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Optimizing the production of green food color from mulberry leaves at different harvest times

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ABSTRACT

Purpose: The white mulberry tree (*Morusalba*) having a low water requirement, is native to Iran. Mulberry leaf extract is a rich chlorophyll source, and natural source of antioxidants and coloring pigments. White mulberry leaf extract was studied in terms of color production ability and antioxidant properties. **Research method:** The extracts of mulberry leaves were extracted by pure solvents of methanol, acetone, ethanol and 96% and 80% ethanol. Color extraction was investigated at beginning, middle and end of the production season. Antioxidant capacity, phenolic compounds, ferric reducing power and color stability were evaluated. **Findings:** 80% ethanol had a higher extraction rate and was cost-effective. In April and May, more total chlorophyll and carotenoid were extracted than in June. To determine the optimized antioxidant capacity, 100% ethanol solvent, sample to solvent ratio of 1 to 10, and microwave power of 500-watt were used. The leaves phenolic compounds extracted were highest in April (24.01 µg/ml) and lowest in June (19.13 µg/ml). The ferric reducing power was higher in the first samples of the season. Color stability decreased with pH from 6 to 3 and temperature increasing from laboratory temperature to 90°C and receiving light. **Research limitations:** The extracted color was not investigated in food products. **Originality/Value:** Chlorophyll, carotenoids, phenolic compounds and ferric reducing power were higher in the leaves at the beginning of the season. Color extraction from leaves harvested in April with 80% ethanol solvent had a higher value while maintaining pH and storage at room temperature in the dark.

INTRODUCTION

The mulberry tree belongs to the genus *Morus* and the family *Moraceae*, whose leaves, roots, and branches are widely used in pharmacology. This tree is native to temperate and subtropical regions of the northern hemisphere (Thabti et al., 2011). The mulberry tree is one of the native and drought-tolerant trees of Iran that grows in most parts of this country (Askari & Talebi, 2020). Scientists and nutritionists are always looking for natural compounds with antioxidant properties. Mulberry is one of the plants with antibacterial, antiviral, antiinflammatory, antiallergic, antiobesity, and anticancer properties. A variety of phenolic compounds have also been identified in the leaves of this plant (Saurabh et al., 2012). Phenolic compounds, as natural antioxidants, play a critical role in scavenging free radicals and diminish their production, and prevent oxidative stress, which leads to damage to the structure of molecules in the body (Fernandez-Panchon et al., 2008). In a study, the highest total phenol was obtained in red mulberry leaves with an average of 994.3 mg of gallic acid per 100 g of dry weight (Thabti et al., 2011).

Leaf pigments of trees generally include chlorophyll a, chlorophyll b, and carotenoids that are produced during the process of photosynthesis in plant leaves and their amounts in different plant species depending on genetic and environmental factors (Sumanta, 2014). Chlorophylls are green plant pigments with antioxidant properties. This pigment is widely used as a colorant (Fatemi, 2015).

A wide range of solid-liquid extraction (SLE) techniques is used to extract and purify plant extracts. In general, these are divided into two general categories, traditional and new techniques. Traditional techniques have been used for decades and are often time-consuming and require large amounts of solvent. Supercritical fluid, microwave, and the use of pressurized solvent are the new extraction techniques that are fast and efficient methods used to extract solids (Beatrice & Christen, 2002). Shaikh and Dongare (2008) reported that chlorophyll and carotenoid levels in plants change with slight changes in climate. Ritchie (2006) reported that the extraction with acetone had the highest extraction efficiency than other solvents, but acetone solvent was not ideal for extracting chlorophyll from all plants because this solvent performed very poorly in some cases. Methanol is also a toxic solvent and is easily absorbed by the skin and inhaled (Porra, 1991). In a study, Sumanta (2014) investigated the effect of different solvents in the extraction and measurement of photosynthetic pigments. According to the results, the extraction of different pigments depended on the chemical structure of the solvent.

Microwave extraction is used to separate bioactive compounds from plants. Microwaves are non-ionized electromagnetic waves that are in the spectrum between radio and infrared waves. The 915 MHz spectrum with greater penetration depth is the most used in industrial activities, while 2450 MHz is used for extraction activities (Tangbin et al., 2012). Different concentrations of solvent also affect the extraction efficiency. In a study, the extraction efficiency increased by increasing the ethanol concentration from 30 to 50%. It has also been observed that both solvent amount and the pH of the solvent have significant effect on the extraction efficiency (Hai Rong et al., 2012).

Hanula et al. (2020) showed that the highest content of total phenolic compounds in the extracts was obtained after the application of a temperature of 45 °C, using ultrasound for 25 min and 45 min, microwaves for 3.16 min and a water bath for 25 min. Ultrasound turned out to be the most effective method of flavonoid extraction. In turn, the highest anthocyanin content was obtained for microwave extraction. Additionally, the application of microwaves for 4.33 min (45 °C) guaranteed the highest ferric-reducing antioxidant activity (FRAP) among the extracts. The results showed that the use of microwaves shortens the açai

extraction time and ensures both a high content of total phenolic compounds and strong antioxidant activity in the extract. Feng et al. (2022) showed that low-temperature ultrasonic-assisted method was used to extract MLP, and through employing the Design-Expert software, the optimal conditions for MLP extraction were ultrasonic power 179.34 W, ultrasonic time 13.92 min, and ratio (v/w, mL/g) of water to raw material 23.55.

Saifullah et al. (2021) used the green extraction technique microwave-assisted extraction (MAE) was applied and the extraction parameters were optimized using response surface methodology (RSM) to maximize the extraction yield of phenolic compound and antioxidant properties. The results showed that the MAE parameters including radiation time, power, and sample to solvent ratio had a significant influence on the extraction yield of phenolic compounds and antioxidant capacity. The optimal MAE conditions were radiation time of 6 min, microwave power of 630 W, and sample to solvent ratio of 6 g/100 mL.

Today, the harms of synthetic colors on human health have been proven. Unlike synthetic colors, natural origin colors do not have toxic, allergenic, or carcinogenic effects. For some of them, beneficial properties such as antioxidant, antimicrobial and anticancer properties have been reported. Considering the harms of synthetic antioxidants used in the food industry, it is critical to find natural alternatives especially from plant sources with coloring and antioxidant properties. Chlorophyll is a compound that chemically meets both of these needs. The mulberry tree is a drought-tolerant plant with a low water requirement that is native to Iran and grows in most parts of this country. It seems that its leaves can be used for coloring. According to the requirements of the Food and Drug Administration for using natural dye instead of artificial color, the possibility of extracting vegetable green color from mulberry leaves for use in food industry and the antioxidant properties of this color at different times of leaf harvest was studied.

MATERIALS AND METHODS

Sampling of mulberry leaves

Leaf samples of white mulberry tree (*Morusalba*) were taken from trees located in the Agricultural and Natural Resources Research Center of Isfahan, Iran in different sizes (small 2×3 and large 3×5 cm^2) in 2019. The samples were taken at the beginning of spring and once every 30 days for three months from different tree parts and dried in the shade at ambient temperature during three days in the laboratory. Then they were grinded by laboratory table grinder with separated grind container (National grinder with speed range up to 10,000 rpm). All chemical solvents used were from Merck Company with analysis grade. The Spectrometer used was Nova spec II made by Pharmacia Biotech, Netherland. A Panasonic microwave oven with model NN-S754WF made by Matsushita Electric industrial from Korea was used for the experiments.

Table 1. Equations for calculating pigment concentration with specific solvents

Ethanol 95%	Methanol 90%
$Chl_a=13.36A_{664}-5.19A_{648}$	$Chl_a=16.82A_{665}-28.9A_{652}$
$Chl_b=43.37A_{648}-8.12A_{664}$	$Chl_b=36.92A_{652}-16.54A_{665}$
$Chl_a+Chl_b=5.24A_{664}+0.1539A_{642}$	$Chl_a+Chl_b=0.28A_{665}+27.64A_{652}$
$Cx+C=\frac{(1000A470-2.13Chla-97.64Chlb)}{209}$	$Cx+C=\frac{(1000A470-1.91Chla-95.15Chlb)}{225}$

Methanol 100%

$Chl_a=16.27A_{665}-6.16A_{652}$
$Chl_b=16.36A_{642}-2.43A_{660}$
$Chl_a+Chl_b=1.44A_{665}+24.93A_{652}$

$$Cx+C=\frac{(1000A470-1.63Chla-104.96Chlb)}{221}$$

Acetone 100%

$Chl_a=11.24A_{661}-2.04A_{644}$
$Chl_b=20.13A_{644}-4.19A_{661}$
$Chl_a+Chl_b=7.05A_{661}+18.09A_{644}$

$$Cx+C=\frac{(1000A470-1.9Chla-63.14Chlb)}{214}$$

Acetone 80%

$Chl_a=12.25A_{663}-2.79A_{646}$
$Chl_b=20.5A_{646}-5.1A_{663}$
$Chl_a+Chl_b=7.15A_{663}+18.71A_{646}$

$$Cx+C=\frac{(1000A470-1.82Chla-85.02Chlb)}{198}$$

Chl_a=chlorophyll a, Chl_b=chlorophyll b, Chl_a+Chl_b=total chlorophyll, C_x+ c =total carotenoids

Extraction of chlorophyll and carotenoids using various solvents

To extract total chlorophyll, chlorophyll a, and chlorophyll b from white mulberry leaves, methods mentioned by Sumanta et al. (2014) and Costache et al. (2012) were used with some modifications. For this purpose, the early leaves of the season were used. First, 0.4 g of the dried and grinded sample was mixed with 10 ml of Merck made solvent (pure acetone, pure methanol, pure ethanol, 96% ethanol, 80% ethanol). After centrifugation for 15 minutes at 4200 rpm, its absorption was read at 517 nm wavelength. Then the concentrations of the pigments were obtained using the relationships in Table 1.

Determination of the best time to extract the color

Chlorophyll and carotenoids of mulberry leaves were extracted three times in April, May, and June using the best-obtained solvent from the previous step to determine the best time to extract the color.

Measurement of antioxidant capacity

Mulberry leaf samples were used in April to measure antioxidant capacity. To measure antioxidant capacity, 10 ml of the solvent (80 and 100% ethanol) was added to 1 gram of sample to obtain a more suitable concentration of ethanol to determine the antioxidant capacity. To obtain the optimum ratio of sample to solvent, extraction was performed in different ratios of sample to solvent, including ratios of 1 to 10, 1 to 15, 1 to 20, and 1 to 25 to obtain the best ratio of sample to solvent. Then It was placed in the dark for 90 minutes. Extraction was performed by using of microwave method proposed by Zoe. To obtain the maximum antioxidant extraction powers of 300, 500, 600, and 700 for 100 seconds were used. (Zou et al., 2012). 50 µl of the extract was added to 5 ml of 0.004% DPPH (2,2-diphenyl-1-picryl hydroxyl) solution in ethanol and after 30 minutes in the dark, its adsorption was read at 517 nm against Blank. The percentage of free radical scavenging was calculated using Equation 1 (HedayatZadeh & EsmaeilZadeh, 2015).

Antioxidant capacity (%) = (A blank-A sample) ×100 /A blank (1)

A: Absorption number

Measurement of total phenol

In this experiment, the effect of sampling time (April, May, and June) on total phenol content was investigated. First, 20 microliters of the extract were mixed with 1.16 ml of distilled water and 100 µl of folin-ciocalteu reagent, and after one to eight minutes, 300 µl of 20% sodium carbonate was added to it. After mixing, it was placed in a 40 °C water bath for 30 minutes and then the adsorption was read at 760 nm. Phenol content was calculated based on the standard curve of gallic acid (Singleton & Rossi, 1965).

The ferric reducing power*α-Amylase activity assay*

This trait was evaluated separately for the three months of April, May, and June. In this experiment, 1 ml of the extract was mixed with 2.5 ml of phosphate buffer (0.2 M) with pH= 6.6 and 2.5 ml of potassium ferricyanide (10 g/l) and placed in a water bath with 50°C for 0.5 hours. Then 2.5 ml of 10% trichloroacetic acid (TCA) was added and centrifuged at 1650 rpm for 10 minutes. After that, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride III (1 ppm), and its adsorption was read at 700 nm. In this method, a higher absorption number indicates more reducing power (Salmanian et al., 2014).

Color stability assessment

The stability assessment of extracted chlorophyll and carotenoids from April samples with changes in pH, heat, and light was carried out according to the methods of Koka et al. (2007) and Fatemi (2015). The range of pH changes was considered from 3 to 6 in laboratory temperature and light. Color samples with pH = 6 were subjected to temperature changes at 50, 70 and 90 °C. The effect of light on color stability was evaluated immediately after extraction (control). Then half of the samples were placed in dark and the rest were placed in light of the laboratory. After 24 hours, the amount of color in the samples was measured (T24).

Data analysis

In this study, each experiment was performed in a randomized complete block design with time effect in three levels and solvent effect in two levels. The experiments were carried out in three replications with five observations in each replication. Data were analyzed using SPSS software (version 16) and the means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

Determination of the most suitable solvent for chlorophyll and carotenoid extraction

The effect of the investigated five solvents in the extraction of chlorophyll and carotenoid pigments are given in Table 2. Pure methanol had the highest extraction efficiency of total chlorophyll, chlorophyll a, and chlorophyll b pigments with averages of 12.646, 6.641, and 6.185 µg/ml compared to other treatments, respectively. Khan et al. (2013) also obtained the highest chlorophyll extraction efficiency using methanol 100%. Due to the chemical nature of chlorophyll, this compound dissolves easily in non-polar solvents. In another study, the highest percentage of chlorophyll extraction was reported by using diethyl ether, while using methanol and acetone was less effective (Sumanta et al., 2014). In the present study, color extraction was carried out for use in food, so methanol is not suitable and recommended, due to the toxicity of this solvent. The highest chlorophyll extraction efficiency after methanol belonged to different concentrations of ethanol and no significant difference was observed between different concentrations of ethanol in chlorophyll extraction, therefore, 80% ethanol

concentration was used to extract the color due to its cost-effectiveness. These results were also true for chlorophyll a and b.

The highest extraction efficiency of carotenoid pigment was obtained with pure acetone with an average of 0.951 µg/ml (Table 2), which was consistent with the results of Sumanta et al (2014). In another study, the highest amount of phenolic compounds and antioxidant activity in apple leaves of Aldas cultivar was obtained from extracts from ethanol (Liaudanskas et al., 2014).

Determination of the best time for color extraction

The results of total chlorophyll and carotenoid extraction using 80% ethanol in April, May and June are given in Table 3. The highest total chlorophyll extraction was in April and May, and no significant difference was observed between these two months. In April, the highest amount of carotenoids was extracted, which was significantly more than the next two months. In both studied pigments, the lowest pigment extraction was observed in June (Table 3). Prolonged harvest time reduced the color intensity of chlorophyll from 5.68 to 3.92 µg/ml and carotenoids from 0.57 to 0.19 µg/ml. Therefore, it can be concluded that in the first two months of the year, the concentration of chlorophyll in the leaves is almost constant, so it is better to extract natural green color in these months. These results were consistent with the results by Pothinuch and Tongchitpakdee (2011). They examined the effect of cultivar and leaf age on melatonin levels and reported the lowest melatonin levels in older leaves with more prolonged harvest times.

Antioxidant capacity

The antioxidant activity of extracted leaves with 80 and 100% ethanol, harvested in April is shown in Fig. 1. The use of 100% ethanol led to the extraction of more antioxidants with an average of 36.85% compared to 50% ethanol with an average extraction of antioxidants of 28.03%. These results were consistent with the results of Hijazi et al. (2015) about the use of different percentages of ethanol to measure the antioxidant percentage in *Eryngium campestre* L. According to their results, increasing the percentage of ethanol increased the percentage of antioxidants by DPPH method. In another study, the antioxidant capacity of mulberry leaves was reported between 12.64 to 10.62 mg/g dry weight by DPPH method (Sanchez-Salcedo et al., 2015). Different amounts of antioxidant activity have been reported in mulberry leaves (Thabti et al., 2014). Factors such as genotype, environmental conditions, sampling time, and extraction protocol are influential in this difference (Sanchez-Salcedo et al., 2015).

Table 2. Effect of solvent on mulberry leaf pigment extraction

	Carotenoid (µg/ml)	Chlorophyll b (µg/ml)	Chlorophyll a (µg/ml)	Total chlorophyll (µg/ml)
Pure methanol	0.642±0.2b	6.185±1.2a	6.641±0.78a	12.646±0.78a
Pure acetone	0.951±0.24a	2.211±0.54b	3.696±0.95c	5.899±1.2c
Ethanol 96%	0.658±0.24b	3.255±1.2b	4.697±0.62b	6.934±1.34b
Ethanol 100%	0.658±0.24b	3.6±0.22b	3.98±0.52b	6.17±0.5b
Ethanol 80%	0.658±0.24b	3.233±1.2b	4.51±0.6b	6.898±0.5b

Similar letters in each column indicate no significant difference (Duncan).

Table 3. Effect of harvest time on mulberry leaf pigment extraction

	Total chlorophyll (µg/ml)	Carotenoid (µg/ml)
April	5.68±0.39a	0.57±0.11a
May	4.37±0.52ab	0.27±0.09b
June	3.92±0.25b	0.19±0.08c

Similar letters in each column indicate no significant difference (Duncan).

In the next study, ethanol 100%, the lowest leaf-to-solvent ratio (1 to 10), and microwave with powers of 500, 600, and 700 watts were examined. According to the results, the power of 500 watts leaded to maximum amount of antioxidants extraction from mulberry leaves (Fig. 2). This result was consistent with the result of another study stating that the most suitable microwave power to extract anthocyanins was about 500 watts for 132 seconds (Tangbin et al., 2012).

In the next study, the percentage of ethanol was constant (100%) and the ratio of leaf to solvent was considered variable, and the ratios of 1:10, 1:15, 1:20, and 1:25 were investigated for extraction of antioxidants at 500 watts of microwave power for 100 seconds. The results showed that a ratio of 1:10 had the highest extraction of antioxidants (Table 4). The results were consistent with the results of Lee and Cho (2012). Thus, the antioxidant extraction method was optimized by the microwave method (100% ethanol, sample to solvent ratio of 1:10, and power of 500 watts).

Total phenol

Phenolic compounds are effective antioxidants and major phytochemicals in food (Jeszka-Skowron et al., 2014). In this experiment, the effect of time on the amount of total phenol (based on dry weight) was evaluated. According to Table 5, the phenolic compounds decreased from 24.01 µg/ml in April to 19.13 in June. Over time, the phenolic compounds and consequently the antioxidant properties of the leaves decreased. The results are consistent with the results of Lee and Cho (2012). They also reported that harvest time affected the amount of polyphenols and with increasing harvest time, the amount of polyphenols decreased. Total phenol in the leaves of Spanish mulberry clones was reported to be 12.81 to 16.13 mg/g dry weight (Sanchez-Salcedo et al., 2015). Radojkovic et al. (2012) reported total phenol content in *M. alba* 66.66 and *M. nigra* 115.23 mg/g. Lower amounts of total phenol have been reported in mulberry leaves ranging from 3.45 to 6.31 mg/g by Thabti et al. (2012). This variation in the amount of phenol in leaves can be due to drought, temperature changes, pollution, UV light, pathogen attack, sampling season, and also different extraction methods (Sanchez-Salcedo et al., 2015). Total phenol content is also affected by plant genotype (Scalzo et al., 2005). Methanol extracts from the leaves of various Sorbus species led to the extraction of smaller amounts of phenolic compounds (Olszewska & Michel, 2009).

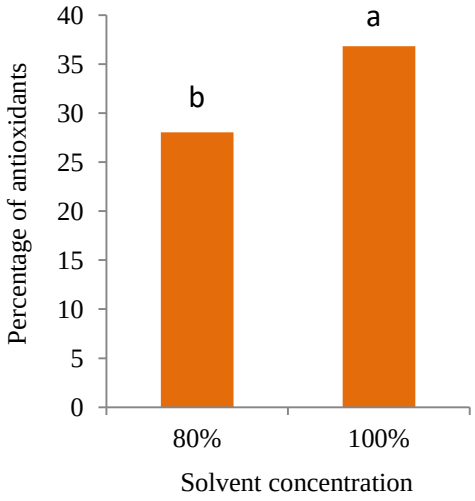

Fig. 1. Effect of ethanol solvent concentration on the extraction of mulberry leaf antioxidants.

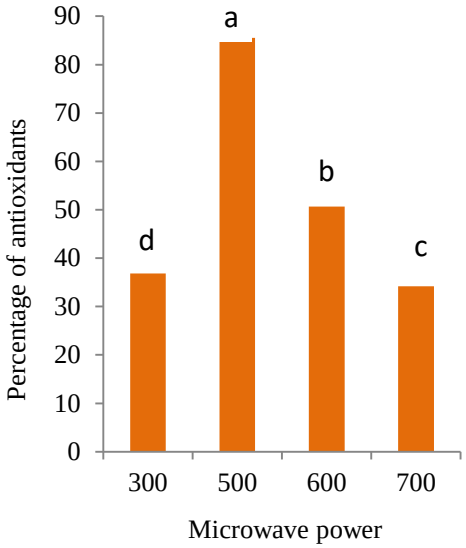

Fig. 2. The effect of microwave power on the extraction of mulberry leaf antioxidants.

Table 4. The effect of solvent ratio on antioxidant extraction

Solvent ratio	1:10	1:15	1:20	1:25
Antioxidant percentage	74.1±0.35a	68.6±0.51b	66.4±0.36d	67.5±0.09c

Similar letters indicate no significant difference (Duncan).

Table 5. The effect of harvest time on total phenol content

Time	April	May	June
Total phenol	24.01±0.93a	18.36±0.38b	19.13±0.93b

Similar letters indicate no significant difference (Duncan).

Table 6. The effect of harvest time on ferric reducing

Absorption number	April	May	June
Ferric reducing	0.977±0.002a	0.89±0.001b	0.885±0.002b

Similar letters indicate no significant difference (Duncan).

Table 7. The effect of pH on the stability of chlorophyll and carotenoid pigments

pH	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Total chlorophyll (µg/ml)	Carotenoid (µg/ml)
6	19.381±0.460a	14.589±0.670a	33.97±0.077a	3.01±0.370a
5	11.441±0.368b	11.334±0.310b	22.775±0.660b	2.88±0.116a
4	6.495±0.101c	9.291±0.395c	15.78±0.493c	2.74±0.324a
3	4.301±0.138d	2.557±0.080d	6.858±0.172d	1.634±0.101b

Similar letters in each column indicate no significant difference (Duncan).

Table 8. Effect of temperature on the stability of chlorophyll and carotenoid pigments

Temperature	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Total chlorophyll (µg/ml)	Carotenoid (µg/ml)
Control	19.381±0.46a	14.589±0.68a	33.97±0.077a	3.15±0.066a
50	17.249±0.287a	14.128±0.58a	30.038±0.32a	3.01±0.37a
70	15.91±0.362b	11.922±0.096b	29.171±0.195b	2.63±0.27a
90	4.301±0.139c	2.557±0.08c	6.857±0.172c	1.634±0.101b

Similar letters in each column indicate no significant difference (Duncan).

Table 9. The effect of light on the stability of chlorophyll and carotenoid pigments

Sample	Chlorophyll a (µg/ml)	Chlorophyll b (µg/ml)	Total chlorophyll (µg/ml)	Carotenoid (µg/ml)
Control	19.381±0.46a	14.589±0.68a	33.97±0.077a	3.15±0.066a
Lighting (T24)	3.877±0.552b	4.254±0.623b	8.132±0.362b	0.41±0.499c
Darkness (T24)	3.91±0.89b	4.797±0.66b	8.371±0.23b	1.95±0.284b

Similar letters in each column indicate no significant difference (Duncan).

The ferric reducing power

The ferric reducing power is used as a criterion for the electron-donating ability of phenolic compounds. This is an important mechanism in the oxidation process of phenolic compounds. This power is related to the antioxidant activity of biological compounds. A higher absorption number indicates more reducing power (Salmanian et al., 2014). At the beginning of the season, the highest amount of ferric reduction (absorption number 0.977) was observed. The value of this trait was not significantly different between May and June and was less than April (Table 6). These results were consistent with the results of Zou et al. (2012), which showed a reduction in ferric reducing power with increasing harvest time.

Effect of pH on color stability

Chlorophyll decomposes easily under acidic, heat, light, and oxygen conditions (Tonucci & Von Elbe, 1992). Because color is an important factor in the acceptance of a product, preventing the decomposition and change of chlorophyll during the thermal process in the food industry is of particular importance. It has been reported that thermal processes and pH changes caused structural and chemical changes in the tissue of vegetables, which eventually led to discoloration of these products (Heaton & Marangoni, 1996; Mosharraf & Keramat, 2000). After color extraction with ethanol 80% in April leaf sample, the effect of pH on color stability was measured. By decreasing the pH from 6 to 3, the levels of total chlorophyll (from 33.97 to 6.858 µg/ml) and carotenoids (from 3.01 to 1.634 µg/ml) significantly decreased (Table 7). Reduction of these pigments by lowering the pH by Koca et al. (2007) have also been reported. In acidic environments, magnesium is replaced in the chlorophyll ring by two hydrogen ions, and the green color of the chlorophyll turns into the olive color of pheophytin (Van Boekel, 2000). If the purpose of the color extraction is to be used in acidic foods (such as beverages, etc.), conditions must be provided that the color remains stable under acidic conditions during the shelf life of the products.

Effect of heat on color stability

According to the results in Table 8, increasing the temperature reduced the color intensity of chlorophyll and carotenoid pigments. Erge et al. (2008) also reported that increasing the temperature from 70 to 100 °C caused loss of chlorophyll and green color in vegetables. The loss of chlorophyll green color during the thermal process is due to the conversion of chlorophyll to pheophytin. The formation of pheophytin during the heat is due to the release of intracellular organic acids and the formation of new acidic compounds (La Borde & Von Elbe, 1990). Further increase in temperature causes formation of pyropheophitin due to the loss of carbomethoxy groups from pheophytins (Mangos & Berger, 1997).

The effect of light on color stability

The concentration of chlorophyll and carotenoid pigments significantly decreased after 24 hours at room temperature under light and dark conditions (Table 9). The effect of light on carotenoid pigment was higher, reducing it more intensely than in the dark. The results of this

study were consistent with the results of Maunders and Brown (1983) that light reduces green pigments. The researchers also found that chlorophyll a decreased more than chlorophyll b.

CONCLUSION

The results of this study showed that the extraction of antioxidants and color pigments from the green leaves of mulberry trees is possible and the early leaves of the growing season have a higher content of antioxidants and pigments. The use of different solvents affects the extraction percentage, but ultimately the use of ethanol solvent is safer than other solvents. Changing temperature, pH, and light conditions affect the stability of this color and with decreasing pH and increasing temperature, its stability decreases. It seems that the application of this natural food coloring is suitable in products with neutral pH and stored at ambient temperature or lower temperatures.

Conflict of interest

The authors hereby declare that there is no conflict of interest.

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