

# The effects of cooking time and sugar on total phenols, hydroxymethylfurfural and acrylamide content of mulberry leather (pestil)

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## RESEARCH ARTICLE

### Abstract

The effects of glucose syrup (0, 20, and 40%), sucrose syrup (0, 20, and 40%) and cooking time (10 and 20 min) on the chemical characteristics of mulberry pestil were investigated by replacing glucose syrup and sucrose syrup with mulberry syrup. Changes in glucose and sucrose syrup concentrations significantly affected invert sugar, sucrose and total soluble solids content of mulberry pestils. The invert sugar values of mulberry pestil increased with the addition of glucose syrup, but decreased with the addition of sucrose syrup. An increase in levels of sucrose or glucose syrup resulted in an increase of the lightness of colour values; however, an increase in cooking time reduced the lightness of colour of mulberry pestil. When sucrose and glucose syrup were added, an increase in pH values of samples but a reduction in titratable acidity was detected. Replacing glucose syrup with mulberry syrup in the formulations resulted in a decrease in the amount of hydroxymethylfurfural and acrylamide formed in mulberry pestils. The reducing effect of glucose syrup on hydroxymethylfurfural and acrylamide formation was much greater than the effect of sucrose syrup.

**Keywords:** acrylamide, hydroxymethylfurfural, glucose syrup, mulberry pestil

### 1. Introduction

Pestil is traditionally thought to have high nutritional value in human nutrition and be a good source of energy; and it can also be stored for a long time without deterioration (Cagindi and Otles, 2005). Production of fruit leather (pestil) and drying is one of the oldest methods of increasing the shelf life of fruits. Pestils are prepared using boiled fruit juice, generally grape or mulberry juice mixed with starch or wheat flour and the mixture is dried in the sun or oven. Dried fruits have become an alternative to fresh fruits because the demand for high-quality dried fruits is increasing all over the world (Bala *et al.*, 2005; Threlfall *et al.*, 2007). They are eaten as candy or snacks, and presented as flexible strips or sheets (Cagindi and Otles, 2005; Erenturk *et al.*, 2004). One of the most important of them is mulberry leather known as mulberry pestil.

There are different production methods for pestil depending on geographical regions, and different components

like sugar, starch, or wheat flour can be used (Kaya and Kahyaoglu, 2005). The consumer trend nowadays is to seek more natural fruit leathers made from natural fruits (Gujral *et al.*, 2013). It is an important product in human nutrition because of its high carbohydrate, mineral and organic acid content.

Adulteration of foods is one of the main problems in the food industry. Generally, foods with a high trade value are adulterated by adding cheap ingredients. Today, the production of adulterated pestil is one of the most serious problems for mulberry pestil producers. Sucrose and glucose syrups are used in the commercial production of pestil. Glucose syrup offers a variety of advantages to commercial users. Since it is liquid, rather than crystalline, like sucrose, the sweetener offers advantages in storage, transportation, and distribution logistics for industrial users (Akbulut and Bilgiçli, 2010).

Recently, two contaminants have gained much interest due to their high toxicological potential and wide occurrence in foods containing sugar: acrylamide and hydroxymethylfurfural (HMF). Acrylamide and HMF are compounds that occur during heating, preservation and storage processes (Gökmen, 2014). These processes may lead to the Maillard reaction and caramelisation of carbohydrates in the acid medium of fruit-based foods (Capuano and Fogliano, 2011; Rada-Mendoza *et al.*, 2002; Vinci *et al.*, 2012). The Maillard reaction and caramelisation have negative consequences, not only on the sensory characteristics of foods, but also due to changes in nutritional value (Boonchiangma *et al.*, 2011). Both acrylamide and HMF are considered as probably or potentially carcinogenic to humans or might be metabolised by humans into potentially carcinogenic compounds (Capuano and Fogliano, 2011; Zhang *et al.*, 2008). As a safety evaluation, Tardiff *et al.* (2010) estimated the tolerable daily intake of acrylamide for neurotoxicity to be 40 µg/kg/day. These toxic compounds can be present in fruit pests. The amount of acrylamide and HMF in foods is directly related to the heat load applied during processing because of the formation of Maillard reaction products which depends directly on the processing temperature and time.

Different food ingredients affect the chemical and sensory properties of food materials. The main objective of this work is to study the effects of adding glucose and sucrose syrup on HMF and acrylamide concentrations in mulberry pestil.

## 2. Material and methods

### Materials

Mulberry syrup, for pestil production with initial total soluble solids (°Brix) of 72 g/100 g, was obtained from Dutpinar Food Trade Industry Limited Company in Malatya, Turkey. Phenolic compounds, pH, HMF and acrylamide values of the mulberry syrup were 35 µg GAE/ml, 5.5, 15 mg/l and <10 µg/l, respectively. Glucose syrup (dextrose equivalent = 71% glucose, 80°Brix, pH=7.30) and sucrose (100°Brix, pH=7.80) were supplied from a local supermarket in Erzurum, Turkey. All syrups (mulberry, glucose and sucrose syrups) used to make the mulberry pestil were from the same concentration (20°Brix) and sugar syrups diluted with drinking water. For mulberry pestil production, three different levels of sucrose syrup (0, 20 and 40%), glucose syrup (0, 20 and 40%) and two different cooking times (10 and 20 min) were used. Wheat flour (8%), with 11.2% protein and 12.5% moisture, was used in the present study. A total of 18 treatments were investigated and derived from a 3×3×2 full factorial design. The formulations of materials used in this investigation are given in Table 1.

The formulations were prepared on the basis of mulberry syrup. Mulberry syrup (1000 g), sucrose syrup (0-200 and 400 g), glucose syrup (0-200 and 400 g) and wheat flour (80 g) were added to the batch. Mulberry syrup levels decreased according to the amount of sugar syrup added. The mixture was cooked for 10 or 20 min after the boiling process. The mixture was then formed into sheets of 6 mm thick and dried in a hot air dryer at 50 °C for 10 h. The mulberry leather was sealed in polyethylene pouches and stored at room temperature. Preparation of the mulberry leather was conducted in three replications.

Total soluble solids (TSS) contents of pests were determined according to Phimprian *et al.* (2011). TSS was measured with an Abbe refractometer (Carl Zeiss, Jena, Germany); mulberry leather (5 g) was finely blended with 45 ml distilled water, the mixture was filtered, and the resulting filtrate was used for TSS measurement. Invert sugar, sucrose and total sugar content were analysed using the Lane-Eynon method according to AOAC method 920.183 (AOAC, 2000). The colour was determined using a Hunter Lab colorimeter (Minolta CR-200; Minolta, Osaka, Japan). Titratable acidity (TA) was determined according to Cemeroglu (1992). Pestil (5 g) was finely blended with 50 ml distilled water, and expressed as a percentage of citric acid, determined with 0.1 N NaOH up to pH=8.1 (INOLAB pH 720; WTW, Weilheim, Germany). The concentrations of total phenols in the samples were determined using the

Table 1. Mulberry pestil formulations.

| Samples | Formulation <sup>1</sup>                 | Cooking time (min) |
|---------|--|--------------------|
| 1       | 1000 g MS + 80 g WF                      | 10                 |
| 2       | 1000 g MS + 80 g WF                      | 20                 |
| 3       | 800 g MS + 200 g GS + 80 g WF            | 10                 |
| 4       | 800 g MS + 200 g GS + 80 g WF            | 20                 |
| 5       | 600 g MS + 400 g GS + 80 g WF            | 10                 |
| 6       | 600 g MS + 400 g GS + 80 g WF            | 20                 |
| 7       | 800 g MS + 200 g SS + 80 g WF            | 10                 |
| 8       | 800 g MS + 200 g SS + 80 g WF            | 20                 |
| 9       | 600 g MS + 200 g SS + 200 g GS + 80 g WF | 10                 |
| 10      | 600 g MS + 200 g SS + 200 g GS + 80 g WF | 20                 |
| 11      | 400 g MS + 200 g SS + 400 g GS + 80 g WF | 10                 |
| 12      | 400 g MS + 200 g SS + 400 g GS + 80 g WF | 20                 |
| 13      | 600 g MS + 400 g SS + 80 g WF            | 10                 |
| 14      | 600 g MS + 400 g SS + 80 g WF            | 20                 |
| 15      | 400 g MS + 400 g SS + 200 g GS + 80 g WF | 10                 |
| 16      | 400 g MS + 400 g SS + 200 g GS + 80 g WF | 20                 |
| 17      | 200 g MS + 400 g SS + 400 g GS + 80 g WF | 10                 |
| 18      | 200 g MS + 400 g SS + 400 g GS + 80 g WF | 20                 |

<sup>1</sup> GS = glucose syrup; MS = mulberry syrup; SS = sucrose syrup; WF = wheat flour.

Folin-Ciocalteu colorimetric method (Gulcin *et al.*, 2002). The calibration curve was performed with gallic acid and the results were expressed as  $\mu\text{g}$  of gallic acid equivalents per milligram of sample ( $\mu\text{g}$  GAE/mg of sample).

### Analysis by HPLC of hydroxymethylfurfural

The HMF content of pestils was determined according to Rada-Mendoza *et al.* (2002). The analysis of HMF was carried out using a high-performance liquid chromatography (HPLC) system (Agilent 1100 series G1322A; Agilent Technologies, Waldbronn, Germany). Samples (2 g) were placed in a 50 ml flask; 4 ml each of Carrez I and II reagents were added while stirring and the volume made up with Milli-Q water. After standing for 30 min, the supernatant was filtered through a filter of 0.45  $\mu\text{m}$  and then injected into the HPLC. Methanol:water (10:90) was used as mobile phase. The flow rate was 1 ml/min and injection volume was 50  $\mu\text{l}$ . The UV-VIS detector and Nucleosil 5C18 (250 $\times$ 4.6 mm ID) column (HICROM, Reading, UK) was set at 280 nm. Quantitation was carried out by the external standard method, using a commercial standard of HMF (Sigma, St. Louis, MO, USA).

### Analysis by GC-MS of acrylamide

The analysis of acrylamide by gas chromatography-mass spectrometry (GC-MS) was performed according to Robarge *et al.* (2003). 1 g of samples was combined with 10 ml water. Samples were mixed for 20 min, and then centrifuged (5,500 rpm). The supernatant was filtered using 0.45  $\mu\text{m}$  nylon syringe filters. 200–300  $\mu\text{l}$  of brominating reagent was added to 3 ml of the filtered sample and

standards, which were gently mixed, and then allowed to react in an ice bath for 1 h. One drop of 1.0 N sodium thiosulphate was added to each sample to decompose any remaining bromine. Samples were extracted with 2 ml ethyl acetate, then centrifuged (5,500 rpm) for 10 min. The supernatant was transferred to vials for analysis. Selected ion monitoring was chosen to increase sensitivity. The oven program was ramped from 40–200  $^{\circ}\text{C}$  at 30  $^{\circ}\text{C}/\text{min}$  and was used in splitless mode for analysis. DB-225 (30 cm  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ; Agilent Technologies, Inc., Santa Clara, CA, USA) as column and helium as the mobile phase were used.

### Statistical analysis

All the experiments were carried out in triplicate and in two different trials. Statistical evaluations were performed using the SPSS package (a completely randomised design procedure by SPSS; SPSS, Chicago, IL, USA). Differences between data were tested using the Duncan's range test ( $P < 0.05$ ).

## 3. Results and discussion

The general effect of cooking time, sucrose and glucose syrup on invert sugar, sucrose, total sugar, pH, TA and TSS values of mulberry pestil were shown in Table 2. Invert sugar, sucrose, total sugar, pH, TA and TSS values of mulberry pestil significantly changed with the addition of sucrose and glucose syrup. Total sugar content of samples was increased by the addition of sucrose and glucose syrup; the highest value was observed when sucrose was added at a ratio of 40%. The invert sugar values of mulberry pestil increased with the addition of glucose syrup, but decreased

**Table 2.** The general effects of cooking time and sugar syrups on invert sugar, sucrose, total sugar, pH, titratable acidity (TA) and total soluble solids (TSS) values of mulberry pestil (mean  $\pm$  standard error).<sup>1</sup>

|                    | n | Invert sugar (%)  | Sucrose (%)       | Total sugar (%)   | pH               | TA (%)            | TSS ( $^{\circ}\text{Brix}$ ) |
|--------------------|---|-------------------|-------------------|-------------------|------------------|-------------------|-------------------------------|
| Sucrose syrup (%)  |   |                   |                   |                   |                  |                   |                               |
| 0                  | 6 | 52.79 $\pm$ 0.28a | 2.12 $\pm$ 0.32c  | 55.01 $\pm$ 0.29b | 5.70 $\pm$ 0.01c | 0.59 $\pm$ 0.001a | 76.21 $\pm$ 0.84a             |
| 20                 | 6 | 43.17 $\pm$ 0.44b | 11.66 $\pm$ 0.30b | 55.45 $\pm$ 0.30b | 5.79 $\pm$ 0.01b | 0.46 $\pm$ 0.001b | 67.90 $\pm$ 0.28c             |
| 40                 | 6 | 35.51 $\pm$ 0.69c | 21.85 $\pm$ 0.44a | 58.52 $\pm$ 1.04a | 5.89 $\pm$ 0.01a | 0.29 $\pm$ 0.001c | 71.72 $\pm$ 0.37b             |
| P-value            |   | **                | **                | **                | **               | **                | **                            |
| Glucose syrup (%)  |   |                   |                   |                   |                  |                   |                               |
| 0                  | 6 | 42.57 $\pm$ 2.29c | 11.85 $\pm$ 2.07a | 55.05 $\pm$ 0.28c | 5.73 $\pm$ 0.01c | 0.58 $\pm$ 0.001a | 70.00 $\pm$ 0.60b             |
| 20                 | 6 | 43.35 $\pm$ 2.24b | 11.67 $\pm$ 2.56a | 55.64 $\pm$ 0.48b | 5.78 $\pm$ 0.01b | 0.45 $\pm$ 0.001b | 72.96 $\pm$ 1.41a             |
| 40                 | 6 | 45.54 $\pm$ 1.89a | 12.10 $\pm$ 2.69a | 58.28 $\pm$ 1.03a | 5.86 $\pm$ 0.01a | 0.31 $\pm$ 0.001c | 72.88 $\pm$ 1.09a             |
| P-value            |   | **                | n.s.              | **                | **               | **                | **                            |
| Cooking time (min) |   |                   |                   |                   |                  |                   |                               |
| 10                 | 9 | 43.29 $\pm$ 1.76b | 12.15 $\pm$ 1.95a | 56.09 $\pm$ 0.64a | 5.81 $\pm$ 0.01a | 0.45 $\pm$ 0.001a | 71.92 $\pm$ 0.84a             |
| 20                 | 9 | 44.35 $\pm$ 1.74a | 11.59 $\pm$ 1.99b | 56.56 $\pm$ 0.64a | 5.78 $\pm$ 0.01b | 0.44 $\pm$ 0.001b | 71.97 $\pm$ 1.02a             |
| P-value            |   | **                | *                 | n.s.              | **               | *                 | n.s.                          |

<sup>1</sup> Means with different letters in the same column are statistically significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; n.s. = not significant.

with the addition of sucrose syrup. TSS of all mulberry pestils ranged from 67.90 to 76.21°Brix and was not affected by cooking time (Table 2).

The cooking time, and sucrose and glucose syrups significantly influenced total phenols and HMF content of the pestil. As shown in Table 3, total phenols and HMF content of the mulberry pestils significantly decreased with the addition of sucrose and glucose syrup. The decrease in acrylamide concentration was more pronounced in the formulations containing glucose syrup. However, the addition of sucrose syrup did not influence its level. On the other hand, HMF and acrylamide concentrations in the samples increased with cooking time. Longer cooking resulted in more HMF and acrylamide formation. Cooking time and temperature are among the most important factors affecting formation of HMF and acrylamide. In general, HMF and acrylamide formation increases with an increase in cooking time.

Factors of cooking time, and glucose and sucrose syrups had a significant effect on pH and TA values of samples (Table 4). Increasing sucrose and glucose syrup from 20 to 40% generally decreased TA values due to increasing pH values of the pestil samples. The replacement of sucrose (pH=7.80) and glucose syrups (pH=7.30) with mulberry syrup (pH=5.50) in the pestil formulations may have caused a decrease in pH values of the samples. Similar results for pH in the rosehip marmalade were obtained by Aksu *et al.* (1997), who determined a decreasing pH trend with the addition of sugar.

According to the results shown in Table 5, while the total sugar values of samples were not significantly influenced by the replacement of glucose syrup with mulberry syrup at 0% sucrose syrup concentration, they increased with the addition of glucose syrup at 20 and 40% sucrose syrup contents. The lowest total sugar value (54.13%) was attained in formulation containing 0% sucrose syrup and 20% glucose syrup, while the highest total sugar value (63.21%) was observed in formulation containing 40% sucrose syrup and 40% glucose syrup. TSS value of samples generally increased with the addition of glucose syrup in three different levels of sucrose syrup (0-20-40%), but it decreased with the addition of sucrose syrup. Similarly, Phimpfarian *et al.* (2011) reported that increased glucose concentrations resulted in increased TSS values in pineapple leather.

Colour is one of the quality parameters of fruit leathers (Maskan *et al.*, 2002). An increase in levels of sucrose or glucose syrup resulted in an increase in the values of lightness of colour; however, an increase in cooking time reduced the lightness of colour of mulberry pestil. Table 6 shows that  $a^*$  values were most significantly affected by cooking time followed by glucose syrup and then sucrose syrup. Similarly, Phimpfarian *et al.* (2011) reported that increased glucose concentration increased colour values ( $L^*$ ,  $a^*$  and  $b^*$ ) in pineapple leather. In general, an increased  $a^*$  value is not desirable because this means a more red product, which is not acceptable for pestil (Maskan *et al.*, 2002). The  $b^*$  values indicate yellowness or blueness of the sample (Gujral and Khanna, 2002). An increase in sugar syrup levels increased  $b^*$  values indicating an increase in yellowness of samples.  $L^*$  values of samples decreased

**Table 3.** The general effects of cooking time and sugar syrups on total phenols, hydroxymethylfurfural (HMF), acrylamide and colour values of mulberry pestil (mean  $\pm$  standard error).<sup>1</sup>

|                   | n | Total phenols ( $\mu$ g GAE/ mg) | HMF (mg/kg)         | Acrylamide ( $\mu$ g/kg) | $L^*$             | $a^*$            | $b^*$            |
|-------------------|---|----------------------------------|---------------------|--------------------------|-------------------|------------------|------------------|
| Sucrose syrup (%) |   |                                  |                     |                          |                   |                  |                  |
| 0                 | 6 | 18.65 $\pm$ 2.09a                | 140.42 $\pm$ 12.3a  | 13.54 $\pm$ 0.37a        | 32.16 $\pm$ 0.34c | 5.33 $\pm$ 0.52c | 0.63 $\pm$ 0.35c |
| 20                | 6 | 11.53 $\pm$ 0.48b                | 79.17 $\pm$ 5.25b   | 13.94 $\pm$ 0.40a        | 32.70 $\pm$ 0.31b | 7.58 $\pm$ 0.49b | 2.82 $\pm$ 0.52b |
| 40                | 6 | 9.99 $\pm$ 0.36c                 | 67.92 $\pm$ 2.92c   | 13.52 $\pm$ 0.31a        | 35.86 $\pm$ 1.17a | 9.41 $\pm$ 0.46a | 8.55 $\pm$ 1.82a |
| P-value           |   | **                               | **                  | n.s.                     | **                | **               | **               |
| Glucose syrup (%) |   |                                  |                     |                          |                   |                  |                  |
| 0                 | 6 | 16.08 $\pm$ 2.44a                | 115.00 $\pm$ 17.06a | 14.35 $\pm$ 0.39a        | 31.64 $\pm$ 0.29b | 5.47 $\pm$ 0.51b | 1.21 $\pm$ 0.32b |
| 20                | 6 | 12.30 $\pm$ 0.82b                | 91.25 $\pm$ 6.10b   | 13.75 $\pm$ 0.36b        | 33.40 $\pm$ 0.42b | 7.64 $\pm$ 0.67b | 2.77 $\pm$ 0.84b |
| 40                | 6 | 11.79 $\pm$ 0.97b                | 81.25 $\pm$ 9.07c   | 12.90 $\pm$ 0.18c        | 35.68 $\pm$ 1.14a | 9.20 $\pm$ 0.38a | 8.03 $\pm$ 1.90a |
| P-value           |   | **                               | **                  | **                       | **                | **               | **               |
| Cooking time(min) |   |                                  |                     |                          |                   |                  |                  |
| 10                | 9 | 15.42 $\pm$ 1.70a                | 86.39 $\pm$ 8.13b   | 13.16 $\pm$ 0.20b        | 34.26 $\pm$ 0.69a | 7.54 $\pm$ 0.51a | 4.01 $\pm$ 1.24a |
| 20                | 9 | 11.36 $\pm$ 0.57b                | 105.28 $\pm$ 11.13a | 14.17 $\pm$ 0.32a        | 32.89 $\pm$ 0.68b | 7.33 $\pm$ 0.61a | 3.99 $\pm$ 1.17a |
| P-value           |   | **                               | **                  | **                       | **                | n.s.             | n.s.             |

<sup>1</sup> Means with different letters in the same column are statistically significant; \* $P$ <0.05; \*\* $P$ <0.01; n.s. = not significant.

Table 4. pH and titratable acidity (TA) contents of different mulberry pestil formulations (mean  $\pm$  standard error).<sup>1</sup>

| Glucose syrup (%) | Cooking time (min) | Sucrose syrup (%) |                   |                   |                   |                   |                   |
|-------------------|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                   |                    | 0                 |                   | 20                |                   | 40                |                   |
|                   |                    | pH                | TA (%)            | pH                | TA (%)            | pH                | TA (%)            |
| 0                 | 10                 | 5.69 $\pm$ 0.005c | 0.72 $\pm$ 0.001b | 5.76 $\pm$ 0.000c | 0.60 $\pm$ 0.005a | 5.78 $\pm$ 0.005e | 0.43 $\pm$ 0.005a |
|                   | 20                 | 5.65 $\pm$ 0.000d | 0.74 $\pm$ 0.000a | 5.73 $\pm$ 0.005d | 0.59 $\pm$ 0.005a | 5.77 $\pm$ 0.005e | 0.40 $\pm$ 0.005b |
| 20                | 10                 | 5.74 $\pm$ 0.005a | 0.59 $\pm$ 0.005c | 5.75 $\pm$ 0.005c | 0.48 $\pm$ 0.000b | 5.90 $\pm$ 0.005c | 0.30 $\pm$ 0.005c |
|                   | 20                 | 5.70 $\pm$ 0.000c | 0.60 $\pm$ 0.005c | 5.73 $\pm$ 0.005d | 0.47 $\pm$ 0.000b | 5.88 $\pm$ 0.005d | 0.29 $\pm$ 0.005c |
| 40                | 10                 | 5.72 $\pm$ 0.005b | 0.43 $\pm$ 0.005d | 5.89 $\pm$ 0.005a | 0.33 $\pm$ 0.005b | 6.02 $\pm$ 0.005a | 0.16 $\pm$ 0.005d |
|                   | 20                 | 5.71 $\pm$ 0.005b | 0.44 $\pm$ 0.000d | 5.87 $\pm$ 0.005b | 0.32 $\pm$ 0.000a | 5.97 $\pm$ 0.005b | 0.16 $\pm$ 0.005d |
| P-value           |                    | **                | **                | **                | **                | **                | **                |

<sup>1</sup> Means with different letters in the same column are statistically significant; \* $P$ <0.05; \*\* $P$ <0.01.

Table 5. Total sugar and total soluble solids (TSS) contents of different mulberry pestil formulations (mean  $\pm$  standard error).<sup>1</sup>

| Glucose syrup (%) | Cooking time (min) | Sucrose syrup (%) |                   |                    |                   |                   |                   |
|-------------------|--------------------|-------------------|-------------------|--------------------|-------------------|-------------------|-------------------|
|                   |                    | 0                 |                   | 20                 |                   | 40                |                   |
|                   |                    | Total sugar (%)   | TSS (°Brix)       | Total sugar (%)    | TSS (°Brix)       | Total sugar (%)   | TSS (°Brix)       |
| 0                 | 10                 | 55.78 $\pm$ 1.08a | 72.45 $\pm$ 0.05c | 54.37 $\pm$ 0.40d  | 67.55 $\pm$ 0.05b | 54.27 $\pm$ 0.14d | 70.02 $\pm$ 0.02b |
|                   | 20                 | 55.41 $\pm$ 1.45a | 72.45 $\pm$ 0.05c | 55.44 $\pm$ 0.13bc | 67.50 $\pm$ 0.00b | 55.03 $\pm$ 0.28c | 70.02 $\pm$ 0.02b |
| 20                | 10                 | 54.45 $\pm$ 0.25a | 77.45 $\pm$ 0.05b | 54.45 $\pm$ 0.25d  | 67.45 $\pm$ 0.05b | 57.56 $\pm$ 0.14b | 72.45 $\pm$ 0.05a |
|                   | 20                 | 54.13 $\pm$ 0.32a | 80.02 $\pm$ 0.02a | 55.10 $\pm$ 0.13cd | 67.45 $\pm$ 0.05b | 58.14 $\pm$ 0.14b | 72.95 $\pm$ 0.45a |
| 40                | 10                 | 54.89 $\pm$ 0.22a | 77.45 $\pm$ 0.05b | 56.17 $\pm$ 0.13b  | 70.05 $\pm$ 0.05a | 62.87 $\pm$ 0.17a | 72.45 $\pm$ 0.05a |
|                   | 20                 | 55.41 $\pm$ 0.05a | 77.45 $\pm$ 0.05b | 57.14 $\pm$ 0.28a  | 67.45 $\pm$ 0.05b | 63.21 $\pm$ 0.17a | 72.45 $\pm$ 0.05a |
| P-value           |                    | n.s.              | **                | **                 | **                | **                | **                |

<sup>1</sup> Means with different letters in the same column are statistically significant; \* $P$ <0.05; \*\* $P$ <0.01; n.s. = not significant.

Table 6. Colour values of different mulberry pestil formulations (mean  $\pm$  standard error).<sup>1</sup>

| Glucose syrup (%) | Cooking time (min) | Sucrose syrup (%) |                  |                   |                  |                  |                  |                  |                   |                   |
|-------------------|--------------------|-------------------|------------------|-------------------|------------------|------------------|------------------|------------------|-------------------|-------------------|
|                   |                    | 0                 |                  |                   | 20               |                  |                  | 40               |                   |                   |
|                   |                    | L*                | a*               | b*                | L*               | a*               | b*               | L*               | a*                | b*                |
| 0                 | 10                 | 31.1 $\pm$ 0.28d  | 3.99 $\pm$ 0.02c | 0.36 $\pm$ 0.03b  | 32.7 $\pm$ 0.01b | 5.95 $\pm$ 0.09c | 0.57 $\pm$ 0.07c | 32.9 $\pm$ 0.05f | 7.70 $\pm$ 0.16b  | 2.83 $\pm$ 0.12e  |
|                   | 20                 | 30.5 $\pm$ 0.19d  | 2.78 $\pm$ 0.26d | 0.03 $\pm$ 0.06bc | 31.7 $\pm$ 0.14c | 5.48 $\pm$ 0.11c | 1.07 $\pm$ 0.06c | 30.6 $\pm$ 0.11e | 6.93 $\pm$ 0.66b  | 2.40 $\pm$ 0.39e  |
| 20                | 10                 | 33.7 $\pm$ 0.15a  | 5.09 $\pm$ 0.22b | -0.72 $\pm$ 0.18d | 33.4 $\pm$ 0.06a | 7.58 $\pm$ 0.01b | 2.75 $\pm$ 0.01b | 35.4 $\pm$ 0.33d | 10.00 $\pm$ 0.37a | 5.64 $\pm$ 0.54d  |
|                   | 20                 | 32.6 $\pm$ 0.13b  | 5.11 $\pm$ 0.03b | -0.27 $\pm$ 0.07c | 30.9 $\pm$ 0.14d | 7.17 $\pm$ 0.12b | 2.41 $\pm$ 0.02b | 34.1 $\pm$ 0.03c | 10.85 $\pm$ 0.17a | 6.84 $\pm$ 0.10c  |
| 40                | 10                 | 33.1 $\pm$ 0.13ab | 7.60 $\pm$ 0.16a | 2.23 $\pm$ 0.08a  | 33.9 $\pm$ 0.02a | 9.53 $\pm$ 0.46a | 5.09 $\pm$ 0.39a | 41.6 $\pm$ 0.15a | 10.40 $\pm$ 0.08a | 17.35 $\pm$ 0.22a |
|                   | 20                 | 31.7 $\pm$ 0.01c  | 7.40 $\pm$ 0.39a | 2.19 $\pm$ 0.17a  | 33.3 $\pm$ 0.38a | 9.77 $\pm$ 0.15a | 5.03 $\pm$ 0.16a | 40.1 $\pm$ 0.08b | 10.51 $\pm$ 0.02a | 16.24 $\pm$ 0.16b |
| P-value           |                    | **                | **               | **                | **               | **               | **               | **               | **                | **                |

<sup>1</sup> Means with different letters in the same column are statistically significant; \* $P$ <0.05; \*\* $P$ <0.01.



with cooking time. These colour changes indicated that a browning reaction had occurred during the leather production. Aksu *et al.* (1997) have reported that decreases in L\* value correlated well with increases in the browning of foods. Also, acrylamide concentration showed a negative correlation with the colour of samples. In general, HMF and acrylamide formation decreased, when L\*, a\* and b\* values increased in the samples containing sugar syrup. A decrease in HMF and acrylamide concentrations and an increase in L\* values of the samples containing sugar syrup may result from reducing the amount of free amino

acid from mulberry syrup. This is because the rate of the Maillard reaction decreases with the decrease in the amount of amino acids. While HMF and acrylamide concentration increased with the cooking time of samples, the L\* value significantly decreased with the cooking time. Intense cooking conditions (time and temperature) lead to a darker product and higher concentrations of acrylamide (Gökmen and Mogol, 2010).

The Maillard reactions take place in three major stages and are dependent on factors such as concentrations of reactants

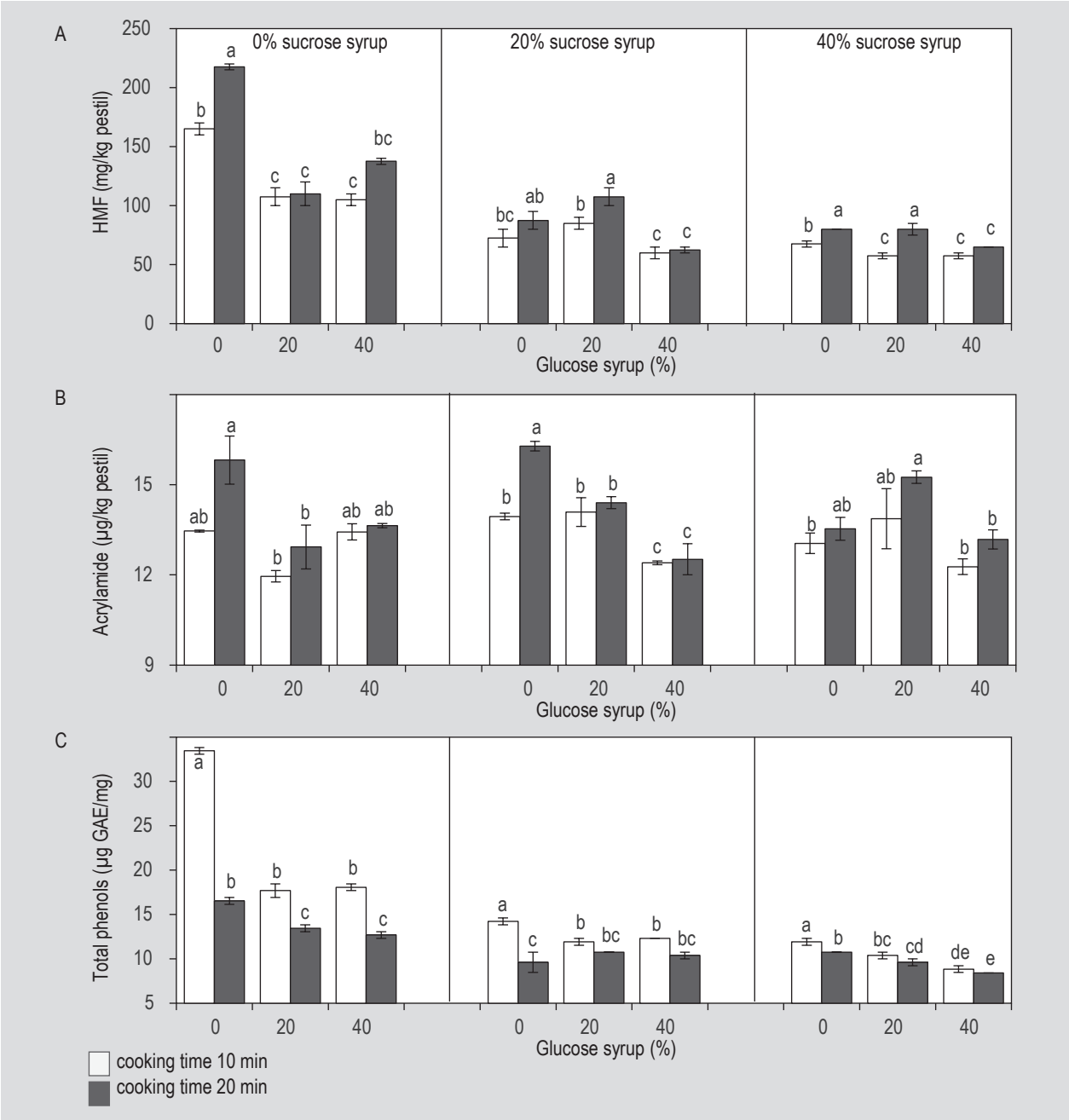


Figure 1. (A) Hydroxymethylfurfural (HMF), (B) acrylamide and (C) total phenols values of different mulberry pestil formulations at different cooking times (white bars: 10 min; black bars: 20 min).

and reactant type, pH, time, temperature and water activity (Lingnert *et al.*, 2002). As shown in Figure 1A and 1B, the type and concentration of sugar syrup had a strong impact on HMF and acrylamide formation in mulberry pestil, with the exception of acrylamide formation with the addition of sucrose syrup. In general, HMF and acrylamide concentrations decreased with the addition of sugar syrup in mulberry pestil. Replacing glucose syrup with mulberry syrup in the formulations resulted in a decrease in the amount of HMF and acrylamide formed in mulberry pestils. The effect of glucose syrup on HMF and acrylamide formation was much greater than the effect of sucrose syrup. On the other hand, acrylamide formation in sucrose syrup was not significantly influenced. The pH values of these samples ranged from 5.71 to 5.89, thus it may be impossible that this pH was greater than the pH formation of HMF. The pH values of these samples may provide unsuitable conditions for the occurrence of HMF. The formation of HMF in foods is highly dependent on pH and it occurs in low pH conditions (Capuano and Fogliano, 2011; Dogan and Toker, 2015). In addition, the low HMF contents in samples containing glucose syrup may be due to the lower levels of free amino acids with a decrease in mulberry syrup concentration in samples containing glucose syrup. On the other hand, the optimum pH value for acrylamide formation is stated to be at a range between 7 to 8 (Mestdagh *et al.*, 2008) but pH values of samples were lower than optimum for acrylamide formation. Acrylamide forms as a result of the reactions between reducing sugars and free amino acids, especially asparagine at high temperatures ( $>120^{\circ}\text{C}$ ) (Gökmen *et al.*, 2005; Ölmez *et al.*, 2008). Furthermore, the highest cooking temperature in pestil production was determined as  $102^{\circ}\text{C}$  in baked pestil samples (20 min). Also, HMF and acrylamide concentration of samples increased with cooking time, because the rate of the non-enzymatic browning reactions depends on the amount of total soluble solids present in the juice or concentrate (Kus *et al.*, 2005).

The acceptability of fruit and vegetables for human consumption may be affected by their phenolic content (Ayaz *et al.*, 2007). Phenolic compounds are susceptible to heat damage and thus are lost during various processing operations (Hojjatpanah *et al.*, 2011). Our results (Figure 1C) with mulberry pestils confirm this, as all the mulberry pestils cooked for 20 min had a lower total phenol content when compared with the samples cooked for 10 min.

#### 4. Conclusions

The study revealed that chemical properties of the mulberry pestil were influenced when sugar syrup (sucrose and glucose syrup) was replaced by mulberry syrup. TA values of mulberry samples generally decreased with the addition of sucrose and glucose syrups. The invert sugar content of samples decreased, while sucrose and total sugar content increased with the addition of sucrose

syrup. Total phenols and HMF content of the mulberry pestils significantly decreased with the addition of sucrose and glucose syrup. The reducing effect of glucose syrup on HMF and acrylamide formation was much greater than the effect of sucrose syrup. As expected, HMF and acrylamide concentrations in the samples increased with cooking time. Finally, it was demonstrated that HMF and acrylamide concentrations in the conditions of this study decreased with the addition of sugar syrup in mulberry pestil production.

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