

Influence of Cultivar and Storage Conditions in Anthocyanin Content and Radical-Scavenging Activity of Strawberry Jams

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Abstract—The strawberry jam is rich in bioactive compounds. It is economically and commercially important and widely consumed. Different strawberries cultivars can be used for its preparation, however, a careful selection should be performed to guarantee the preservation of bioactive compounds during jam storage. Two strawberry cultivars (Camarosa and American 13) were analyzed by HPLC, three anthocyanins: cyanidin-3-glucoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside were quantified. Camarosa strawberries presented significantly higher concentration of anthocyanins ($p < 0.05$), and greater radical-scavenging activity.

During jam storage period significant changes occurred regarding anthocyanin composition, at 15 and 60 days. Jams stored in dark presented slightly higher levels of anthocyanins. No significant changes were observed in jam's radical-scavenging activity between 15 and 60 days of storage under dark and light conditions. Camarosa cultivar is more appropriate for strawberry jam preparation because it contains higher levels of anthocyanins and higher radical-scavenging activity.

Keywords—American 13, Anthocyanin, Camarosa, Radical-Scavenging Activity, Strawberry Jams

I. INTRODUCTION

STRAWBERRIES are an important source of bioactive compounds, namely phytochemicals and vitamins that have physiological effects [1], [2]. Among the bioactive compounds, anthocyanins contribute to both the sensorial organoleptic attributes, the nutritional value [3], and influence its quality. Moreover, strawberries are economically and commercially important and widely consumed fresh or in processed forms, such as jams, juices, and jellies. These fruits are among the most studied berries from the agronomic, genomic, and nutritional points of view. The selection of strawberry cultivars to produce jam should also take in consideration health-promoting compounds as they may play a significant role in the prevention of chronic diseases.

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An attractive red color is one of the most important quality characteristics for the strawberry jam industry and influences consumers' acceptance of the product. The anthocyanin content has a strong influence on typical jam red color and is the main source of its antioxidant capacity. However, the storage conditions should be such that ensure the stability of its color and radical-scavenging activity. The color loss is due to the degradation of anthocyanin pigments and the formation of browning compounds such as Maillard reaction products. The antioxidant capacity can decrease, remain unchanged or even increase during processing or storage [4]-[7].

Several factors affect the color of strawberry products, namely, storage temperature, pH, structure and concentration of anthocyanins, and cultivars [8]-[11]. The effect of cultivar and storage temperature on quality of strawberry jam was evaluated [9], [12]. The reported optimum storage temperature for jam is 4 °C. Greater degradation of anthocyanins at higher temperature was observed in other types of jam, such as, raspberry jam stored at room temperature [14]. The degradation of sucrose and anthocyanin during heating may affect both the color and antioxidant capacity. However, to the best of our knowledge, no report exists on the effect of processing and storage (in the dark and in the light) on the anthocyanin content and antioxidant capacity of homemade jams prepared with Camarosa and American 13 cultivars.

Hence, the objectives of this study were the (i) characterization of anthocyanin composition of two strawberry cultivars (Camarosa and American 13) and their radical-scavenging activity, (ii) evaluation of anthocyanin content and radical-scavenging activity after jam preparation, and (iii) study of the evolution of their composition during storage with and without light exposure.

II. MATERIALS AND METHODS

A. Materials

All solvents, reagents and standards used in this study were of analytical grade. Methanol (LiChrosolv) and formic acid (purity=98-100%) were provided by Merck (Darmstadt, Germany). Hydrochloric acid 32% PA-ISO was purchased from Panreac (Barcelona, Spain). 1,1'-Diphenyl-2-picrylhydrazyl (DPPH) and cyanidin-3-O-glucoside chloride and pelargonidin 3-O-glucoside chloride standards were purchased from Sigma-Aldrich (Steinheim, Germany). Ultra-pure water (0.055 $\mu\text{S}/\text{cm}$) was obtained by using a Seral Milli-Q system from Millipore. Standard solutions were prepared in methanol and kept refrigerated in the dark.

B. Sampling Procedure

Strawberry fruits from two different cultivars (cv Camarosa and American 13) were harvested at the commercial maturity stage. Immediately after harvesting, strawberries were frozen at storage at -18°C until analysis. Homemade jams from fresh strawberries of each cultivar were prepared using 500 g of chopped strawberries and 500 g of sugar.

The mixture was heated in a gas stove until ebullience (85°C), and left boiling during 15 minutes in low heat and cooled at room temperature. Jams were analysed after preparation and the remaining amounts were divided in two batches which were stored at room temperature. Two batches of each cultivar were stored in the dark (D), and the others were stored under light exposure (L). Samples were analysed after 15 (T1) and 60 days (T2) of storage.

C. Extraction of Anthocyanins from Strawberries and Strawberry Jams

Samples containing 5g of strawberries were grounded to paste with an electric chopper and strawberry jams were homogenized and weighed, followed by addition of 20 mL of 0.1 % (v/v) hydrochloric acid in methanol. The obtained mixture was shaken during 20 min on a magnetic stirrer and filtered through a 0.45 µm filter.

The extraction process was repeated once more and the pooled filtrates were diluted to 50 mL with 0.1% (v/v) hydrochloric acid in methanol.

As the intensity and stability of the anthocyanins pigments depends of the presence or absence of light, all the analytical steps were carried out in subdued light and under controlled conditions.

D. HPLC-DAD Conditions

Chromatographic analysis were carried out in an analytical HPLC-DAD unit (Jasco, Tokyo, Japan) equipped with Jasco PU-2080 HPLC pumps, an MD-2010 Plus multiwavelength detector, and a type 7725i Rheodyne injector with a 20µL loop.

The column was an ACE C18 column (5 µm; 250mm length; 4.6mm internal diameter). Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France) was used for the acquisition and processing of the HPLC absorbance data. Chromatographic separation was performed using a 10% formic acid (eluent A) and formic acid/water/methanol (10:40:50 v/v/v, eluent B).

The linear gradient program used for anthocyanin separation was 0-5 min, 10% B in A; 5-7.5 min, 10-25% B in A keeping this proportion for 5 min; 12.5-30 min, 25-75% B in A; 30-40 min, 75-10% B in A; and 40-45 min, 10% B in A for column rinse and re-equilibration. Detection of anthocyanins was set at 520 nm, flow-rate was 1.0 mL/min, and separations were carried out at ambient temperature.

E. Quantification of anthocyanins in Strawberries and Strawberry jams

An external calibration method was used for quantification of anthocyanins.

Commercially available anthocyanin standards were diluted in methanol to obtain diluted standard solutions with concentrations ranging from 0.00024 to 0.00095 mg/mL for cyanidin-3-O-glucoside chloride and 0.00053 to 0.034 mg/mL for pelargonidin 3-O-glucoside chloride. Concentration of anthocyanins was expressed as mg/100 g for strawberries and strawberry jams.

The method validation was accomplished by testing the linearity, the detection limit (LOD), the quantification limit (LOQ), the precision (repeatability and reproducibility) and accuracy of the method. The linearity of the method was checked through the calibration standards, and obtained by linear regression of the peak area versus concentration.

The LOD values were calculated as the concentration corresponding to three times the standard deviation of the background noise. Precision was evaluated by estimating the repeatability and reproducibility of the method. One sample of strawberry and of strawberry jam was analysed daily, repeating the analysis over two days.

The repeatability was studied by running three consecutive replications of the same sample in three different concentrations and calculating the RSD for retention times and peaks areas. The reproducibility was calculated as the RSD of retention times and peaks areas across days.

F. Radical-Scavenging Activity

Antioxidant activity was determined using a DPPH-based assay. The radical scavenging activity was determined spectrophotometrically in a Biotek ELX808 plate reader (Biotek Corporation, Broadview, IL).

Six dilutions of each tested sample with two replicates were prepared in 96-well plates. Each well contained 100 µL of diluted methanolic extract and 100 µL of 300 µM DPPH 96 % (v/v) ethanolic solution. The absorbance was measured at 515 nm for 30 minutes. DPPH scavenging percentage was calculated using the following formula:

$$DPPH\ scavenging\ effect\ (\%) = 100 - \left(\frac{A_{extract} - A_{blank1}}{A_{control} - A_{blank2}} \times 100 \right) \quad (1)$$

with $A_{extract}$ as the absorbance of the extract against the DPPH solution, $A_{control}$ the absorbance of the DPPH solution, A_{blank1} the absorbance of the extract alone and A_{blank2} the absorbance of methanol in the well, all obtained after 30 min reaction.

The percentage of remaining DPPH against the extract was then plotted to obtain EC_{50} , the equivalent extract concentration able to scavenge 50 % of the DPPH radicals present in the reaction medium.

G. Statistical Analyses

The averages of duplicate analysis and standard deviations were calculated for each experimental parameter. Mean differences were evaluated with ANOVA, with Duncan test ($p < 0.05$) as post-hoc. Statistical analysis was performed with SPSS for Windows, version 15 (SPSS, Chicago, IL).

III. RESULTS AND DISCUSSION

A. Validation of HPLC-DAD method for quantification of anthocyanins

External calibration curves were established for cyaniding-3-glucoside and pelargonidin-3-glucoside. The linear range was set to concentrations between 0.00095 and 0.0024 mg / ml for cyanidin 3-glucoside and 0.034 and 0.00053 mg / ml for pelargonidin-3-glucoside. All calibration curves showed good linear regression ($r^2 \geq 0.9991$) within test ranges. The LOD was 0.00006 and 0.00007 mg/mL for cyanidin 3-glucoside and pelargonidin-3-glucoside, respectively, while LOQ was 0.00024 and 0.00053 mg/ml for cyanidin 3-glucoside and pelargonidin-3-glucoside, respectively. The developed method provided good precision with intra-day and inter-day variations with RSD lower than 2% for retention times and lower than 5% for peaks area.

B. Characterization of two strawberry cultivars, through the anthocyanin composition and radical-scavenging activity

Three anthocyanins were identified and quantified in the two strawberry varieties: cyanidin-3-glucoside (Cy-3-gl), and pelargonidin-3-glucoside (Pg-3-gl) were identified by comparison with standard retention time and spectra, whereas pelargonidin-3-rutinoside (Pg-3-rt) was identified by comparison with literature and quantified using Pg-3-gl calibration curves.

Table I summarizes results from anthocyanin content and radical-scavenging activity presented as EC_{50} in two strawberry varieties. Camarosa strawberries presented significantly higher concentration of anthocyanins ($p < 0.05$), and greater radical-scavenging activity (lower EC_{50} although differences were not significant) when compared with the American 13 strawberries.

TABLE I
ANTHOCYANIN CONTENT AND RADICAL-SCAVENGING ACTIVITY OF STRAWBERRIES

	Camarosa	American 13
Cy-3-gl	0.76±0.12	0.18±0.01
Pg-3-gl	22.26±1.97	6.80±0.73
Pg-3-rt	3.90±0.94	0.92±0.01
Total anthocyanins	26.38±1.66 ^a	7.96±0.86 ^b
EC_{50}	39.13±2.39 ^a	46.67±2.11 ^a

Individual and total anthocyanins are expressed as mg/100g; EC_{50} as mg/ml. Values represent mean ± standard deviation. Different letters in the same row indicate significant differences ($p < 0.05$)

Cerezo et al, [15] obtained a total concentration of 28.51 mg/100g for the same anthocyanins in strawberry and Buendía et al, [16] quantified 47.4 mg of anthocyanins/ 100g of Camarosa strawberries. The variation observed results from environmental factors such as growing region or ripening season.

No significant differences were found by other authors between the content of anthocyanins in strawberries grown during summer or winter, although the strawberries grown during the summer had higher content than strawberries grown during winter [17].

According to other studies, during the harvest, the content of flavonoids and antioxidant activity were significantly higher in strawberries grown at higher temperatures [18].

C. Anthocyanins and radical-scavenging activity of homemade jams prepared with two cultivars of strawberry and influence of storage time

Figures 1 and 2 show typical chromatograms of fruits and jams from Camarosa and American 13 cultivars, respectively.

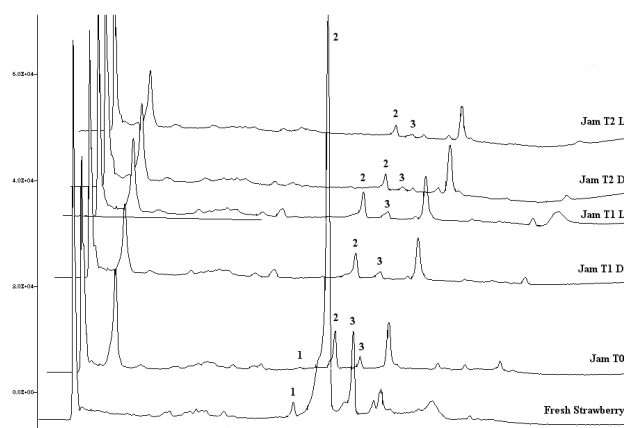


Fig. 1 Chromatograms of fresh strawberry of Camarosa cultivar, and jams. T0-jam after preparation; T1D-jam stored in dark during 15 days; T1L-jam stored in light during 15 days; T2D-jam stored in dark during 60 days; T2L-jam stored under light exposure during 60 days

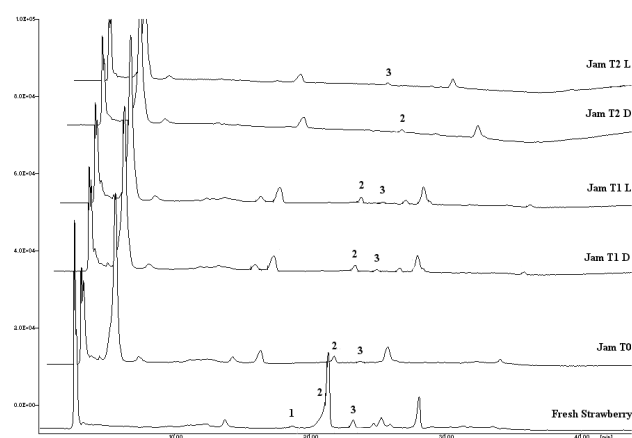


Fig. 2 Chromatograms of fresh strawberries of American 13 and jams. T0-jam after preparation; T1D-jam stored in dark during 15 days; T1L-jam stored in light during 15 days; T2D-jam stored in dark during 60 days; T2L-jam stored under light exposure during 60 days

The experimental results obtained for anthocyanin contents and radical scavenging activity obtained as a function of storage treatment (days) and light/dark conditions are shown in Table II for Camarosa jam and in Table III for American 13 jam.

TABLE II
ANTHOCYANIN CONTENT AND RADICAL-SCAVENGING ACTIVITY OF
CAMAROSA STRAWBERRY JAMS DURING STORAGE TIME

	Cy-3-gl	Pg-3-gl	Pg-3-rt	Total anthocyanins	EC ₅₀
T0	n.d.	2.49±0.03	0.84±0.03	3.33±0.05 ^a	44.33±2.47 ^a
T1D	n.d.	2.15±0.07	1.32±0.12	3.40±0.11 ^a	70.79±4.43 ^b
T1L	n.d.	1.88±0.06	0.78±0.06	2.64±0.08 ^{a,b}	69.12±2.35 ^b
T2D	n.d.	1.21±0.11	0.65±0.04	1.89±0.20 ^b	71.86±8.74 ^b
T2L	n.d.	0.93±0.07	0.59±0.07	1.60±0.11 ^b	81.09±8.16 ^{b,c}

Individual and total anthocyanins are expressed as mg/100g; EC₅₀ as mg/ml. Values represent mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$). n.d.: not detected.

T0-jam after preparation; T1D-jam stored in dark during 15 days; T1L-jam stored in light during 15 days; T2D-jam stored in dark during 60 days; T2L-jam stored under light conditions during 60 days.

TABLE III
ANTHOCYANIN CONTENT AND RADICAL-SCAVENGING ACTIVITY OF
AMERICAN 13 STRAWBERRY JAMS DURING STORAGE TIME

	Cy-3-gl	Pg-3-gl	Pg-3-rt	Total anthocyanins	EC ₅₀
T0	n.d.	3.77±0.23	0.80±0.03	4.58±0.25 ^a	105.6±6.2 ^a
T1D	n.d.	0.86±0.12	0.48±0.14	1.51±0.22 ^{b,c}	100.2±5.4 ^{a,b}
T1L	n.d.	0.86±0.16	0.46±0.11	1.49±0.28 ^{b,c}	109.7±19.9 ^a
T2D	n.d.	0.77±0.09	n.d.	0.77±0.09 ^c	132.0±19.6 ^c
T2L	n.d.	0.53±0.13	n.d.	0.53±0.13 ^c	135.8±8.7 ^c

Individual and total anthocyanins are expressed as mg/100g; EC₅₀ as mg/ml. Values represent mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$). n.d.: not detected. T0-jam after preparation; T1D-jam stored in dark during 15 days; T1L-jam stored in light during 15 days; T2D-jam stored in dark during 60 days; T2L-jam stored in light during 60 days

The results obtained indicate that anthocyanin loss is high in strawberry jams when compared with fruits composition (Fig. 1 and 2). Although anthocyanin composition of the fruits from the two cultivars is different the respective jams after preparation (T0) presented similar composition.

However, during storage different behavior was observed. Jam from Camarosa cultivar maintained its anthocyanin content during the first 15 days of storage and after 60 days of storage around 50% of anthocyanin composition was still quantified, whereas jam from American 13 cultivar suffered a significant reduction of its anthocyanin content during the first 15 days of storage and after 60 days the anthocyanin content was very low.

The results of the radical-scavenging activity of the strawberry jams, using the DPPH assay, highlight significant differences between jams from the two cultivars. EC₅₀ observed for American 13 jam was twice the value observed for Camarosa jam indicating a much lower antioxidant activity. No significant differences were observed in radical-scavenging activity of jams stored at room temperature under dark and light conditions between 15 and 60 days.

The evolution of radical-scavenging activity (expressed as 1/EC₅₀) of strawberry fresh fruits and respective jams over 60 days storage period is highlighted in Fig.3.

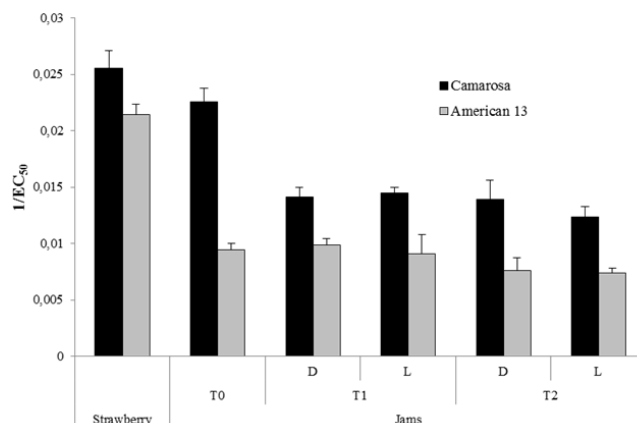


Fig. 3 Radical-scavenging Activity of Camarosa and American 13 Jams with different storage conditions

These results are in agreement with those obtained by other authors that reported a decrease by 50–60 % of initial value of radical scavenging activity of bayberry, blackcurrant, blackberry, blueberry, raspberry, red currant and strawberry jams [5].

IV. CONCLUSION

The strawberry cultivar used to prepare jam should be carefully selected to guarantee highest amount of health-promoting compounds and antioxidant properties. During the storage period significant changes occur in jam composition at 15 and 60 days, concerning anthocyanin composition. Jams stored in dark presented slightly higher levels of anthocyanins. No significant changes were observed in jams' radical-scavenging activity between 15 and 60 days of storage under dark and light conditions. Owing to its high anthocyanin content and radical-scavenging activity Camarosa cultivar is preferred than American 13 for strawberry jam preparation.

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