COLOR STABILITY OF STRAWBERRY JAM FORTIFIED BY PURPLE CARROT PUREE

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Abstract

The discoloration of strawberry products due to the rapid degradation of pigments is considered as one of the most quality defects that affect their acceptance. Therefore, in the present study, the objective was to investigate the possibility to improve the color stability of strawberry jam by using purple carrot puree. The obtained results declared that purple carrot puree characterized by high content of anthocyanins and total phenols compared to strawberry puree. Furthermore, the contents of individual anthocyanins were analyzed by HPLC. The highly stable acylated anthocyanins pigments represented 66.64% of total anthocyanins in purple carrot compared to 20.6 % from strawberry. Cyandin-3-xylosyl-glucosyl-galactoside acylated with Ferulic acid was the predominant anthocyanin in purple carrot while Pelargonidin 3-glcosside was the major component in strawberry. Purple carrot puree was adding by 15, 20 and 30% to strawberry puree during jam processing. The jams were monitored over a period of 6 month of storage under normal conditions of light exposure and room temperature. The results revealed that pH value, viscosity, total anthocynin and total phenol contents were increased gradually by increasing the amount of purple carrot and markedly decreased the degradation of anthocyanin during storage period by 53.2, 52.9, 18.0 and 7.3%in control,15, 20 and 30% samples respectively. Furthermore, the color of jams were controlled during the storage period. L^*, a^* , b^*, hue ° and chroma values showed that the color of strawberry jams containing purple carrot was improved and showed stability. The results of sensory evaluation revealed that the jam of 20% purple carrot treatment obtained the highest overall acceptability score at the end of storage period.

Finally, from all results obtained, it can be concluded that the addition of purple carrot up to 20% during strawberry jam processing maintained the color of jam without any effect on their acceptance.

INTRODUCTION

Besides flavor, texture and economic considerations, color is one of the most important attributes affecting consumer acceptance of food. Color stability, particularly after heat and light exposure, remains a challenge. Therefore, both natural and synthetic colorants are added to processed food to compensate for varying quality and to restore initial appearance, but also to color food, which would otherwise be unattractive or unappealing (Stintzing *et al.*, 2002).

Strawberries (*Fragaria ananassa*), that are one of the most popular fruits worldwide, are rich in nutrients such as amino acids, vitamins and anthocyanins. Loss of quality in this fruit is mostly due to its relatively high metabolic activity and sensitivity to fungal decay. Strawberries are also susceptible to water loss, bruising and mechanical injuries due to their soft texture and lack of a protective rind (Garcia *et al.,* 1998). Strawberries are consumed mainly as fresh fruit. In addition, many other strawberry products such as juice, nectar, puree, and juice concentrate as well as jam are commercially available. Whereas fresh strawberries exhibit brilliant red colors, the processed fruits are characterized by rapid color loss and pigment degradation (Espin *et al.,* 2000). Factors which affect the color and stability of anthocyanins include structure and concentration, pH value, temperature, light, presence of co pigments, self association, metallic ions, enzymes, oxygen, ascorbic acid, sugar and their degradation products, proteins and sulphur dioxide (Mazza and Miniati, 1993).

Natural red colorants permitted in foods include betanin, cochineal (carmine and carminic acid) , carotenoids and anthocyanins (Askar,1993). Anthocyanins(ACs) are glycosides of polyhydroxy and polymethoxy derivatives of flavylium cation. Recent studies demonstrated that polyphenolic flavonoids exhibit a wide range of biological, pharmacological and chemoprotective properties as free radical scavengers preventing oxidation and cancer initiation (Kong *et al.,* 2003). Commercial anthocyanin colorants are mostly derived from fruits and vegetables. These sources include red grape, elderberry, black currant, blackberry, raspberry, black chokeberry, red cabbage, purple carrot , purple corn, red radish and purple sweet potato. The largest natural commercial source for anthocyanins is red grape skin, followed by elderberry, purple carrot and red cabbage (Downham and Collins, 2000).

Carrot (*Daucus carota L.)* is one of the main vegetable crops grown in temperate climate regions. Carrot originates from the wild forms growing in Europe and southwestern Asia. The first cultivated carrot types were purple or violet. Later yellow and orange types we rederived from this anthocyanin type, by selection process (Banga 1984). Purple carrot (or black carrot as it sometimes referred to) is an all natural food colorant, offering a final color which can vary from deep violet to bright red. The color can be controlled by simply controlling the pH value. Purple carrots are a good source of anthocyanin pigments . The anthocyanin content of black carrots was reported to be 1750 mg/ kg fresh weight (Mazza and Miniati, 1993). Anthocyanins from purple carrot were relatively stable to heat and pH change compared to anthocyanins from other sources due to diacylation of anthocyanin structure. Purple carrots also contain high amounts of acylated anthocyanins. Stintzing *et al.* (2002) identified four major anthocyanins in purple carrot extract and found 41% of anthocyanins to be acylated.

The usage of purple carrot anthocyanins as a natural colorant in the production of confectionery, jellies, jams, preserves and frozen desserts was discussed by Birks (1999). Alasalvar *et al*. (2001) reported that black carrots contain a high amount of nutraceutical components. Furthermore, Netzel *et al.* (2007) proved that anthocyanin rich extract from purple carrot inhibited proliferation of the both types of human cancer cells (colorectal adenocarcinoma and promyelocytic leukemia) in a dose dependent manner. Utilization of anthocyanin sources rich in (cyanidin, delephindin and petunidin) for food application may enhance health preventive effects. Due to the presence of cyanidinbased pigments purple carrot seems to be a suitable candidate for the development of health promoting foods.

The objectives of this study were to evaluate the effects of adding purple carrot puree on the color stability of strawberry jam.

MATERIALS AND METHODS

Materials

a) Purple carrot (*Daucus carota L*. *sp*. *Sativus var. atrorubens*.) and Strawberries (*Fragaria ananassa*) used in this study (season 2013) were purchased from the local market in Giza governorate, Egypt.
b) The solvents used for spectral and HPLC analysis were of HPLC grade and all other solvents were of ACS grade.
c) Chemicals used in this study were of analytical grade and purchased from El-Gomhoria Co.
d) The anthocyanin standards from Carl Roth GmbH (D-76185 Karlsruhe, Germany).

Methods

Purple carrot puree preparation:

Carrots were trimmed and washed by tap water, the roots cut into halves by stainless knife. The endoderm parts removed while the cortex were collected and blanched in boiling water for 5 minutes then pureed using an electrical mixer blender and screened by a piece of muslin cloth to obtain homogenized puree.

Strawberry puree preparation:

Strawberry fruits were sorted, the hulls and stem were carefully removed by stainless knife and washed by tap water then pureed using an electrical mixer blender and screened by a piece of muslin cloth to obtain homogenized puree.

Strawberry jam preparation:

Strawberry jam was processed according to the Egyptian Standard (ES) No.129 part 2 (2005). Different strawberry jams were prepared using different ratios of purple carrot puree and other materials, listed in Table (1). The mixture of strawberry , carrot puree and sugar was cooked in an open pan with continues manual stirring till total soluble solids reached to 65° Brix. The hot jams were filled into glass jars (400 ml) then tightly closed and stored at room temperature.

Table 1. Recipes of strawberry jams fortified by purple carrot puree.

Ingredients / Treatments	Sucrose (g)	Strawberry puree (g)	Carrot puree (g)	Citric acid (g)/kg	Commercial citrus pectin (g)/kg
Control	1100	1000	----	4.0	5.0
Jam with 15%carrot	1100	850	150	4.0	5.0
Jam with 20% carrot	1100	800	200	4.0	5.0
Jam with 30% carrot	1100	700	300	4.0	5.0

Analytical methods

Moisture content, total soluble solids, total titratable acidity, pH values, ascorbic acid and ash were determined according to AOAC (2005).

Browning index was determined according to the method of Meydev *et al,* (1977), as its light absorbance at 420 nm

Viscosity was measured using Brookfield Viscometer DVIII Ultra Programmable Rheometer at rotation speed of 10 rpm, using spindle No. HA-0 7 at 25°C following the method of Askar and Treptow (1993).

The yield was calculated from the amount of puree obtained from 100 g raw fruit

Total anthocyanin:

The total anthocyanin was determined as reported by Mondello et.al.(2000). Ten gm of sample were filtered through glass wool, and the pulp washed with 90 ml of a Ethanol : HCl mixture previously prepared mixing 79.7 ml of anhydrous ethyl alcohol with 20.3 ml of HCl (37%). The volume was made up 100 ml by solvent. The absorbance has been measured at 535 nm, by spectrophotometer (Jenway 6405 UV/visible), using 1 cm cells. The qualification was done with respect to standard curve of cyaniding-3-glucoside. The results were expressed as cyaniding-3-glucoside equivalent (mg per 100 ml of sample).

Analysis of anthocyanins of strawberry and purple carrots extract:

Purification of anthocyanins:

The anthocyanins concentrated extract was purified according to the method of (Attoe and Van-Elbe, 1981). The concentrate of anthocyanin was purified with petroleum ether and with ethyl acetate to remove non polar impurities. After phase separation; residual solvent was removed from the aqueous phase with rotary evaporator.

Identification of anthocyanins pigments by High-Performance Liquid Chromatography (HPLC) :

The purified anthocyanin of purple carrots was identified by HPLC according to the method reported by (Andersen, 1989) using suplecosil LC 18 column .For both HPLC column of two solvents were used for elution the first was formic acid, water (1:9) and the second formic acid, water, methanol (1:4:5). The flow rate was 1.5ml/min. The elutes were monitored by visible spectrometry at max. wavelength 520 nm.

Color measurement:

The color of samples (L*, a* and b*) was measured with a Minolta Chroma Meter (CM- 3600d, Minolta, and Ramsey, NJ). To prepare the samples for color measurement, purees and jam samples were poured into a 35-mm Petri dish and carefully covered with a Saran Wrap transparent film which was carefully pressed against surface to remove air bubbles as reported by Abonyi *et. al*(2001). Color of the samples was measured by contacting the color meter with the film-covered sample. Measurements were taken at 5 different locations on the sample. At each location 5 readings were taken. The mean of 25 readings was reported. A darkness factor b*/a* was used to quantify possible color changes (Tulasidas *et al.*, 1993). The hue angle(H*) and chroma (C*) which are given by $H^* = \tan^{-1}(b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{1/2}$ were also calculated.

Determination of total phenols content:

Total phenol contents (TPC) of samples was determined spectrophotometrically by a spectrophotometer (Jenway 6405 UV/visible) using the Folin & Ciocalteu assay described by Vinson *et al.*(1995) . One gm of sample was mixed with 1 ml of 6 M HCl and 5 ml of 75% methanol / water solution in a screw-capped tube. The tube was vortexed and placed in a 90ºC water bath and shaken for 2 h. Then, the tube was allowed to cool to room temperature and diluted to a 10 ml volume with distilled water. One milliliter of this solution was mixed with 5 ml of previously tenfold diluted Folin & Ciocalteau reagent. Fifteen milliliters of Na_2CO_3 (7 g/100 ml) were added to this mixture to produce basic conditions. The mixture was diluted to 100 ml with distilled water. The absorbance versus prepared blank was read at 760 nm until it reached steady state. The same procedure was applied for six standard solutions of Gallic acid (50–300 mg/100 ml). Final results were expressed as mg Gallic equivalent per 100 ml of juice.

Sensory evaluation:

Sensory evaluation of the different tested juices were carried out using the method of Howard and Dewi (1995) by ten staff members for color (10), texture (10), taste (10), and aroma (10) .The overall acceptability was calculated from the total scores of the tested attributes.

The organoleptic data were statistically analyzed using the ANOVA procedure of the SPSS statistical package for IBM computer (SPSS, 1990).

RESULTS AND DISCUSSION

Chemical properties of purple carrot and strawberry puree:

Results in Table (2) show comparison between some physicochemical properties of purple carrot and strawberry purees. The obtained results declared that strawberry puree was higher in TSS, total acidity, ascorbic acid while purple carrot puree was the slightly higher in yield, moisture content and pH value but the highest by two or three folds in viscosity, total anthocyanins and total phenols.

Table 2. Physicochemical composition of purple carrot and strawberry purees.

Parameter	Purple carrot	Strawberry
Yield %*	88.7	82.3
Moisture content %	93.4	92.6
TSS (°Brix)	6.7	7.3
Total acidity	0.08	0.72
pH value	6.1	3.4
Ascorbic acid (mg/100g)	15.5	17.8
Viscosity (centipoises)	88.0	34.7
Total anthocyanin (mg/100g)	172.4	38.3
Total phenols (mg/100g)	465.3	243.7

Identification of purple carrot and strawberry purees anthocyanins:

The anthocyanin pigments of both purple carrot and strawberry purees were analyzed and identified by HPLC as shown in Fig.1(a and b) and Table (3). Purple carrot anthocyanins comprise high amount of acylated cyanidin derivatives which exhibit remarkable stability to pH value changes and heat treatment (Stintzing *et al.,*2002). The acylated anthocyanin pigments from purple carrot puree represented about 66.64% of total anthocyanins compared to 20.6 % from strawberry. Yoshida *et al*. (1991) reported that acylated anthocyanins are protected from the hydrophilic attack of water molecules by the acyl moieties (intramolecular effect).

Beside improving the stability, the acylated anthocyanins display enhanced biological activities, anthocyanins possess strong radical-scavenging, antimutagenic activities and anti-hypertensive effects (Suda *et al.,*2003). Cyandin-3-xylosyl-glucosyl-galactoside acylated with Ferulic acid was the predominant compound (33.65) followed by Cyandin-3-xylosyl-glucosyl-galactoside acylated with Coumaric acid (29.85) in purple carrot while Pelargonidin 3-glcosside was the major component which represented about 68.2% of total chromatogram area in strawberry. However these results in general were in accordance with Kammerer *et al.* (2007).

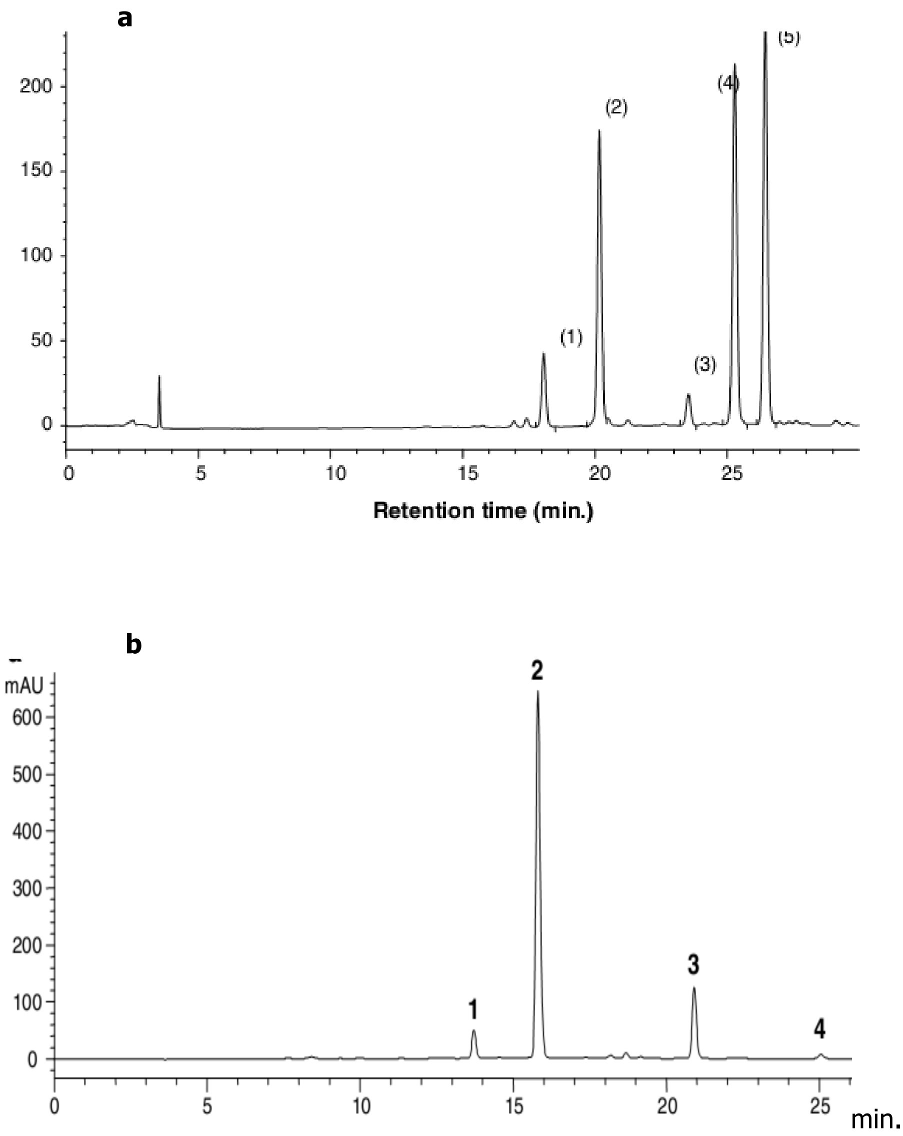

Fig. 1. HPLC chromatogram of anthocyanin pigments extracted from purple carrots (a) and strawberry (b).

Table 3. Identification of anthocyanin pigments extracted from purple carrots and strawberry purees.

Identified anthocyanin	Area %	
	Purple carrot	Strawberry
Cyandin-3-xylosyl-glucosyl-galactoside	4.65	-
Cyandin-3-xylosyl-galactoside	28.70	-
Cyandin-3-xylosyl-glucosyl-galactoside - acylated with sinapic acid	3.24	-
Cyandin-3-xylosyl-glucosyl-galactoside acylated with ferulic acid	33.65	-
Cyandin-3-xylosyl-glucosyl-galactoside acylated with coumaric acid	29.85	-
Cyandin 3-glcoside	-	13.5
Pelargonidin 3-glcosside	-	68.2
Pelargonidin-3-glcoside- acylated with malic acid	-	19.7
Pelargonidin-3-glcoside- acylated with succinic acid	-	0.9

Physical and chemical properties of strawberry - purple carrot jam :

The influence of using different levels of purple carrot puree as a colorant agent on the quality parameters of prepared strawberry jam during 6 month of storage are shown in Table (4). The results indicate that pH values were gradually increased by both increasing the level of carrot puree and storage period on the other hand viscosity was also increased as increasing carrot puree but decreased during the storage. Data in Table indicate that browning index was increased during storage in all treatments but with lower rates in case of adding purple carrot may be to reducing the formation of Millard reaction products (Kammerer *et al.,* 2007).

Initially, data indicated that the total anthocyanins concentration was increased significantly with increasing the level of adding purple puree which degraded gradually during the storage period, much faster degradation rate of anthocyanin was noticed in control and 15% purple carrot treatments . Furthermore, the anthocyanins in the jams of strawberry fortified by purple carrot showed a greater stability than those from strawberry one. These results are closed with those reported by Kirca, *et al.* (2006) and Stintzing *et al.* (2002) who indicated that the higher stability of anthocyanins is due to the high degree of acylation enabling intra-molecular copigmentation effects. The same tendencies were also observed for total phenols**,** they increased by increasing the amount of purple carrot and the degradation rate of total phenols from control jam was higher compared with the other purple carrot treatments. Concerning to total phenols, it is will know that phenols play role as antioxidant agent and correlated with the antioxidant capacity of fruits (Prior *et al.,* 1998). This result proved that mixing the purple carrot puree with strawberry decrease the degradation rate of pigment during storage and promote the hidden quality indices of strawberry jam.

Table 4. Effect of adding purple carrot on some properties of strawberry jam.

Parameter	Storage period (month)	Level of carrot puree %			
		control	15%	20%	30%
PH value	0	3.2	3.4	3.4	3.5
	3	3.5	3.6	3.6	3.7
	6	3.9	3.8	3.8	4.0
Viscosity (Centipoises)	0	72.4	96.5	112.6	140.3
	3	58.7	82.4	98.7	113.4
	6	46.9	65.5	82.9	94.6
Color index	0	0.246	0.242	0.247	0.256
	3	0.278	0.274	0.268	0.259
	6	0.315	0.298	0.278	0.264
Total anthocyanin (mg/100g)	0	26.7	34.3	39.4	58.9
	3	17.8	19.6	35.6	56.4
	6	12.5	16.2	32.3	54.6
Total phenols (mg/100g)	0	156.8	179.3	196.4	212.5
	3	138.7	168.1	187.5	204.8
	6	114.5	155.4	180.6	198.4

Color changes:

The color changes in strawberry jams samples as affected by different levels of purple carrots during 6 months of storage at room temperature are shown in Fig.(2 and 3). The control strawberry jam (prepared without any purple carrot) had a brighter color (highest L*) than the other treatments contained purple carrot. A gradual increase during storage in L* values was observed in control sample compared to a slight differences in colored jams due to occurring a pigment degradation resulting in color bleaching as mentioned by Espin *et al.* (2000). Otherwise, the addition of purple carrot puree during strawberry jam processing enhanced both a* (redness) and b* values and showed a slight decrease throughout storage.

Hue angles (Fig.2) of the control samples were significantly increased compared to other treatments which, in combination with a comparatively low chroma value, describes the pale-brownish color of the processed jam. Besides thermal pigment degradation, Maillard reactions may also be responsible for the formation of brown compounds as explained by Kammerer *et al.* (2007) . The strawberries mixed with purple carrot anthocyanins exhibited a slightly lower hue◦ value. This effect was more pronounced when the jams were stored. Accordingly, chroma values of the control samples recorded lower value compared with purple carrots treatments. Since chroma reflects color brilliance or purity and is correlated with the degree of anthocyanin acylation (Kammerer,2007). The loss of brilliance was less pronounced when purple carrot were used for color promotion.

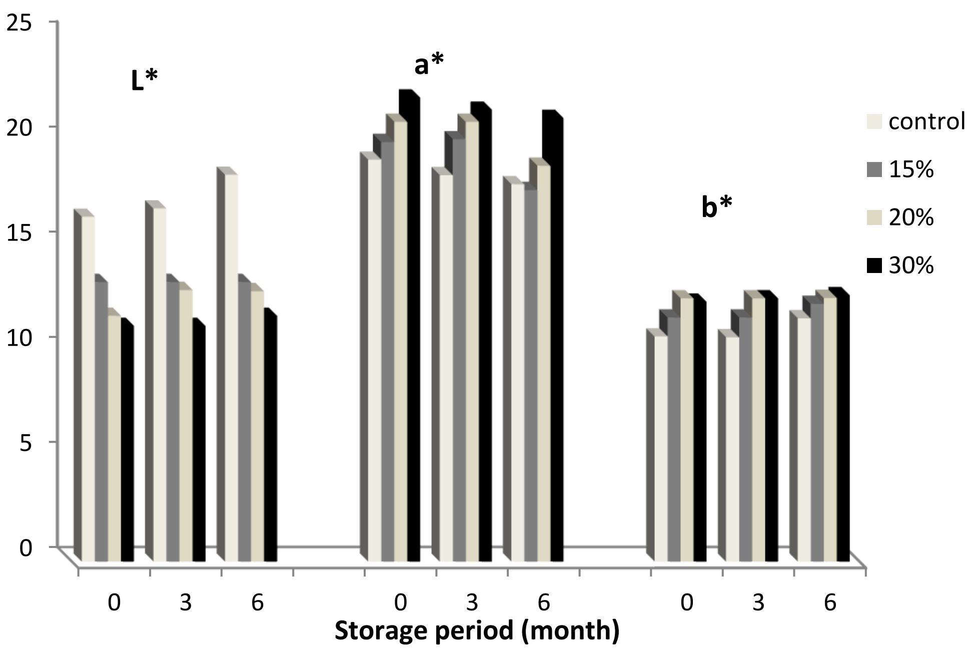

Fig 2. L*, a∗ and b∗ of strawberry jams mixed with purple carrot.

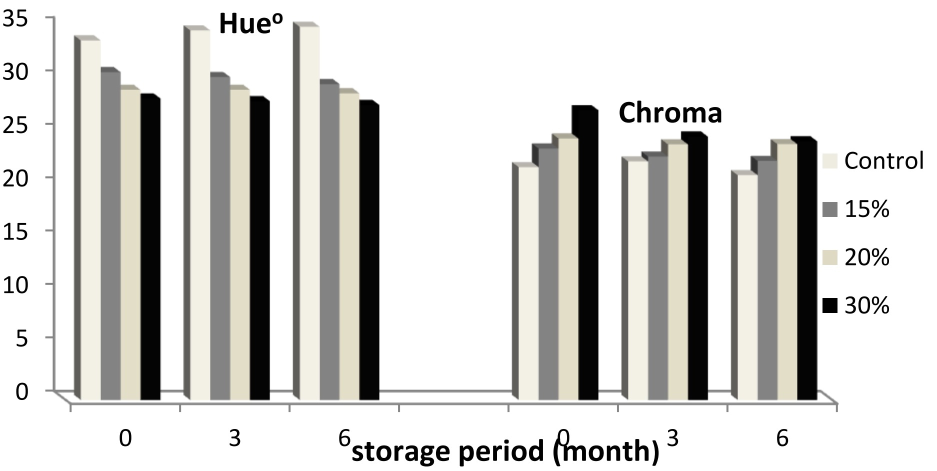

Fig. 3. Hue ° and chroma of strawberry jams mixed with purple carrot.

Organoleptic properties:

Results in Table (5) represent the effect of using purple carrot for promotion of color stability of strawberry jam. It could be observed that sensory attributes decrease gradually after storage for six months at room temperature in all tested samples. The highest overall acceptability score after six months of storage was noticed for jam of strawberry mixed with 20% purple carrot. Data showed no significant differences between the color, texture, taste, aroma and overall acceptability in both control and 15% purple carrot samples at zero time. In contrast, after six months of storage the addition of purple carrot with different levels significantly affected color and texture where the highest score were recorded in both 20 and 30% purple carrot samples while taste and aroma reduced slightly, no significant differences, were achieved between control and purple carrot addition up to 20% .

Table 5. Sensory evaluation of strawberry jam fortified by purple carrot.

Treatment	Storage period	Color	Texture	Taste	Aroma	Overall acceptability
Control	0	9.2^{ab}	9.4^{a}	9.6^{a}	9.7^{a}	9.5^{a*}
	3	7.8^{c}	8.7^{b}	8.7^{b}	8.6^{bc}	8.5^{bc}
	6	6.2^{e}	7.9^{c}	8.5^{bc}	8.2^{c}	7.7^{c}
Strawberry + 15% purple carrot	0	9.3^{a}	9.4^{a}	9.5^{a}	9.6^{a}	9.5^{a}
	3	8.4^{bc}	8.8^{b}	8.7^{b}	8.5^{bc}	8.6^{bc}
	6	8.2^{c}	8.6^{bc}	8.6^{bc}	8.2^{c}	8.4^{bc}
Strawberry + 20% purple carrot	0	9.5^{a}	9.8^{a}	9.2^{ab}	9.3^{ab}	9.5^{a}
	3	9.2^{ab}	9.4^{a}	8.6^{b}	8.6^{b}	9.0^{ab}
	6	8.7^{b}	8.8^{b}	8.4^{bc}	8.4^{bc}	8.6^{bc}
Strawberry + 30 % purple carrot	0	9.6^{a}	9.6^{a}	8.6^{b}	8.4^{bc}	9.1^{a}
	3	9.4^{a}	9.2^{ab}	8.2^{c}	8.0^{c}	8.7^{b}
	6	8.8^{b}	8.9^{b}	7.5^{cd}	7.4^{d}	8.2^{c}

** a-d values with different letters in the same column are significant different at P<.0.05.*

CONCLUSION: Strawberry products like jam suffer from discoloration or color bleaching due to rapid degradation of pigments. Results from this study clearly showed that purple carrot puree have a high concentration of acylated anthocyanins made it more stable and suitable for mixing with strawberry during jam processing . Adding up to 20% of purple carrot puree during strawberry jam processing enhanced the color stabilization during and after six month of storage at room temperature without any effects on the other organoleptic attributes.

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ثبات لون مربى الفراولة المدعمة بيوريه الجزر البنفسجى

مجدى عبد الهادى ، جميلة يوسف عطية وعفاف محمد على

معهد بحوث تكنولوجيا الأغذية – مركز البحوث الزراعية – جيزة – مصر

يعتبر تدهور لون منتجات الفراولة كنتيجة لحدوث تكسير فى الصبغات احد اهم عيوب الجوده التى تؤثر على قبولها لذا فإن الهدف من هذه الدراسة هو بحث مدى امكانية تحسين ثبات لون مربى الفراولة باستخدام بيوريه الجزر البنفسجى.

وقد اوضحت النتائج ان بيوريه الجزر كان متميزا فى ارتفاع محتواة من الفينولات الكلية و الأنثوسانينات الكلية مقارنة بالفراولة كما أنه عند تحليل صبغة الأنثوسيانين بجهاز ال HPLC وجد ان نسبة الانثوسيانينات المؤستلة العالية الثبات تصل إلى ٦٦,٦٤ % فى بيوريه الجزر بينما تصل إلى ٢٠,٦ % فى الفراولة. وكان مركب Cyandin-3-xylosyl-glucosyl-galactoside acylated with Ferulic acid هو السائد فى الجزر بينما مركب Pelargonidin 3-glcosside هو المكون السائد فى الفراولة.

تمت اضافة بيوريه الجزر بتركيزات ١٥ ، ٢٠ و ٣٠ % اثناء تصنيع مربى الفراولة مع تخزين المربى لمدة ٦ شهور تحت الظروف الطبيعية من حرارة وإضاءة. وقد ادت هذه الإضافة إلى زيادة تدريجية بزيادة نسبة الإضافة فى محتوى المربى من كل من ال PH ،اللزوجة ، الأنثوسيانينات الكليه والفينولات الكليه كما قللت الفاقد من صبغة الأنثوسيانين خلال فترة التخزين حيث انخفض محتوى الأنثوسيانين بنسب 53.2 ، 52.9 ، 18.0 و7.3 % فى عينات المربى الكنترول ، ١٥ ، ٢٠ و ٣٠ % جزر على التوالى وذلك بعد ٦ اشهر من التخزين. كما تم قياس اللون اثناء فترة التخزين حيث اظهرت النتائج المتحصل عليها إلى تحسن اللون كما كان اكثر ثباتا فى المربيات المحتوية على بيوريه الجزر. ومن ناحية أخرى أظهرت نتائج التقييم الحسى ان عينة المربى المحتوية على ٢٠ % بيوريه جزر كانت الأكثر قبولا عند نهاية فترة التخزين.

وفى النهاية من خلال النتائج السابقة يمكن الاستنتاج ان إضافة بيوريه الجزر البنفسجى بنسبة تصل إلى ٢٠ % اثناء تصنيع مربى الفراولة قد حافظت على اللون بدون اى تأثير على القبول العام.