa ecotypes (i.e., Ancovinto and Cancosa). Thus, saponin-free quinoa showed lower ($p<0.05$) total polyphenols, total flavonoids and γ -tocopherol contents and lower ($p<0.05$) antioxidant capacity (DPPH assay). However, when a heated (50 °C for 30 days) marine-oil model system was evaluated, addition of saponin-free quinoa extracts led to a marked lipid oxidation inhibition (assessment of conjugated diene, peroxide, TBARS and polyene contents) in the oil, this result being similar to the rancidity inhibition produced by saponin-including extracts for both quinoa ecotypes. It is probable that water-washing process would have extracted

hydrophilic polyphenols and flavonoids, whose preservative effect in the actual lipophilic-type system would hardly be expected to occur. Consequently, saponin-free quinoa has shown to provide a suitable product including lipophilic-type antioxidant molecules, susceptible to develop an active preservative role in lipid-food systems with high degree of unsaturation.

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FIGURE LEGENDS

Figure 1: Peroxide value assessment (meq active oxygen kg⁻¹ oil)* in marine-oil systems in the presence of Ancovinto quinoa extracts**

* Mean values of three replicates (n=3); standard deviations are indicated by bars. For each storage time, values accompanied by different letters (a-e) denote significant differences (p<0.05). Initial marine-oil value: 12.19±0.13.

** Abbreviations employed as expressed in Table 2.

Figure 2: Peroxide value assessment (meq active oxygen kg⁻¹ oil)* in marine-oil systems in the presence of Cancosa quinoa extracts**

* Mean values of three replicates (n=3); standard deviations are indicated by bars. For each storage time, values followed by different letters (a-d) denote significant differences (p<0.05). Initial marine-oil value: 10.74±0.13.

** Abbreviations employed as expressed in Table 2.

Figure 3: Thiobarbituric acid value assessment* in marine-oil systems in the presence of Ancovinto quinoa extracts**

* Mean values of three replicates (n=3); standard deviations are indicated by bars. Units as expressed in the Materials and Methods section. For each storage time, values accompanied by different letters (a-d) denote significant differences (p<0.05). Initial marine-oil value: 8.55±0.21.

** Abbreviations employed as expressed in Table 2.

Figure 4: Thiobarbituric acid value assessment* in marine-oil systems in the presence of Cancosa quinoa extracts**

* Mean values of three replicates (n=3); standard deviations are indicated by bars. Units as expressed in the Materials and Methods section. For each storage time, values accompanied by different letters (a-d) denote significant differences ($p < 0.05$).

Initial marine-oil value: 10.70 ± 0.14 .

** Abbreviations employed as expressed in Table 2.

TABLE 1: Chemical determinations* related to composition and antioxidant behavior of lyophilized Ancovinto and Cancosa quinoa extracts**

Chemical analysis	Quinoa extract			
	ANS	AN	CAS	CA
TS (g 100 g ⁻¹ dry quinoa)	1.15 b (0.05)	ND	0.84 a (0.06)	ND
TP (mg GAE g ⁻¹ dry quinoa)	201.61 c (3.18)	142.85 b (0.08)	147.93 b (0.59)	111.48 a (0.54)
TF (mg QE g ⁻¹ dry quinoa)	51.24 c (2.91)	31.83 b (0.20)	31.75 b (0.53)	25.26 a (1.02)
α-tocopherol (mg kg ⁻¹ dry quinoa)	63.11 b (2.34)	58.43 b (2.76)	48.56 a (3.71)	44.00 a (1.87)
β-tocopherol (mg kg ⁻¹ dry quinoa)	ND	ND	ND	ND
γ-tocopherol (mg kg ⁻¹ dry quinoa)	58.71 d (1.65)	51.29 c (2.39)	43.18 b (1.17)	35.97 a (1.94)
δ-tocopherol (mg kg ⁻¹ dry quinoa)	10.34 c (0.56)	9.25 bc (0.61)	8.85 ab (0.25)	7.80 a (0.57)
DPPH assay (μmoles TE g ⁻¹ dry quinoa)	189.77 d (1.19)	114.99 b (0.75)	155.67 c (2.29)	101.51 a (1.20)

- * In each row, mean values followed by different letters (a, b, c, d) denote significant differences ($p < 0.05$) among quinoa samples.
- ** ANS and CAS denote Ancovinto and Cancosa lyophilized samples including saponins. AN and CA denote Ancovinto and Cancosa lyophilized samples not including saponins. Other abbreviations employed: TS (total saponins), TP (total polyphenols), TF (total flavonoids), GAE (gallic acid equivalent), QE (quercetin equivalent), TE (trolox equivalent), DPPH (2,2-diphenyl-1-picrylhydrazyl) and ND (not detected).

TABLE 2: Conjugated diene formation* in cod-liver-oil system in the presence of Ancovinto and Cancosa quinoa extracts**

Sample	Storage time (days)				
	2	9	16	24	30
Control- ANC	0.82	0.85	0.91 b	0.99 d	1.8 d
	(0.03)	(0.03)	(0.01)	(0.04)	(0.05)
BHT-ANC	0.83	0.84	0.87 a	0.90 bc	0.89 ab
	(0.01)	(0.03)	(0.01)	(0.02)	(0.02)
ANS-1	0.82	0.84	0.85 a	0.93 c	0.97 c
	(0.01)	(0.01)	(0.02)	(0.00)	(0.01)
AN-1	0.82	0.84	0.87 a	0.84 a	0.91 b
	(0.06)	(0.04)	(0.01)	(0.01)	(0.02)
ANS-2	0.83	0.84	0.87 a	0.85 a	0.87 a
	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)
AN-2	0.85	0.86	0.87 a	0.84 a	0.87 a
	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)
ANS-3	0.84	0.87	0.86 a	0.85 ab	0.87 a
	(0.01)	(0.01)	(0.03)	(0.03)	(0.01)
AN-3	0.84	0.88	0.86 a	0.87 ab	0.89 ab
	(0.01)	(0.02)	(0.02)	(0.02)	(0.01)
Control-CAN	0.81	0.84	0.86	0.93 d	0.98 b
	(0.03)	(0.01)	(0.02)	(0.01)	(0.05)
BHT-CAN	0.82	0.80	0.83	0.86 bc	0.84 a
	(0.02)	(0.03)	(0.02)	(0.01)	(0.02)

CAS-1	0.84 (0.01)	0.83 (0.02)	0.83 (0.03)	0.90 cd (0.03)	0.94 b (0.01)
CA-1	0.81 (0.02)	0.81 (0.03)	0.82 (0.02)	0.84 ab (0.02)	0.92 b (0.02)
CAS-2	0.84 (0.04)	0.84 (0.00)	0.82 (0.03)	0.81 a (0.02)	0.81 a (0.03)
CA-2	0.84 (0.01)	0.85 (0.01)	0.83 (0.02)	0.84 ab (0.02)	0.83 a (0.01)
CAS-3	0.85 (0.02)	0.84 (0.01)	0.86 (0.02)	0.83 ab (0.01)	0.82 a (0.01)
CA-3	0.82 (0.01)	0.84 (0.02)	0.84 (0.01)	0.83 ab (0.02)	0.86 a (0.02)

* Mean values of three replicates (n=3); standard deviations are indicated in brackets. Units as expressed in the Material and Methods section. For each quinoa ecotype and storage time, mean values followed by different letters (a, b, c, d) denote significant differences ($p < 0.05$). No letters are included when no significant differences ($p > 0.05$) are found. Initial oil values: 0.80 ± 0.01 (day 0; Ancovinto experiment) and 0.81 ± 0.02 (day 0; Cancosa experiment).

** According to the Material and Methods section, different quinoa extract/marine oil ratios (0.2 mg/0.5 g, 1.0 mg/0.5 g and 5.0 mg/0.5 g) were tested for lyophilized extracts obtained from Ancovinto and Cancosa quinoa with (ANS-1, ANS-2, ANS-3 and CAS-1, CAS-2, CAS-3, respectively) and without (AN-1, AN-2, AN-3 and CA-1, CA-2 and CA-3, respectively) saponin compounds. Marine-oil (Control-ANC and Control-CAN) and positive (BHT-ANC and BHT-CAN) controls as expressed in the Materials and Methods section.

TABLE 3: Polyene index assessment* in cod-liver-oil system in the presence of Ancovinto and Cancosa quinoa extracts**

Sample	Storage time (days)	
	16	30
Control-ANC	1.60 a	1.54 a
	(0.00)	(0.01)
BHT-ANC	1.63 b	1.61 b
	(0.01)	(0.02)
ANS-1	1.63 b	1.59 b
	(0.01)	(0.02)
AN-1	1.64 b	1.61 b
	(0.00)	(0.03)
ANS-2	1.63 b	1.63 b
	(0.01)	(0.02)
AN-2	1.63 b	1.63 b
	(0.01)	(0.01)
ANS-3	1.62 ab	1.61 b
	(0.03)	(0.00)
AN-3	1.64 b	1.62 b
	(0.01)	(0.00)
Control-CAN	1.61 a	1.58 a
	(0.01)	(0.02)
BHT-CAN	1.65 b	1.63 bc

	(0.01)	(0.01)
CAS-1	1.65 b (0.01)	1.62 abc (0.03)
CA-1	1.65 b (0.01)	1.61 ab (0.01)
CAS-2	1.66 b (0.00)	1.66 c (0.01)
CA-2	1.65 b (0.00)	1.69 cd (0.03)
CAS-3	1.66 b (0.00)	1.66 c (0.01)
CA-3	1.66 b (0.03)	1.65 c (0.01)

* Mean values of three replicates (n=3); standard deviations are indicated in brackets. For each quinoa ecotype and storage time, mean values followed by different letters (a, b, c, d) denote significant differences ($p < 0.05$). Initial oil values: 1.65 ± 0.01 (day 0; Ancovinto experiment) and 1.63 ± 0.01 (day 0; Cancosa experiment).

** Sample names as expressed in Table 2.

Figure 1

Figure 2

Figure 3

Figure 4