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ORIGINAL ARTICLE

Bioactive compounds and quality parameters of natural cloudy lemon juices

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Abstract In this study, bioactive compounds (phenolic and carotenoid) and some quality parameters (color, browning index and hydroxymethylfurfural (HMF)) of natural cloudy lemon juice, pasteurized (90 °C/15 s) and storage stability of concentrated lemon juice (−25 °C/180 days) were carried out. Fifteen phenolic compounds were determined in the lemon juice and the most abounded phenolic compounds were hesperidin, eriocitrin, chlorogenic acid and neoeriocitrin. In generally, phenolic compound concentrations of lemon juice samples increased after the pasteurization treatment. Four carotenoid compounds (β-carotene, β-cryptoxanthin, lutein and zeaxanthin) were detected in natural cloudy lemon juice. Lutein and β-cryptoxanthin were the most abounded carotenoid compounds in the lemon juice. Color values of the lemon juices were not affected by processing and storage periods. HMF and browning index of the lemon juices increased with concentration and storage. According to the results, storing at −25 °C was considered as sufficient for acceptable quality limits of natural cloudy lemon juice.

Higlights
- Lemon fruit has a strong commercial value for the fresh products market and food industry.
- Pasteurization is essential to improve the shelf-life and safety of lemon juice.
- Natural cloudy lemon juice has rich bioactive components.

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Keywords Natural cloudy lemon juice · Phenolic and carotenoid compounds · Color · HMF · Storage

Introduction

Citrus fruits are of the most important horticultural crops, and lemon (*Citrus limon*) is the third most important citrus crops. Lemon fruit has a strong commercial value for the fresh products market and food industry (Gonzalez-Molina et al. 2010). Lemons ranked the third among the citrus industry in the world, with a total annual production of about 9 % in the citrus production (Mu et al. 2012). Lemons are cultivated in many countries all over the world (13861411tons) and Turkey is the sixth in the production of lemon (790,211 tons) in 2011 (FAO 2013).

The beneficial effects of the dietary citrus fruits can be attributed, not only to the vitamin C, minerals, dietary fiber, essential oils, organic acids and carotenoids, but also to the antioxidant activity of their flavonoids (Bermejo et al. 2011; Guimarães et al. 2010; Gonzalez-Molina et al. 2010; Goodwin 1980). Natural antioxidants of citrus products can reduce oxidative damage in the human body (Abeysinghe et al. 2007). Taking into account the bioactive composition of lemon, a wide range of beneficial effects on the prevention of different kinds of cancer, cardiovascular diseases, glucose, lipid metabolism and obesity have been reported (Adibelli et al. 2009). Lemon juice is characterized by the presence of significant amounts of the flavanones, hesperidin and eriocitrin (Gattuso et al. 2007). Fanciullino et al. (2006) reported that orange and mandarin juices accumulated high contents of several carotenoids (violaxanthin, lutein, zeaxanthin, and α-cryptoxanthin), whereas lemon juice was poor in these components.

The composition of fresh citrus juice is adversely affected by industrial processing and/or storage conditions. Industrial processing involves a number of different stages that result in some alterations from the original composition of fresh citrus juice (Jordan et al. 2003). Pasteurization is essential to improve the shelf-life and safety of fruit juice. The delicate fresh composition of citrus juices is easily changed by pasteurization, as the juice undergoes various compositional changes. Pasteurization has some impacts on the quality of lemon juice, such as the loss of color, flavour, nutritional value and taste (Espachs-Barroso et al. 2006). Ibarz et al. (2009) reported that nonenzymatic browning caused by Maillard reaction (formation of HMF and furfural) affects the quality of fruit juices negatively, affecting its organoleptic properties, such as texture, color, taste and flavor, and nutritional quality, including the contents of vitamins, sugars and other minority compounds (Toribio and Lozano 1986; Johnson et al. 1995; Urbicain et al. 1999; Buedo et al. 2001).

However, limited references have been found for the effects of pasteurization, concentration and storage on bioactive components and some quality parameters of lemon juices. In order for processors to understand better the changes that take place during the processing of lemon juices, quantitative information on the components present in fresh, pasteurized, concentrated and stored lemon juice is needed. Therefore, the objectives of this study were to evaluate bioactive components and some quality parameters of naturally cloudy lemon juice and concentrated lemon juice during storage (at −25 °C for 180 days).

Materials and methods

Production of natural cloudy lemon juices

Lemons (*Citrus limon* (L.) Burm. f. cv. Interdonato) were purchased from a local market (Adana, Turkey) and were washed with tap water. The lemon juice was extracted by a citrus extractor machine (Can Can, Citrus Extractor Machine, Turkey) and then rapidly strained through a stainless steel sieve with pore diameter of 1 mm to accomplish separation of most of the suspended matter from lemon juice. The some quality properties of lemon juice which was used in that study had 8.75 ± 0.25 % soluble solid content, 2.66 ± 0.07 pH, and 7.07 ± 0.78 g/100 mL (as citric acid) titratable acidity. The lemon juice was pasteurized at 90 °C for 15 s, and the juice was concentrated using a rotary vacuum evaporator until it reached about 45° brix. Each experiment was carried out in three replications. Samples of concentrate lemon juice were stored at −25 °C for 180 days in brown bottles and were analyzed in 2 month intervals. Lemon juice concentrates (concentrate samples were diluted to 9° brix and then analyses were made) produced in the laboratory were subjected to the following analyses:

Color measurement

Color (CIE L^*, a^*, b^*) analysis were conducted by the Color Flex HunterLab Instrument. 50 mL of juice was transferred into 20 mm Glass Optical Cell Light Path and then analyzed. The results were given according to the CIELAB color system. In this system, L^* defines lightness (0: black; 100: white), a^* denotes the red/green value ((+): red; (−): green) and b^* the yellow/blue value ((+): yellow; (−): blue). In addition, the following formulas were used for the calculations of Hue*, C*:

$$Hue^* = arctan\left(\frac{b^*}{a^*}\right)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

Determination of browning index

5 mL of lemon juice sample was mixed with 5 mL ethyl alcohol (95 %) in teflon tubes and then centrifuged (4000 rpm, 10 min, at 4 °C). The supernatant was passed through a 0.45 μm teflon membrane filter and the absorbance of the supernatant was obtained at 420 nm in a spectrophotometer (Perkin Elmer Lambda 25-UV/VIS, USA) (Meydav et al. 1977).

Determination of hydroxymethylfurfural (HMF)

HMF extractions of samples were carried out according to the method reported by Gökmen and Acar (1996). HPLC analyses were done by means of a Shimadzu LC-20AT (Japan) system. The best chromatographic conditions were determined as a result of preliminary experiments as follows: 20 μL of supernatant was injected into the C18 ACE (4.6 × 250 mm) column; the column was maintained at 30 °C with a flow rate of 0.5 mL/min; and the photodiode array detector was set at 285 nm. Methanol/water/acetic acid (20/79/1, v/v/v) was used as mobile phases.

Determination of total phenolic content

Total phenolic content in lemon juice samples were measured by the previously reported Folin-Ciocalteu method with some modifications (Abdullakasim et al. 2007). For measurement of the total phenolic content, 5 mL of lemon juice was mixed with 5 mL of 80 % methanol in teflon tube and then the tubes were centrifuged at 4000 rpm for 20 min at 4 °C (Heraeus Bofuge Primo R, Germany). For analysis, 100 μL

appropriately diluted sample or standard solution at various concentrations was mixed with 100 μL Folin-Ciocalteu reagent and 3000 μl deionised water and mixed thoroughly. After incubation for 10 min at room temperature, 100 μL of 20 % Na_2CO_3 solution was added with immediate mixing and was further incubated at room temperature for 2 h in the dark. The mixture absorbance was then measured at 765 nm using a spectrophotometer (Perkin Elmer Lambda 25-UV/VIS, USA). Gallic acid was used as standard and total phenolic contents of the samples were expressed in milligrams per L as gallic acid equivalents (mgGAE/L).

Determination of phenolic compounds

For the measurement of phenolic compounds, 5 mL of lemon juice was mixed with 10 mL of 80 % methanol in the teflon tube and sonicated (Bandelin Sonerex, Germany) at room temperature (~25 °C) for 15 min. Then, tubes were centrifuged with 4000 rpm 10 min at 4 °C (Heraeus Bofuge Primo R, Germany). Finally, the supernatant was passed through a 0.45 μm teflon membrane filter and injected into the HPLC instrument (Agcam et al. 2014). HPLC analyses were carried out by means of a Shimadzu LC-20AT (Japan) system, consisting of a quaternary pump, a column temperature control oven (CTO-10AS), an autosampler unit (SIL-20A), a degasser module (DGU-20A_5) and a photodiode array detector (SPD-M20A). 20 μL of supernatant was injected into the C18 XTerra (Waters, 4.6 × 250 mm) column. The column was kept at 30 °C and the flow rate was 1 mL/min. The photodiode array detector was set at 280 and 320 nm. 5 % formic acid (A) and 20 % A + 80 % ACN (B) were used as mobile phases. According to the preliminary experiments for lemon juice phenolics, the best gradient elution was determined as follows: 0 min: A 100 %; 10 min: 95 % A + 5 % B; 25 min: 90 % A + 10 % B; 55 min: 80 % A + 20 % B; 70 min: 55 % A + 45 % B; 90 min: 100%B; 95 min: 100 % A.

The phenolic compounds were identified by comparing their UV–visible spectra and retention times with that of corresponding standards. Quantification of phenolic compounds was carried out at 280 and 320 nm using external standard method. Calibration curves were obtained using the commercial standards of the concentrations normally present in lemon, obtaining regression coefficients (R^2) above 0.996 in all cases.

Determination of total carotenoid content

Total carotenoid determination was carried out according to the previously described method of Lee and Castle (2001) with some modifications. 5 mL of juice and 10 mL of hexane solution (hexane/methanol/acetone, 50/25/25, v/v with 0.1 % BHT) were mixed and then centrifuged for 10 min 4000 rpm at 4 °C. The supernatant phase was used for absorbance measuring (450 nm) by spectrophotometer (Perkin Elmer Lambda 25-UV/VIS, USA). Total carotenoids were calculated using the extinction coefficient of β-carotene ($E^{1/2} = 2505$).

Determination of carotenoid compounds

Pigment extraction from juices and saponification procedures were carried out according to the previously reported method of Melendez-Martinez et al. (2007). HPLC analyses were carried out by means of a Shimadzu LC-20AT (Japan) system, consisting of a quaternary pump, a column temperature control oven (CTO-10AS), an autosampler unit (SIL-20A), a degasser module (DGU-20A_5) and a photodiode array detector (SPD-M20A). 50 μL of supernatant was injected into the C30 ProntoSIL (5.0 μm, 4.6 × 250 mm) column. The column was kept at 20 °C and the flow rate was 1 mL/min. Photodiode array detector was set to 450 nm. Methanol (A), methyl-*tert*-butyl ether (B) and ultrapure water (C) were used as mobile phases. According to the preliminary experiments, the best gradient elution was as follows: 0 min: A 90 % + 5 % B + 5 % C; 5 min: 95 % A + 5 % B; 40 min: 75 % A + 25 % B; 55 min: 55 % A + 45 % B; 60 min: 90 % A + 5 % B + 5 % C; 65 min: 90 % A + 5%B + 5 % C.

The carotenoid compounds were identified by comparing their UV-visible spectra and retention times with that of corresponding standards. Quantification of carotenoid compounds was carried out at 450 nm using external standard method.

Antioxidant activity

The antioxidant activity of the lemon juices was evaluated using the DPPH* free radical-scavenging method. DPPH* free radical-scavenging activity measurements were carried out according to the procedure of Klimczak et al. (2007) with some modifications. 5 mL of lemon juices were mixed with 5 mL of methyl alcohol (80 %) in teflon tubes and then centrifuged (4000 rpm, 10 min, at 4 °C). Briefly, 0.1 mL of supernatant was added to 2.46 mL of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH*; 0.025 gL^{-1} in 80 % methyl alcohol) and mixed by vortex. After incubating for 10 min in the dark, absorbance of the samples was measured at 515 nm using the spectrophotometer. Antioxidant activity was expressed as the percentage decline of the absorbance:

$$\text{Antioxidant activity}(\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Where, $A_{control}$ was the absorbance of the control, and A_{sample} was the absorbance of the sample.

Statistical analysis

The software SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis of variance (ANOVA) and Duncan's multiple comparison test in order to determine significant differences among the treatments. Each experiment was repeated at least three times.

Results and discussion

Effects of processing and storing on total phenolic content and phenolic compounds

Changes in total phenolic content and phenolic compounds of lemon juice samples are given in Table 1. Total phenolic contents were detected as 250.4 and 264.9 mg GAE/L after the treatments of the extraction and pasteurization, respectively. In addition, the lowest value of total phenolics was determined as 246.2 mg GAE/L after the treatment pulp removing. Xu et al. (2008) were reported that total phenolics in juices from 15 citrus varieties (seven mandarins, four sweet oranges, one lemon, one grapefruit, and two pummeloes) of China were determined in the range of 751.82–1555.49 mg GAE/L. Total phenolic content of lemon juice was reported as 751.82 mg GAE/L. Khosa et al. (2011) studied on total phenolics in juices from four different varieties of citrus lemon, indigenous to Pakistan and the total phenolic contents from juices of citrus lemon determined following the Folin-Ciocalteu assay were found in the range of 690.62–998.29 mg/L. Determined in previous studies are greater than our lemon juice results. Phenolic content of lemon juices could be influenced by several factors such as growing conditions, geographical origin, and fruit maturity.

Three hydroxybenzoic acids (gallic acid, vanillic acid and protocatechuic acid ethyl ester (PAEE)), five hydroxycinnamic acids (*o*-coumaric acid, ferrulic acid, caffeic acid, *p*-coumaric acid, chlorojenic acid,), five flavanons (hesperidin, eriocitrin, neoeriocitrin, neohesperidin, naringin) and two flavanols (rutin, quersetin) were detected in the lemon juice samples (Table 1). Totally, fifteen phenolic compounds were determined and hesperidin, eriocitrin, neoeriocitrin (as flavonoids) and chlorogenic acid (as phenolic acid) were the most abounded ones in the lemon juice. Concentration of these compounds were identified between 81.5–117.4 mg/L, 42.5–63.2 mg/L, 9.0–13.4 mg/L and 9.7–14.3 mg/L in the lemon juice samples, respectively. Similarly, Gattuso et al. (2007) reported that lemon juice is characterized by the presence of significant amounts of the flavanones, hesperidin (20.5 mg/100 mL) and eriocitrin (16.7 mg/100 mL). Caristi et al. (2003) studied on the flavonoid profile in lemon juices obtained from the main Sicilian cultivars (Femminello comune, Monachello and Interdonato). In three types of lemon juice, eriocitrin and hesperidin amounts were detected as 84–298 mg/L and 88–197 mg/L, respectively. Our hesperidin results were in harmony with the Caristi et al. (2003) findings. However; other researchers indicated that eriocitrin (eriodictyol 7-O-β-rutinoside) was the main flavonoid in lemon fruit (Tripoli et al. 2007; Miyake et al. 2006). Gonzalez-Molina et al. (2012) studied on new beverages of lemon juice (Fino) with elderberry and grape concentrates as a source of bioactive compounds. They detected eriocitrin (6.26 ± 0.06 mg/100 mL) and hesperidin (6.14 ± 0.09 mg/100 mL) in the lemon juice obtained from fino lemon varieties. Syringic acid, sinapic acid, naringenin, isoquercetin, kamferol, luteolin and apigenin could not be detected in lemon juice samples. Mellisho et al. (2011) reported that high flavonoid contents in the lemon juice were determined in the range of 800–1500 mg/L with eriocitrin, hesperidin, and apigenin. Tounsi et al. (2011) studied on phenolic compounds in the lemon juice and those compounds were determined as gallic acid with 6.93 ± 0.32 mg L^{-1}, chlorogenic acid (0.64 ± 0.19 mg L^{-1}), p-coumaric acid (1.00 ± 0.14 mg L^{-1}), rutin (0.77 ± 0.15 mg L^{-1}) and quercetin (0.34 ± 0.00 mg L^{-1}).

In generally, total phenolic content and phenolic compounds of lemon juice samples increased by pasteurization. But, the differences among the production stages were not significant, statistically. Xu et al. (2007) reported that free fraction of phenolics increased, whereas ester, glycoside, and ester-bound fractions decreased after heating. Moreover, there was a decrease of total phenolic acid content after heat treatment and the content of four flavanone glycosides (narirutin, naringin, hesperidin, and neohesperidin) declined with heating time and temperature. Hayat et al. (2010) stated that the total phenolic acid content in mandarin pomace decreased with increasing microwave power and treatment time. In this study, the amounts of extractable phenolic substances increased with pasteurization. According to the results, it could be said that membrane of plant cell could be affected destructively with heat treatment and on the cell membrane are formed pores. Consequently, pores formed on the cell membrane enhance mass transfer out of the cells (Agcam et al. 2014).

Changes in phenolic content of lemon juice concentrates during storage period are given in Table 2. Total phenolic contents of the lemon juice concentrates ranged from 1314.6 to 1370.7 mg/L. Changes in total phenolic content and phenolic compounds in lemon juice concentrates were not significant during storage (at −25 °C, 180 days). The most abundant phenolic compounds in lemon juice concentrates, hesperidin ranged from 501.5 to 567.9 mg/L with storage period, eriocitrin from 254.7 to 284.6 mg/L, neoeriocitrin from 58.3 to 64.7 mg/L and chlorojenic acid from 57.1 to 70.1 mg/L. According to the results, storage at −25 °C was considered as sufficient for protection of these components.

Table 1 Effect of processing stages on bioactive compounds and antioxidant activity of lemon juice

	Extraction	Pulp removing	Pasteurization
Total phenolic content (mg GAE/L)	250.4 ± 38.6[a]	246.2 ± 16.1[a]	264.9 ± 23.4[a]
Hydroxybenzoic acids (mg/L)			
Gallic acid	3.7 ± 0.4[a]	3.1 ± 0.5[a]	4.5 ± 1.4[a]
Vanillic acid	0.34 ± 0.1[b]	0.31 ± 0.1[b]	0.87 ± 0.1[a]
PAEE[a]	6.1 ± 1.0[a]	4.2 ± 0.7[a]	6.3 ± 1.8[a]
Syringic acid	n.d.		
Hydroxycinnamic acids (mg/L)			
o-coumaric acid	1.3 ± 0.1[a]	0.9 ± 0.2[a]	1.4 ± 0.4[a]
Ferrulic acid	0.2 ± 0.0[a]	0.1 ± 0.0[a]	0.2 ± 0.0[a]
Caffeic acid	4.5 ± 0.7[a]	3.4 ± 0.5[a]	4.7 ± 1.4[a]
p-coumaric acid	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]
Chlorogenic acid	12.0 ± 1.7[a]	9.0 ± 1.6[a]	13.4 ± 4.1[a]
Sinapic acid	n.d.		
Flavanones (mg/L)			
Hesperidin	113.6 ± 11.0[a]	81.5 ± 12.7[a]	117.4 ± 32.5[a]
Eriocitrin	53.9 ± 13.7[a]	42.5 ± 6.7[a]	63.2 ± 18.0[a]
Neoeriocitrin	12.5 ± 2.6[a]	9.7 ± 1.5[a]	14.3 ± 4.1[a]
Neohesperidin	1.3 ± 0.4[a]	0.9 ± 0.2[a]	1.6 ± 0.6[a]
Naringin	3.8 ± 0.8[a]	2.8 ± 0.6[a]	4.1 ± 1.3[a]
Naringenin	n.d.		
Flavonols (mg/L)			
Rutin	3.4 ± 0.4[a]	2.3 ± 0.5[a]	3.5 ± 1.3[a]
Quercetin	2.5 ± 0.4[a]	1.5 ± 0.4[a]	2.6 ± 1.4[a]
Isoquercetin	n.d.		
Kamferol	n.d.		
Flavones (mg/L)			
Luteolin	n.d.		
Apigenin	n.d.		
Total carotenoid content (μg/100 mL)	44.72 ± 3.19[a]	44.35 ± 2.36[a]	36.74 ± 3.40[b]
Zeaxanthin	0.66 ± 0.09[a]	0.65 ± 0.04[a]	0.49 ± 0.02[b]
Lutein	5.05 ± 0.06[a]	5.10 ± 0.10[a]	5.01 ± 0.02[a]
β-carotene	0.55 ± 0.14[a]	0.51 ± 0.33[a]	0.42 ± 0.01[a]
α-carotene	n.d.		
β-cryptoxanthin	3.80 ± 0.15[a]	3.11 ± 0.05[b]	2.68 ± 0.02[c]
Antioxidant activity (%)	81.20 ± 0.75[a]	82.01 ± 0.89[a]	81.53 ± 1.27[a]

Small letters on the same row show the difference between the stages of production (level of 0.01 importance)

n.d. not detected

[a] Protocatechuic acid ethyl ester

Effect of processing and storing on total carotenoid content, carotenoid compounds and antioxidant activity

Total carotenoid contents of lemon juice samples are given in Table 1. The highest value of total carotenoid content was detected as 44.35 μg/100 mL after the treatments of extraction. The lowest value of total carotenoids was determined as 36.74 μg/100 mL after pasteurization. Concentration of total carotenoids was significantly lower ($p < 0.01$) in the pasteurized lemon juice than in the untreated (extracted) juice (decrease of 17.84 %). Five carotenoid compounds (α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin) were investigated in naturally cloudy lemon juices (Table 1). Lutein (5.01–5.10 μg/100 mL) and β- cryptoxanthin (2.68–3.80 μg/100 mL) were detected as the most abounded carotenoid compounds in lemon juice. α-carotene could not be detected in lemon juice samples. In generally, carotenoid compounds of lemon juice decreased after pasteurization ($p < 0.01$).

Table 2 Effect of storage periods on bioactive compounds and antioxidant activity of concentrated lemon juice

	0. Day	60. Day	120. Day	180. Day
Total phenolic content (mg GAE/L)	1370.7 ± 101.4[a]	1314.6 ± 84.2[a]	1342.9 ± 31.1[a]	1357.2 ± 97.9[a]
Hydroxybenzoic acids (mg/L)				
Gallic acid	21.0 ± 2.5[a]	21.5 ± 2.6[a]	19.4 ± 0.2[a]	19.8 ± 0.9[a]
Vanillic acid	3.39 ± 0.3[a]	3.35 ± 0.7[a]	3.38 ± 0.5[a]	3.57 ± 1.2[a]
PAEE[a]	26.8 ± 3.6[a]	30.4 ± 1.6[a]	29.7 ± 0.8[a]	25.6 ± 0.6[a]
Hydroxycinnamic acids (mg/L)				
o-coumaric acid	5.9 ± 0.8[a]	6.7 ± 0.4[a]	6.5 ± 0.2[a]	5.9 ± 0.4[a]
Ferrulic acid	0.6 ± 0.2[a]	0.6 ± 0.0[a]	0.5 ± 0.0[a]	0.6 ± 0.0[a]
Caffeic acid	20.7 ± 4.6[a]	24.8 ± 2.5[a]	20.7 ± 0.4[a]	19.9 ± 1.1[a]
p-coumaric acid	0.5 ± 0.1[a]	0.4 ± 0.1[a]	0.5 ± 0.0[a]	0.4 ± 0.1[a]
Chlorogenic acid	57.1 ± 11.1[a]	70.1 ± 5.1[a]	58.1 ± 1.5[a]	56.9 ± 4.1[a]
Flavanones (mg/L)				
Hesperidin	523.2 ± 96.7[a]	567.9 ± 54.9[a]	534.3 ± 3.6[a]	501.5 ± 22.5[a]
Eriocitrin	274.7 ± 46.7[a]	284.6 ± 26.3[a]	266.2 ± 1.8[a]	254.7 ± 11.4[a]
Neoeriocitrin	61.9 ± 9.2[a]	64.7 ± 4.1[a]	58.3 ± 1.9[a]	58.3 ± 4.0[a]
Neohesperidin	12.8 ± 4.7[a]	11.1 ± 0.3[a]	12.9 ± 2.1[a]	13.6 ± 0.6[a]
Naringin	17.4 ± 3.0[a]	20.6 ± 1.4[a]	14.9 ± 2.8[a]	15.4 ± 3.9[a]
Flavonols (mg/L)				
Rutin	16.2 ± 3.6[a]	16.5 ± 2.4[a]	16.9 ± 0.6[a]	15.7 ± 1.5[a]
Quercetin	13.1 ± 4.1[a]	11.6 ± 1.8[a]	11.6 ± 0.2[a]	10.1 ± 1.7[a]
Total carotenoid content (μg/100 mL)	201.1 ± 9.3[a]	194.3 ± 12.2 [a]	192.5 ± 22.1 [a]	190.7 ± 22.4 [a]
Zeaxanthin	2.33 ± 0.4[a]	2.45 ± 0.6[a]	2.14 ± 0.03[a]	2.11 ± 0.01[a]
Lutein	31.65 ± 0.5[a]	30.81 ± 1.1[a]	30.66 ± 1.9[a]	30.51 ± 0.2[a]
β-carotene	4.09 ± 1.2[a]	3.44 ± 0.6[a]	3.82 ± 0.1[a]	3.28 ± 0.4[a]
β-cryptoxanthin	20.88 ± 1.56[a]	20.26 ± 1.86[a]	18.61 ± 1.97[a]	17.94 ± 1.35[a]
Antioxidant activity (%)	79.85 ± 1.2[b]	81.42 ± 1.4[ab]	83.06 ± 0.6[a]	82.5 ± 1.0[a]

Small letters on the same row show the difference between the stages of production (level of 0.01 importance)

[a] Protocatechuic acid ethyl ester

Xu et al. (2008) studied on citrus which cultivated in China and total carotenoid content of lemon juice was reported as 0.08 ± 0.04 mg/L. According to a study, total carotenoid contents were determined as 0.05–0.08 mg/L in lemon juice (Khosa et al. 2011). Total carotenoid contents were reported as 0.45 ± 0.11 mg/L in lemon (Eureka) juice. β-carotene and β- cryptoxanthin amounts were detected as 0.11 ± 0.01 mg/L and 0.34 ± 0.02 mg/L, respectively (Bassene et al. 2009). Fanciullino et al. (2006) reported that total carotenoid content was 1.26 mg/L in lemon juice. In previous studies, carotenoid contents were reported on different values by several researchers. This case can be explained by ecological factors like phenolic contents.

In several studies (Lee and Coates 1999a; Lee and Coates 2003; Gama and Sylos 2005; Cortes et al. 2006), losses of β-carotene or β-cryptoxanthin were very low during pasteurization or the thermal concentration of different citrus juices. Sanchez-Moreno et al. (2005) reported that cases of thermal treated orange juice (90 °C for 1 min) led to an increase in β-cryptoxanthin (19.19 %) and zeaxanthin (37.49 %) and to a decrease in lutein (23.10 %). In this study, concentrations of zeaxanthin (25.75 %), lutein (0.79 %), β-carotene (23.63 %) and β-cryptoxanthin (29.47 %) decreased in the pasteurized lemon juice ($p < 0.01$).

Total carotenoid contents of lemon juice concentrates ranged from 190.7 to 201.1 μg/100 mL (Table 2). Storage periods (at −25 °C, 180 days) of the concentrated lemon juice showed that the total carotenoid concentrations slightly decreased during storage period. But, those changes of total carotenoid contents in lemon juice concentrates were not significant, statistically. Lutein and β-cryptoxanthin concentrations ranged from 30.51 to 31.65 μg/100 mL and 17.94–20.88 μg/100 mL in the lemon juice concentrates with storage periods, respectively. Differences in carotenoid compounds were not significant statistically for storage periods.

Antioxidant activities of lemon juice samples were determined between 81.20 and 82.01 % (Table 1). According to the results, effects of production stages were statistically insignificant on antioxidant activity. Antioxidant activities of lemon juice concentrates increased and decreased during storage

Table 3 Effect of processing stages on color, browning index and HMF content of lemon juice

	Extraction	Pulp removing	Pasteurization
*L**	67.96 ± 3.52[a]	71.98 ± 6.31[a]	73.50 ± 2.90[a]
*a**	0.43 ± 0.18[a]	0.40 ± 0.34[a]	0.33 ± 0.17[a]
*b**	20.04 ± 0.48[a]	19.15 ± 0.91[a]	20.40 ± 0.13[a]
*C**	20.05 ± 0.48[a]	19.15 ± 0.92[a]	20.40 ± 0.13[a]
Hue*(°)	88.77 ± 0.55[a]	88.83 ± 0.92[a]	89.08 ± 0.50[a]
Browning index (420 nm, abs)	0.132 ± 0.01[a]	0.127 ± 0.00[a]	0.142 ± 0.01[a]
HMF (μg/L, ppb)	9.73 ± 0.66[b]	8.47 ± 1.72[b]	21.92 ± 4.90[a]

Small letters on the same row show the difference between the stages of production (level of 0.01 importance)

($p < 0.01$). Arena et al. (2001) reported that during processing or storage of orange juice a number of chemical changes can occur and these changes can have a significant effect on the overall antioxidant activity. Many naturally occurring antioxidants that are relatively unstable can be substantially lost (such as ascorbic acid degradation) (Lee and Chen 1998; Lee and Coates 1999b). Ascorbic acid is considered as a most important water-soluble antioxidant. It protects compounds in extracellular and intracellular spaces in most biological systems and reduces tocopherol radicals back to their active form at the cellular membranes (Kaur and Kapoor 2001). It can directly scavenge superoxide radical, singlet oxygen, hydrogen peroxide and hydroxyl radical. Citrus juices are a rich source of ascorbic acid, which is an important antioxidant in these juices (Klimczak et al. 2007). Ascorbic acid contents were already conducted in other study for samples processed under the same conditions. Further, ascorbic acid results have been also published by Ucan et al. (2014) for similar processing stage lemon juice samples. They reported that ascorbic acid contents were determined as 419.91 ± 24.3, 361.64 ± 16.3 mg/L and 344.91 ± 40.0 mg/L for cloudy lemon juice samples obtained after the treatments of the extraction, pulp removing and pasteurization, respectively. Also they showed that ascorbic acid content of concentrated samples (1682.00 ± 135.10 mg/L) was decreased more than 16 % (1409.53 ± 150.47 mg/L) during storage. Processing or storage can sometimes improve the antioxidant activity of naturally occurring antioxidants. For example, polyphenols at an intermediate oxidation state can exhibit higher radical scavenging activity than the completely nonoxidized ones (Nicoli et al. 1999; Polydera et al. 2004). Xu et al. (2007) assumed that many antioxidant phenolic compounds in plants are usually presented as the covalently-bound form; therefore, some processing methods were employed to liberate them in order to enhance their antioxidant capacity. Seok-Moon et al. (2004) reported that heat treatment may liberate some low molecular weight in phenolic compounds and increase the antioxidant capacity of citrus peel as a result. Hayat et al. (2010) expressed that, after microwave treatment, the free fraction of phenolic acids increased, whereas the bound fractions decreased and antioxidant activity was increased at all.

Effect of processing and storing on color, browning index and HMF contents

Color values of fresh and pasteurized lemon juices are given in Table 3. L* values were determined 67.96, 71.98 and 73.50 for extraction, pulp removing and pasteurization, respectively. L* values of pulp removed and pasteurized lemon juices were increased. The a* value of lemon juice was 0.43 for extraction and 0.33 for pasteurization. a* values of pulp removed and pasteurized lemon juices decreased. Changes in b* values of lemon juices with production stages were not statistically important. C* values were ranged from 19.15 to 20.40 for lemon juices. Hue* values were calculated as 88.77, 88.83 and 89.08° for extraction, pulp removing and pasteurization lemon juice samples, respectively. Changing of all color values with processing was not statistically significant. Our

Table 4 Effect of storage periods on color, browning index and HMF of concentrated lemon juice

	0. Day	60. Day	120. Day	180. Day
*L**	67.18 ± 1.10[a]	68.17 ± 1.02[a]	68.17 ± 0.55[a]	67.89 ± 0.86[a]
*a**	3.83 ± 0.35[a]	3.76 ± 0.43[a]	3.73 ± 0.37[a]	3.73 ± 0.34[a]
*b**	58.63 ± 1.53[a]	59.24 ± 1.71[a]	58.80 ± 1.79[a]	58.49 ± 1.42[a]
*C**	58.76 ± 1.55[a]	59.36 ± 1.73[a]	58.92 ± 1.81[a]	58.61 ± 1.43[a]
Hue*(°)	86.27 ± 0.27[a]	86.37 ± 0.32[a]	86.38 ± 0.25[a]	86.36 ± 0.25[a]
Browning index (420 nm, abs)	0.139 ± 0.01[b]	0.143 ± 0.01[b]	0.170 ± 0.02[a]	0.177 ± 0.01[a]
HMF (μg/L, ppb)	235.87 ± 13.60[b]	440.73 ± 31.67[a]	503.20 ± 79.95[a]	493.60 ± 103.73[a]

Small letters on the same row show the difference between the stages of production (level of 0.01 importance)

results are similar with Lee and Coates (2003) findings. They reported that a color shift toward positive b* and negative a* directions indicate more yellow and less red in pasteurized juices. After pasteurization, they showed slight increase in L* value that indicated a lightening of juice surface color, a chroma(C*) increase from 17.70 to 20.19. Lee and Coates (1999a) reported that a similar observation of small increases in L* values was also reported with thermal processing of red grapefruit juices. And also, the color shift during thermal processing was attributed to the degradation of chromoplasts and solution of carotenes in other cellular lipids. Furthermore, Genovese et al. (1997) speculated that since juice color was reflected by suspended pulp particles (juice sacs), changes in suspended pulp particles after thermal pasteurization probably would also affect color changes in juices.

In this study, a*, b*, C* and Hue* values increased whereas L* values decreased after concentration of samples. During storage L* values of lemon juice concentrates (Table 4) ranged from 67.18 to 68.17, a* values 3.83 to 3.76, b* values 58.63 to 59.24, C* values 58.61 to 59.36, Hue* values 86.27 to 86.38°. According to the statistical analysis, changes in the color values during storage were not significant.

Browning index values of extraction, pulp removing and pasteurization samples were determined as absorbance 0.132, 0.127 and 0.142, respectively (Table 3). Changing of browning index values in processing was not statistically important. Browning index values determined as absorbance between 0.139 and 0.177 during storage (Table 4). The browning index values increased with storage and these increases were found significant ($p<0.01$). Bull et al. (2004) reported that browning index value was determined as 0.097 in fresh Valencia orange juice after 4 week storage at 4 and 10 °C was determined as 0.136 and 0.135, respectively. On the other hand, in fresh navel orange juice was determined as 0.050 after 4 week storage at 4 and 10 °C was determined as 0.136 and 0.135, respectively. Rodriguez-Saona et al. (1999) showed that the rate of polymeric color formation at refrigerated temperature was very low.

HMF content of lemon juice was found 21.92 μg/L after pasteurization ($p<0.01$) (Table 3). HMF content for concentrated of lemon juice increased to 235.87 μg/L and changed between 235.87 and 503.20 μg/L during storage (Table 4). The increases were found statistically significant ($p<0.01$) after 60th day. Burdurlu et al. (2006) reported that after 8 week storage, HMF contents of citrus juice concentrates at 28 °C ranged from 3.01 to 28.32 mg/kg and the variation of HMF values at 37 °C were between 521.52 and 1141.99 mg/kg, while those values for 45 °C ranged from 1401.1 to 3252.3 mg/kg. According to this data, the increase of HMF at 45 °C was approximately 2.7 times higher than that of at 37 °C. They expressed that HMF accumulation of citrus juice concentrates increased depending on the storage temperature. Because of our storage temperature was −25 °C, HMF values were found lower about 1000 fold.

Conclusions

According to analysis results, total phenolic content of lemon juice samples increased after pasteurization treatment. Fifteen phenolic compounds were determined in the lemon juice, and also hesperidin, eriocitrin, neoeriocitrin and chlorogenic acid were the most abounded phenolic compounds. Total carotenoid content of lemon juice samples decreased because of pasteurization stage. Lutein and β- cryptoxanthin were detected as the most abounded carotenoid compounds in lemon juice. Browning index values of concentrated lemon juice increased but changes in all color values were not significant during storage. In conclusion, natural cloudy lemon juice had higher bioactive compounds and quality parameters investigated in comparison to pasteurized and concentrated ones. However, pasteurized and concentrated lemon juices (when processed and stored under suitable conditions) can be considered as good sources in terms of bioactive compounds which are necessary to protect human health.

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