Phenolic Composition, Colour and Anthocyanin Stability of Black Mulberry and White Mulberry Jam as Affected by Cultivar and Storage Temperature

Siyah ve Beyaz Dut Reçellerinin Fenolik İçerik, Renk ve Antosiyanin Kararlılığı Üzerine Çeşit ve Depolama Sıcaklığının Etkisi

Research Article

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ABSTRACT

Jams were prepared from two mulberry cultivars; '*Morus nigra* L. (black mulberry)', '*Morus alba* L. (white mulberry).' Jams were stored at 5 and 25˚C, in darkness and under daylight (750 lux). The quality parameters assessed were Hunter L*;a*;b*, anthocyanin pigments and the contents of total phenolics. During storage, eventhough amount of total phenolics and anthocyanins decreased; this reduction was insignificant by statistical. Jam produced from both of cultivars stored at 5˚C had better colour qualities than jam stored at 25˚C. The light conditions during storage did not affect the quality parameters of the product during four months of storage.

Key Words
Mulberry, anthocyanins, phenolics, color.

ÖZ

Reçeller, *Morus nigra* L. (siyah dut), *Morus alba* L. (beyaz dut) iki dut çeşitinden hazırlanmıştır; Reçeller 5 ve 25˚C'de karanlık ve gün ışığı (750 lüks) altında depolanmıştır. Kalite parametreleri olarak Hunter L*;a*;b antosiyaninler ve toplam fenolikler değerlendirilmiştir. Depolama boyunca, antosiyaninlerin ve toplam fenoliklerin miktarı azalmış bu azalma istatistiksel olarak önemsiz bulunmuştur. 5˚C'de depolanan reçeller 25˚C'de depolananlardan daha iyi renk kalitesine sahiptir. Dört ay boyunca depolama sırasındaki ışık şartları reçellerin kalite parametrelerini etkilememiştir.

Anahtar Kelimeler
Dut, antosiyaninler, fenolikler, renk.

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INTRODUCTION

Mulberry belongs to the genus Morus of the family Moraceae. There are 24 species of Morus and one subspecies, with at least 100 known varieties [1]. Mulberry (Morus species) is grown by wild or cultivated in many countries for its foliage, which is a primary source of food for silkworms (*Bombyx mori* L.). Generally, there are three cultivars of mulberry, including white (*Morus alba*), black (*Morus nigra*), and red (*Morus rubra*) [2]. The South East Anatolia region of Turkey has suitable growing conditions for cultivating high quality mulberry fruits. The amount of mulberry production in Turkey was 78.860 tonnes in 2011 [3] and its cultivation in Turkey has been known for more than 400 years. The main mulberry production areas of Turkey are Black Sea region as well as south eastern and central Anatolian regions where the harvesting period is between June and August. Moreover, 95% of the mulberry trees grown in Turkey are white (*M.alba*), whereas 3% are red (M. rubra) and 2% are black (*M. nigra*) [4]. The red colored fruits are eaten fresh and are also used in marmalades, jams, juices, liquors, natural dyes, and in the cosmetics industry [5].

During the decades years the antioxidants in fruit and vegetables have been emphasized and especially the colour derived from anthocyanins has been recognized as an important component in reducing the risk of several chronic diseases such as cancer, coronary heart disease, diabetes type 2, hypertension and cataract [6]. Reduction in antioxidant activity during processing and storage [7] may reduce the health beneficial effects of such food products.

Attractive red colour is one of the most important quality characteristics for the mulberry jam processing industry, beside of typical sweet-sour mulberry flavour and convenient jam consistency. Cultivar and degree of ripeness are major factors determining taste and colour of mulberry jams [8].

Colour stability of red fruit products is affected by temperature, pH, oxygen, sugar content, ascorbic acid and metals [9]. The degradation of pigments results in discolouration of the product. During processing the pigments can be hydrolysed, and degraded to anthocyanidin and sugar. The anthocyanidins are unstable when exposed to light and are more easily oxidized than the anthocyanins, and consequently more susceptible to browning reactions [10].

The monomeric anthocyanins are red at pH 1.0, while they are colourless at pH 4.5 [11]. In general temperature is the most important factor for degradation of the colour pigments. The total amount of pigments in mulberries is also important for the stability of the colour of the jams [12]. The most common anthocyanins are cyanidin 3-O-rutinoside (60%) and cyanidin 3-O-glucoside (38%).in mulberry pigments. The minor anthocyanins (totally 2%) are pelargonidin 3-O-glucoside and pelargonidin 3-O-rutinoside [13].

The jam recipe, processing procedures, jar type, storage conditions are the most important factors for the jam quality. The shelf life of jam is normally 6–12 months. Studies on the degradation of pigments in jam stored at different temperatures show that degradation increases at higher temperatures [14]. The optimal storage temperature for jam is believed to be 4°C [15].

Although white and black mulberries are wild fruit that has a great importance in nutrition and traditional medicine in Turkey; there are no detailed studies concerning white and black mulberries and its jams.

The goals of this study were to evaluate anthocyanins and total phenolics content of mulberries jam and its colour development during storage at different temperature and light regimes, as well as the influence of cultivar on jam production.

MATERIALS and METHOD

Short Description of Cultivars

Morus alba L.: Older and early maturing Turkey cultivar, with white coloured and aromatic berries. *Morus nigra* L.: with dark red coloured, good taste and aroma, suitability for jam industry is known. *Morus alba*, *Morus nigra* were used on this study since they are the most commonly cultivated in

Table 1. Storage conditions for the samples.

Sample no	Light (Lux)	Temperature (˚C)
1	0 (darkness	25
2	0 (darkness	5
3	daylight (750)	25
4	daylight (750)	5
5	0 (darkness)	25
6	0 (darkness)	5
7	daylight (750)	25
8	daylight (750)	5

1,2,3,4 *Morus nigra* L., 5,6,7,8 *Morus alba* L.

Turkey. Fully ripe mulberry fruits were harvested and then stored at 5˚C until jame processing.

Jam Processing and Storage Conditions

The jam was processed at the Adıyaman University Food depertmant Laboratuary. The equipment consists of two stainless-steel pans with steam heating. Before the fresh mulberries (4 kg) filled into the pan with the water (450 ml) and sugar (4.2 kg) was added. Mulberries and sugar were mixed before heating to 80˚C. Pectin solution (40 g in 400 ml water) was then added and the temperature raised to 90˚C and held there for 3 min before cooling down to 80˚C and citric acid (100 g) was added and the jam was stirred for 10 min before cooling to 60˚C and filling in transparent glass jars. The jam was kept room temperature and then moved to the different storage conditions (Table 1). The jam was stored for four months at these conditions;

Analysis of the Jam

Extraction of Anthocyanins Mullberry Jams

Samples containing 5 g of mulberry jams (three replicates from each glass jar) were homogenised and weighted, 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 by adding 0.1% (v/v) hydrochloric acid in methanol [16].

Total Momoneric Anthocyanin Analysis

Total monomeric anthocyanin content of mulberry jam was determined using the pH-differential method described by [17] using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M), and sodium acetate buffer, pH 4.5 (0.4 M). The samples were diluted in a ratio of 1:10 with twice distilled water. A 0.4 ml of diluted sample was mixed with 3.6 ml of corresponding buffers and allowed to equilibrate for 15 min at room temperature. The absorbance of each equilibrated solution was measured at 515 nm and 700 nm, using UV–VIS Lambda 25 spectrophotometer [17]. Total monomeric anthocyanins were calculated as mg / 100 cyaniding-3-glucoside according to the following equation:

$$\text{Total anthocyanins (mg/L)} = A \times MW \times DF \times 1000 \times l$$

where $A = (A_{515} - A_{700})\text{pH } 1.0 - (A515 - A700)\text{pH } 4.5$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor (10) as final volume per initial volume; l = path length in cm; ϵ= 26,900 molar extinction coefficient in L/mol/cm for cyanidin-3-glucoside; 1000= conversion factor from g to mg. All analyses were done in triplicate (n= 3). Glass cuvettes of 1 cm path-length were used and all measurements were carried out at room temperature (~22°C). Absorbance readings were made against twice distilled water as blank.

Colour Measurements

Color of mulberry jams was measured by a colorimeter (CR-400, Konika Minolta, Japan) and recorded in the L*, a*, b* color system.

Colorimeter was calibrated throughout the study by using a standard white ceramic reference (L* = 97.91, a* = -0.68 and b* = +2.45). Three samples were measured for mulberry jams and the measurements were averaged [18].

Extraction of Total Phenolic Content

The jams extract were prepared as described by [19]. with some modification. About 2 g of each sample was weighed out, and extracted with 50 mL of methanol. Extraction was carried out under stirring for 60 min at 60°C. Each extract was filtered out using Whatman No. 3 filter paper, filled accordingly in a 50 mL volumetric flask, and allowed to set in the dark until analysis.

Determination of Total Phenolics

The total phenolic content in mulberry jam samples were determined colourimetrically by the Folin–Ciocalteu method [20]. 100 μL of sample (diluted 1:5 (v:v) with methanol) was mixed with 6 ml of twice distilled water and 500 μL of Folin–Ciocalteu reagent was added. After waiting 5 min at room temperature, 1.5 mL of sodium carbonate (20% w/v) was added to adjust optimum pH for the reaction. The mixture was vortexed and incubated at room temperature (~22°C) for 2 h and then the absorbance was measured at 765 nm using a UV–VIS Lambda 25 spectrophotometer [17]. Gallic acid was used as a standard and total phenolic content was expressed in mg gallic acid equivalents (GAE) per litre. A mixture of water and reagents was used as a blank. All analyses were done in triplicate (n = 3).

Storage of Mulberry Jams

Mulberry jams samples were stored at different temperatures (5°C and 25°C) and light regimes (daylight and darkness). Both types of muberry jam (black mulberry jam and white mulberry jam) were analysed after preparation and then after 0, 30, 60, 90 and 120 days of storage.

Statistical Analysis

Two different type of mulberry, two temperatures and two different type of light and five storage period were assigned to forty groups of mulberry in 2x2x2x5x1x5 factorial design. All data subjected to statistical analysis using the GLM procedures of statistical program SAS [21]. Significant differences were tested further using a Tukey's HSD multiple ranges test to determine the differences among treatments.

RESULT and DISCUSSION

At the end of the project, increment in L degrees and reduction in total anthocyanin content were detected for all black mulberry samples. During storage, black mulberry samples were bleached and corresponding to the bleaching in the black colour, L degrees were increased. And also reduction in the a and b degrees support this situation. For white mulberry samples same results were achieved. But, due to the colour substances, increment in b degrees were also detected in some of the white mulberry samples. And all this changes in colour values were insignificant by statistically.

When jams were stored at 25°C, anthocyanin and phenolic contents went down quickly in both black and white mulberry jams. Regarding those jams stored at 5°C daylight and darkness, there were a 32%, 25% pigment degradation followed 7%, 2% reduction in BMJ ones and a 35%, 22% followed 22%, 11% reduction in WMJ after 120 days. These results completely agreed with those obtained by [19] and [22] mulberry jams showed lower anthocyanin and phenolic contents degradation at 5°C than at 25°C after 120 days storage.

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References

1. A.M. Pawlowska, W. Oleszek, A. Braca, Quali-quantitative analyses of flavonoids of *Morus nigra* L. and *Morus alba* L. (Moraceae) fruits. Journal of Agricultural and Food Chemistry, 56 (2008) 3377–3380.
2. P. Aramwit, N. Bang, and T. Srichana, The properties and stability of anthocyanins in mulberry fruits. Food Research International, 43 (2010) 1093–1097.
3. FAO 2012. faostat.fao.org.
4. Y. Elmaci, T. Altug, Flavour evaluation of three black mulberry (*Morus nigra*) cultivars using GC/MS, chemical and sensory data. Journal of the Science of Food & Agriculture, 82 (2002) 632–635.

5. S. Ercisli, and E. Orhan, Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. Food Chem., 103 (2007) 1380-1384.
6. J. W. Lampe, Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. American Journal of Clinical Nutrition, 70 (1999) 475–490.
7. M. G. Lindley, The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. Trends in Food Science and Technology, 9 (1998) 336–341.
8. W. Sundfor, Evaluation of strawbeery cultivars for jam with focus on colour characteristics. Master Thesis, Agricultural University of Norway, Aas, 58 (2001).
9. L.M. Withy, T.T. Nguyen, R.E. Wrolstad, D.A. Heatherbell, Storage changes in anthocyanin content of red raspberry juice concentrate. J. Food Sci., 58 (1993) 190–192.
10. K. Hermann, Anthocyanins in Food. Zeitschrift fur Lebensmittel-Untersuchung und Forschung., 148 (1972) 290-302.
11. Wrolstad, . Color and Pigment Analysis in Fruit Products. S Bulletin 624, Agricultural Experiment Station, Organ State University, Coruallis 1976.
12. F.J. Francis, Food colorants: anthocyanins. Crit. Rev. Food Sci. Nutr., 28 (1989) 273–341.
13. C. Qin, Y. Li, W. Niu, Y. Ding, R. Zhang, X. Shang, Analysis and Characterisation of Anthocyanins in Mulberry Fruit. Czech Jornal of Food Sciences, 28 (2010) 117-126.
14. C. García-Viguera, P. Zafrilla, F. Artés, F. Romero, P. Abellán, F.A. Tomás-Barberán, Colour and anthocyanin stability of red raspberry jam. J. Sci. Food Agric., 78 (1998) 565–573.
15. M.R. Ochoa, A.G. Kesseler, M.B. Vullioud, and J.E. Lozano, Physical and chemical characteristics of raspberry pulp: storage effect on composition and color. Lebensm. Wiss. Technol., 32 (1999) 149-153.
16. F. Queiroz, C. Oliveira, O. Pinho, and I.M.P.L.V.O. Ferreira, Degradation of anthocyanins and anthocyanidins in blueberry jams/stuffed fish. Journal of Agricultural and Food Chemistry, 57 (2009) 10712–10717.
17. M.M. Giusti, RE. Wrolstad, Unit F1.2: anthocyanins. Characterization and measurement with UV-visible spectroscopy. In: Wrolstad, RE, editor. Current protocols in food analytical chemistry. New York: John Wiley & Sons., (2001) 12-13.
18. K. Haffner, M.B. Finstad, H.J. Rosenfeld, and G. Skrede, Colour of raspberry jam as influenced by cultivar, temperature and light during storage. Acta Horticulturae, 628 (2003) 829–834.
19. T.M. Rababah, N.S. Hettiarachchy, and R. Horax, Total phenolics and antioxidant activity activity of fenugreek, green tea, black tea, grape seed, vinger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. J. Agric. Food Chem., 52 (2004) 5183–5186.
20. V. L. Singleton, J. A. Rossi, Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am. J. Enol. Vitic. 16 (1965) 144-158.
21. SAS Institute, SAS /STAT.SAS Inst Inc, Cary, NC, USA, 2000.
22. C. García-Viguera, P. Zafrilla, F.A. Tomás-Barberán, Determination of authenticity of fruit jams by HPLC análisis of anthocyanins. J. Sci. Food Agric., 73 (1997) 207–213.