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ORIGINAL ARTICLE

Effects of convective drying assisted by ultrasound and osmotic solution on polyphenol, antioxidant and microstructure of murtilla (*Ugni molinae* Turcz) fruit

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Abstract The effects of pretreatment with ultrasound and an osmotic solution combined with hot air convection drying on the total polyphenol content (TPC), antioxidant activity and microstructural of murtilla skin fruit were evaluated. The effects of ultrasound frequency (0 and 130 kHz), osmotic solution concentration (0 and 70 °Brix) and time (60 or 120 min) on the TPC and the antioxidant activities as measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays were evaluated. The TPC and DPPH antioxidant activity decreased significantly ($p < 0.05$) when ultrasound was applied at 0 °Brix for 60 min. Higher FRAP activity was obtained upon treatment with ultrasound and an osmotic solution for 60 min. The ORAC values did not significantly differ based on the pretreatment methods but decreased when an osmotic solution was applied for 120 min without ultrasound. When ultrasound and the osmotic solution were applied, the skin cells of the dried murtilla fruit became more distorted, resulting in larger spaces between them and causing loss of shape. Although the application of pretreatment procedures before murtilla fruit drying did not positively affect the TPC, DPPH or ORAC individually, the application of a Global Standardized Response based on the followed by a mathematical model adjustment indicated that a 70 °Brix osmotic solution applied for 60 min was the best treatment for preparing murtilla fruit aiming a high antioxidant activity in dried product.

Keywords Dehydration · Sonicate · Osmosis · Murta · Phenolics · Microscopic

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Introduction

In the food industry, hot air drying under forced convection is the most common method for dehydration because it is reasonably economical. However, the removal of internal moisture takes a relatively long time. The color, taste and nutritional characteristics of the rehydrated product could be damaged by high temperatures (Ramos et al. 2003). Thus, alternative methods, such as microwave drying, freeze drying, osmotic drying, vacuum drying, spray drying and infrared drying, have been investigated (Reyes et al. 2011; Yi et al. 2017; Coklar et al. 2018). These methods can avoid the disadvantages of hot air drying but there are drawbacks: freeze drying is an expensive method, and spray drying is only applicable to fluids (Reyes et al. 2010). Complementary technologies, such as ultrasound-assisted dehydration in a liquid medium, have been implemented as pretreatment-associated drying procedures (Rodrigues and

Fernandes 2007; Garcia-Noguera et al. 2010). The use of ultrasonic waves may enhance the effectiveness of convective hot air drying, improving the quality of the dried product (Kek et al. 2013). Drying methods must ensure product stability and should not affect bioactive components, such as polyphenolic compounds. The application of ultrasound decreases the drying time and hence the processing costs, but it increases the rate of mass transfer during the drying of various fruits and vegetables (Mothibe et al. 2011). In addition to changes in the nutritional quality observed after drying, microstructural changes due to water removal have been reported in strawberries (Garcia-Noguera et al. 2010) and apples (Ramírez et al. 2011). An alternative to removing water before drying is the use osmotic solutions. The equilibrium with the solid will be influenced by the type of solution, concentration, temperature, and time (Sablani et al. 2002). An improvement in the effective water diffusivity and a decrease in the processing time were obtained by simultaneous pretreatment with ultrasound and an osmotic solution prior to the drying of pineapple and strawberry (Fernandes et al. 2009; Garcia-Noguera et al. 2010).

Murtilla (*Ugni molinae* Turcz.) is a Chilean native plant from the Myrtaceae family that grows in the forest edges of the Andes coastal mountains and produces an edible berry (Pastenes et al. 2003; Scheuermann et al. 2008). The fruit is usually eaten fresh but is also commercially available in canned forms and in jam, juice and liquor. Due to its pleasant fruity aroma and high content of polyphenolic compounds, murtilla fruit is recognized for its antioxidant properties, and it presents potential for commercialization as a dry product (Scheuermann et al. 2008; Alfaro et al. 2013; Augusto et al. 2014; Jofré et al. 2016). Murtilla fruit has a thick peel, which constitutes a barrier to mass transfer during convective drying with hot air. Pretreatment with ultrasound and an osmotic solution may improve the drying speed, as reported for other fruits (Rodrigues and Fernandes 2007; Garcia-Noguera et al. 2010; Mothibe et al. 2011). This study evaluated the effects of ultrasound and osmotic solution as pretreatment methods before drying by convective air on the polyphenols content, antioxidant activity and microstructure of dried murtilla fruits.

Materials and methods

Fruit material

Fresh murtilla (*Ugni molinae* Turcz) fruit, genotype INIA 23-02, was harvested in the experimental field of the Institute of Agricultural Research (INIA-Carillanca) located in Tranapuente (38° 70′ S; 73° 35′ W), Chile. The fruits were immediately transported to the Food Science Laboratory at the Universidad de La Frontera. A sieve system was used to select the fruit with a diameter between 1.5 and 1.0 cm, in order to form a homogeneous sample in relation to the diameter of the individual fruits.

Experimental design

A factorial experimental design was applied to analyze the effects of pretreatment methods and time on the total polyphenol content and antioxidant activities of dried murtilla fruits. The independent variables were the ultrasound frequency (0 and 130 kHz), the osmotic solution concentration (0 and 70 °Brix), and the holding time (60 and 120 min). The selection of variable levels was based on conditions reported by Fernandes et al. 2009; Garcia-Noguera et al. 2010; Almeida et al. 2015; and Romero and Yépez 2015. A two-level factorial design was applied (Table 1). The dependent variables were the total polyphenol content (TPC), the antioxidant activities as measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays and the reactive oxygen species (ROS) scavenging activity measured by the oxygen radical absorbance capacity (ORAC) assay using peroxyl radical (ROO·). The experiments were carried out in triplicate, which means that for each point of the factorial design (Table 1), three different samples of dried murtilla fruit were produced. The TPC and antioxidant activity were determined for each of the three samples. Statistica software, 12.0 (StatSoft Inc. 2014, Tulsa, OK, USA) was used for data analysis.

Ultrasound and osmotic solution pretreatment procedures

Fresh murtilla fruit were pretreated with ultrasound in an ultrasonic bath (JAC-2010, Kodo Technical Research Co. Ltd., Korea) using an ultrasound frequency of 130 kHz. Other murtilla fruit samples were not pretreated with ultrasound (0 kHz). Distilled water (0 °Brix) and an osmotic solution (70 °Brix) prepared from concentrated apple juice (Diana Naturals, Chile) were used as the liquid media. The ratio of fruit to liquid medium was 1:4 (w/v) based on previous work (Fernandes et al. 2006; Oliveira et al. 2006). The murtilla fruits were immersed in the liquid medium in a glass jar and then placed into an ultrasonic bath for 60 or 120 min. After completing each pretreatment procedure, the murtilla fruits were removed from the glass jars and absorbent paper was used to remove excess liquid.

Convective drying

A forced circulating air oven (Memmert model UFE 400, Germany) was used to dry the murtilla fruit after

Table 1 Factorial design of the pretreatments applied before murtilla fruit dehydration

Run	Independent variables					
	Real value			Coded value		
	Ultrasonic frequency (kHz)	Osmotic solution concentration (°Brix)	Time (mins)	Ultrasonic frequency (kHz)	Osmotic solution concentration (°Brix)	Time (mins)
1	0	0	60	− 1	− 1	− 1
2	130	0	60	1	− 1	− 1
3	0	70	60	− 1	1	− 1
4	130	70	60	1	1	− 1
5	0	0	120	− 1	− 1	1
6	130	0	120	1	− 1	1
7	0	70	120	− 1	1	1
8	130	70	120	1	1	1

pretreatment with ultrasound and osmotic solution. The drying conditions were fixed according to Nowacka et al. (2012), Alfaro et al. (2014) and Augusto et al. (2014). The oven was set at 70 °C, and the air flow was 2 m/s. A fruit charge density of 3 kg/m^2 was used. Each sample was arranged on the trays in a single layer. The drying process lasted 5–6 h, being interrupted when the samples reach a final moisture content of 7% of wet base (b.p.). The dry matter of the dried murtilla fruit was determined to establish the moisture content, and this value was used to calculate the TPC and antioxidant activity in 100 g of dry weight.

After pretreatment procedures and convective drying, the samples were extracted for determination of the total polyphenol content and antioxidant activities. Also, microstructure was analyzing for the murtilla dried fruits.

Total polyphenol content (TPC) and antioxidant activities

Dried murtilla extracts were prepared from 1 g of dried sample minced in a mortar and mixed with 20 mL of methanol (99.9%, Merck, Germany). The mixture was held at 30 °C whlile stirring at 170 rpm for 20 min in an incubator (GFL-3032, Germany) and then filtered (Whatman N°1) under vacuum (Reyes et al. 2011; Alfaro et al. 2013). The extracts were subjected to the following determinations.

Determination of the total polyphenol content (TPC)

The Folin–Ciocalteau method was used for TPC determination (Wong et al. 2006). A 40-µL aliquot of murtilla extract was mixed with distilled water (3.16 mL), added of 200 µL of Folin–Ciocalteau reagent, and, after 5 min, added of 600 µL of 20% Na_2CO_3 solution. Samples were kept at 20 °C for 120 min in the dark. The absorbance was measured at 765 nm using a spectrophotometer (Spectronic Genesys 5, Sweden), and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g dry weight (Reyes et al. 2011; Alfaro et al. 2013).

Determination of the 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging capacity

The DPPH antioxidant activity was determined based on the radical scavenging by the compounds in the fruit extracts (Brand-Williams et al. 1995). A 50-µL aliquot of the methanolic murtilla extract was added to 1950 µL of a DPPH methanolic solution. Absorbance at 515 nm was measured after 30 min. The results were expressed as µmol Trolox equivalents (TE) per 100 g dry weight (Reyes et al. 2011; Alfaro et al. 2013).

Determination of the ferric reducing antioxidant power (FRAP)

The FRAP assay was conducted according to Al-Duais et al. (2009). A 20-µL aliquot of the extract was mixed with 30 µL of distilled water in a 96-well microplate. After the addition of 200 µL of FRAP reagent (prepared fresh daily: 10 volumes of 300 mmol/L acetate buffer (pH 3.6), 10 volumes of 20 mmol/L $FeCl_3$ and one volume of 10 mmol/L TPTZ (2,4,6-tripyridyl-striazine) in 40 mmol/L HCl) the absorbance at 595 nm was registered by a microplate reader (Spectra Max M3, Molecular Devices). Water was used as blank. Ferrous sulfate solutions (0.25–2.5 mmol/L) were used for calibration curve and results were expressed in mmol of Fe^{2+} per 100 g dry weight.

Determination of the ROS scavenging activity by an oxygen radical absorbance capacity (ORAC) assay

The ROO· scavenging activity was determined by the ORAC method, as described by Melo et al. (2015). ROO· was generated by the thermal decomposition of AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride). The reaction mixtures in the sample wells contained the following reagents at the indicated final concentrations (in a final volume of 200 µL): 60 µL of fluorescein (508.25 mM); 30 µL of the standard, control or murtilla extract; and 110 µL of the AAPH solution (76 mM). Solutions of fluorescein were diluted in 75 mM phosphate buffer (pH 7.4) that had been previously prepared and stored at 4 °C. The AAPH was dissolved in phosphate buffer. The mixture was kept in a microplate reader (Molecular Devices, LLC, USA) at 37 °C for 120 min. The fluorescence signal was monitored at an emission wavelength of 528 nm and an excitation wavelength of 485 nm every minute until the fluorescence fully decayed. Trolox was used as the standard control, and the results were expressed in µmol of Trolox equivalents (TE) per 100 g dry weight.

Determination of the global standardized response (GSR)

To comprehensively evaluate the antioxidant activity of the extracts produced under different conditions, a Global Standardized Response was calculated following De Camargo et al. (2014). Standardized responses (in relation to the maximum observed value of each assay) of antioxidant activity by DPPH, FRAP, and reactive oxygen species determined using the ORAC method were used to indicate a global value including three different patterns of antioxidant activity. The GSR was determined by the following equation:

$$GSR = \sum_{i=1}^{mn} m\left[\left(\frac{Vi}{HVi}\right) * \frac{100}{n}\right]$$

where V_i = experimental response from each run, HVi = highest observed value in the response group, m = method number, n = number of methods to adjust the global response.

The GSR was used as a depend variable to adjust the first-order mathematical model, including linear parameters and interactions between independent variables.

Microstructure analysis

One random sample was collected after each pretreatment experimental essay and fixed with a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) and 1% glutaraldehyde for 24 h at ambient temperature. After fixation, samples were dehydrated, immersed in a series of graded ethanol solutions and embedded using a historesin embedding kit. Then, the dehydrated and embedded murtilla fruits were sectioned into 5 µm-thick layers with a Leica RM 2065 microtome (Leica, Germany). Photomicrographs of the layers showing the cellular structure were taken using a ZEISS Primo Star light microscope (ZEISS, Germany) with a digital image capture system (Garcia-Noguera et al. 2010).

Statistical analysis

All determinations were carried out in triplicate, and the results are expressed as the means ± standard deviations (SDs). Statistical analyses were conducted using Statistica software, 12.0 (StatSoft Inc. 2014, Tulsa, OK, USA). Data were analyzed via ANOVA ($p < 0.05$), and Duncan's test was used to determine significant differences ($p < 0.05$) in the TPC, DPPH, FRAP and ORAC assays. The quality of the fit of the adjusted model for GSR was evaluated in terms of the coefficient of determination (R^2) and the F-test. The lack of fit F-value ($p < 0.05$) was obtained from an analysis of variance (ANOVA) (Rodrigues and Iemma 2015).

Results and discussion

Total polyphenol content and antioxidant activity

The antioxidant activities of fruits have mainly been related to their phenolic compounds (Alfaro et al. 2013; Jofré et al. 2016). Some studies have shown that the polyphenolic compounds in murtilla fruits and their antioxidant activities are affected by the drying process (Reyes et al. 2010; Augusto et al. 2014). Convective air drying can reduce nutrients in fruits due to the long drying times and high temperatures (Sablani 2006). Thus, ultrasound pretreatment is expected to be the best way to preserve nutrients during drying due to its ability to reduce drying period at ambient temperatures (Mothibe et al. 2011). Osmotic dehydration reduces the moisture in fruits while maintaining their bioactive compounds, and also intensifying the fruit color (Almeida et al. 2015). TCP of fresh murtilla used in this study was 715.3 ± 119.7 mg/100 g dry weight. The effects of pretreatment with ultrasound and osmotic solutions on the TPC and antioxidant activities of dried murtilla fruit by convective drying according to the factorial design (Table 1) are shown in Fig. 1.

The TPC decreased significantly ($p < 0.05$) when samples immersed in distilled water as the liquid medium was subjected to ultrasound for 60 min. When the same conditions were applied for 120 min, the observed TPC was not significantly different. In some cases, the decrease in TPC is attributed to oxidative degradation caused by the oxygen in the pores of the tissue and the intensification of enzymatic activity, especially after long treatment periods (Wiktor et al. 2016). Meanwhile, with 60 min using the osmotic solution (70 °Brix), ultrasound-assisted pretreatment resulted in a significantly higher TPC compared to no sonication treatments. Studies conducted with kasturi lime juice (25 kHz for 30 and 60 min) and grapefruit juice (28 kHz for 30, 60 and 90 min) as raw material also showed that ultrasound treatments resulted in increase in the total phenolic content (Bhat et al. 2011; Aadil et al. 2013). This observation could be attributed to the release of polyphenolic compounds following the rupture of the cell wall caused by the cavitation pressure exerted during ultrasound treatment. Likewise, the incorporation of hydroxyl groups into the aromatic rings of the polyphenolic compounds due to ultrasonication could contribute to this increase (Aadil et al. 2013). Conversely, when the osmotic solution was applied for 120 min, no significant differences in the TPC values were observed among the samples subjected to ultrasound and those that were not.

To evaluate the antioxidant activity of the dried murtilla fruit subjected to different pretreatment procedures (Table 1), DPPH and FRAP assays were conducted. Based on the DPPH free radical assay (Fig. 1), the antioxidant activities of the fruit samples subjected to ultrasound in the two different liquid media (distilled water and osmotic solution) and for the two treatment durations (60 and 120 min) were not significantly different ($p < 0.05$). However, when distilled water was applied (0 °Brix) for 60 min without ultrasound treatment (0 kHz), the DPPH antioxidant activity was higher than that observed following the other conditions studied. The decrease in DPPH antioxidant activity due to pretreatment with an osmotic solution and ultrasound is consistent with the results reported by Romero and Yépez (2015). These authors observed a decrease in the antioxidant activity of Andean blackberry when the fruit was sonicated at 24 kHz for 30 min utilizing distilled water as the liquid medium before drying. This decrease was attributed to the migration of the antioxidant compounds from the fruit to the liquid medium and the modifications that occur in the fruit skin, which favor the transfer of the components in the fruit to the liquid by decreasing resistance.

Concerning the FRAP assay, when distilled water was used, no significant differences ($p < 0.05$) were observed between the fruit subjected to ultrasound for the two pretreatment periods (Fig. 1). However, the application of the osmotic solution (70 °Brix) resulted in a significant difference that was influenced by the treatment time. Almeida

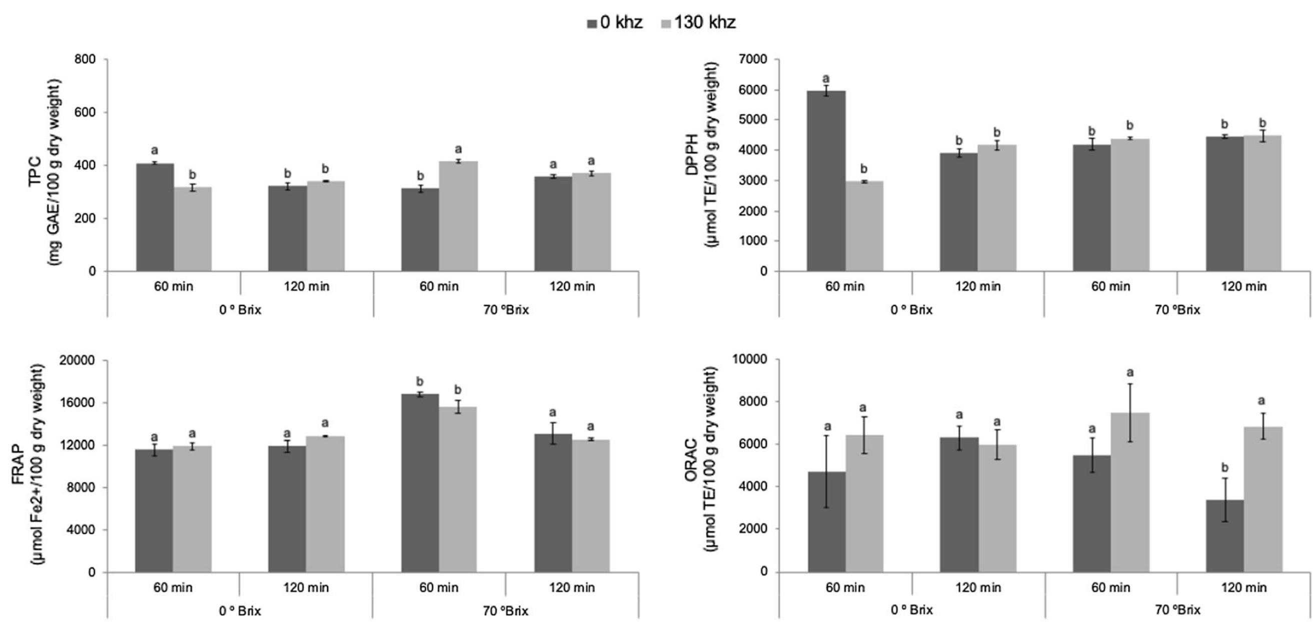

Fig. 1 Total polyphenol content (TPC) 2,2-diphenyl-1-picryhydrazyl antioxidant activity (DPPH), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) of dried murtilla fruit submitted to ultrasound (0 and 130 kHz) for 60 and 120 min in an osmotic solution (0 and 70 °Brix). Data represent the means of triplicate analyses for each assay, and bars with different letters are significantly different ($p < 0.05$)

et al. (2015) suggested that this behavior is probably due to the incorporation of sugar into the product, which prevents the loss of antioxidant compounds. This hypothesis could explain the higher antioxidant activities in the samples for which the osmotic solution was applied for 60 min compared to the activities of the samples that were sonicated for 60 min in distilled water.

No significant differences ($p < 0.05$) in the ORAC values were observed when distilled water was applied (Fig. 1). When the osmotic solution was tested during 60 min, treatment with ultrasound showed no significant impact on the results. However, when the osmotic solution was applied for 120 min, a lower ORAC activity was observed in the fruit samples that were not subjected to ultrasound compared with those that were subjected to ultrasound. The variables of ultrasonic frequency, osmotic solution concentration and pretreatment time did not affect the ORAC values of dried murtilla fruit probably because of the low concentration of phenolic compounds following all treatments, which prevented a significant effect on ROO· (Cao et al. 1995).

Therefore, to fit a preliminary model that includes the different applied methods the GSR was calculated, and the first-order coded model was adjusted:

$$Y_2 = 71.08 + 1.25\,X_1 + 2.67\,X_2 - 2.50\,X_3 + 3.33\,X_1X_2 + 2.17\,X_1X_3 - 2.92\,X_2X_3$$

where Y_2 is the GSR, X_1 is the ultrasonic frequency, X_2 is the osmotic solution concentration, and X_3 is time.

The model was evaluated based on the R^2 value and F-test (Table 2). Significant F value indicate that the model can be used to predict the GSR matching of the DPPH, FRAP, and ORAC values. Additionally, the lack of fit was not significant ($p < 0.05$), and 79% (R^2) of the variation can be explained by the regression. The surface responses are presented in Fig. 2 and corroborate previous observations based on the effects of the studied variables on individual antioxidant activities. A higher ultrasonic frequency applied with the osmotic solution for a shorter period of time results in dehydrated murtilla fruit with a desirable global antioxidant activity.

According to Fig. 2 the use of a 70 °Brix osmotic solution during 60 min allows to achieve a high antioxidant activity. Due to the significant interactions effects included in the mathematical model, expressed graphically in Fig. 2, the application of ultrasound at 20 kHz for 60 min, combined to the osmotic solution, indicates that it is possible to improve the GSR by matching independent variables values. Although it is not the aim of this work, results pointed the possibility of reducing the time and energy used in the drying process of murtilla fruits, reflecting in cost reduction.

Microstructural changes

The morphology of murtilla fruit skin tissue before and after the application of ultrasound is presented in Fig. 3. These results were obtained using the two liquid media with ultrasound application for 60 min in all cases. This samples were selected because in a previous work, differences in the drying curves were only observed with a treatment time of 60 min. The microstructure analysis shows that cells of murtilla fruit skin presented normal morphology, with thin walls and without visible intercellular spaces before ultrasound pretreatment using distilled water (Fig. 3a). After being subjected to ultrasound treatment (60 min), the skin cells showed alteration in their structure when immersed in distilled water (Fig. 3b). They became more distorted and showed decreased adhesion, which resulted in large spaces among the cells. The same effect was observed in pineapples, and these changes encouraged the internal water of the fruit to flow outward (Fernandes et al. 2009). These changes have been uniquely attributed to cell separation and breakdown as a result of ultrasound application in distilled water (Fernandes et al. 2009). When murtilla fruit was subjected to the osmotic solution (70 °Brix) before the ultrasound pretreatment, the skin cells showed normal morphology, with thin walls. However, differences were not clearly observed in the size of the intercellular spaces (Fig. 3c). Similar behavior was reported for strawberry osmotic drying; the texture was changed by the pectin solution, and the cells were ruptured after being treated for 30 min (Prinzivalli et al. 2006). Prosapio and Norton (2017) observed that when strawberries were subjected to osmotic dehydration prior to oven drying, some cells were still present, even if they were

Table 2 Analysis of variance (ANOVA) of the GSR for the antioxidant activities of dried murtilla fruit

Variation source	Sum of squares	Degrees of freedom	Mean square	Fcalc	p value
Regression	941.66	6	156.94	10.9	0.00005
Residuals	244.16	17	14.36		
Lack of fit	13.50	1	13.50	0.9	0.34760
Pure error	230.66	16	14.42		
Total	1185.83	23			

$R^2 = 0.79$

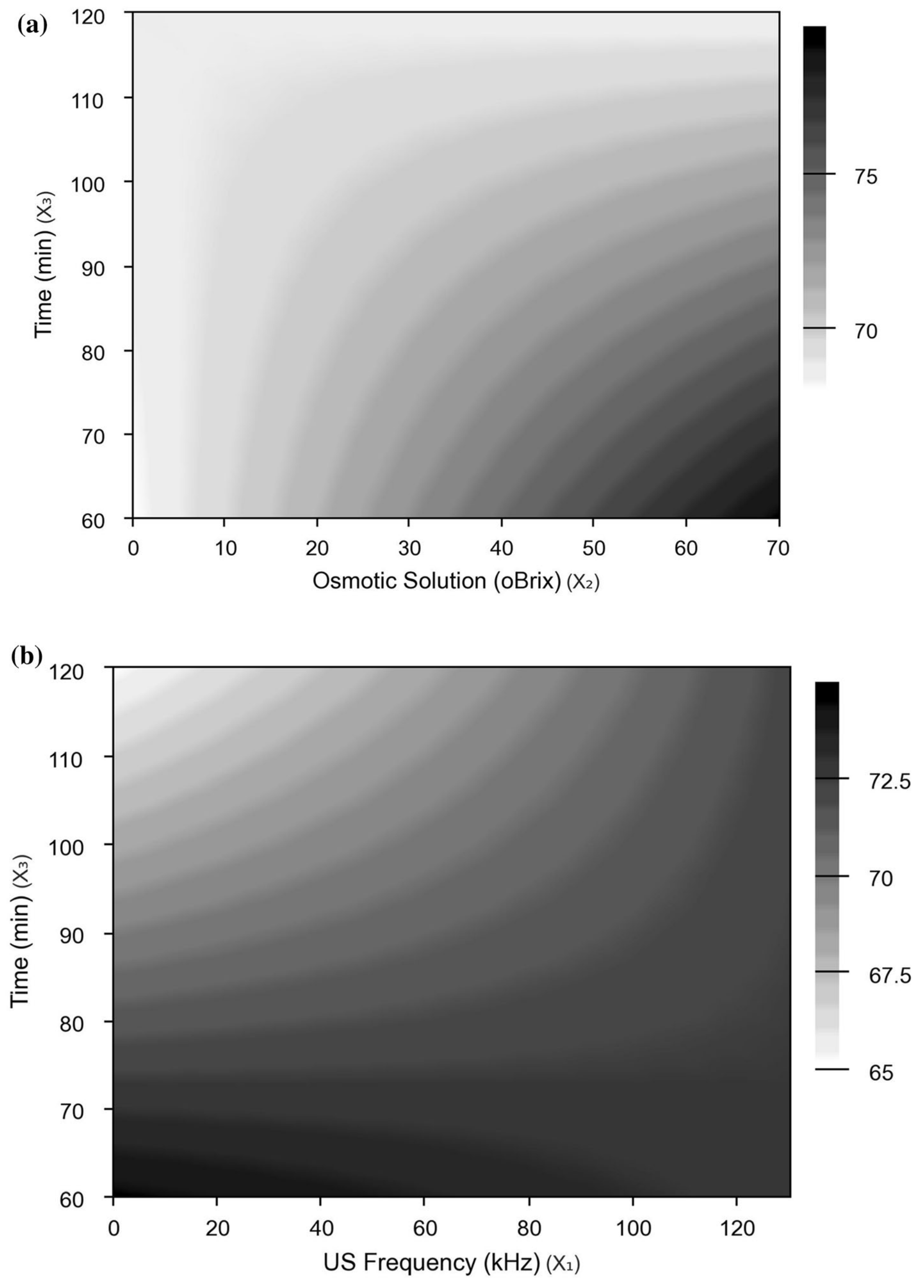

Fig. 2 Contour plot of the GSR of dried murtilla fruit with respect to the variation in the **a** treatment time and osmotic solution concentration and **b** ultrasonic frequency and treatment time

broken in some locations. When the murtilla fruits were sonicated for 60 min with an osmotic solution as the liquid medium (Fig. 3d), the cells lost their shape, and in some cases, they were ruptured, resulting in the formation of spaces between cells. The alterations in the cellular structure of pineapple, melon and apple caused by osmotic dehydration increase with increasing treatment time (Fernandes et al. 2009; Nowacka et al. 2012). However, although cell modification was observed for dried murtilla fruit pretreated with ultrasound and an osmotic solution (Fig. 3c, d), no effect on the release of TPC was observed (Fig. 1) even though an application duration of 60 min had a beneficial effect on the FRAP antioxidant activity and GSR (Figs. 1, 2a).

Conclusion

Pretreatment with ultrasound and an osmotic solution before murtilla fruit drying did not affect directly the TPC, DPPH antioxidant activity or ROS scavenging activity measured by the ORAC method. However, by using the osmotic solution (70 °Brix) as the liquid medium for 60 min positive effect on the FRAP antioxidant activity and the Global Standardized Response, which matched the

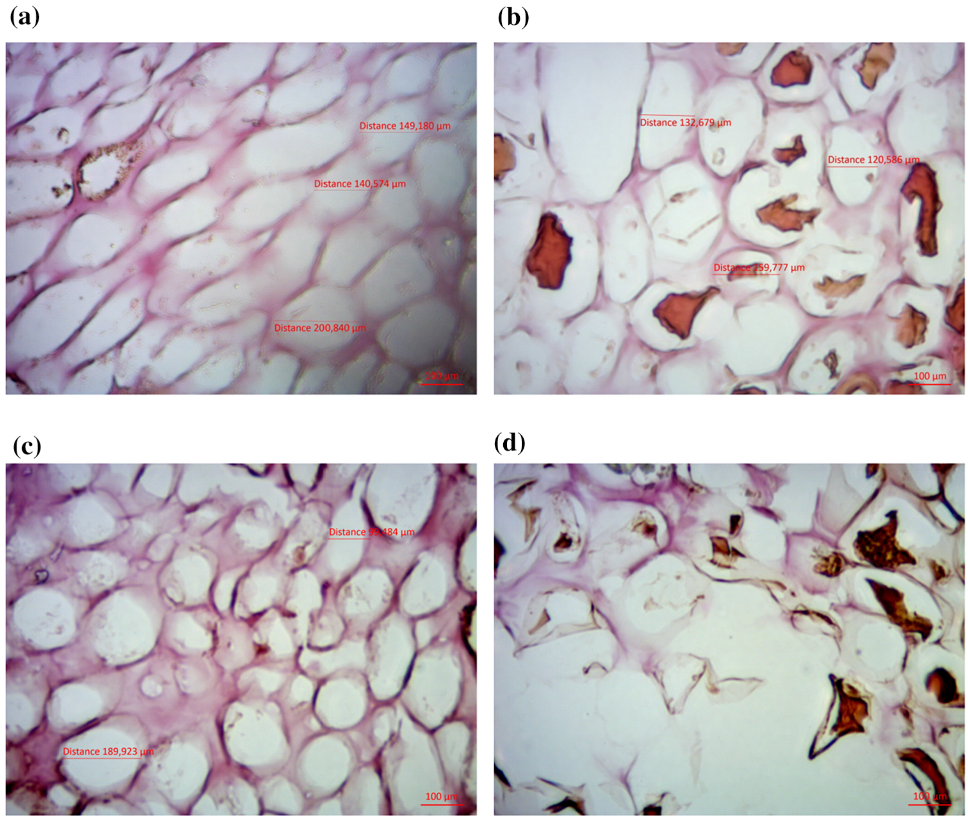

Fig. 3 Photomicrographs of dried murtilla fruit skin **a** before ultrasound pretreatment in distilled water, **b** after ultrasound pretreatment in distilled water, **c** before ultrasound pretreatment in an osmotic solution, and **d** after ultrasound pretreatment in an osmotic solution

DPPH, FRAP, and ORAC results. Pretreatment of murtilla with ultrasound immersed in either distilled water or the osmotic solution affected the fruit skin microstructure. The cells became more distorted, and the loss of cell adhesion resulted in large spaces among the cells. Pretreatment procedures based on different ultrasound conditions and osmotic solution concentrations combined with convective drying should be evaluated to confirm the skin cell damage and to improve the release of polyphenolic components from murtilla fruit and increase its antioxidant activity after drying.

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