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AGRONOMY
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2nd INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY

14-15 March 2024

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PREFACE

*"From the mouth of my immortal teacher Pasteur, I heard these words:
It is true that science is international, but every scientist must be a man who in
his scientific work is warmed by love for the people from which he sprang and
to whom he owes all his strength".*

*Prof. Dr. Milan Jovanović Batut (1847-1940)"
The first dean of the Faculty of Medicine in Belgrade*

Agricultural science and agriculture as a profession monitor and study changes occurring in this area, point out problems in agricultural practice, and find solutions. The Faculty of Agronomy in Čačak, in addition to educating students, 29y traditionally organizes the Symposium on Biotechnology (SYMBIOTECH) every year. The main goal is to acquaint the wider scientific and professional public with the results of the latest scientific research, and bring together domestic and foreign scientists in the fields of primary agricultural production, food processing, and environmental protection. We work tirelessly in pursuit of excellence.

At the 2nd International Symposium on Biotechnology, a total of 80 papers were presented in the 7 sections: Field, Vegetable and Forage Crops, Pomology and Viticulture, Livestock Production, Plant Protection, Food Safety and the Environment, Food Technology, Nutritionism, and Applied Chemistry.

We owe great gratitude to the **Ministry of Science, Technological Development and Innovation of the Republic of Serbia** and the **City of Čačak** for their traditional financial support and patronage of SYMBIOTECH24. We thank companies, entrepreneurs, stakeholders and all long-time friends of the Faculty of Agriculture for their material and organizational support.

In Čačak, March 2024

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University of Kragujevac



is organizing

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29th Symposium on Biotechnology with International Participation
14-15 March 2024

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FORMATION OF HYDROXYMETHYLFURFURAL DURING THE INDUSTRIAL PRODUCTION OF PLUM PEKME

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Abstract: It was analyzed the industrial plum pekmez production: blanching, mashing, cooking blanched plum pulp with invert sugar solution and caramel, final heat treatment, cooling pekmez and surface treatment. The aim was to determine the critical stages in which hydroxymethylfurfural (HMF) forms. HMF is synthesized by the thermal sugar decomposition or as one product of the Maillard reaction and can be an indicator of the thermal degree of food processing. Also, HMF is a quality parameter of food due to its potentially harmful effect on consumer health. The cooking blanch m pulp was a critical stage when the HMF content was significant increased.

Keywords: plum, pekmez, hydroxymethylfurfural

Introduction

The hydroxymethylfurfural or 5-hydroxymethyl-2-furaldehyde (HMF), is a cyclic aldehyde built from a furan ring with an aldehyde and an functional group. HMF can be formed in various reactions, including Maillard reactions, when the carboxyl group of reducing sugars react with amino acids, peptides or proteins or by the dehydration of hexoses, in an acidic environment (caramelization) during thermal processes (Lee et al, 2019). Data on the impact of HMF on human health vary. There are reports about cytotoxic, genotoxic and carcinogenic effects, but also positive health effects like antioxidant anti-carcinogenic and anti-hypoxic action (Choudhary et al, 2021).

The pekmez is a product with an appropriate thickened consistency, produced by cooking fruit pulp and/or fruit pulp/puree of one or more types of fruit, with or without added sugar with a maximum of up to 25% in relation to the total amount of fruit (Official Gazette, 2015). The applied heat treatment during pekmez production causes changes in primary fruit chemical

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composition and the loss of many naturally presented nutrients (Kim and Padilla-Zakour, 2004), and the formation of potentially harmful by-products such as HMF. The amount of HMF depends on the applied method of processing and storage condition Kuşçu and Bulantekin, 2016; Oral et al, 2012). The higher temperature and duration of fruit thermal treatment causes the formation of a larger amount of HMF. It has shown that vacuum evaporation can be used effectively in pekmez production, which prevents HMF formation (Kuşçu and Bulantekin, 2016)

Therefore, the HMF content can be used as an indicator of food thermal processing (Radovanović et al, 2017) and also as a quality parameter Đurović et al, 2022), with the view that it is considered potentially harmful to human health. The aim of this research was to determine the critical stages in the industrial production of plum pekmez, in which HMF forming.

Materials and methods

Materials

Samples of frozen plums, invert sugar solution, blanched plum pulp, semi-pekmez and final product-pekmez were sampled in the local fruit processing factory during the production of plum pekmez with invert sugar solution. The invert sugar solution was prepared by sucrose hydrolysis with citric acid as a catalyst. All chemicals were p.a., except those of HPLC grade methanol (J. Baker, Netherlands) and the HMF standard (Dr. Ehrenstorfer GmbH, Germany)

Production of plum pekmez

The industrial production of pekmez implies a series of related and well-coordinated processes: from the reception of fresh fruit to the final product, with certain specificities (Fig. 1). It used plum fruits mix of the Stanley, Čačanska rodna and Požegača varieties. Defrosted fruits were transported for blanching, 85–90 °C for 15 to 30 min. The heated plum was then transported to a sieve (diameter 3.5 mm), where the stones were separated. This was followed by fine mashing on sieves with diameters of 1.2 mm and 0.6 mm. After mashing plum pulp was cooked at 70 °C and 0.85 bar, until the content of dry matter of 54 to 57% was reached. Halfway through the cooking, invert sugar solution was drawn into the system. Towards the end of the cooking, caramel (E-150d) was

added, by first dissolving it in a small amount of pulp, and only after that it is drawn into the steamer and the cooking process continues.

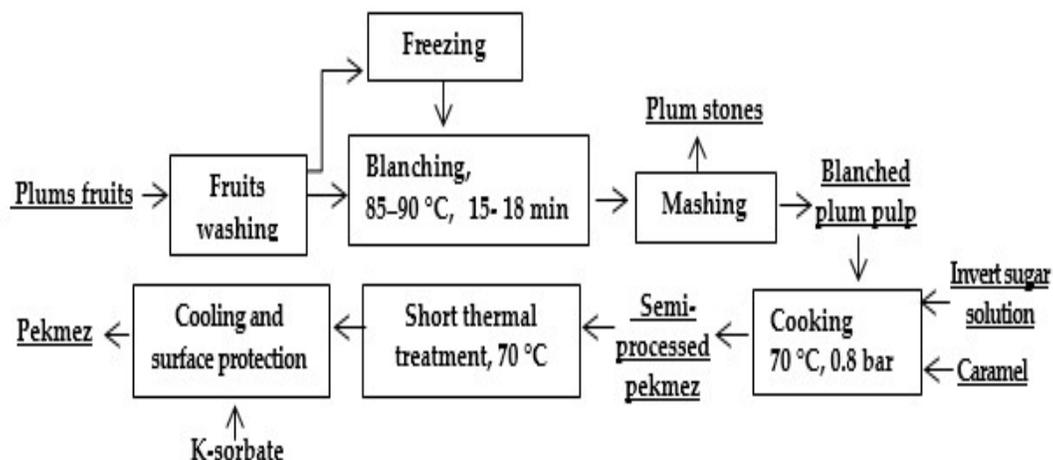


Fig. 1. Scheme of plum pekmez production in local fruit processing factory

The s mi-processed pekmez then was heated to 80 °C, and cooled to a temperature of 20 to 25 °C. The cooled pekmez was exposed to surface protection with a 6% solution of potassium sorbate and transporte refrigerator where it was stored at a temperature of 4 to 6 °C until distribution to the customer.

Determination dry matte

Dry matter was determined by Abbe refractometer (A. Krüss-Optronic Germany), with a precision of ± 0.00 . Stones were removed from the defrosted plum fruits, then pl ms were crushed and homogenized with the liquid phase that was released after defrosting. Other samples did not require special preparation for measurement.

Determination of HMF content

The HMF content was determined by the liquid chromatography –HPLC (Waters, USA), as described by the authors Vorlova et al. (2006). The concentrations of Hmf standards were in the range from $0.015 \text{ mg}\cdot\text{mL}^{-1}$ to $0.236 \text{ mg}\cdot\text{mL}^{-1}$. There were weighted $2\pm 0.001 \text{ g}$ samples, dissolved in a small amount of deionized water and 2 mL of Carrez I and Carrez II solutions were added to

each sample and filled up to 50 mL volumetric flasks with deionized water, wellhomogenized and left for 30 minutes to clear. Before injection, each sample was filtered through an ALLPURE NY membrane filter (0.45 μm) and homogenized by shaker (Ika MS 3 digital, China). Separation of components in the sample was performed on a Spherisorb ODS2 column (4.6 mm \times 250 mm, 5 μm), at 30 °C. It used an isocratic mode with a mobile phase flow rate of A: water 0.9 mLmin⁻¹, B: methanol 0.1 mLmin⁻¹ (Binary Waters pump 1525). 20 μL of the standard or sample solution was injected each time (Autosampler 717 plus). The measurement was performed at 285 nm (Dual λ Absorbance Detector 2487). The retention time of the HMF standard peak and HMF in samples that occurred is at 7.7 and 8 min, respectively.

Determination of total acidity in plum

This method is based on the titration of the test sample with NaOH solution in the presence of a phenolphthalein indicator until a persistent light pink color appears (Official Gazette,1983). Total acidity is expressed as malic acid.

Polarimetric determinat

A polarimeter POI 1, (Optica, Italy) with sodium lamp (589.44 nm) was used. Solutions of invert sugar were prepared in the concentration range 0,05 g·L⁻¹ to 2 g·L⁻¹ by adding five drops of NH₄OH (conc.25%) in a 100 mL volumetric flask to prevent sugar mutarotation. The specific angle of optical rotation was determined graphically from function: $\alpha=f(c)$ and Biot's law.

Results and discussion

Properties of plum fruit and invert sugar solution

According to Official Gazette (2021) the minimum content of soluble dry matter for plums used for processing, except for variety Požegača, is 12%, and for Požegača is 14%. A mixture of three varieties: Požegača, Stanley and Čačanska rodna was used for the production of pekmez and the sample of defrosted plum had a dry matter of 14.5% (Table 1). The acidity of plum fruits depends on the variety, climatic, growing and storage conditions. It was analyzed defrosted plum sample, so the acidity can be changed during the freezing process (Van der Sman, 2020).

Table 1. Properties of plum fruit and invert sugar syrup

Defrosted plum		Invert sugar solution		
Soluble dry matter, %	Total acidit (%, w/w)	Dry matter %	HMF, mgkg ⁻¹	[α], °cm ³ ·g ⁻¹ ·dm ⁻¹
14.5±0.0	0.3±0.025	71±0.0	1.55	47.17*

*obtained from the calibration curve: $\alpha=f(c)$, $R^2=0.9746$

Invert sugar solution was produced in the industry by hydrolysis of sucrose with citric acid at 60 °C and under 0.8 bar. The content of dry matter in a sample of invert sugar solution was in accordance with the Official Gazette (2017), where it is defined as a minimum content of dry matter of 62%. The invert sugar solution was obtained by partial hydrolysis of sucrose, which confirms the specific rotation of the plane of polarized light. The specific rotation of pure sucrose solutions (66.5 °cm³·g⁻¹·dm⁻¹) has decreased (47.17 °cm³·g⁻¹·dm⁻¹) due to the formation of monosaccharides, whereby fructose turns the plane of polarized light to the left and glucose to the right

There was recorded the HMF presence in invert sugar solution as a consequence of the carbohydrate breakdown under the thermal treatment in acid media, but the application of lower temperature and pressure (60 °C, 0.8 bar) during hydrolysis probably contributed to the formation of HMF in a small amount, 1.55 mgkg⁻¹.

Formation HMF during plum pekmez produc

Comparing the chromatograms of all analyzed samples (Figure 2) and amounts (Table 2) can be observed that the least amount of HMF was formed during the production of the invert sugar solution. It is expected, because the cooking time was the shortest and the temperature during the process was the lowest. A slightly higher content of HMF was observed in the blanched pulp, because the fruits were treated at a significantly higher temperature during blanching in the thermobrick. Although this process takes significantly less time than sucrose hydrolysis, the high temperature and composition of the medium most likely contributed to these results. It was observed that the resulting semi-processed pekmez had a higher content of HMF, due to the effect of longer exposure to high temperature. The continuous addition of blanched pulp during cooking can mask the real content of HMF in semi-processed pekmez.

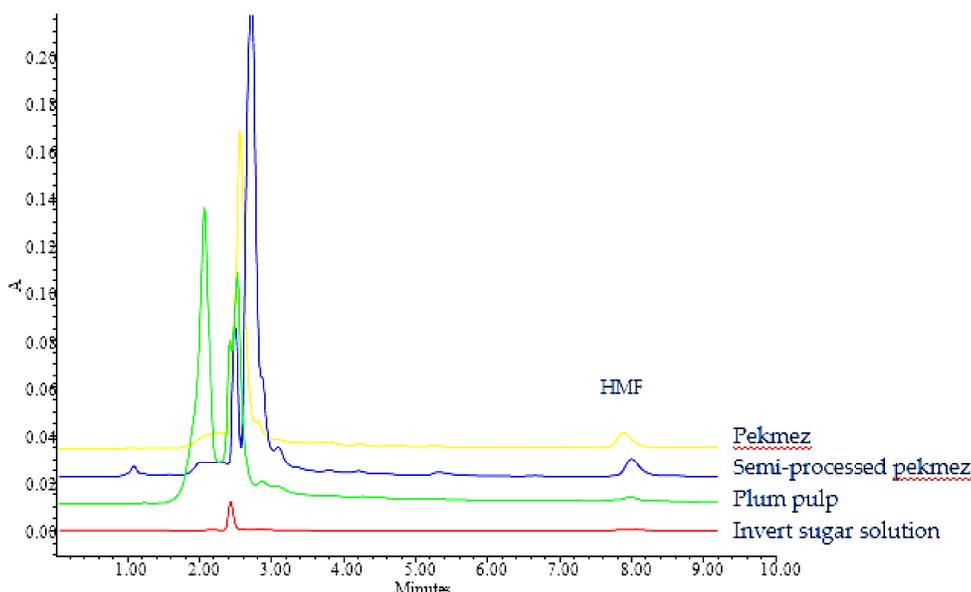


Fig.2. Comparative chromatograms of: invert sugar solution, blanched pulp, semi-processed pekmez and pekmez

The highest content of HMF was determined in the pekmez which is expected, if we take into account that the cooking time was the longest, and that the product was briefly exposed to a slightly higher temperature towards the end of cooking. Mendoza et al (2002) found HMF in all samples of jams, regardless of the pH, sugar or dry matter, from traces to 7.17 mg/100 g product (mean value close to 1.35 mg/100 g product).

The content of dry matter in the pulp (Table 2) was below the optimal values (from 16 to 23%) provided by the recipe, which was probably a consequence of losses during defrosting and the use of plum fruits with an almost low content of dry matter.

Table 2. Content of dry matter and HMF in pulp, semi-product and finished product – pekmez

Parameter	Pulp	Semi-pekmez	Pekmez
Dry matter, %	14.75±0.0	54.25±0	57.00±0.0
HMF, mgkg ⁻¹	5.55	18.10	18.60

Therefore, longer cooking was applied, so that the final product has a needed content of dry matter. In the semi-processed pekmez, the expected

significantly higher content of dry matter was observed than in the pulp, which was a consequence of the cooking process lasting several hours.

Conclusion

The industrial production of plum pekmez is a complex process that includes several stages: washing plum fruits, freezing, blanching, separating stones, fine mashing, cooking, cooling and protecting pekmez surface with K-sorbat. The thermal treatment applied during production invert sugar solution and plum pekmez influenced the formation of hydroxymethylfurfural (HMF) was observed in invert sugar solution, blanched pulp, semi-processed pekmez and the final product-pekmez. The content of HMF in the invert sugar solution, produced with citric acid, was the lowest due to the sucrose hydrolysis performed at 60 °C and under 0.8 bar. In the blanched plum pulp the HMF was quantified in the amount of 5.5 mgkg⁻¹. The cooking blanched plum pulp was a critical stage when the HMF content was increased more than 3 times. Based on the results, it is clear that the HMF is an indicator of the thermal treatment of plums during pekmez production.

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