

# Free Radical Scavenging Activity and Phenolic Content in Strawberry Fruit and Jam

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## Summary

Fresh fruit of three strawberry cultivars *Fragaria ananassa* × Duch. (cvs. 'Clery', 'Honeoye' and 'NF 421' (Asia)) were investigated and used to produce jams, which were analyzed before and after dark storage at 20 °C for six months. The aim of this investigation was to characterize all the samples in relation to the total phenolics, flavonoids, nonflavonoids, total anthocyanins and antioxidant activity.

Among investigated strawberry fruit cv. Clery showed the highest amount of total phenolics, while regarding to total anthocyanins, cvs. 'Honeoye' and 'NF 421' showed higher amounts of total anthocyanins. The jam processing under applied conditions had appreciable effects on preserving stability of polyphenolics.

Our results showed that total phenolics were more stable during processing in comparison with total anthocyanins. Furthermore, in all investigated samples flavonoids were predominant. Relevant differences among cultivars were observed when total phenolics, total anthocyanins, flavonoid and nonflavonoid contents in jams were compared. After six months storage, in all investigated samples anthocyanins were at least stable, without marked influences of cultivar.

Compared to the strawberry fruits, the jams also represented a significant source of antioxidant compounds, even considering the lower content of phenolic compounds. Six month storage had influence on further decrease of antioxidant activity. Among investigated samples, the highest correlation was found in the total anthocyanins contents and the DPPH. Hence, the obtained results showed that besides fresh strawberry fruit, the strawberry jams also possess noticeable content of important bioactive compounds with considerable antioxidant activity.

## Key words

strawberry, jam, phenolics, antioxidant activity, storage

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## Introduction

Strawberries (*Fragaria x ananassa* Duch.) are one of the most popular fruits in Croatia and their consumption has increased with the development of new cultivars available at almost all seasons. The majority of fruits are sold on the fresh fruit market and only small proportion is processed, usually by small producers. The most common strawberry products are jams traditionally cooked according to the old national recipes that contain the greater amounts of sugar without adding pectin. Whereas technology plays an important and dynamic role in consumer food choices, increasing attention has been paid by consumers to the health and nutritional aspects (vitamins content, mineral elements, antioxidants, etc.) of fruit and vegetables products (Scalzo et al., 2005). Fruits and vegetables contain significant levels of biologically active components that impact health benefits beyond basic nutrient (Oomah and Mazza, 2000). Numerous studies have suggested that the phytochemical content and corresponding antioxidant activity of fruits and vegetables contribute to their protective effect against chronic and degenerative diseases (Record et al., 2001, Heinonen et al., 1998).

Strawberries are one of the richest sources of natural antioxidants among fruits (Cordenunsi et al., 2002; Wang and Lin, 2000). Several studies have shown that the strawberry generally possesses a high level of antioxidant activity, which is linked to the levels of phenolic compounds in the fruit (Sun et al., 2002; Vinson et al., 2001; Heinonen et al., 1998). Wang and Jiao (2000) showed that strawberry juice exhibited a high level of antioxidant capacity against free radical species including superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen.

The polyphenolic composition and antioxidant properties of different strawberry cultivars have been the subject of many investigations. Several strawberry cultivars have been found to display significantly higher levels of antioxidant activity than others (Meyers et al., 2003; Wang et al., 2002), and the individual flavonoid and phenolic acid compounds have been found to differ among cultivars (Häkkinen and Törrönen, 2000). However, little research has been done on the polyphenolic composition and antioxidant properties of low sugar strawberry jams and no comparison has been undertaken of the contribution of total phenolic, flavonoid and anthocyanin content on antioxidant activities of Croatian strawberries. Thus, it is of interest to further explore the differences in phenolic content and antioxidant activity between different strawberry cultivars in order to provide a more complete characterization of their benefits.

This study is undertaken to investigate the stability of phenolics during processing of three strawberry cultivars Clery, Honeoye and NF 421 (Asia), as in their jams before and after six months of dark storage at 20 °C. As antioxidant activity is becoming an increasingly important parameter with respect to fruit and vegetable quality, it is important to know in which extent they are affected by processing and storage.

## Material and methods

### Strawberry samples

Full-ripened strawberry fruit of the cultivars Clery, Honeoye and NF 421 (Asia), grown nearby Zagreb, Croatia, were hand-harvested and delivered to the Laboratory where they were manually washed and carefully sorted to remove any damaged ones. The fruits were frozen at -18 °C in polyethylene bags. These samples were used for jam preparation within four weeks from freezing. The strawberry purées were prepared from whole thawed strawberries using commercial blender (Vaxy Zepter hand blender).

### Preparation of low sugar jam

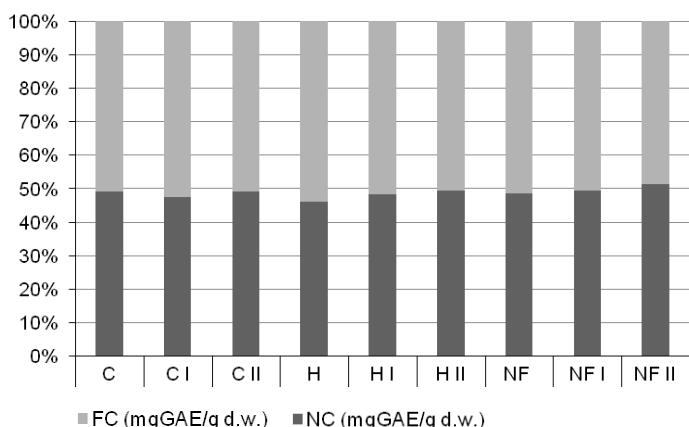
Jams cooked under vacuum were prepared in the pilot plant (Pecon, Croatia). The jam formulation was 64.4% fruit, 35% sugar, 0.6% pectin and 45 °Brix. Fruit purée blended with sugar (sucrose) was placed in a vacuum cooker and stirred and boiled. The cooking temperature did not exceed 80 °C and the vacuum did not exceed 0.8 bar. The mixture was allowed to boil for 20 min, after which soluble solids were measured by a hand type refractometer (LEICA 7531L). Pectin solution (Grinsted TM Pectin LA 410, Danisco Ingredients, Denmark) was added when the nearly cooked mass achieved a defined solid content of 45° Brix. When the cooked mass reached 45 °Brix the cooking was finished and the jams were filled into hot glass jars, capped and pasteurized at 80 °C for 10 min. Afterwards, they were allowed to cool at room temperature and stored in the dark at 4 °C for about one week, until analysis. Other jam samples were stored in the dark at 20 °C until analysis.

### Methods

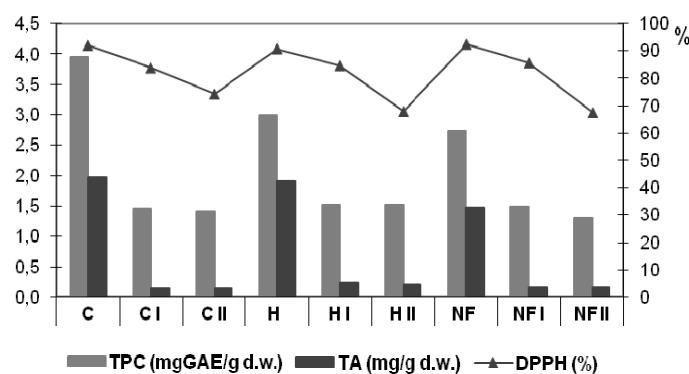
The total phenol content (TPC), flavonoid content (FC) and nonflavonoid content (NC) were determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). The results were expressed as mg gallic acid equivalent (GAE)/ g of dry weight. The amount of flavonoids was calculated as difference between total phenols and nonflavonoids and expressed as mg gallic acid equivalent (GAE)/ g of dry weight. Total anthocyanins (TA) were determined according to method proposed by Ough and Amerine (1989). The results were expressed as mg gallic acid equivalent (GAE)/ g of dry weight. DPPH Radical Scavenging Assay (1,1-Diphenyl-2-picrylhydrazyl) was made by method of Brand-Williams et al. (1995). The results were expressed in% of antioxidant activity. All spectrophotometric measurements were performed by UV-VIS spectrophotometer UV-Vis Unicam β.

## Results and discussion

Strawberries are good sources of polyphenolics such as flavonoids, especially anthocyanins, to which have been attributed many beneficial effects (Hannum, 2004). This study showed that all investigated samples contained very similar quantities of flavonoids and nonflavonoids with slight predominant quantities of flavonoids (>50%) in all samples, except stored 'NF 421' (Asia) jam. In this work, relevant differences among cultivars were observed when FC and NC



**Figure 1.**  
Flavonoid (FC) and nonflavonoid (NC) content in fresh strawberry fruit of cultivars 'Clery' (C), 'Honeoye' (H) and 'NF 421' (NF), their jams (I) and jams stored for six months (II)



**Figure 2.**  
Total phenolic content (TPC), total anthocyanins (TA) and DPPH values in fresh strawberry fruit of cultivars 'Clery' (C), 'Honeoye' (H) and 'NF 421' (NF), their jams (I) and jams stored for six months (II)

in fresh samples were compared (Figure 1). The highest FC and NC contents were determined in 'Clery', followed by 'Honeoye' and 'NF 421'. 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 10-36%. The most stable were flavonoids in 'NF 421' (Asia) jam with losses of 10%, followed by 'Honeoye' jam with losses of 17%. The greatest flavonoid losses of 37% were observed in 'Clery' jam. In comparison with flavonoids, nonflavonoids showed similar stability with losses of 7-40%. The same as it was approved among stability investigation of flavonoids, the most stable nonflavonoids were also detected in 'NF 421' (Asia) and 'Honeoye' jams, with losses of 7% and 10%, respectively. The greatest nonflavonoid losses of 40% were observed in 'Clery' jam.

During six month of dark storage, nonflavonoids were rather more stable than flavonoids. 'Clery' and 'Honeoye'

jams even showed little increase of nonflavonoids during storage by 1.3% and 1.4%, respectively, while 'NF 421' (Asia) jam showed nonflavonoid loss of 3.8%. In comparison with nonflavonoids, flavonoids were slightly more unstable with loss of 2% ('Honeoye'), 4.8% ('Clery') and 11% ('NF 421' (Asia)). However, obtained results showed that the processing procedure had greater impact on flavonoid and nonflavonoid decrease than storage time.

TPC in fresh samples ranged from 3.96 ('Clery') to 2.74 ('NF 421') mg GAE/g of dry weight (Figure 2). These results are in accordance with previous reports (Da Silva Pinto et al., 2008; Da Silva Pinto et al.; 2007, Cordenunsi et al., 2002). Obtained results for TPC fell in the range of 300 to 341 mg GAE/100 g fresh weight reported by Aaby et al. (2005). A recent study (Scalzo et al. 2005) with six cultivars of *F. ananassa* ('Don', 'Idea', 'Camarosa', 'Wave', 'Sveva' and 'Patty') reported phenolic contents between 181.4 and 212.8 mg GAE/100 g fresh fruit.

The highest TPC among investigated fresh strawberries was observed in cultivar 'Clery' (3.96 mg GAE/g d.w.), followed by 'Honeoye' (3.00 mg GAE/g d.w.), and 'NF 421' (2.74 mg GAE/g d.w.). During processing fruit into jams TPC decreased in all jam samples by 45-63%. 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, Gil et al., 1997). During the processing the most unstable total phenolics were observed in 'Clery' jam with the loss of 63%. The stability of TPC in 'NF 421' (Asia) and 'Honeoye' jams was almost equal to the loss of 45% and 49%, respectively.

After six months of storage, total phenols showed similar stability as flavonoids with the decrease range of 0.5% - 12%. However, the most stable were total phenolics in 'Honeoye' and the most unstable in 'NF 421' (Asia) jams.

Unfortunately, the attractive color of fresh strawberries does not normally prevail during processing and storage (Garzón and Wrolstad, 2002). Compared to other berries, strawberries are relatively low in pigment content, ranging in TA from 10 to 80 mg/100 g of fresh weight (Clifford, 2000; Skrede et al., 1992). For eight cultivars of *F. ananassa* analyzed by Meyers et al. (2003), the anthocyanin content ranged from 22.0 to 48.0 mg/100 g fresh fruit. 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.

Regarding to TA in fresh samples, it was observed that cultivars 'Clery' and 'Honeoye' showed higher, while 'NF 421' (Asia) showed the lowest amounts of TA. Degradation during processing and storage can have a major impact on the color of finished products. A study about the evolution of anthocyanins in raspberries during jam making, in which heat was used, showed that 17-40% of anthocyanins were lost (García-Viguera et al., 1998). During jam processing at atmospheric pressure, anthocyanin losses in the final product varied from 10% to 80% when boiling time was ranging from 10 min to over 15 min (García-Viguera and Zafrilla, 2001).

Under vacuum pressure conditions, loss was approximately 40% during a 15 min process (García-Viguera et al., 1999). Boiling time in our processing was about 45–60 min which may had influence on the greater losses of anthocyanins. Considerable differences among investigated cultivars were not found when stability of anthocyanins during the processing was observed.

Six months storage had not a clear effect on the further degradation of anthocyanins. 'Honeoye' jam showed the largest loss of TA (11%), while 'Clery' and 'NF 421' (Asia) showed degradation of TA with the losses of 1.5% and 2%, respectively.

Some strawberry cultivars produce jams with more acceptable and stable colour than others. Loss of red colour during production and/or storage of processed food is influenced by many factors. Various transformations occur during the processing or domestic cooking of foods containing anthocyanins, producing yellowish or brownish pigments which are largely uncharacterized (Clifford, 2000). Anthocyanins losses are probably due to complex formation with other compounds during jam processing. According to Clifford (2000), the nature of the transformation products is not known but there is clear evidence of the involvement of sugars and ascorbic acid (or their thermal degradation products), metal ions and hydrogen peroxide derived from ascorbic acid. Kim and Padilla-Zakour (2004) reported that jam processing caused a 90% decrease in anthocyanins from cherry.

Compared to fresh strawberry fruit, whose antioxidant activity were in the range of 90.74% to 92.41%, the jams also represent a noticeable source of antioxidant compounds, even considering the lower content of phenolic compounds, with the antioxidant activity of 83.76% to 85.65%. 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 antioxydant activity among strawberry jams, in spite of the differences in their TPC and TA contents. Using the same assay Wang and Jiao (2000) reported DPPH values in the range from 57% to 69% for juices of six *F. x ananassa* cultivars.

In the study of Kalt et al. (1999) antioxidant activity was strongly correlated with the content of total phenolics (0.83) and anthocyanins (0.90). Our results indicated that the highest correlation (0.8180) was found in TA contents and the DPPH activity of all investigated samples. In addition, TPC also showed good correlation with the DPPH (0.8022) while FC and NFC showed considerable correlations. Hence, these results were considered to show that the influence on the DPPH activity was mostly due to anthocyanins and total phenolics. These findings are in keeping with previous observations (Heinonen et al., 1998; Wang and Lin, 2000) and suggested that associations between the antioxidant properties and the proportion of phenolics present as anthocyanins are generally very evident in strawberry.

## Conclusions

Strawberry represents one of the most important sources of bioactive compounds with antioxidant activity, together with other berries. Results obtained in this study can be considered of particular interest to better define the variations among three different cultivars grown in Croatia.

There are considerable differences in the contents of all investigated bioactive compounds among strawberry cultivars. 'Clery' had the highest values for total anthocyanins, total phenolics, flavonoid and nonflavonoid content and also elevated antioxidant capacity and, as a result, this cultivar seems to be the most suitable for the selection of promising cultivars in relation to beneficial effects for human health. Jam processing retains evident content of important bioactive compounds present in the starting strawberry fruit. However, in comparison with 'Honeoye' and 'NF 421', 'Clery' showed the lowest stability of bioactive compounds during processing. Obtained results show that the processing procedure had greater impact on decreasing of bioactive compounds than storage time.

Although some losses could have occurred, the present results suggest that jams may still represent important sources of bioactive compounds in the diet.

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