

THE EFFECT OF ENZYMATIC TREATMENT ON BIOACTIVE COMPOUNDS AND ANTIOXIDANT CAPACITY OF PASTEURIZED APPLE JUICE

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Abstract

In this study the effect of enzymatic treatment of pasteurisation apple juice derived from two apple cultivars, on bioactive compounds and antioxidant capacity was evaluated. The enzymatic treatment with pectinase was used to obtain the clear juice. The total polyphenols content was determined by Folin-Ciocalteu method, the total flavonoids was determined by colorimetric method and vitamin C was determined by titrimetric method. The antioxidant activity was determined by DPPH method. The enzymatic treatment decrease significantly the content in polyphenols and flavonoids. The vitamin C drastic decreases during the pasteurisation process, because is heat labile. The antioxidant capacity was not affect by the enzymatic treatment in the case of Golden juices but it was low in the case of Florina juices by 17.72%.

Key words: apple juice, polyphenols, flavonoids, antioxidant capacity, pectinase

INTRODUCTION

The apples are one of the most fruits consumed in the world. The benefits of consumption of the apples are derived from its secondary metabolites composition, like phenolic compounds. This class include a large group of bioactive compounds, from simple phenol molecules to polymeric structures. The type and the level of these compounds varied with species, the variety, the physiological stage, tissue type and also, the environmental conditions (Carbone et al., 2011; Scalbert & Williamson, 2000). Epidemiological studies (Malin et al., 2003; Joshipura et al., 2001) shown a direct correlation between the high consumed fruits and decrease of appearance of different type of cancer and cardiovascular disease. From this reason, the adage "an apple of day keep the doctor away" is quite popular.

The most commonly phytochemical compounds present in whole apple with antioxidant effect are quercetin conjugates, catechin, epicatechin, procyanidin, coumaric, chlorogenic and gallic acid, phloridzin (Figure 1).

The high content in bioactive compounds are found in apple peels, compared with apple flesh.

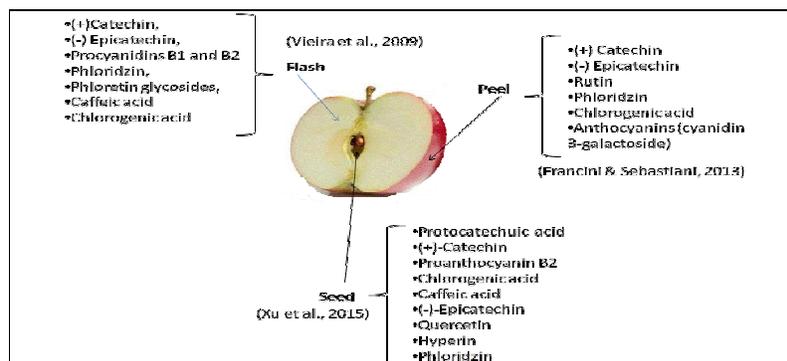


Figure 1. Distribution of bioactive compounds in the apple fruit

The aim of this study was to evaluate the effect of enzymatic treatment of pasteurisation apple juices (80°C, 20 minutes) on bioactive compounds such as total polyphenols, total flavonoids, vitamin C and antioxidant capacity.

MATERIALS AND METHODS

Apple cultivars and enzymatic treatment of apple juice

The experiments were carried out using 2 apple cultivars (Florina and Golden). The apple fruits were harvested at commercial maturity, weighed and measured and immediately processed for the analyses described below. To obtain the juice, the seeds of fruits were removed manually and the flesh was introduced into a commercial juice extractor. From apple juices were determined the fruit quality indices (total soluble solids, titrable acidity and pH). Then, the fruit juice samples were divided into two portions. A portion was treated with enzymes called pectinase (Gammapect LC), and maintained at 40°C for 20 minutes. The samples treated with enzymes were called GE and FE, originating from Golden and Florina apple cultivars respectively. Another portion was untreated with enzyme, and was called G and F (derived from Golden and Florina apple cultivars). Then, both samples (treated and untreated) were pasteurised at 80°C, for 20 minutes, centrifuged and from supernatants, the bioactive compounds and antioxidant capacity were recorded.

Determination of physical-chemical parameters of juices

Total soluble solid content (SSC), expressed in °Brix was determined for the juice of each cultivar using a digital hand-held refractometer (DR201-95). The pH of diluted fruit pulp (1:10, w/v) was measured using a pH-meter (WTW GmbH). Titrable acidity (TA) was determined by titrating 10 ml of diluted fruit-juice (1:10, w/v) with 0.1 M NaOH. Three titration analyses per cultivar were performed, and the data were expressed in g malic acid/100 g fresh weight (FW).

Determination of bioactive compounds

- ***Total Polyphenols Content***

Total phenolic content was determined by the Folin-Ciocalteu method (Singleton *et al.*, 1999). The fruit juice (100 µl) was mixed with 1700 µl distilled water, 200 µl Folin-Ciocalteu reagent (dilution 1:10, v/v) and 1000 µl of 15% Na₂CO₃ solution, and the mixture was incubated at room temperature, in the dark, for 2 hours. The absorbance was measured at 765 nm using a spectrophotometer (Shimadzu 1240 mini UV-Vis). The results were expressed in mg gallic acid equivalents (GAE)/100 ml juice.

- ***Total Flavonoids Content***

The total flavonoids content was determined using a colorimetric method [Kim *et al.*, 2003]. Firstly, 1 ml of the extract was mixed with 4 ml water and introduced in a volumetric flask (10 ml). Then, 3 ml of NaNO₂ (5%) solution were added, shaken up and standing for 5 minutes. Secondly, 0.3 ml of the AlCl₃ (10%) solution was added to the volumetric flask, shaken, and was left to stand for 6 minutes. Finally, 2 ml of the NaOH (1M) solution was added to the volumetric flask, shaken and left to stand for 15 minutes before determination. The absorbance was recorded at 510 nm, using a spectrophotometer Shimadzu mini UV-Vis and the results were expressed as mg quercetin equivalent (QE)/100 ml juice.

- ***Vitamin C***

The content of vitamin C in apple juices was determined using titrimetric method with 2,6-dichlorophenolindophenol reagent [Contreras-Calderón *et al.*, 2011] 10 g of homogenized fresh sample was mixed with 20 ml of 2% solution oxalic acid. The mixture was homogenized, diluted to 100 ml with 2% oxalic acid solution and filtered. Ten ml of filtrated solution was titrated with 0.01% of 2,6-dicloro-phenol-indophenol solution and the final point was considered when the solution had a pink colour. Results were expressed as mg of ascorbic acid equivalents /100 ml juice.

Determination of antioxidant capacity by DPPH method

The DPPH radical-scavenging activity was determined using the method proposed by Singleton *et al.* (1999). Briefly, an aliquot of 100 µl sample was mixed with 1.4 ml of DPPH solution (80 µM) and 1 ml ethanol. The homogenate was shaken vigorously and the decrease in the absorbance of the resulting solution was monitored at 515 nm for 5 min on a spectrophotometer (Shimadzu 1240 mini UV-Vis). The percentage of scavenging effect of different extracts against DPPH radicals, was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{[(A_0 - A_5) \times 100]}{A_0}$$

Where, A_0 is absorbance of the blank, and A_S is absorbance of the samples at 515 nm

Statistical analysis

All extraction assays were carried out in triplicate. Results were expressed as means \pm standard deviation (SD). For the comparison between apple samples, one-way analysis of variance, ANOVA (Tukey's Multiple Comparison Test), with GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA, www.graphpad.com) was applied.

RESULTS AND DISCUSSION

The fruit quality parameters, such as total soluble solids, titrable acidity and pH, tested were shown in Table 1. The highest SSC values were observed in Golden apple juice, comparative with Florina apple juice (18.09 ± 0.12 , respectively 14.13 ± 0.11). The Florina apple cultivar showed the highest titrable acidity values, and the lowest value of pH.

Table 1

The fruit quality parameters in two apple cultivar, Florina and Golden

CULTIVAR	Origin	SSC	pH	TA	RI = SSC/TA
Florina	RO	18.09 ± 0.12	3.44 ± 0.02	1.29 ± 0.03	14.023 ± 0.37
Golden	RO	14.13 ± 0.11	3.76 ± 0.02	1.12 ± 0.01	12.61 ± 0.17

The ripening index ratio (RI = SSC/TA) is an important indicator of taste quality aspect of the mature fruit (Hegedus et al. 2010).

The total polyphenols content and flavonoids content in apple juices are presented in Figure 2 (a,b).

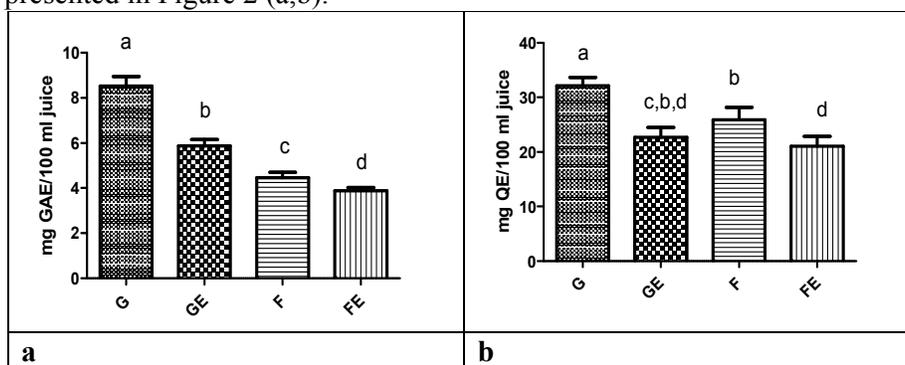


Figure 2. Total polyphenolic content (a) and total flavonoid content of Golden and Florina apple juices untreated with enzymes (G, respectively F) and treated with enzymes (GE, respectively FE). Mean values denoted with the different letters denotes significantly statistically, $p \leq 0.05$.

The highest content in polyphenols was recorded in Golden apple juice comparative with Florina juice. After the treatment of juices with pectinase, the content in polyphenols compounds was significantly decreases. Regarding to the content of total flavonoids, also the Golden apple juice has the highest content comparative with Florina juices. As in case of total polyphenols the content in flavonoids was lowest in the samples treated with enzymes.

The Golden juice has the highest content in Vitamin C comparative with Florina apple juice (Figure 3). After pasteurization, the content in vitamin C significantly decrease, with 73.56% in the case of Golden juice

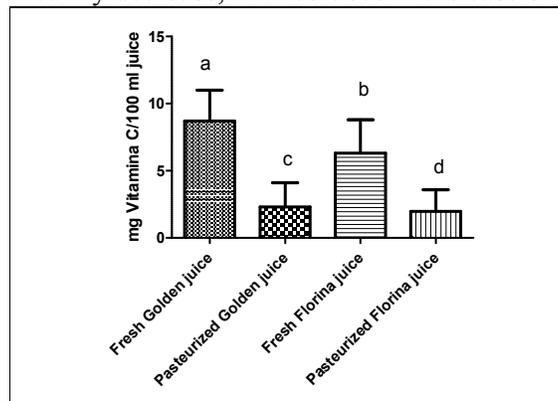


Figure 3. The changes in vitamin C content in fresh juices and pasteurized juices. Mean values denoted with the different letters denotes significantly statistically, $p \leq 0.05$.

Antioxidant capacity of apple juice was determined by DPPH method and the results are shown in Figure 4.

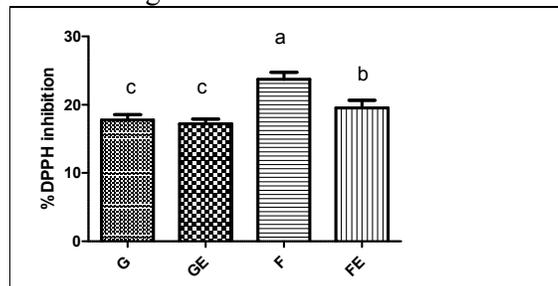


Figure 4. The antioxidant capacity of enzymatic treated (GE, FE) and untreated (G, F) apple juices. Mean values denoted with the different letters denotes significantly statistically, $p \leq 0.05$.

CONCLUSIONS

Our results shown that the treatment of apple juice with pectinase in order to obtain the clarified juice decrease the content in total polyphenols with

31.17% and 12.96% for Golden and Florina apple juices respectively. Regarding to the content of total flavonoids, also the enzymatic treatment, decreased by 29.30% and 18.76% of these compounds in Golden, respectively Florina juices. During the pasteurization, the content in vitamin C decreases significantly, because this vitamin is heat labile. The antioxidant capacity of Golden juices was not affected by enzymatic treatment, instead it was significantly decreased in the case of enzymatic treatment of Florina juices.

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