



ORIGINAL SCIENTIFIC PAPER

Influence of Jam Processing Upon the Contents of Phenolics and Antioxidant Capacity in Strawberry fruit (*Fragaria ananassa* × Duch.)

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Summary

Seven cultivars of strawberry (*Diamante*, *Elsanta*, *Honeoye*, *Madeleine*, *Marmolada*, *Miranda* and *Miss*) were analyzed for total phenols (TP), total flavonoids (TF), total nonflavonoids (TN), total anthocyanins (TA) and antioxidant capacity (AC) before and after low sugar jam production to evaluate their changes after thermal processing. The content of TP, TF and TN were determined according to the Folin-Ciocalteu assay. Anthocyanins were determined by spectral method based on the bisulfite bleaching of monomeric anthocyanins. The antioxidant capacity was evaluated by 2,2-diphenyl-1-picrylhydrazil radical (DPPH).

Fresh fruits had total phenolics ranging from 251.97 to 713.06 mg gallic acid equivalent GAE/g of dry weight. In all investigated samples, fresh strawberries and jams, nonflavonoids were predominant phenols. Cultivar *Honeoye* had the highest amounts, while cultivar *Miss* had the lowest amounts of TP and TN. Cultivar *Miranda* had TF in the highest concentrations, while the lowest concentrations were found in cultivar *Marmolada*. TA for investigated cultivars was 63.55-177.71 mg Cy-3-G/100 d.w. with strong differences among cultivars. On the basis of dry weight the processing and heating during jam making generally decreased the contents of TP for 37-70 %. During processing, cultivar *Madeleine* showed the greatest stability of TP, TN and TF, while cultivar *Elsanta* showed the greatest anthocyanins stability. In comparison with fresh strawberry fruit, whose antioxidant capacity were in the range of 0.23 mmol TE/kg f.w. to 0.67 mmol TE/kg f.w., the jams also represent a noticeable source of antioxidant compounds, even considering the lower content of phenolic compounds, with the antioxidant capacity of 0.20 mmol TE/kg f.w. to 0.62 mmol TE/kg f.w.

Hence, the obtained results showed that besides fresh strawberry fruit, the strawberry jams also possess noticeable content of important bioactive compounds with considerable antioxidant capacity.

Keywords: *Fragaria x ananassa* Duch., strawberry, phenolics, anthocyanins, jam, antioxidant capacity

Introduction

Strawberry (*Fragaria x ananassa* Duch.) is widely consumed, both as fresh fruit and as an ingredient in processed products. It is a very rich source of bioactive compounds including vitamin C, E and phenolic compounds (Aaby et al., 2005, Sun et al., 2002), which have proved useful as markers of strawberry cultivars (Oszmianski and Wojdylo, 2009). The health beneficial effects of strawberry fruits have been attributed to their high levels of a wide variety of phytochemicals, of which phenolics constitute the greatest proportion, contributing to both their sensorial-organoleptic attributes and their nutritional value (Scalzo et al., 2005, Proteggente et al., 2002, Deighton et al., 2000). Furthermore, several studies have shown that the strawberry generally possesses a high level of antioxidant capacity, which is linked to the levels of phenolic compounds in the fruit (Aaby et al., 2005, Rababah et al., 2005, Sun et al., 2002, Vinson et al., 2001).

The increasing demand for dietary compounds with antioxidant action has focused interest on fruits as natural sources of these compounds, but also fruit products, such as jams, can be good source of biologically active compounds with considerable antioxidant potential (Wicklund et al., 2005, Kim and Padilla-Zaokur, 2004, Amakura et al., 2000). To manufacture fruit jams, both at the household and commercial levels, fruits and sugar are combined in similar ratios, followed by cooking, to produce a tasty product of sufficiently high sugar content with satisfactory keeping qualities (Downing, 1996). In the aspect of human health the low calorie character of processed fruit is recommended and for the past decade production of low

calorie food has expanded. Reduced calorie food is a nutritionally altered product that contains at least 30 % less of sugar than the reference food (National Regulations NN 17/09, NN 76/06).

Whereas strawberry is the most important cultivated berry in Croatia, the major part of the crop is sold on the fresh fruit market and only small part is processed, usually by small producers according to old recipes which contain the greater amount of sugar. During the production of jam the fruits undergo to a long heating. Above all, the extended heating could influence in meaningful way the content in phenolic substances (Mikkelsen and Poll, 2002, García-Viguera et al., 1999). The processed strawberry fruits are characterised by rapid colour loss and pigment degradation. The quality, then, would be irreproachable and equivalent to that of the raw fruit through not necessarily presenting the same aspect.

Since, the fruit is not available all the year and reduced calorie strawberry jams are not present in the market, the objective of this work was to determine whether the jams could also represent a good source of bioactive compounds. As antioxidant content is becoming an increasingly important parameter with respect to fruit quality, it is of great interest to evaluate changes in antioxidant status during processing into jam.

In this paper, we studied the total phenolic contents (total flavonoids, total nonflavonoids, total anthocyanins) in fresh strawberries and in strawberry low calorie jams. The scavenging capacity on the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical was measured, too, and the relationship between the phenolic contents and the radical scavenging effect was estimated and discussed.



Materials and Methods

Materials

Strawberry samples

Strawberries (*Fragaria × ananassa* Duch.), of the cultivars *Diamante*, *Elsanta*, *Honeoye*, *Madeleine*, *Marmolada*, *Miranda* and *Miss*, were hand-harvested at full maturity (full red colour) on the same commercial orchard near by Zagreb, Croatia, and brought by car to the Laboratory within 4 h after harvesting. On arrival, the fruits were manually washed, after removal of the sepals and elimination of damaged fruits, and then frozen to -18 °C in polyethylene bags for about 4 weeks, until analysis and jam preparation.

Preparation of jam

Jams were prepared in laboratory conditions under atmospheric pressure. The jam formulation was 64.2 % fruit, 35 % sugar, 0.8 % pectin and 45 °Brix.

Firstly, fruit purée was prepared by blending thawed strawberry fruit (Vaxy Zepter hand blender). Thence, fruit purée blended with sugar (sucrose) was stirred and boiled. The mixture was allowed to boil for 10 minutes after soluble solids were measured by a hand type refractometer (LEICA 7531L). Pectin solution (Grinsted TM Pectin LA 410, Danisco Ingredients, Denmark) was added nearby cooked mass achieve defined solid content of 45°. When cooked mass was made up to 45 °Brix the cooking was finished and jams were filled into hot glass jars, capped and kept pasteurized at 80 °C/10 min. Afterwards, the jams allowed to cool at room temperature and stored in the dark at 20 °C until analysis.

Methods

Determination of total phenolics, flavonoids, nonflavonoids and anthocyanins

The amounts of total phenolics (TP), total flavonoids (TF) and total nonflavonoids (TN) in all samples were determined

spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). The amount of flavonoids was calculated as difference between total phenols and total nonflavonoids. The results are expressed as mg gallic acid equivalent (GAE)/ 100 g of dry weight of edible part of fruits or jam.

Total anthocyanins (TA) were also determined by using spectrophotometric method (Ough and Amerine, 1988). The molar absorption coefficient for cyanidin-3,5-diglucoside was used as a standard value. Results were expressed as mg of cyanidin-3,5-diglucoside equivalents (Cy-3,5-DG) per 100 g of dry matter of edible part of fruits or jam.

Determination of antioxidant capacity

The antioxidant capacity of all samples was evaluated by 2,2-diphenyl-1-picrylhydrazil radical (DPPH[•]) using the method proposed by literature (Brand-Williams et al., 1995). The extracts of total phenolics were also used for DPPH assay. The results are expressed as mmol TE (Trolox equivalent)/kg fresh weight.

All experiments were carried out in duplicate whereas all spectrophotometric measurements were performed by UV-VIS spectrophotometer UV-Vis Unicam β.

Results and Discussion

Strawberries are rich source of polyphenols such as flavonoids, especially anthocyanins, to which many beneficial effects have been attributed (Aaby et al., 2005, Hannum, 2004, Sun et al., 2002). Seven of the most common strawberry cultivars grown in Croatia, grown at the same place and under the same conditions, were compared in relation to the contents of phenolic compounds (Table 1).

Regarding to phenols, this study showed that in all investigated samples, fresh strawberries and jams, nonflavonoids were predominant phenols. Relevant differences among cultivars were observed when TN and TF in fresh samples were

Table 1. Nonflavonoid, flavonoid and total phenols content in fresh strawberry fruit and strawberry jams

Strawberry cultivar	Sample	Nonflavonoids (mg GAE/100 g d.w.)	Flavonoids (mg GAE/100 g d.w.)	Total phenols (mg GAE/100 g d.w.)
<i>Diamante</i>	Fresh	296.78	42.21	338.99
	Jam	275.43	14.08	289.51
<i>Elsanta</i>	Fresh	379.15	59.51	438.66
	Jam	320.29	36.47	356.82
<i>Honeoye</i>	Fresh	656.47	56.59	713.06
	Jam	231.00	31.94	262.94
<i>Madeleine</i>	Fresh	319.06	45.73	364.79
	Jam	294.18	38.91	383.19
<i>Marmolada</i>	Fresh	394.89	33.43	428.32
	Jam	258.39	28.66	287.05
<i>Miranda</i>	Fresh	347.51	62.28	409.79
	Jam	261.08	27.83	288.96
<i>Miss</i>	Fresh	208.85	43.12	251.97
	Jam	191.44	21.34	212.78

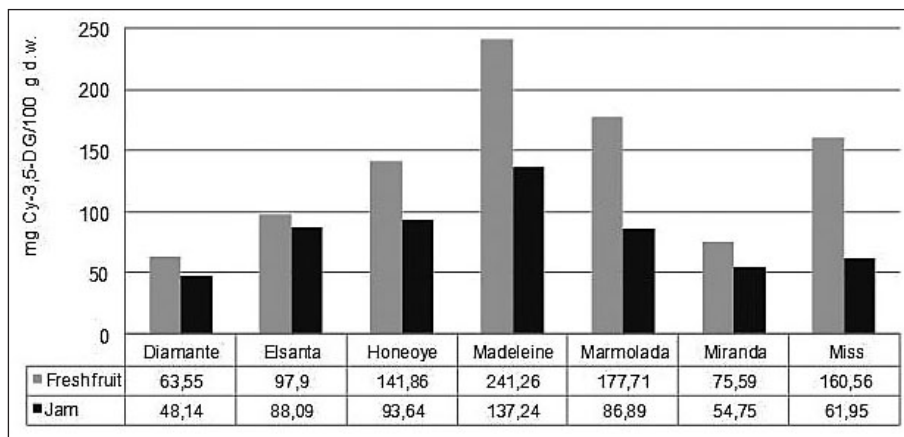
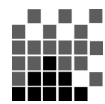


Figure 1. Total anthocyanins in fresh strawberry fruit and strawberry jams

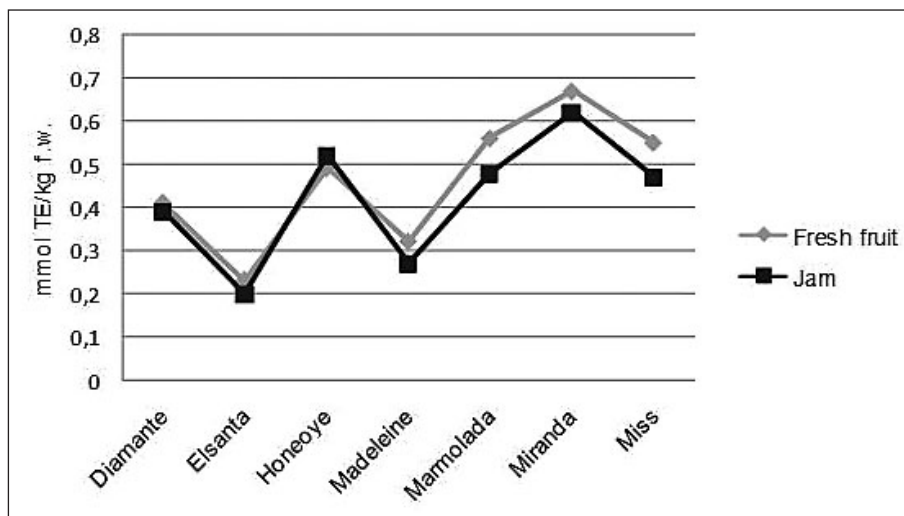


Figure 2. DPPH values in fresh strawberry fruit and strawberry jams

compared. The highest TN was determined in *Honeoye* (656.47 mg GAE/100 g d.w.), while in other cultivars was determined in the range from 208.85 mg GAE/100 g d.w. (*Miss*) to 394.89 mg GAE/100 g d.w. (*Marmolada*). The highest TF contents were determined in *Miranda* (62.28 mg GAE/100 g d.w.) and the lowest, almost two time less, in *Marmolada* (33.43 mg GAE/100 g d.w.). Da Silva Pinto et al. (2008) reported the contents of total flavonoids in seven strawberry cultivars ranged from 27.1 to 48 mg GAE/100 g f.w. what is in agreement with obtained results.

TP in fresh samples ranged from 251.97 mg GAE/100 g d.w. (*Miss*) to 713.06 mg GAE/100 g d.w. (*Honeoye*). Phenolic compounds measured in strawberries confirmed results from other studies. Proteggente et al. (2002) determined the total phenolic content of strawberries with 330 mg GAE/100 g f.w., and Heinonen et al. (1998) measured only half of that result (161 mg GAE/100 g f.w.). Klopotek et al. (2005) found in strawberry 257 mg GAE/100 g f.w. while Skupieñ and Oszmiański (2004) and Pineli et al. (2010) found values from 174 to 443 mg GAE/100 f.w. However, different cultivars contain varying concentrations of total phenolics (Meyers et al., 2003).

The strawberry jam processing alters the content of important bioactive compounds present in starting strawberry fruit. Häkkinen et al. (2000) reported that strawberry jam processing decreased flavonoids by 15-20 %. Our results showed that processing into jam influenced flavonoid losses by 14-66 %. The most stable were flavonoids in *Madeleine* and *Marmolada* jams while the greatest flavonoid losses were observed

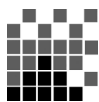
in *Diamante* jam. In comparison with flavonoids, nonflavonoids showed similar stability with losses of 7-64 %. The same as it was approved among stability investigation of flavonoids, the most stable nonflavonoids were also detected in *Madeleine* together with *Diamante*, and *Miss* jams, with losses of 7.87 %, 7.19 % and 8.34 %, respectively. The greatest nonflavonoid losses were observed in *Honeoye* jam.

During processing fruit into jams TP decreased in all jam samples by 37-70 % and concentrations varied between 212.78 (*Miss*) and 383.19 mg GAE/100 g d.w. (*Madeleine*). These results are partially in accordance with the literature showing total phenols losses by 20 % and even more during the processing of strawberries to jam (Amakura et al., 2000, Häkkinen et al., 2000). Although the highest amount of TP was determined in cultivar *Honeoye*, during processing into jam, the most unstable were TP in *Honeoye* jam with the lost of 70.37 %. The stability of TP in *Diamante*, *Elsanta*, *Marmolada*, *Miranda* and *Miss* was similar with the losses of 48.98 % to 56.06 %. The most stable were TP in cultivar *Madeleine* with lost of 37.51 %. Da Silva Pinto et al. (2007) reported for five strawberry jams from a local market level of total phenolics in the range of 58-136 mg GAE/100 g f.w. what is in accordance with obtained results.

Anthocyanins are quantitatively the most important type of polyphenols

in strawberry. The amount of anthocyanins is important for the attractiveness and maturity assessment of strawberries. Regarding to TA in fresh samples, it was observed that cultivar *Madeleine* showed considerably higher value of TA (241.26 mg Cy-3,5DG/100 g d.w.) in comparison to others (63.55-177.71 mg Cy-3,5DG/100 g d.w.) (Fig 1). Cultiva rs *Diamante* and *Miranda* produced jams with lower amounts of TA compared to other strawberry cultivars. Obtained results are in accordance with previous report from Kähkönen et al. (2001) where total anthocyanins for three strawberry cultivars was 184-232 mg Cy-3-G/100 d.w. Meyers et al. (2003) in a study carried out with eight different strawberry cultivars, reported average anthocyanin concentrations of 41.1 mg/100 g f.w., with strong differences among cultivars, the richest one having twice the concentration than the poorest. Similar variation was found by Cordenunsi et al. (2002) for six strawberry cultivars with anthocyanin contents from 13.0 to 55.0 mg/100 g fresh fruit. Pineli et al. (2010) found total anthocyanins for two strawberry cultivars from 22.64 to 29.29 mg/100 g f.w. The influence of cultivar on total anthocyanin content in six strawberry cultivars was approved by Ngo et al. (2007) where the levels of TA was 37,1-122,3 mg Cy-3-G/100 g f.w.

The attractive red colour of fresh strawberries does not normally prevail during processing and storage (Garzón and Wrolstad, 2002). Processing strawberries by manufacturing into jam necessitates high-temperature treatments that can alter and damage color of the finished product. Some strawberry cultivars produce jams with more acceptable and stable colour



than others. The jam recipe, processing procedures, jar type, storage conditions and duration are important factors for the jam quality. Obtained results show that cultivars with higher levels of anthocyanins approved the greater degradation during processing. *Miss*, *Marmolada* and *Madeleine* jams showed the largest lost of TA (61.42 %, 51.11% and 43.12%, respectively), while in other jams degradation of TA was appreciably lower (10.02-43.12 %). The most stable during processing were anthocyanins in cultivar *Elsanta*.

During jam manufacture at atmospheric pressure, anthocyanin losses in the final product varied from 10 % to 80 % when boiling time ranging from 10 min to over 15 min (García-Viguera and Zafrilla, 2001). Processing blueberries into various forms resulted in significant losses of anthocyanins (28-59 %) (Brownmiller et al., 2008). A study about the evolution of anthocyanins in raspberries during jam making showed that 17-40 % of anthocyanins were lost (García-Viguera et al., 1998). Kim and Padilla-Zakour (2004) reported that jam processing caused a 90 % decrease in anthocyanins from cherry. The anthocyanin lost of 70.44 % during processing strawberries into jam was observed in work of Ngo et al. (2007).

Compared to fresh strawberry fruit, whose antioxidant capacity were in the range of 0.23 mmol TE/kg f.w. to 0.67 mmol TE/kg f.w., the jams also represent a noticeable source of antioxidant compounds, even considering the lower content of phenolic compounds, with the antioxidant capacity of 0.20 mmol TE/kg f.w. to 0.62 mmol TE/kg f.w. (Fig 2). This fact could be at least partially explained by the formation of antioxidant Maillard products during jam processing (Klopotek et al., 2005). The results showed that there were no remarkable differences in antioxidant capacity among strawberry jams, in spite of the differences in their TP, TF, TN or TA contents. When looking correlations between TF, TN, TP or TA and DPPH values, our results indicated that the highest correlation (0.731) was found in TP contents and the DPPH capacity in fresh strawberry samples, while jams did not show good correlations. Hence, these results were considered to show that the influence on the DPPH capacity was mostly due to total phenolics. High correlation levels (0.9495-0.9685) were indicated in the total phenolic contents and the DPPH capacity of the berries (Amakura et al., 2000). These findings are in keeping with previous observations (Wang and Lin, 2000, Heinonen et al., 1998) and suggested that associations between the antioxidant properties and the proportion of phenolics present are generally very evident in strawberry.

Conclusions

Strawberries (*Fragaria ananassa* × Duch.) have received much attention due to their positive role in human health and disease prevention. The protective effects of strawberries have generally been attributed to the wide array of phenolics present in the fruit which are responsible for the high free radical scavenging capacity. Results obtained in this study can be considered of particular interest to better define the variations among seven different strawberry cultivars most frequently grown in Croatia. There are considerable differences in the contents of all investigated bioactive compounds among strawberry cultivars.

Cultivar *Honeoye* had the highest amounts of TP and NF, while cultivar *Miranda* had the highest concentrations of TF. In cultivars *Marmolada* and *Miss*, the highest concentrations of TA was determined. Processing procedure had greater impact on decreasing of all bioactive compounds, while it did not have marked impact on decrease of antioxidant capacity. Cultivar *Madeleine* showed the highest stability of TP, TF and TN,

while *Elsanta* showed the highest stability of TA. Although some losses could have occurred, the present results suggest that jams may still represent important sources of bioactive compounds in the diet with noticeable antioxidant capacity.

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