

## Effect of Freezing and Processing Technologies on the Antioxidant Capacity of Fruit Pulp and Jelly

Cristine Maso Jeusti Bof<sup>1</sup>, Roselei Claudete Fontana<sup>2</sup>, Luciani Tatsch Piemolini-Barreto<sup>1</sup> and Ivana Greice Sandri<sup>1\*</sup>

<sup>1</sup>Centro de Ciências Exatas e Tecnologias; Curso de Engenharia de Alimentos; Universidade de Caxias do Sul; Rua Francisco Getúlio Vargas, 1130; C. P.: 1352; 95070-560; Caxias do Sul - RS - Brasil. <sup>2</sup>Instituto de Biotecnologia; Universidade de Caxias do Sul; Rua Francisco Getúlio Vargas, 1130; 95070-560; Caxias do Sul - RS - Brasil

### ABSTRACT

*The effect of freezing and processing technology on the antioxidant capacity of grape (*Vitis vinifera*), apple (*Malus domestica*), strawberry (*Fragaria x Anassa*), pear (*Pyrus communis* L.), guava (*Psidium guajava* L.), and fig (*Ficus carica* L.) was evaluated for 90 days. Under a storage temperature of -15 °C, there was no significant difference in the antioxidant capacity of grape and fig pulp, and a higher antioxidant capacity was found for guava pulp (27 µmol/g). While the technological processing did not affect the antioxidant capacity of pear and apple jellies, all other jellies studied showed a reduced antioxidant capacity. The processing reduced the antioxidant capacity of grapes in 45%. Among the fruit products, the highest antioxidant activities were found for guava pulp and jelly (27 and 25 µmol/g, respectively), followed by grape pulp (22 µmol/g).*

**Key words:** functional foods, pulps, jellies, free radicals, storage

### INTRODUCTION

Natural antioxidants, particularly those found in the fruits and vegetables, have attracted the interest of consumers and the scientific community. Epidemiological studies have shown that there is a positive association between the intake of both vegetables and fruits and a reduction in cardiovascular diseases (Hu 2003; Bonerz et al. 2006; Ikram et al. 2009; Labiós et al. 2011), certain cancers (Ikram et al. 2009; Riboli and Norat 2003), immune system problems, arthritis, inflammation and brain dysfunction (Leong and Shui 2002; Greenspan et al. 2005), and risk of Alzheimer's disease (Dai et al. 2006).

Antioxidants are substances that, if present in low concentrations, significantly prevent the oxidation

of a substrate. Among the antioxidants, there are enzyme systems such as superoxide dismutase, catalase, and glutathione reductase, while vitamins, uric acid, glutathione, melatonin, polyphenols, amongst others, are non-enzymatic antioxidants (Packer and Colman 1999; Kim and Chung 2002; Halliwell and Gutteridge 2007).

Human body produces reactive species of carbon, sulfur, nitrogen and oxygen, but the most important due to their reactivity and damage they can cause are the reactive oxygen species, such as superoxide, hydrogen peroxide, and hydroxyl radical (WHO 2003).

The presence of phenolic compounds, such as flavonoids, phenolic acids, and anthocyanins, in addition to those already known, such as vitamins C and E, and carotenoids, contributes to the beneficial effects of these foods (Silva et al. 2004;

\* Author for correspondence: igsandri@ucs.br

Ajaikumar et al. 2005). In recent years, there is a growing interest in determining the total phenolic contents and antioxidant capacities of vegetables and fruits (Ayala-Zavalaa et al. 2004; Zheng et al. 2007; Çelik et al. 2008; Rui et al. 2011; Prasad et al. 2011).

Phenolic compounds content can be influenced by the factors such as maturity, species, type of cultivation, geographic origin, level of growth, harvest conditions and storage process (Kim et al. 2003). In addition, the bioactive compounds are susceptible to oxidation reactions during the processing and storage of food (Robards et al. 1999), because some of these compounds are unstable under thermal processing (Samaniego-Sánchez et al. 2011), cold storage (Cilla et al. 2011; Ibrahim et al. 2011). The objective of this study was to evaluate the effect of freezing and processing technologies on the antioxidant capacity of the pulp and jelly of fruits (grape, apple, strawberry, pear, guava, and fig).

## MATERIAL AND METHODS

### Raw materials

The fruits used in this study were grape (*Vitis vinifera*) Isabela variety, apple (*Malus domestica*) Gala variety, strawberry (*Fragaria x Anassa*) Diamante and Oso Grande varieties in equal proportions, pear (*Pyrus communis* L.) Williams variety, guava (*Psidium guajava* L.) Rica and Século XXI varieties, in equal proportions, and fig (*Ficus carica*, L.) Roxo de Valinhos variety. They were acquired from a market at Caxias do Sul, RS, Brazil. All the fruits were harvested during the summer 2009-2010. They were first selected and washed with tap water, before being processed.

In order to obtain the pulp, the fresh fruits were processed in a horizontal pulp-processing machine (Tomasi, Brazil). Pulp (1000g) of each type of fruit was frozen and stored at  $-15^{\circ}\text{C}$  for three months.

To obtain the jelly, 45% of fruit pulp and 55% of sugar were taken as follows. The pH of the fruit was adjusted to 3.2 by adding citric acid (Merck, Germany). The fruit pulp was transferred to a jacketed steam kettle, mixed with cane sugar and citrus pectin (CP Kelco S.A., Brazil). The mixture was heated until boiling and reaching 68 Brix. The finished product was pasteurized in 100-mL jars with lids. Afterwards, the jars were inverted for 3 min to pasteurize the lids, allowed to cool, and

then stored in an upright position at room temperature. Jelly (1000 g) of each type of fruit was stored at room temperature ( $25 \pm 2^{\circ}\text{C}$ ) for three months.

The antioxidant activity of the pulp and jelly of each fruit was determined after 0, 30, 60, and 90 days. The antioxidant activity was measured in triplicate for each fruit pulp and jelly.

### Obtaining the extracts

The extraction was performed according to Larrauri et al. (1997), with modifications. The extracts were prepared with 10g of sample using 40 mL of methanol (50w/v). After homogenization, the mixture was left to stand for 60 minutes at room temperature and after this period, the material was centrifuged. The supernatant was transferred to a 100-mL volumetric flask. Forty milliliter of acetone (70 w/v) were added to the precipitate of the first extraction. After homogenization, the mixture was allowed to stand for 60 minutes at room temperature. The mixture was centrifuged, and the supernatant was transferred to the flask containing the first supernatant and the volume was completed with 100 mL of distilled water.

### Determination of antioxidant activity

The antioxidant activity was determined according to the method described by Nenadis et al. (2004). The absorbance was read exactly 6 min after initial mixing of 3.0 mL of ABTS<sup>•+</sup> (2,20-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) and 30  $\mu\text{L}$  of extracts or Trolox standards. The absorbance was measured at 734 nm. Standard curve was prepared using different concentrations of Trolox (6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid) and the results were expressed as Trolox equivalent antioxidant capacity (TEAC) ( $\mu\text{mol TEAC} / \text{g sample}$ ).

### Analysis of results

The statistical tests were performed by using the analysis of variance (one-way ANOVA) and Tukey's test, with probability level below 5 % ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

The antioxidant activity of the fruit pulp and jelly of guava, grape, fig, strawberry, apple, and pear

right after their preparation are shown in Table 1. A variation of 4.0–27.0  $\mu\text{mol/g}$  in the antioxidant activity was observed in the studied fruits. When comparing the antioxidant activity between the pulp and the jelly of each fruit, a decrease was observed in the grape, apple, and pear jellies.

According to Dávalos et al. (2005) and Ruberto et al. (2007), the antioxidant activity of the fruits and fruit jellies was directly related to the content of phenolic compounds, and these compounds could be degraded by physical-chemical factors related to food processing.

**Table 1** - TEAC values (Trolox equivalent antioxidant capacity) of fruit pulp and fruit jelly right after preparation.

Fruit	TEAC ( $\mu\text{mol/g}$ )	
	Pulp	Jelly
Guava	27.0 $\pm$ 0.07 <sup>a</sup>	25.0 $\pm$ 0.04 <sup>a</sup>
Grape	22.0 $\pm$ 0.03 <sup>b</sup>	10.0 $\pm$ 0.05 <sup>c</sup>
Fig	5.1 $\pm$ 0.03 <sup>f</sup>	5.0 $\pm$ 0.02 <sup>e</sup>
Strawberry	16.0 $\pm$ 0.03 <sup>c</sup>	15.2 $\pm$ 0.05 <sup>b</sup>
Apple	11.1 $\pm$ 0.02 <sup>d</sup>	5.5 $\pm$ 0.01 <sup>d</sup>
Pear	7.3 $\pm$ 0.01 <sup>e</sup>	4.0 $\pm$ 0.03 <sup>f</sup>

Values correspond to the average of three tests. Values followed by same letters in each column do not differ statistically at a 5% level ( $p < 0.05$ ).

As expected, apple and pear showed the lowest antioxidant capacity (Kevers et al. 2007). As known, the antioxidant capacity of fruits is attributed to vitamin C and phenolic compounds such as flavonoids and phenolic acids (Lee et al. 2003, Müller et al. 2010). Vitamin C was found to account for 65–100% of the antioxidant potential of beverages derived from the citrus fruits, but less than 5% of apple and other non-citrus fruit juices (Gardner et al. 2000). The vitamin-C portion was responsible for more than 15% of the antioxidant capacity in the samples known as low anthocyanin fruits (acerola, apple, orange) (Müller et al. 2010). The phenolic compounds change in contact mainly with oxygen. According to Zardo et al. (2009), the reaction of enzymatic browning is the major factor responsible for the loss (up to 83%) of the antioxidant activity in apple juice. Besides enzymatic browning, phenolic compounds present in apple and pear change during the process of obtaining pulp and jelly.

In general, the products from fruit with higher intensity of red color (guava, grape, and strawberry) had higher antioxidant capacity (Table 1). This was in agreement with other studies suggesting that amongst all the common fruits and vegetables in the diet, those with dark blue or red colors have the highest antioxidant capacity (Liu et al. 2002; Wu et al. 2006; Solomon et al. 2006; Çelik et al. 2008).

The high antioxidant activity in these products might be attributed to the presence of anthocyanins. Abe et al. (2007) studied the phenolic compounds and antioxidant capacity of

varieties of *Vitis labrusca* and *Vitis vinifera* grape and found a higher correlation between total anthocyanins and antioxidant capacity in comparison to total phenolics and antioxidant capacity. Kalt et al. (2001) and Stojanovic and Silva (2007) also concluded that anthocyanins could make a greater contribution to antioxidant activity than other phenolic compounds.

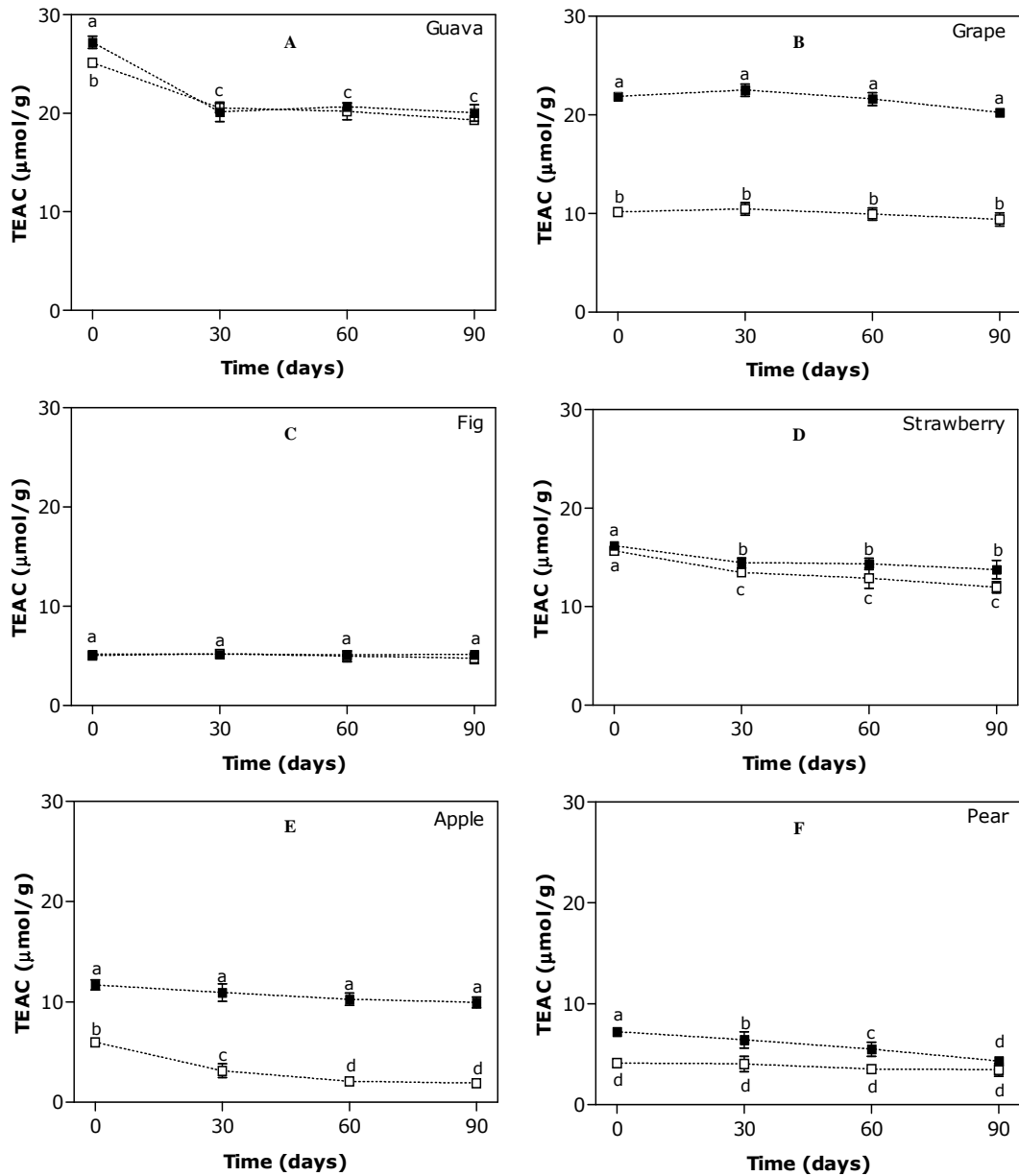
Figure 1 shows the antioxidant activity of the pulp and jelly of each fruit after 0, 30, 60, and 90 days of storage. In general, the heat processing caused a decrease in antioxidant capacity. Heat treatments have been shown to significantly decrease the concentration of polyphenols in apple juice (Aguilar-Rosas et al. 2007). Khanal et al. (2010) reported lower contents of proanthocyanidins from grape and blueberry pomaces heated at 60, 105 °C and 125 °C. The components with antioxidant capacity present in strawberry and fig jelly showed to be heat-resistant. This result could be related with monomeric pigment concentrations during the storage (Withy et al. 1993), stability of anthocyanins (García-Viguera et al. 1999), growing season, geographical origin, and agricultural practices (Chun et al. 2005).

In contrast, storage time in the freezer and contact with light did not affect these compounds. According to Wicklund et al. (2005), jelly stored at 4 °C had a higher content of anthocyanins and total antioxidant capacity than the samples stored at 20 °C, while there were no significant differences between dark and light storage.

A decrease in antioxidant activity was found after 30 days of storage for guava jelly and for guava

pulp. Even showing a decrease of 26% (pulp) and 20% (jelly), this fruit showed higher antioxidant activity than other fruits tested (Fig. 1A). The high presence of antioxidant activity in guava may be related to levels of polyphenols and flavonoids.

While Hassimotto et al. (2009) measured 3.2  $\mu\text{mol}$  equiv. BHT/g of antioxidant activity in red guava pulp, the present study found values eight times higher. Some factors such as maturity, material preparation, and analysis methods might cause the difference (Xu et al. 2008).



**Figure 1** - TEAC values (Trolox equivalent antioxidant capacity) ( $\mu\text{mol/g}$ ) of fruit pulp and jelly of guava, grape Isabel variety, fig, strawberry, pear, and apple Gala variety for 90 days of storage. The values correspond to the average of three tests. Values followed by same letters do not differ statistically at the level of 5% ( $p < 0.05$ ) (filled square, pulp, open square, jelly).

Grape pulp and grape jelly showed significant differences between each other, but the antioxidant activity did not change during the storage period. The fruit pulp showed 50% more antioxidant activity than the jelly (Fig. 1B). Falcão et al. (2007) evaluated the antioxidant activity of grape jelly made of the Isabel and Refosco varieties, and the values ranged between 3.9–10.2  $\mu\text{mol/g}$ . These values were similar to those obtained in this study. Kuskoski et al. (2005) evaluated the antioxidant activity in grape pulp and obtained a value of 9.2  $\mu\text{mol/g}$ , lower than the values found in this study. The differences between the values quoted in the literature and those found herein were probably due to grape variety, climatic conditions and weather at the growing site, which might have interfered in the phenolic compound content and, consequently, in the antioxidant properties.

Figure 1C shows the antioxidant activity in fig pulp and jelly. Among the evaluated fruit pulps, fig pulp showed lower antioxidant activity, but in fig jelly, its initial activity remained stable. Veberic et al. (2008) evaluated the content of phenolic compounds in figs in the northern Mediterranean area and found that phenol content varied according to harvest dates and the variety, which might justify the low antioxidant activity when compared with other fruits evaluated. The jelly was prepared with the skin, which contained healthful nutrients that should not be discarded. Solomon et al. (2006) reported that fig fruit skin was a major source of anthocyanins and polyphenols.

Strawberry pulp and strawberry jelly showed an initial antioxidant activity of 16.0 and 15.2  $\mu\text{mol/g}$  (Fig. 1D), respectively, similar to the values obtained by Kuskoski et al. (2005), who obtained 12  $\mu\text{mol/g}$  in strawberry pulp. The antioxidant activity of strawberry is due to the presence of anthocyanins and gallic acid (Zheng et al. 2007). The antioxidant activities of apple pulp and apple jelly are shown in Figure 1E. The pulp and jelly presented significant differences between each other, and higher activities were found for the pulp, indicating again that compounds with antioxidant activity present in apple were affected when processed in jelly. Storage time did not affect the antioxidant activity of the pulp significantly, indicating that the temperature of  $-15\text{ }^{\circ}\text{C}$  had a protective effect on antioxidant activity. The jelly that was kept at room temperature lost 50% of its antioxidant activity.

The antioxidant activity of the pear pulp (Fig. 1F) showed a significant decrease during the period of study, and this behavior was not observed for the antioxidant activity of pear jelly. According to Halliwell (1997), this decrease observed for the pulp could be explained by the oxidation of the antioxidant compounds in contact with light and oxygen, as well as by the differences in antioxidant activity between the pulp and jelly, resulting from the handling of the jelly.

Xu et al. (2008) found that the contribution of ascorbic acid to the total antioxidant capacity of citrus juices was more than 50%. These results were in agreement with previous reports (Arena et al. 2001), which suggested that ascorbic acid, not the phenolic compounds, was the major contributor of total antioxidant capacity. However, some studies suggested that phenolic compounds dominated total antioxidant capacity of citrus fruits (Rapisarda et al. 1999).

Previous published data suggested that the antioxidant properties of fruits could be influenced by various external factors, including the cultural system or the storage temperatures (Ayala-Zavalaa et al. 2004; Cordenunsi et al. 2005). Jin et al. (2011) reported that strawberries stored at  $10\text{ }^{\circ}\text{C}$  had higher antioxidant enzyme activities, higher phenolic and anthocyanin contents, and higher oxygen radical scavenging capacities than those stored at 0 or  $5\text{ }^{\circ}\text{C}$ . This suggested that secondary metabolites, such as phenolics or anthocyanins, could be manipulated by postharvest storage conditions, including storage temperature.

## CONCLUSIONS

All the studied fruit pulps and jellies showed antioxidant capacity, although of different intensities. Of all the fruit pulps and jellies studied, guava pulp and jelly had highest antioxidant activity. The processing and storage time might have contributed to the loss of antioxidant activity in the pulp and jelly during the evaluation period. Results showed that the fruit pulp and jelly could be used as sources of natural antioxidants, and they could be more effective and economical than using dietary supplements to protect the human body against oxidative damage. Therefore, it could be concluded that the consumption of these products should be encouraged.

## ACKNOWLEDGEMENTS

The authors are thankful to the Universidade de Caxias do Sul (UCS) for their financial support of this work.

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Received: February 07, 2011;

Revised: June 06, 2011;

Accepted: December 12, 2011.