

Influence of processing parameters on physicochemical properties of Iranian mulberry (*Morus alba* L.) molasses

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Abstract: The white mulberry is a native fruit to China. The white mulberry as a fruit with high water content and sensitive texture, is a perishable fruit with high-level waste. To reduce the losses of the white mulberry, it used to be processed traditional dried fruit and mulberry molasses. The purpose of this study was to determine chemical and physical properties of white mulberry molasses made up of different cultivars, neutralizers and concentration methods. Two examples of cultivars were Bokhara and Khardar mulberry (local cultivars), two neutralizing compounds were white soil (traditionally special soil can be used as neutralization) and calcium carbonate, the concentration conditions were concentration under vacuum conditions and concentration under atmospheric pressure to achieve the desired final concentration (70 Brix). The experimental design was factorial in the frame of completely randomized design which was performed in three replications. The results showed that the neutralization and concentration methods had significant effects on acidity, pH and color (L^* , a^* , b^* indexes). The selected sample for preparing mulberry molasses was the concentration of Bokhara mulberry in vacuum condition with calcium carbonate as neutralizing agent.

Keywords: acidity, concentration, mulberry molasses, neutralization

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1 Introduction

Morus alba, known as white mulberry, is a short-lived, fast-growing, small to medium sized mulberry tree, which grows to 10–20 meters tall. The species are native to northern China, and is widely cultivated and adapted naturalized elsewhere (Wu et al. 2013). The trees are generally deciduous in temperate regions, but trees grown in tropical regions can be evergreen (Hunter, 1995). The fruit is 1–2.5cm long in the species, in the wild it is deep purple, but in many cultivated plants it varies from white to pink; it is sweet and bland. The fruits are also eaten, often dried or made into wine or vinegar (Wu et al. 2013). In traditional Chinese medicine, this fruit is used to treat prematurely grey hair and treat constipation and diabetes.

Molasses is one of the traditional food products in Turkey and other countries. It can also be produced from fruits containing high amounts of sugar like grape, mulberry, date, apple, plum, watermelon, apricot, sugar beet and fig by concentration of juices up to 70–80 soluble dry matter content. However, the grape and mulberry are the most common fruits used in processing molasses (Batu, 1991c). Recently, the molasses produced from mulberry is more popular because of its medicinal properties. Mulberry molasses is highly recommended for colds, stomach, complaints and anemia. Therefore the price of mulberry molasses is higher than other molasses types. Fresh and dried mulberry is used as a raw material to produce mulberry molasses (Cakmakci and Tosun, 2010).

Concentrated fruit juice is mostly manufactured in industrial conditions, although traditionally and still today farmers produce small amounts of fruit in order to supply their own requirements and the market. Molasses was consumed generally for breakfast instead of jam,

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marmalade or honey particularly in winter (Alpaslan and Hayta, 2002), since fruit molasses contains high amounts of sugar, mineral and organic acid, it is a very important food product in human nutrition (Demiröz ü et al. 2002; Ustün and Tosun, 1997). Fruit molasses easily passes into the blood without digestion because most of its carbohydrate is in the form of monosaccharides like glucose and fructose. This is nutritionally important, especially for babies, children and sportsmen and in situations demanding urgent energy. Molasses has an important function in the working of the brain in which glucose is an energy source. Furthermore, molasses (pekmez) confers approximately 1225 kJ/100g of energy and also has important organic acids and mineral materials. There are many publications about production, composition, standardization and health benefits of molasses (Aksu and Nas, 1996; Batu, 1991a; Batu, 1991b; Batu, 1991c; Batu, 1993; Batu and Aktan, 1993; Batu and Yurdagel, 1993; Bozkurt et al., 1999; Ustun and Tosun, 1997). Fruit molasses has been produced with different techniques considering species of fruits used in production. There is a number of research about chemical properties of grape molasses (Batu, 1993; Batu and Yurdagel, 1993; Bozkurt, et al., 1999; Demiröz ü et al., 2002; Karakaya and Artık, 1990; Tosun and Ustün, 2002; Ustün and Tosun, 1997). Grape juice is used for the production of grape molasses. Acidity is decreased with the application of CaCO₃ containing white molasses soil, or CaCO₃ only, and the grape juice is left to sediment. Acid-decreased and clarified grape juice is concentrated to the desired brix degree under various conditions.

In the food industry, type of fruit used, neutralizing compounds and concentration conditions of fruit juice are important factors in order to make fruit molasses with different chemical and physical properties.

However, there is a little information about chemical properties of mulberry molasses. Therefore, the purpose of this study was to determine chemical and physical properties of mulberry molasses produced from different

mulberry cultivars, two neutralizing compounds and different concentration conditions.

2 Materials and methods

2.1 Materials

Two cultivars mulberry named Bokhara and Khardar were used in this study. Berries were collected from the gardens around Mashhad (Iran). White molasses soil was supplied from local market (White soil is made predominantly from clay and calcium carbonate, to decrease the acidity of fruit juice and also produce new materials then suspended solids deposited, so acidity decreases and the final product clarifies). Other chemicals such as calcium carbonate were reagent grade, Merck, Germany.

2.2 Sample preparation

For mulberry molasses production, fresh mulberry was used as raw material. Firstly, mulberry was placed in boiling vessels after cleaning. After adding water to mulberry, the mixture was completely stirred, and boiled for some minutes. It was shown light brown color depending on the process time and mulberry variety, then the mixture was filtered by using smooth cloth during two steps. Then for removing acidity of the mixture, two neutralizing agents such as white molasses soil and calcium carbonate each 3% were used. After one hour, the mulberry juice was left to sediment. Acid-decreased and clarified mulberry juice was concentrated to the desired brix degree (65-72) under atmospheric pressure, or under vacuum condition (rotary vacuum evaporator). The final product was cooled and packed in the glass bottles. The bottles were kept in room temperature and away from the sunlight for further experiments. Several types of mulberry molasses obtained from this study are given in Table 1.

Table 1 Treatments in mulberry molasses formulations

| Treatments on each formulation | Type of molasses |
|---|------------------|
| Bokhara mulberry+whitesoil+concentrated under atmospheric condition | 1 |
| Bokhara mulberry+whitesoil+concentrated under vacuum | 2 |

| | |
|---|---|
| condition | |
| Bokhara mulberry+calcium carbonate+ concentrated under atmospheric condition | 3 |
| Bokhara mulberry+calcium carbonate+ concentrated under vacuum condition | 4 |
| Khardarmulberry+whitesoil+concentrated under atmospheric condition | 5 |
| Khardarmulberry+whitesoil+concentrated under vacuum condition | 6 |
| Khardar mulberry+ calcium carbonate+ concentrated under atmospheric condition | 7 |
| Khardar mulberry+ calcium carbonate+ concentrated under vacuum condition | 8 |

General parameters were measured following official methods (AOAC, 2005).

2.3 Acid Value (Acidity)

Titrateable acidity was measured with 0.1 N, NaOH and expressed as percentage of citric acid.

2.4 pH Value

The pH was measured with a pH meter (model: Metrohm 691, Switzerland).

2.5 Brix (soluble solids)

Total soluble solid content of molasses samples (°Brix) were measured with a table refractometer (model: Shou chittangliang, China).

2.6 Color measurement

The color index is one of the most important factors in the quality of fruit products such as molasses produced by heat treatment. Manufacturing processes such as concentration, drying and cooking can affect final product color (Guine et al., 2012). Color measurement of the molasses was carried out using the colorimeter (CR 400, Chromometer, Minolta, Japan). Colorimeters give measurements that can be correlated with human eye-brain perception, and give tristimulus (L, a and b) values directly (HunterLab, 1995). The measurements were recorded as Hunter L, a and b color values (Alpaslan and Hayta, 2002). Color values for each sample were computed by using three measurements from different positions. The color values were expressed as L for darkness/lightness (0 black, 100 white), -a greenness, +a redness, -b blueness, and +b yellowness (Yildiz and Alpaslan, 2012). The pigments in white mulberry include carotenoids and some little anthocyanins. The fruits are seen white, pink and pale red.

Color is derived from the natural pigments in fruits and vegetables, many of which change as the plant proceeds through maturation and ripening. The primary pigments imparting color quality are the fat soluble chlorophylls (green) and carotenoids (yellow, orange, and red) and the water soluble anthocyanins (red, blue), flavonoids (yellow), and betalains (red). In addition, enzymatic and non-enzymatic browning reactions may result in the formation of water soluble brown, gray, and black colored pigments. The enzymes involved in browning reactions include polyphenol oxidase, which catalyzes the oxidation of polyphenolic compounds, and phenylalanine ammonialyase, which catalyzes the synthesis of precursors to phenolsubstrates. Carotenoids are sensitive to light and oxidation but relatively stable to heat. Carotenoids may be bleached by an enzyme called lipoxygenase, which catalyzes the oxidation of lipid compounds. Anthocyanins are sensitive to both pH and heat (Patras et al., 2010).

2.7 Statistical analysis

Results were reported as mean of three replications. Analysis of variance (ANOVA) accompanied with Duncan test (Mstat-C) was conducted to identify the significant difference between samples ($p < 0.01$).

3 Results and discussion

Some of the characteristics of mulberry fruits used in this study, and presented in Table 2.

Table 2 Chemical properties of two mulberry cultivars fruit

| Fruit | Brix | Acidity, % | pH |
|------------------|-----------|------------|------------|
| Bokhara mulberry | 24.5±0.76 | 0.98±0.037 | 6.58±0.055 |
| Khardar mulberry | 23±0.65 | 1.45±0.07 | 6.85±0.036 |

3.1 Effects of mulberry fruits used on evaluated attributes

Table 3 evaluated the properties of mulberry molasses samples from two mulberry cultivars.

Table 3 The mean acidity, pH and color of mulberry molasses for two mulberry cultivars

| Cultivar | Acidity, % | pH | L* | a* | b* |
|------------------|--------------------|--------------------|---------------------|---------------------|--------------------|
| Bokhara mulberry | 0.368 ^b | 5.853 ^a | 17.788 ^a | 0.964 ^a | 1.302 ^a |
| Khardar mulberry | 0.848 ^a | 5.486 ^b | 17.118 ^b | -0.127 ^b | 0.842 ^b |

* Different lower case superscripts in the column indicate significant difference (P<0.01)

The results showed the cultivar of the fruit were significantly correlated ($p < 0.01$) on acidity and pH of two kinds of prepared mulberry molasses.

The acidity percent was determined in terms of citric acid which varied significantly among cultivars (0.368% to 0.848%). The acidity of molasses of Bokhara mulberry was lower than the molasses of Khardar mulberry (Table 3). Both of the molasses mulberry differed significantly from each other. Because of the acidity of Bokhara mulberry was lower than Khardar mulberry (Table 2). Elmac and Altuq (2002) observed 1.51% to 1.79% acidity in black mulberry genotypes while Koyuncu et al. (2004) estimated 1.35% to 1.86% acidity in black mulberry genotypes. However, Ercisli and Orhan (2007) reported 0.25%-1.40% acidity in white mulberry genotypes. Besides, the present results coincide with earlier findings due to cultivars and agro-climatic conditions (Iqbal et al., 2010).

pH mulberry cultivars molasses also differed significantly (5.48-5.85) being maximum in Bokhara mulberry molasses and minimum in Khardar mulberry molasses (Table 3). The significantly higher pH in Bokhara mulberry molasses rendered it less acidic than the other one. These results are in line with earlier findings (Ercisli and Orhan, 2007), which revealed that white mulberry genotypes possessed pH 3.52 to 5.60. Elmac and Altuq (2002) reported a pH range of 3.60-3.80 for black mulberry genotypes. Therefore, the results supported each other. The molasses of Khardar mulberry had less sweet than Bokhara mulberry molasses.

From a technological point of view, fruit characterization by species and varieties is performed on the basis of physical as well chemical properties: shape,

size, texture, flavor, color/ pigmentation, dry matter content (soluble solids content), pectin substances, acidity, vitamins, etc. These properties are directly correlated with fruit utilization (Dauthy, 1995).

Color is one of the important physical factors in evaluating products. Carotenoids and anthocyanins are responsible for mulberry and mulberry products color. The amount of these components depends on the variety and growth conditions. Color values of L, a,b and a/b from Hunter-Lab were used to analyze the effects of variety and harvest time on color changes of mulberry molasses (Tehrani et al., 2006).

In the present study, there were significant differences in color values of two types of molasses. Based on this comparison, the effects of sample variety on color values were statistically analyzed. The results of statistical analysis showed that produced molasses samples of Bokhara mulberry had color values of L*, a*, b* higher than produced molasses samples of Khardar mulberry. The cause was probably related to the type and amount of pigments in mulberry fruits. The intensity and clarity of color product result of the color pigments product and non-enzymatic browning process was carried out during caramelization process.

Due to the high sugar content of mulberry, much of the color of mulberry molasses was resulted from caramel-related reactions during the concentration.

In order to produce molasses from different fruits, the neutralization process is necessary. For this purpose fruit juice is mixed with a type of neutral agent such as white soil and calcium carbonate for neutralization process. There was a significant difference between the acidity of two kinds of produced molasses. Calcium carbonate was more effective in decreasing acidity than white soil. Neutralization agent, similarly increased pH of mulberry molasses. There was a significant difference between pH of two kinds of molasses. Calcium carbonate was more effective in increasing pH than white soil (Table 4).

Batu (2005) reported, for production of liquid molasses from grape, used of sterile white soil containing

70.4% CaCO_3 . Sedimentation was accomplished. After filtration, a clarified grape juice was produced containing pH 4.90 and 6.76 g/kg total acidity (grape juice had pH 4.25 and 6.15 g/kg total acidity).

Table 4 The mean acidity, pH and color of mulberry molasses for two neutralization agent

| Neutral Compound | Acidity, % | pH | L* | a* | b* |
|-------------------|---------------------|-------------------|---------------------|-------------------|-------------------|
| White soil | 0.661 ^{a*} | 5.49 ^b | 17.43 ^{ns} | 0.25 ^b | 0.94 ^b |
| Calcium carbonate | 0.556 ^b | 5.85 ^a | 17.47 ^{ns} | 0.59 ^a | 1.2 ^a |

* Different lower case superscripts in the column indicate significant difference ($P < 0.01$)

The effects of neutral agents on L* index of mulberry molasses, were not significant. But a* and b* indexes of molasses color showed significant differences. The results showed calcium carbonate was more effective than white soil in decreasing acidity of mulberry juice. The mulberry juice is concentrated in two ways. The first case, mulberry juice was boiled up to 70 ° brix. The second case, mulberry juice was concentrated with rotary vacuum evaporation up to similar brix. Table 5 shows the effects of various concentration conditions namely atmospheric condition and vacuum condition (rotary vacuum evaporation) on evaluated properties in samples of mulberry molasses.

Table 5 Mean acidity, pH and color of mulberry molasses for two concentration conditions

| Concentration condition | Acidity, % | pH | L* | a* | b* |
|-------------------------|---------------------|-------------------|--------------------|-------------------|-------------------|
| Atmospheric condition | 0.631 ^{a*} | 5.62 ^b | 17.27 ^b | 0.28 ^b | 0.96 ^b |
| Vacuum condition | 0.586 ^b | 5.72 ^a | 17.64 ^a | 0.56 ^a | 1.19 ^a |

* Different lower case superscripts in the column indicate significant difference ($P < 0.01$)

The results indicated that the concentration methods (atmosphere or vacuum conditions) were significantly correlated ($p < 0.01$) on acidity, pH and color (L*, a*, b* indexes) of the samples of two kinds of mulberry molasses. The differences were significant at level 1%.

The use of concentration method in vacuum caused avoiding undesirable changes in color of final product. The long-term thermal processes with high temperatures

caused darkening of final product color during caramelizing the sugars in fruits.

There are many reports indicating that many reactions during thermal processes have affected the color of the product. Pigments analysis, specially carotenoids, anthocyanins, chlorophylls and browning reactions such as Millard, caramelyzation, oxidation of ascorbic acid and enzymatic browning are the most common reactions (Assawarachan and Noomhorm, 2010). The color changing from two different methods of concentration were measured by lightness values (L*), redness values (a*) and yellowness (b*) values. The results have been widely used to describe the color changes during thermal processing of fruit and vegetable products. The work on the concentration of pomegranate juice (Maskan, 2006) was reported that Hunter color values changed during heat treatments. In addition, researchers observed similar results in their studies such as thermal treatment of concentrated mango puree, peach puree and tomato paste (Ahmed et al., 2002; Barbosa et al., 1983).

In the present study, color indexes (L*, a*, b*) of samples that are concentrated under vacuum condition were significantly higher than concentrated samples under atmospheric condition. It means that, the molasses produced under vacuum condition showed clearer and brighter red and yellow colors than molasses samples produced under normal conditions. Concentration of samples under vacuum condition, resulted in better color than atmospheric condition.

In vacuum condition the boiling point is decreased, and concentration process is done in lower temperatures therefore caramelization reactions decrease, finally the products are obtained with brighter and clearer colors (Yasaii et al., 2011).

The samples were concentrated in vacuum conditions had lower acidity and higher pH, because the acidic compounds can be evaporated during concentration process (Elhadad et al., 2013).

The interaction effects between mulberry cultivars and neutral compounds on mean of acidity, pH, color the

samples of mulberry molasses are reported in Table 6. The results showed the influences of two variables on the acidity were significant at 1% level. The treatment contained Khardar mulberry and white molasses soil had maximum acidity while the treatment contained Bokhara mulberry and calcium carbonate showed minimum acidity. The treatment contained Bokhara mulberry and calcium carbonate and the treatment contains Khardar mulberry and white molasses soil showed the highest and lowest pH, respectively. The indexes L*, a*, b* in the sample of mulberry molasses containing Bokhara mulberry and Calcium carbonate were maximum and the sample of Khardar mulberry and Calcium carbonate revealed the lowest color indexes.

Table 6 Interactive effects of mulberry cultivars and neutral agents on mean acidity, pH and color of mulberry molasses

| Treatment | Acidity, % | pH | L* | a* | b* |
|---------------------------------------|---------------------|-------------------|--------------------|--------------------|-------------------|
| Bokhara mulberry+ white soil molasses | 0.434 ^{c*} | 5.55 ^b | 17.67 ^b | 0.59 ^b | 1.02 ^b |
| Bokhara mulberry + Calcium carbonate | 0.303 ^d | 6.15 ^a | 17.91 ^a | 1.34 ^a | 1.58 ^a |
| Khardar mulberry +white soil molasses | 0.888 ^a | 5.42 ^c | 17.2 ^c | -0.09 ^c | 0.86 ^c |
| Khardar mulberry +Calcium carbonate | 0.809 ^b | 5.55 ^b | 17.04 ^d | -0.17 ^d | 0.82 ^d |

* Different lower case superscripts in the column indicate significant difference (P<0.01)

The interactive effects of mulberry cultivars and concentration methods on mean acidity, pH, color the samples of mulberry molasses are reported in Table 7. The results showed the effects of two variables on evaluated properties were significant at 1% level. The treatment containing Khardar mulberry and atmospheric condition showed maximum acidity while the one with Bokhara mulberry and atmospheric condition showed minimum acidity. The treatment contained Bokhara mulberry and vacuum condition and the treatment contained Khardar mulberry and atmospheric condition showed the highest and lowest pH, respectively. The indexes L*, a*, b* in the samples of mulberry molasses contained Bokhara mulberry and vacuum condition were maximum and the samples containing Khardar mulberry

and atmospheric condition showed the lowest color indexes.

Table 7 Interactive effects of mulberry cultivars and concentration conditions on mean acidity, pH, color of mulberry molasses

| Treatments | Acidity, % | pH | L* | a* | b* |
|--|---------------------|-------------------|--------------------|--------------------|-------------------|
| Bokhara mulberry + atmospheric condition | 0.391 ^{c*} | 5.78 ^b | 17.43 ^b | 0.69 ^b | 1.08 ^b |
| Bokhara mulberry + vacuum condition | 0.346 ^d | 5.92 ^a | 18.15 ^a | 1.24 ^a | 1.53 ^a |
| Khardar mulberry + atmospheric condition | 0.871 ^a | 5.45 ^d | 17.11 ^d | -0.12 ^c | 0.84 ^c |
| Khardar mulberry + vacuum condition | 0.825 ^b | 5.52 ^c | 17.13 ^c | -0.13 ^c | 0.84 ^c |

* Different lower case superscripts in the column indicate significant difference (P<0.01)

The interaction effects of neutral agents and concentration methods on mean of acidity, pH, color the samples of mulberry molasses were evaluated in Table 8. The results showed the effects of two cultivars on experimented properties were significant at 1% level. The treatment contains calcium carbonate and vacuum condition showed minimum acidity value, maximum pH value and the highest L*, a*, b* indexes.

Table 8 Interactive effects of neutral compounds and concentration conditions on mean acidity, pH and color of mulberry molasses

| Treatments | Acidity, % | pH | L* | a* | b* |
|---|---------------------|-------------------|--------------------|-------------------|-------------------|
| white molasses soil + atmospheric condition | 0.673 ^{a*} | 5.47 ^d | 17.27 ^b | 0.42 ^b | 1.02 ^b |
| white molasses soil + vacuum condition | 0.649 ^b | 5.50 ^c | 17.59 ^a | 0.09 ^d | 0.86 ^d |
| calcium carbonate + atmospheric condition | 0.590 ^c | 5.76 ^b | 17.26 ^b | 0.15 ^c | 0.89 ^c |
| calcium carbonate + vacuum condition | 0.522 ^d | 5.94 ^a | 17.68 ^a | 1.02 ^a | 1.51 ^a |

* Different lower case superscripts in the column indicate significant difference (P<0.01)

4 Conclusion

The white mulberry fruit is a perishable fruit with high-level waste. Molasses is one of the traditional food to be produced from fruits with high amounts of sugar like mulberry by concentration to achieve the desired final concentration (70 Brix). In this study, the treatments were two kinds of mulberry (cultivar), two neutralization agents and two concentration conditions for preparing

mulberry molasses. The results showed, Bokhara mulberry was suitable cultivar for mulberry molasses making in its color and taste. Calcium carbonate for neutralization mulberry juice was more effective than white soil molasses. The concentration in vacuum condition (rotary vacuum evaporator) was better than atmospheric condition in clarifying and taste of mulberry molasses.

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