



Research article

Effect of geographical location, processing and simulated digestion on antioxidant characteristics of quince (*Cydonia oblonga*)

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ABSTRACT

Quince fruit (*Cydonia oblonga* Miller) is an important source of bioactive compounds, especially of phenolic compounds, that's why it is considered a good source of antioxidants. However, it is known that the antioxidant characteristics and the beneficial effect of foods are affected by the variety, geographical origin, processing and gastrointestinal digestion. In this work, the effects of the geographical origin, processing, and simulated digestion of quince jam on its antioxidant characteristics were studied. Phenolic composition and antioxidant capacity were determined in quince fruit and jam from four different locations in San Juan, Argentina. The results showed that quince fruit samples from St. Lucia had the highest total phenolic content (5.13 mg GAE/g; $p < 0.05$) and *in vitro* antioxidant capacity measured by ferric-reducing antioxidant power assay (FRAP) and radical-scavenging capacity assay (DPPH[•] and ABTS^{•+}). With regard to quince jam, a positive effect was observed on its antioxidant characteristics after processing. Twenty-one phenolics were detected in jam, being most of them derivatives of hydroxycinnamic acids (eg. 5-caffeoylquinic acid; 4-caffeoylquinic acid and quinic acid). Studies on stability and bioaccessibility of quince phenolics showed that less than 25% of the initial phenolics in jam were detected after digestion being quinic acid and hydroxycinnamic acids the most resistant. The *in vitro* antioxidant capacity showed, in general, a similar trend to the phenolics content throughout the digestion process. The results obtained showed that the antioxidant activity of quince and jam is related to the type and amount of phenolics in the samples, which depends on the geographical origin, processing, and gastrointestinal digestion. These variables are not always taken into account when studying the probable antioxidant activity of food, even though they should be considered for a complete nutritional evaluation of a food.

1. Introduction

Quince is a fruit tree from the ancient wild *Cydonia* species of the Caucasus region, located in warm Southwest Asia (Iran and Turkey). It has been cultivated in different regions due to the resistance of the species, and the excellent nutritional, culinary and therapeutic properties of its fruit. Quince (*Cydonia oblonga* Miller) belongs to the family Rosaceae and its fruits are not generally consumed without prior processing, because of hardness, acidity, bitterness, and astringency of the pulp. The fruit is cooked with sugar and used to make different jams, such as marmalades, compotes, jellies and liqueurs (Silva et al., 2000, 2004).

From the chemical point of view, the edible part of the quince fruit is important, due to its health promoting properties. The pulp of fruits is

composed of sugars, organic acids, polysaccharides, and, in smaller proportion, other compounds such as proteins, lipids, vitamins and phenolics. Beneficial effect of quince phenolics to human health are recognized (Silva et al., 2002, 2004; Baroni et al., 2018; Sut et al., 2019; Khan and Ahmad, 2021; Ashraf et al., 2016). Hydroxycinnamic acids (caffeoyl and coumaroyl derivatives) and flavonols (quercetin and kaempferol derivatives) are the major phenolic compounds, while flavanols (catechin and epicatechin) are found in smaller proportion (Silva et al., 2002, 2004; Baroni et al., 2018). It has been shown that the content of polyphenolics, besides the variety, may be affected by many other factors, such as geographical origin (soil characteristics, climate condition), among others (Cascales and García, 2021; Rasheed et al., 2018).

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The production of quince fruits and their different processing products is a tradition in the province of San Juan, Argentina. The excellent development of *Cydonia oblonga* Miller cultivars in mentioned province is possible thanks to the plant versatility and adaptation to saline soils, since it is capable of adapting itself to warm as well as dry zones. Given

the soil and climatic characteristics of San Juan, quince has high pectin content; consequently, the fruit and jam are different from those produced in other geographical areas (Alimentos Argentinos, 2019).

Raw quince is recognized for its high content of phenolics (Legua et al., 2013; Szychowska et al., 2014). As published in a recent study,

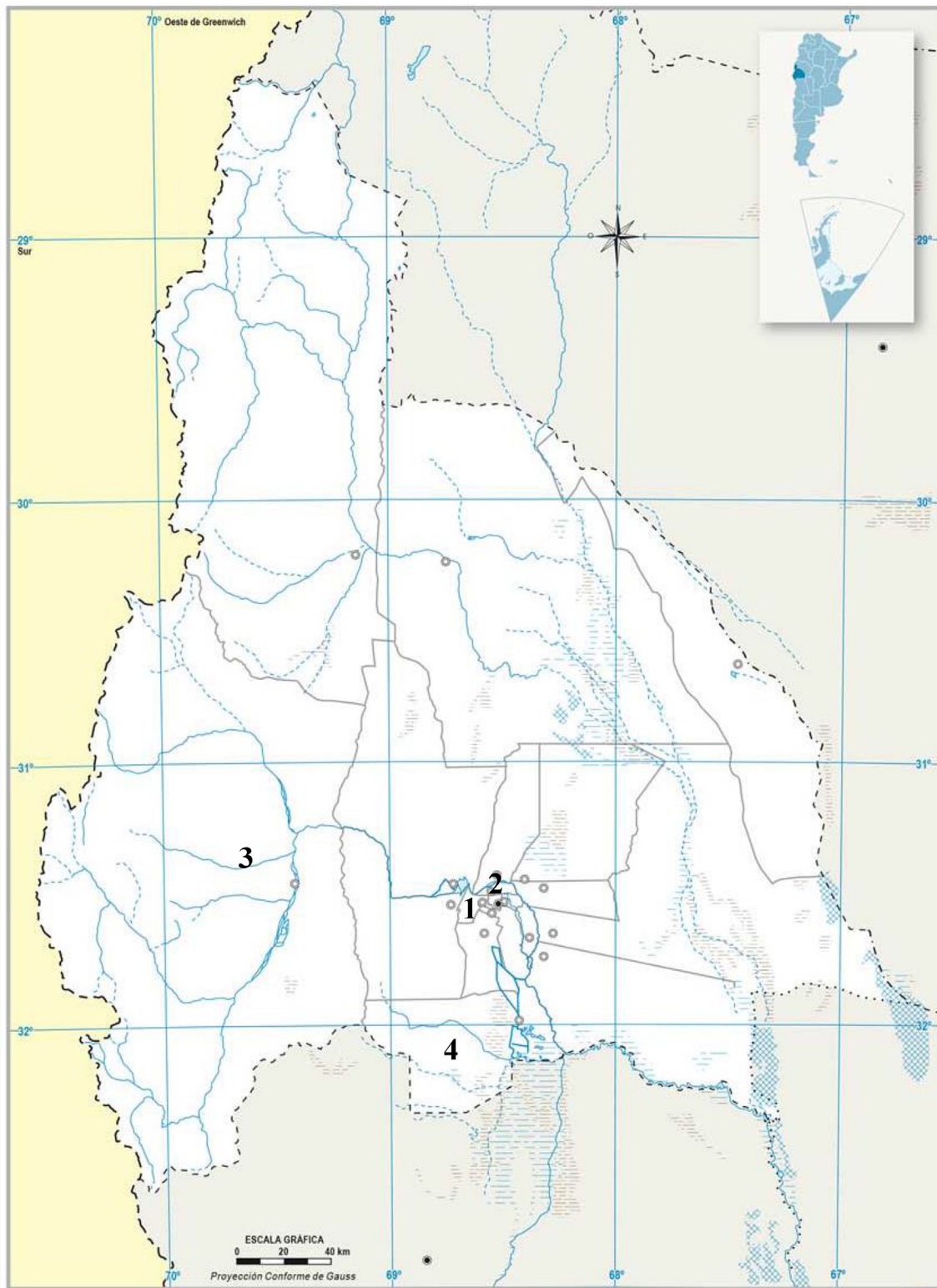


Figure 1. Location of sampling areas of quince fruits in the province of San Juan. 1- Department of Rivadavia, 2 - Department of Santa Lucía; 3 – Department of Calingasta and 4 – Department of Sarmiento. Source: Instituto Geográfico Nacional. <https://www.ign.gob.ar/AreaServicios/Descargas/MapasEscolares#nanogallery/gallery21/0/78>, <https://www.ign.gob.ar/AreaServicios/Biblioteca/citar>.

jam processing did not produce a decrease in phenolic content, and, in some cases, the phenolic content even increased (Baroni et al., 2018) in agreement with the results showed in other foods such as peppers, pumpkin, peas, leeks, spinach, broccoli, garlic and onions (Williamson and Manach, 2005).

Although it is true that the presence of bioactive compounds in the diet is associated with health benefits, their effects depend on the biochemical state in which they reach the bloodstream, and therefore the tissues. On the other hand, the digestion process itself involves a series of changes in macro and micronutrients affecting their final bioavailability (bioaccessibility and bioabsorption) to be later used by the organism (Parada and Aguilera, 2007). At the present time, different models simulating *in vitro* gastrointestinal digestion have been developed and used in different food matrices like orange juice, whole-wheat flour pasta, cookies, red grapes, wine, among other foods (Gil-Izquierdo et al., 2001; Lingua et al., 2018; Podio et al., 2019; Lucini Mas et al., 2020). However, to our knowledge, no studies on the *in vitro* bioaccessibility of quince jam phenolics have been published.

Thus, the general aim of this research was to evaluate the antioxidant characteristics of quince fruit and jam collected from different locations in San Juan, Argentina. Moreover, the effects of processing and simulated digestion on antioxidant characteristics of quince jam were also evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Methanol (HPLC grade) and formic acid (puriss. p.a. for mass spectroscopy) were obtained from J. T. Baker (State of Mexico, Mexico) and Fluka (Steinheim, Germany), respectively. Commercial standard of (+) catechin were purchased from Extrasynthese (Genay, France). Chlorogenic acid, kaempferol and quercetin were purchased from Fluka (Dorset, U.K.). Filters (0.45 μm , HVLP04700) were obtained from Millipore (São Paulo, Brazil). ABTS (2,2'-azino-bis-(3-thylbenzothiazolone-6-sulfonic acid) diammonium salt), DPPH (1,1-diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-tripyridyl- S-triazine), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2- carboxylic acid), Folin-Ciocalteu Reagent, pepsin (P-7000, from porcine stomach mucosa), pancreatin (P-1750, from porcine pancreas) and bile extract (from porcine, B-8631) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). SnakeSkin dialysis bags with a molecular weight cut-off of 10 kDa and a width of 22 mm were obtained from ThermoFisher SCIENTIFIC. All other reagents were of analytical grade.

2.2. Samples

Samples of fresh quince fruit were collected from local farms in the province of San Juan, Argentina: Media Agua (Department of Sarmiento), Calingasta, La Bebida (Department of Rivadavia) and Santa Lucia in March–April 2017 (Figure 1). The different sampling locations were selected based on their geographic and climatic conditions. The Calingasta region is located at the bottom of the Andes Mountain Range, so it has a clearly mountainous relief climate. The Sarmiento region belongs to the pre-mountain section and, therefore, it has a mountainous relief climate, being mainly at the foot of the mountain. The Rivadavia region has two distinct geographical regions, one mountainous part, and another flat part belonging to the Tulum Valley, an area where mainly agricultural activities are developed. Finally, the Santa Lucia area also belongs to the Tulum Valley area. It is located in the alluvial cone of the San Juan River, which produces waterlogging and salinization of the land, due to the geological and climatic characteristics of the region (arid, semi-arid with salt flats).

A total of 30 fruits per locality were collected to make quince jam, of which $n = 8$ randomly selected fruits were used for fresh analysis. Fruits were harvested by hand in September and October, when the fruit has

acquired a greenish-yellow colour and gives off an intense aroma. The fruit is pear-shaped, yellowish-green in colour, with a hard skin covered with greyish pilosity, which gradually fades as it ripens in autumn. All samples were stored at 20–25 °C in the dark until analysis within 2–5 days after sampling. The quinces were washed, weighed, and manually peeled.

2.3. Preparation of quince jam

Prior to the preparation of the jam, the fruit was scalded for 5–10 min and then crushed. Quince jam was prepared in the laboratory by boiling the ground fruit with sugar (50:50 ratio), for approximately 40–50 min (65–68 °Brix). It was subsequently placed in molds and stored at 20–25 °C in the dark at room temperature (25 ± 2 °C) until it reaches a controlled consistency, soft and firm but not hard (4–5 days), so that it can be cut with a knife with a clean cut.

2.4. Phenolic compound extraction

The extraction of phenolic compounds from quince fruit and jam was carried out as described by Baroni et al. (2018). Briefly, lyophilized samples were extracted in triplicate with methanol acidified with 0.1% HCl v/v in a blender (T 18 digital ULTRA-TURRAX, IKA Labortechnik, Germany). These extracts were used for phenolic profile and antioxidant capacity determinations.

2.5. Simulated *in vitro* gastrointestinal (GI) digestion

For jam samples, the assay was performed according to Lingua et al. (2018) and Minekus et al. (2014). Mouth, stomach (gastric) and small intestine (duodenal) digestion steps were performed (Minekus et al., 2014; Podio et al., 2019).

To simulate mouth digestion, four grams of fresh quince jam were homogenized with freshly collected human saliva (3 mL) for 30 s at 24,000 rpm in an Ultra-Turrax T18 blender (Ika-Labortechnik, Germany).

For gastric digestion, the pH was adjusted to 2 with 6M HCl in samples obtained from the mouth digestion. Then, they were incubated in the dark for 2 h at 37 °C in the presence of 500 μL of a pepsin solution (20 mg of pepsin in 500 μL of 0.1 M HCl pH = 2). Finally, for the small intestine digestion a dialysis step was included in order to simulate passive absorption. In this sense, samples obtained from de gastric digestion were incubated with a solution of a pancreatin/porcine bile (15 mg of porcine pancreatin plus 75 mg of porcine bile extract in 3 mL of 0.1 M NaHCO_3 , pH = 7.5). This mixture was placed inside a dialysis bag and dialyzed against 0.1M NaHCO_3 , pH = 7.5 (25 mL of 0.1 M NaHCO_3) for 2 h at 37 °C. The non-dialyzable fraction remaining inside the bag, represents the fraction that would be colon available and the dialysate would be the fraction that would potentially be absorbed by passive diffusion. In order to inactivate enzymes and neutralize NaHCO_3 , both fractions were acidified with formic acid to pH = 2.

All samples were centrifuged at 13,000 g for 10 min and stored at -80 °C until analysis.

Three independent experiments were conducted. Simultaneously, two blank samples (with distilled water instead of jam) were processed and analyzed.

2.6. Analysis of phenolic compounds

2.6.1. Determination of total phenolics (TP)

The TP content of samples was determined by the Folin Ciocalteu method, according to Singleton and Rossi (1965) and Lingua et al. (2018); using gallic acid as standard. Results are expressed as mg of phenolics (equivalent to gallic acid) per g of fruit or jam fresh weight (mg GAE/g FW). All samples were analyzed in triplicate.

2.6.2. Determination of phenolic profile

The phenolic profile was analyzed by HPLC-MS/MS according to Lingua et al. (2018).

For polyphenol identification, retention times, UV/Vis spectra, high-resolution MS and MS/MS spectra were used and compared to authentic standards when available or compared to data reported in literature. For polyphenol quantification, the area of extracted ion chromatograms were used with external calibration plots, constructed by linear regression from available phenolic standards. When reference compounds were not available, a calibration plot from a structurally related compound was used. Phenolic acids were quantified as chlorogenic acid; quercetin and quercetin derivatives as quercetin; kaempferol and kaempferol derivatives as kaempferol; and flavanol compounds as (+)-catechin. The calibration plots were prepared by appropriate dilution from stock solutions in methanol (containing 1 g/L of the pure compound). The limits of detection (LOD) and quantification (LOQ) of the method were calculated considering the signal-to-noise ratio (LOD: $S/N \geq 3$; LOQ: $S/N \geq 10$). LOQ ranged from 0.023 to 0.066 mg/L. All samples and standard solutions were diluted (when required), filtered (0.45 μm), and injected in the HPLC-DAD-MS/MS system. All HPLC runs were performed in triplicate. The results are expressed as mg/100 g jam fresh weight (FW). Recovery % for digestion samples, are calculated as % of original amount in quince jam.

2.7. Estimation of the in vitro antioxidant capacity (AC)

The *in vitro* antioxidant capacity was measured through the evaluation of the reducing power (FRAP) and the radical-scavenging capacity (DPPH and ABTS⁺). Results are expressed in mmol Trolox equivalents per g of fruit or jam fresh weight (mg Trolox/g FW). All samples were analyzed in triplicate.

2.7.1. Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to Benzie and Strain (1996). The working FRAP reagent was prepared as required by mixing 25 mL acetate buffer (300 mM, pH = 3.6), 2.5 mL of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and 2.5 mL 20 mM FeCl₃·6H₂O solutions. Briefly, 100 μL of an appropriately diluted sample was combined with the appropriate reagent and detected at 593 nm.

2.7.2. ABTS⁺ cation radical-scavenging capacity assay

The ABTS⁺ assay, developed by Re et al. (1999), was used in this study. Briefly, the ABTS radical was produced by reacting 7 mM ABTS and 2.45 mM potassium persulfate (final concentration in 10 mL of water), keeping the mixture in the dark at room temperature for 16 h before use. The aqueous ABTS⁺ solution was diluted with methanol to an absorbance of 0.80 ± 0.02 at 734 nm. 100 μL of an appropriately diluted sample was combined with the corresponding reagent and detected at 734 nm.

2.7.3. DPPH radical-scavenging capacity assay

The DPPH assay was performed in accordance with Brand-Williams et al. (1995). DPPH reactive was dissolved in methanol (60 μM) and prepared daily. Briefly, 100 μL of an appropriately diluted sample was combined with the corresponding reagent and detected at 515 nm.

2.8. Statistical analysis

The data are presented as mean \pm SD. ANOVA with mixed models (Di Rienzo et al., 2010) was performed with each variable to assess variations in findings. In the event of significance ($p < 0.05$), a DGC test (Di Rienzo et al., 2002) was used to identify differences between the means. ANOVA analyses were performed using the Infostat software package. In all figures and tables, different letters mean statistically significant differences. To study the relationship between the samples and variables, a principal

component analysis (PCA) was performed using MetaboAnalyst 5.0 software. Data were normalized by sum and auto-scaled before analysis.

3. Results and discussion

3.1. Phenolic content and antioxidant activity of quince fruit from different geographical origins

The total phenol content (TP) and antioxidant capacity (AC) for the quince fruit from different geographical origins are presented in Figure 2. TP showed values between 2.8 and 5 mg of GAE/g of fruit (fresh weight). The samples from La Bebida and Santa Lucia areas possessed significantly higher values ($p < 0.05$) than the rest. Hamauzu et al. (2006) reported similar results for TP in quince fruits from Japan. Regarding the AC, the values obtained were 2–5 mg Trolox/g fruit; 1.15–3 mg Trolox/g fruit and 1.3–3.5 mg Trolox/g fruit for FRAP, DPPH and ABTS⁺, respectively. The results for antioxidant capacity showed a similar trend to those obtained for total phenolics, with the samples from La Bebida and Santa Lucia being the ones with significantly higher values ($p < 0.05$). Similar results for AC were obtained by Wojdylo et al. (2013a) for quince fruits of thirteen different varieties from Poland.

3.2. Phenolics content and antioxidant activity of quince jam from different geographical origins

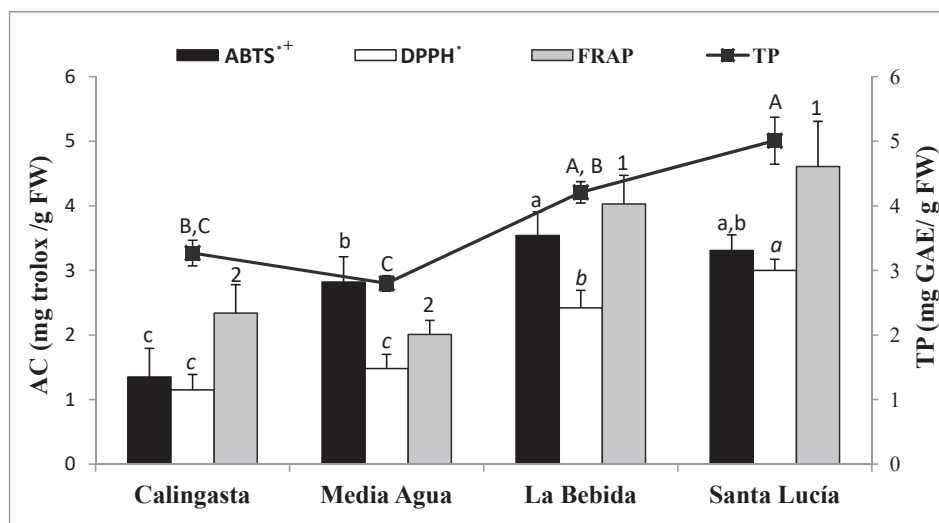
As explained in the introduction, due to the astringent characteristics of this fruit, its main form of consumption is not fresh but processed. So, the antioxidant characteristics of jam produced in different locations in order to understand the effect of processing on their characteristics were evaluated.

The results for TP and AC for quince jam are presented in Figure 3. The total polyphenol content showed values between 2.4 and 4 mg of GAE/g of jam. Quince jam from Santa Lucia possessed significantly higher values ($p < 0.05$) than the rest of the samples. The results obtained for TP are in accordance with results previously published by other authors (Silva et al., 2002, 2004; Wojdylo et al., 2013b).

With regard to antioxidant capacity, the values obtained were 2.2–3 mg Trolox/g of jam, 1.4–2.3 mg Trolox/g of jam, and 1.1–2 mg Trolox/g of jam for FRAP, DPPH, and ABTS⁺, respectively. DPPH assay did not show differences between the origins of fruit. Jams produced from fruits from Media Aqua and Santa Lucia were, in general, the ones that presented a higher antioxidant capacity (ABTS⁺ assay). Similar results for antioxidant capacity assays were previously reported by other authors in quince jam (Wojdylo et al., 2013b; Baroni et al., 2018).

According to phenolic content and antioxidant activity (ABTS⁺ assay), the fruit and jam from Santa Lucia stands out above the rest. Climatic factors within the place of fruit origin mainly rainfall, temperature, light intensity, and relative humidity have great influence on the nutritional quality of fruits (Rasheed et al., 2018). Some of the main differences are the location in the alluvial cone of the San Juan River and the effect of climate, which records high temperatures during the day and notable drops at night, because of the thermal amplitude that characterizes the area.

On the other hand, taking into account that 50 g of fruit is used for every 50 g of sugar for the production of quince jam, the phenolics content and antioxidant capacity is recovered in 160% on average. This increase in phenolics content from fruit to jam could be due to the production process, which facilitates the extraction of phenolics from the fruit. Some phenolics may be bound to nondigestible components of the food matrix, and disruption of cellular structure by processing could cause their release and solubilization (Rothwell et al., 2015; Kårlund et al., 2014; Fares et al., 2010). It has been found that there is no simple cause-effect relationship between processing and the phenolic content and antioxidant activity of food (Cilla et al., 2011). Regarding the effect of heat treatment on phenolics, contradictory results have been reported. On one way, a positive effect on the total phenolic content and



TP: Total Phenolic Content. AC: Antioxidant Activity. Different letters and numbers for each variable indicate significant difference ($p < 0.05$) according to the place of origin. Capital letters for TP, lower letters for ABTS⁺⁺, italic for DPPH⁺ and numbers for FRAP.

Figure 2. Total phenolic content and antioxidant capacity in quince fruit samples.

antioxidant capacity of grapes, orange juice, green tea, plum, carrot and beet jams has been observed after thermal treatment (Miletić et al., 2013; Kamiloglu et al., 2015; Guldiken et al., 2016; He et al., 2016). This is probably due to the alteration of the integrity of the cell wall and membranes, as well as to the degradation of complexes formed with proteins (Cilla et al., 2009). On the other way, a negative effect of thermal treatment has been observed in fruit milks, beet marinades, plums and cabbage (Cilla et al., 2011; Guldiken et al., 2016; Kaulmann et al., 2016), possibly due to the degradation of the compounds by heat.

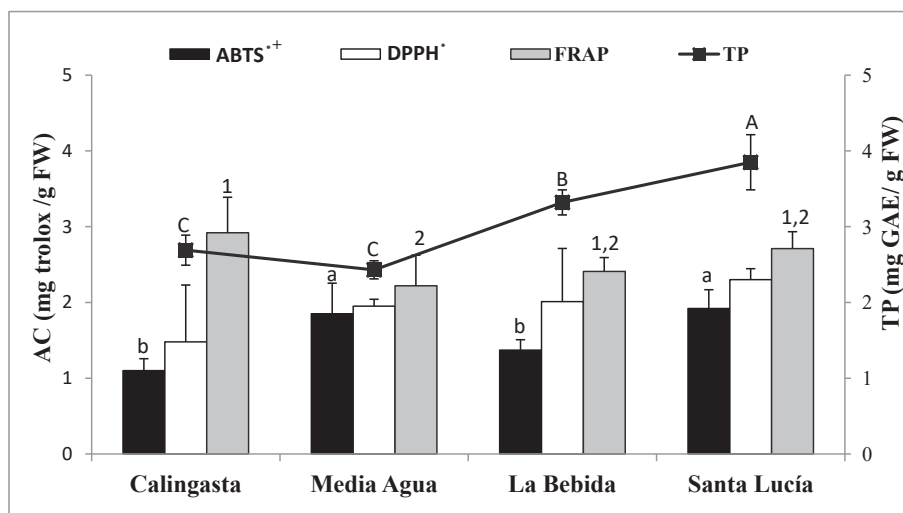
3.3. Effect of simulated gastrointestinal digestion on the phenolics profile and antioxidant activity of quince jam

Bioactive compounds that come from food must be able to withstand the conditions of the gastrointestinal tract as well as to cross the small intestine

membrane in order to exert a beneficial effect on human health (Manach et al., 2004, 2005). In recent years, the *in vitro* gastrointestinal digestion models have been widely used to study the stability and bioavailability of polyphenolic compounds present in food (Minekus et al., 2014). Since quince jam from Santa Lucia showed the highest TP content and high antioxidant activity, it was selected to be used in this part of the study.

The effect of the gastrointestinal digestion on TP and AC of quince jam are shown in Figure 4 and Table 1.

After mouth digestion, only 30% of the phenolics originally contained in jam were detected (Figure 4). In addition, after gastric digestion, an increase in the release of polyphenols was observed, detecting 44% ($p < 0.05$) of polyphenols with respect to the original content in jam. This suggests that during incubation with gastric enzymes phenolics continue to be extracted from the food matrix, so both mouth and gastric digestion are fundamental stages that allow the extraction of polyphenolic compounds,



TP: Total Phenolic Content. AC: Antioxidant Activity. Different letters and numbers for each variable indicate significant difference ($p < 0.05$) according to the place of origin. Capital letters for TP, lower letters for ABTS⁺⁺, italic for DPPH⁺ and numbers for FRAP.

Figure 3. Total phenolic content and antioxidant capacity in quince jam.

increasing their bioaccessibility (Lingua et al., 2018; Tagliacozchi et al., 2010). However, after the intestinal digestion, a significant decrease in phenolics detected was observed ($p < 0.05$). Compared to gastric digestion, 56% of the TPs were degraded under the conditions of intestinal digestion. The degradation of phenolics during intestinal digestion has been previously observed by other authors (Podsedek et al., 2014; Lingua et al., 2018; Podio et al., 2019), who attributed the chemical instability of phenolics to the intestinal alkaline medium ($pH = 7.5$) as well as to the possible interactions with other components of the *in vitro* digestion assay, such as pancreatic enzymes. In the non-dialyzable fraction (intestine lumen), only 24% of the original phenolics in the quince jam were found. Furthermore, 2.7% were detected in the dialyzable fraction (i.e. those that passed the dialysis membrane and would be potentially bioavailable).

Table 1 shows the polyphenol profile determined and quantified by HPLC-DAD-MS/MS in quince jam and in the dialyzable and non-dialyzable fractions. In quince jam, 21 compounds were identified according to retention time, fragmentation patterns and exact mass (Table 1). All the compounds identified have been previously described in quince fruit by other authors (Silva et al., 2000, 2004; Karar et al., 2014; Baroni et al., 2018).

The concentrations found in quince jam for the different compounds (Table 1) are in line to those previously published (Silva et al., 2000, 2004; Wojdyło et al., 2013a; Karar et al., 2014; Baroni et al., 2018).

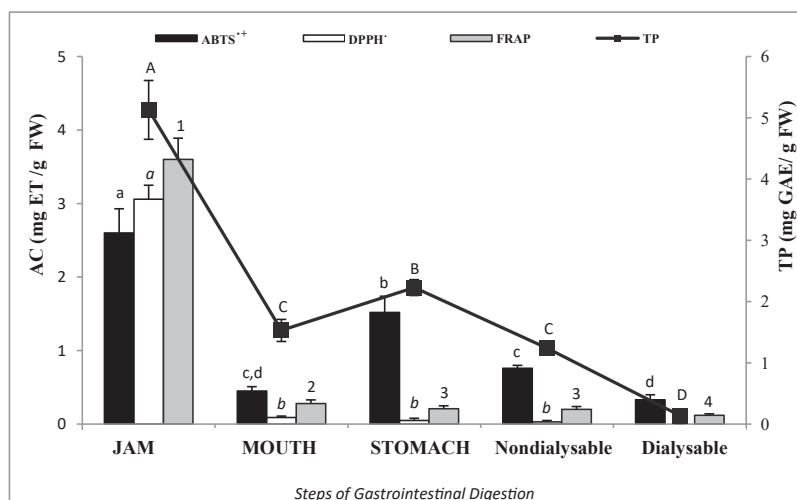
Quinic acid was detected as the only organic acid present in the samples. On the other hand, hydroxycinnamic acids were the main compounds detected in jam samples, representing 65% of the total polyphenols quantified. Five derivatives of caffeoylquinic acid and two of coumaroyl quinic acid were determined, being 5 caffeoylquinic acid the most abundant in the samples. The flavonols were presented as a mixture of aglycones and glycosylated derivatives. Quercetin and kaempferol were detected as aglycones and 6 glycosylated derivatives thereof. In addition, a glycosylated derivative of isorhamnetin was identified. Rutin (quercetin-rutinoside) was the flavonol quantified in the largest proportion. Flavonols represented 15.6% of the total phenolics quantified.

Flavanols were the compounds detected in the lower proportion in jam samples, representing only 3% of the total polyphenols determined. They were represented by catechins and procyanidins, with epicatechin being the most abundant.

As it can be seen in Table 1, of the 21 compounds detected in jam, only 13 ($p < 0.05$) were able to pass through the dialysis membrane, representing the fraction of potentially bioavailable compounds. Among them, the most

abundant ones were those derived from hydroxycinnamic acid and quinic acid. Quinic acid showed a recovery percentage with respect to the initial content in jam of 12%, 4-caffeoylquinic acid of 36%; 3-coumaroylquinic acid of 34%; and 3-caffeoylquinic acid showed a recovery of 16%. The rest of the compounds showed recoveries equal to or lower than 6%. In the non-dialyzable fraction (i.e. potentially viable colon), only 14 compounds were recovered ($p < 0.05$). 3-coumaroylquinic acid was the one recovered in the highest proportion with a percentage close to 31%. However, there were other compounds with low recovery percentages, which are important due to their concentrations in this fraction. Among them are quinic acid, some flavonols and derivatives of caffeoylquinic acid and coumaroylquinic acid.

The decrease of the content of phenolics compounds during gastrointestinal digestion ($p < 0.05$) has already been reported by other authors (Nagar et al., 2020). It has also been established that the bioavailability of polyphenols depends on the chemical structure of the compounds and also on the composition of the surrounding matrix (Jakobek and Matić, 2019; Nagar et al., 2020). In this sense, phenolics can interact with food components such as fibers, polysaccharides, and proteins, which can modify the bioavailability of the same compound in different food matrices. These types of bonds can be covalent/non-covalent and often can only be broken by colonic fermentation (Jakobek and Matić, 2019). This is why the global composition of the food should be taken into account, when comparing the results of bioaccessibility assays. As far as we know, there are no published studies that evaluate the effect of gastrointestinal digestion on quince phenolics. However, there are some published works that study the bioaccessibility of apple polyphenols, which is a fruit of the same family, with a similar phenolic composition to quince. In these works, published by Bouayed et al. (2011; 2012), they observed that flavon-3-ols such as catechin, epicatechin, and procyanidins decreased their concentration drastically from the gastric to the intestinal digestion stage, probably due to the instability of this type of compound to the change of pH between both stages (Bouayed et al., 2012; Nagar et al., 2020). It agrees with our results since the compounds belonging to flavon-3-ols were not detected in the digested fractions except for catechin in a low proportion ($p < 0.05$). In the same sense, these authors report that chlorogenic acid is also sensitive to these pH changes (Bouayed et al., 2012; Quan et al., 2018) and tends to trans isomerizes (Bouayed et al., 2012). Although in this work we see a decrease in chlorogenic acid ($p < 0.05$), we are not able to see the effect of trans-isomerization since chlorogenic isomers were originally already present in our samples.



TP: Total Phenolic Content. AC: Antioxidant Activity. Different letters and numbers for each variable indicate significant difference ($p < 0.05$) according to the stage of digestion. Capital letters for TP, lower letters for ABTS^{•+}, italic for DPPH[•] and numbers for FRAP.

Figure 4. Changes in Total phenolic content and antioxidant capacity of the quince jam throughout the simulated process of gastro-intestinal digestion.

Table 1. Identified and quantified polyphenols in quince jam and digested samples (mg/100 g jam). Values are reported as means \pm SD.

no.	RT (min)	Tentative identification	Molecular Formula	[M – H] [–] (m/z) calc	[M – H] [–] (m/z) exp	error (ppm)	MS ² (m/z)	Jam	Non-dialyzable	Dialyzable
1	7.0	Quinic acid*	C ₇ H ₁₁ O ₆	191.0561	191.0568	3.5	173.0653	24.89 \pm 2.57 ^a	2.18 \pm 0.50 ^c	2.95 \pm 0.49 ^b
2	11.7	4-Caffeoylquinic acid	C ₁₆ H ₁₇ O ₉	353.0878	353.0871	1.9	191.0562	16.05 \pm 2.19 ^a	2.09 \pm 0.48 ^c	5.77 \pm 3.22 ^b
3	11.7	Procyanidin dimer	C ₃₀ H ₂₅ O ₁₂	577.1351	577.1361	1.7	289.0692, 425.3569, 407.7843	1.20 \pm 0.46	<LOD	<LOD
4	11.8	Procyanidin trimer	C ₄₅ H ₃₇ O ₁₈	865.1985	865.1983	-0.3	577.1350, 425.3568, 289.0692	0.80 \pm 0.31	<LOD	<LOD
5	11.9	(+) Catechin*	C ₁₅ H ₁₃ O ₆	289.0718	289.0690	9.2	245, 205	0.81 \pm 0.19 ^a	0.0120 \pm 0.0002 ^b	0.053 \pm 0.003 ^c
6	12.0	Caffeoylquinic acid hexoside	C ₂₂ H ₂₇ O ₁₄	515.1406	515.1352	-10.6	179.0336, 191.0569	5.10 \pm 0.77 ^a	0.031 \pm 0.001 ^b	0.10 \pm 0.02 ^b
7	12.5	3-Coumaroylquinic acid	C ₁₆ H ₁₇ O ₈	337.0929	337.0894	-10.3	191.0559	1.30 \pm 0.15 ^a	0.41 \pm 0.13 ^b	0.44 \pm 0.09 ^b
8	12.7	5- Caffeoylquinic acid	C ₁₆ H ₁₇ O ₉	353.0878	353.0871	-2	191.0563	53.11 \pm 1.52 ^a	3.67 \pm 1.54 ^b	1.22 \pm 0.31 ^c
9	13.2	(–) Epicatechin	C ₁₅ H ₁₃ O ₆	289.0718	289.0709	5.3	245, 203	1.24 \pm 0.49	<LOD	<LOD
10	13.7	3-caffeoylquinic acid*	C ₁₆ H ₁₇ O ₉	353.0878	353.0842	10.3	191.0563	4.85 \pm 2.16 ^a	0.030 \pm 0.001 ^b	0.24 \pm 0.11 ^b
11	14.3	5-coumaroylquinic acid	C ₁₆ H ₁₇ O ₈	337.0929	337.0907	-6.6	191.0568	5.07 \pm 1.01 ^a	0.8 \pm 0.25 ^b	0.8 \pm 0.2 ^b
12	15.0	Methyl caffeoylquinic acid	C ₁₇ H ₁₉ O ₉	367.1035	367.0997	10.1	179.0339	6.23 \pm 0.47	<LOD	<LOD
13	18.9	Quercetin-3-O-rutinoside*	C ₂₇ H ₃₀ O ₁₆	609.1461	609.1457	0.7	301.0337	14.43 \pm 2.35 ^a	1.17 \pm 0.19 ^b	0.67 \pm 0.26 ^c
14	19.4	Quercetin-3-O-glucoside*	C ₂₁ H ₁₉ O ₁₂	463.0882	463.0900	-3.9	301.0337	3.44 \pm 0.68 ^a	0.37 \pm 0.13 ^b	0.12 \pm 0.08 ^c
15	21.0	Kaempferol hexoside	C ₂₁ H ₁₉ O ₁₁	447.0933	447.0941	-1.8	285.0403	0.46 \pm 0.09 ^a	0.05 \pm 0.02 ^b	0.02 \pm 0.01 ^c
16	21.3	Kaempferol rutinoside	C ₂₇ H ₂₉ O ₁₅	593.1512	593.1524	2	285.0407	2.34 \pm 0.44 ^a	0.24 \pm 0.1 ^b	0.10 \pm 0.05 ^c
17	21.4	Isorhamnetin rutinoside	C ₂₈ H ₃₂ O ₁₆	623.1618	623.1635	2.8	315.0280	0.04 \pm 0.02 ^a	0.01 \pm 0.0031 ^b	<LOD
18	21.4	Kaempferol hexoside	C ₂₁ H ₁₉ O ₁₁	447.0933	447.0954	4.8	285.0395	0.93 \pm 0.21 ^a	0.13 \pm 0.04 ^b	0.04 \pm 0.02 ^c
19	23.0	Quercetin feruloyl hexoside	C ₃₁ H ₂₇ O ₁₅	639.1355	639.1362	-1	463.0887, 301.0337	0.09 \pm 0.01	<LOD	<LOD
20	24.7	Quercetin*	C ₁₅ H ₉ O ₇	301.0354	301.0324	9.9	179.0336, 151	0.27 \pm 0.04	<LOD	<LOD
21	28.1	Kaempferol*	C ₁₅ H ₁₀ O ₆	285.0405	285.0389	-5.6		0.31 \pm 0.09	<LOD	<LOD
Total Phenolics								142.96	11.19	12.52

RT, Retention Time; [M–H][–] (m/z), molecular ion; MS² (m/z), fragments from fragmentation of [M–H][–]. *Compounds identified using standards. Other compounds are tentatively identified based on RT, exact mass, fragmentation pattern (MS/MS) according to literature. Different letters for each compound indicate significant difference ($p < 0.05$) between dialyzable and non dialyzable samples.

In the case of glycosylated flavonols, some authors report an increase in the proportion of aglycones postulating the hydrolysis of sugar moiety by digestive enzymes (Bouayed et al., 2012). In our case, we did not observe an increase in the corresponding aglycones; although, a decrease in the glycosylated derivatives was observed ($p < 0.05$) (Bouayed et al., 2012; Quan et al., 2018). This may also be due to the low concentration of these compounds in the original food and therefore the increase in the respective aglycones may be below the detection limit of the technique.

In addition, those compounds determined in the non-dialyzable fraction are those that reach the colon and would exert their beneficial effect there. The microbiota found in the colon is capable of using polyphenols by degrading or transforming them, so they could serve as substrates for the microbial community of the colon (Possemiers et al., 2011). There is growing scientific evidence that suggests a positive effect of dietary phenolics on modulating the composition and activity of colon microbiota (Cardona et al., 2013; Lucini Mas et al., 2020).

Furthermore, the modification of the antioxidant capacity (FRAP, ABTS⁺ and DPPH[•]) of quince jam throughout the digestive process was also studied (Figure 4). For the three assays, values obtained after mouth digestion were substantially lower ($p < 0.05$) than those obtained for quince jam. ABTS⁺ results showed the same tendency as the total polyphenol content. FRAP results did not show significant differences between the mouth, gastric and non-dialyzable fractions, while the dialyzable fraction had significantly lower values ($p < 0.05$). The DPPH[•] values obtained were very low throughout the digestion, demonstrating that the compounds that resist the digestive process would not be acting through this mechanism. Moreover, the dialyzable fraction showed values below the detection limit for DPPH[•].

Taking into account the TP and AC results, the stability and, consequently, the bioaccessibility of the different families of polyphenolic compounds were widely influenced by changes in pH and the activity of digestive enzymes throughout the process. Hydroxycinnamic acids were the most resistant phenolics to gastrointestinal digestion.

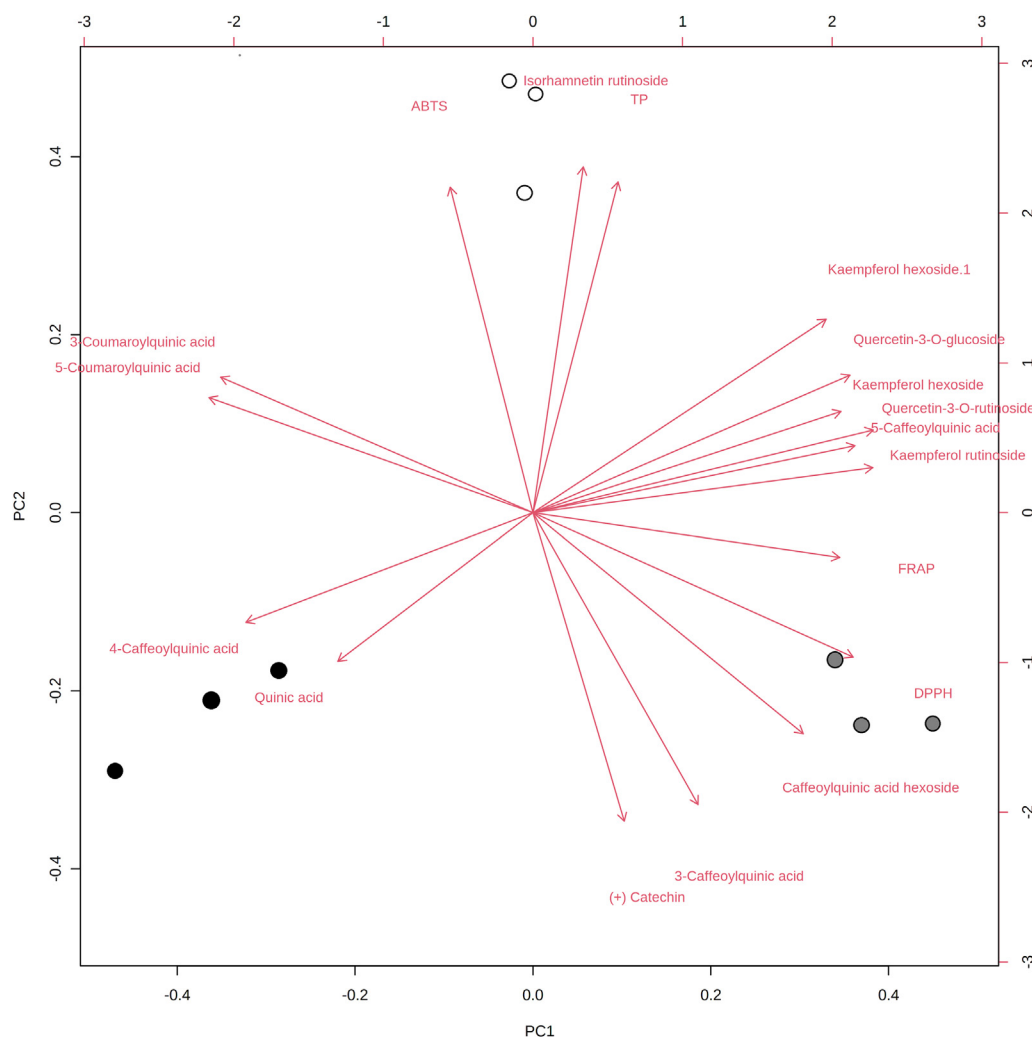


Figure 5. Principal Component Analysis of phenolic compounds and antioxidant activity of quince jam and digested fractions. Score Plot of samples (dots) and Loading plot of variables (vectors). Quince Jam. Dialyzable. Nondialyzable.

3.4. Correlation evaluation between antioxidant activity and phenolic content

Principal component analysis (PCA) was performed to investigate the correlation between antioxidant activity (DPPH, ABTS⁺ and FRAP) and the phenolic composition of jam and gastrointestinal digestion samples (non-dialyzable and dialyzable samples). Figure 5 shows the results for principal component analysis showing both loading plots for variables and score plot for samples. PCA results showed that the first two principal components accounted for 85,9 % of the total variance. PC1 shows a clear differentiation between dialyzable and quince jam samples; while PC2 shows a clear differentiation between non dialyzable and the rest. PC1 and PC2 explained the 54.4% and 31.5% of the variability between samples, respectively. Quince jam samples had a positive correlation with caffeoylquinic acid hexoside, (+) catechin, 3-caffeoylquinic acid and with antioxidant activity determined by FRAP and DPPH methods. Furthermore, most of the quercetin and kaempferol derivatives and 5 caffeoylquinic acid are on that side of PC1. This probably means that Antioxidant activities measured by DPPH and FRAP methods, can be explain by the content of these compounds. Cirak et al. (2022), also showed a high correlation of caffeoylquinic acid and quercetin hexosides with FRAP results in *Achillea arabica*. Dialyzable samples had high positive correlation with quinic acid and 4-caffeoylquinic acid, while non dialyzable samples had correlation with isorhamnetin rutinoside and ABTS⁺ results. Jia et al. (2018) and Arimboor and Arumugan (2012)

also found that isorhamnetin rutinoside exhibits better antioxidant activity by ABTS⁺ method than DPPH method.

These results demonstrate that the AC can be explained from the phenolic profile and that these compounds may act through different mechanisms.

4. Conclusion

Quince fruit collected in Santa Lucia was characterized by having the highest total polyphenol content and antioxidant capacity.

Regarding quince jam, a positive effect was observed on its antioxidant characteristics after processing. Twenty-one phenolics were detected in jam being mostly derivatives of hydroxycinnamic acid, which are known for their biological activity.

Mouth digestion showed that only 30% of quince jam phenolics were bioavailable. After stomach digestion, that percentage increased to 44%. Finally, a significant decrease was observed after the small intestine digestion and absorption, being only 2.7% and 24% of the original phenolics detected in the dialyzable and non-dialyzable fraction, respectively. Quinic acid and 4 and 5-caffeoylquinic acids were the most resistant to digestion. *In vitro* antioxidant capacity throughout the digestion showed, in general, a similar trend to the total polyphenol content.

In brief, the results reported in this work showed that geographical origin, processing and gastrointestinal digestion affect the antioxidant characteristics of quince jam. These variables are not always taken into

account when studying the probable antioxidant capacity of a food commodity and should be included, since there is a direct relationship between nutrition and health.

Declarations

Author contribution statement

María V. Baroni: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

María Paula Fabani: Contributed reagents, materials, analysis tools or data.

Florencia Adan: Performed the experiments; Analyzed and interpreted the data.

Natalia S. Podio: Analyzed and interpreted the data.

Daniel A Wunderlin: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare the following conflict of interests: Daniel Alberto Wunderlin; [is Associate Editor of Heliyon Environment].

Additional information

No additional information is available for this paper.

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