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Determination of Benzoic Acid, Sorbic Acid and HMF in Grape Molasses Produced by Traditional and Modern Methods

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Sorbic acid and benzoic acid have long been widely used in the food industry to inhibit the growth of various bacteria, yeasts and fungi, especially in acidic media. The health effects have led to limitation on the concentrations that can be used in food. In most studies, HMF has been found to have carcinogenic effects such as cytotoxicity toward mucous membranes, the skin and the upper respiratory tract; mutagenicity; chromosomal aberrations; and carcinogenicity toward humans and animals. Because of these reason, the analytical determination of these compounds is important for consumer interest and protection. The aim of this study is to determine the concentration of sorbic acid, benzoic acid and HMF in grape molasses samples by using HPLC. For HPLC analysis, a Zorbax SB RP18 (150 × 4.60 mm i.d. × 5 µm) column was selected as the stationary phase at 25°C. In sixteen grape molasses samples (produced by traditional and modern method), sorbic acid, benzoic acid and HMF concentrations were determined by HPLC method. HMF was detected in all of the commercial and traditional type molasses samples. Only two commercial molasses sample, sorbic acid and benzoic acid were detected.

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1. INTRODUCTION

Preservative additives are substances used to prevent undesirable changes in foods during storage and transportation. Consumer demand tends to nutritious, artificial additive-free, ready-to-eat, and long shelf-life foods. This situation is the reason for pressure on researchers to study the level of additives and harmful components to obtain safe and healthy foods [1].

In these days, benzoic and sorbic acids and their respective sodium, potassium, and calcium salts are widely used as a chemical preservatives. Especially they are generally used to for the extension of shelf life and enhancing of food quality. Sorbic acids and benzoic acids are generally active in foods of low pH value and essentially ineffective in foods at neutral pH values [2,3].

Accrued interest is given to preservatives as recent studies have reported serious side effects associated with these substances. The topical usage of potassium sorbate and sodium benzoate-containing product resulted in skin reactions such as rash, urticaria, and contact dermatitis [4,5].

5-Hydroxymethylfurfural (5-hydroxymethyl-2-furfuraldehyde, HMF, CAS No. 67-47-0) is formed during the thermal treatment of carbohydrate-containing foods, is considered as a potential carcinogen for humans. The previous literature has shown that it was concluded that sugary food heated under household cooking conditions could act as an initiator and promoter of colon cancer because of the presence of HMF [6]. The formations of HMF were inevitable in vinegar and soy sauce, which were seen as essential liquid seasonings for home cooking. Theobald et al. [6] also indicated that it was difficult to estimate the HMF content in commercial samples which was prepared in households [7].

Since the 1950s there have been reports of HMF in food. It has been identified in a wide variety of heat processed foods. Depending on production technology and storage, levels in food vary considerably. While HMF is practically not present in fresh food, high levels in the g/kg range can be found in dried fruits, coffee and caramel products. In honey and some other food, concentrations of HMF can be used as an indicator of heating and storage changes. For example, the Codex Alimentarius standard sets a maximum limit for HMF in honey of 40 mg/kg (80 mg/ kg in tropical honey) as a way of assuring that the product has not undergone heating during processing [8].

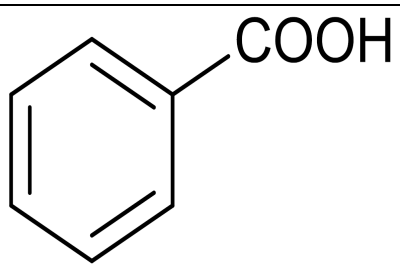
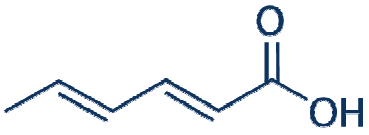
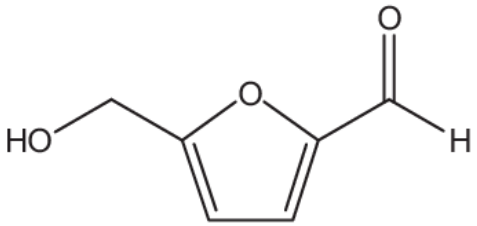
Molasses is one of our traditional foods, although it is industrial production, it is also produced in small family businesses and traditional methods. Grape molasses is Turkey's oldest sweetener used before cane sugar and honey were introduced to the Middle East and Mediterranean regions. In the past years molasses, which is one of the basic nutrients of human beings, has become less productive in the changing world conditions. In Turkey molasses is made between at the end of September and the beginning of October when the grapes are mature and this time is called molasses time.

In order to protect human health, determination of maximum permitted amounts of these additives and amount of HMF in food products is very important. Consequently, easy and reliable analysis methods for detection of these additives in foodstuffs are required for food safety.

There are various methods for analysis of sorbic acids, benzoic acid and HMF in food products, such as ultraviolet (UV) spectroscopy [9], high-performance liquid chromatography (HPLC) [7,10-13], GC [14,15] , and LCMS/MS [16,17]. HPLC detection has become the most widely applied analytical separation technique because of its superior performance and reliability, especially in the pharmaceutical, environmental, forensic, clinical, food, and flavor sciences.

The objective of the present study was to measure the concentration of benzoic acid, sorbic acid and HMF (Table 1) in food products such as grape molasses produced by traditional and modern methods by using of HPLC technique. HPLC detection has become the most widely applied analytical separation technique because of its superior performance and reliability, especially in the food, pharmaceutical, environmental, forensic, clinical, and flavor sciences [7].

Table 1. Chemical structure of studied compounds

Compounds	Chemical structure
Benzoic Acid	
Sorbic Acid	
5-Hydroxymethylfurfural	

2. METHODOLOGY

2.1 Chemicals

All chemicals in this study were used without further purification. The standard of sorbic acid, benzoic acid and HMF were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Sodium hydroxide and acetic acid were also obtained from Sigma-Aldrich. HPLC-grade acetonitrile (ACN), methanol (MeOH Darmstadt, Germany), acetic acid were purchased from Merck. Ultrapure water, with conductivity lower than 0.05 µS/cm, was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Stock solutions of sorbic acid were prepared by dissolving in water (50 mL) to make a 100 mg/L solution. HMF and benzoic acid standards were prepared in methanol. All solutions were protected from light, and all solutions were stored at 4° C for short time usage (daily) and at − 20°C for long-term usage (between days).

2.2 Apparatus

The HPLC analysis was carried out on an Agilent 1260 series HPLC system with ternary solvent pump, online degasser, automatic injection system, column heater, and multi wavelength detector [7]. UV detection was used (235 nm for sorbic acid and benzoic acid; 284 nm for HMF). Analyses were run at a flow rate of 1.0 mL·min^{-1}. Because of the peak shape and analysis time, an Zorbax SB RP18 (150 × 4.60 mm i.d. × 5 µm) column was selected as stationary phase at 25°C. 35% (v/v) ACN-water containing acetate buffer at pH 4.7 was used as a mobile phase for sorbic acid and benzoic acid. For HMF determination, %10 (v/v) MeOH-water containing 0.1 (v/v) acetic acid was selected as a mobile phase. Isocratic elution was used for all analysis.

2.3 Preparation of Molasses Samples for HPLC Analysis

Sixteen grape molasses samples were collected from Turkey, Usak (Eight commercial type and eight traditional type). Five grams of grape molasses samples were dissolved in 25 ml of water, transferred quantitatively into a 50 ml volumetric flask, added by 0.5 ml of Carrez solution I and 0.5 ml of Carrez II and make up to 50 ml with water. The resulting filtrate was used for 20 µL per injection for chromatographic

analysis. The peak area was obtained for each solution on the ordinate against the compounds concentration, in milligrams per liter, on the abscissa.

3. RESULTS AND DISCUSSION

The use of sorbic acid and benzoic acid in processed foods is extremely important. Not using this antimicrobial agent may cause microbial activities that lead to food poisoning [18]. However, there are some limitations [7]. Fermented products are the foremost food groups that have limitations for food additives because of their importance in healthy nutrition, prevention, and curing effects [7]. Turkish Food Codex that is prepared considering scientific truths and conclusions of Codex Alimentarius and in accordance with European Union directives is effective in such applications in Turkey [19].

Hydroxymethylfurfural (HMF) is an intermediate product in the Maillard reaction [20,21] and is also formed from the degradation of sugars at high temperatures [22]. Furfural and Methylfurfural are also furanic compounds formed in non-enzymatic browning reaction during thermic treatments.

In this paper sorbic acid, benzoic acid and HMF contents of sixteen grape molasses sample were determined by HPLC method.

A set of sorbic acid, benzoic acid and HMF standards were tested to determine the validation parameters (linearity, range, detection limit, and quantitation limit) [23]. The linearities were calculated by plotting the peak area versus concentration of compounds. Five standard solutions were prepared for claibration of compounds. Each solution was injected in duplicate. The calibration curves were obtained by linear leastsquares regression. The validation data are reported in Table 2. The method exhibited good linearity based on a correlation coefficient >0.999 for all compounds. The LOD and LOQ were calculated as 3.3 $s \cdot m^{-1}$ and 10 $s \cdot m^{-1}$, respectively, where s is the standard deviation of the response and m is the slope of the corresponding calibration curve [24,25].

In Table 3, sorbic and benzoic acids contents of eight commercial type grape molasses samples were given. For six grape molasses samples, sorbic acid and benzoic acid could not be determined. In only two commercial molasses samples, they could be detected and calculated as a highest value 1970 $mg.kg^{-1}$ for sorbic acid, 988 $mg.kg^{-1}$ for benzoic acid.

Table 2. Statistical evaluation of the calibration data of compounds by HPLC

	Sorbic Acid	Benzoic Acid	HMF
Linearity range (µg mL^{-1})	5.00 – 120.0 (n=5)	5.00-120.0	0.25-10.0
Slope	126.4	54.25	1376.7
Intercept	0.312	-4.66	2.65
SE of slope	$2.05.10^{-1}$	$1.01.10^{-1}$	$2.2.10^{-1}$
SE of intercept	$4.01.10^{-1}$	$1.04.10^{-1}$	$3.11.10^{-1}$
Correlation coefficient (*r*)	0.999	0.999	0.999
Detection limit (LOD) (µg.mL^{-1})	0.031	0.061	0.0047
Quantitation limit (LOQ) (µg.mL^{-1})	0.091	0.184	0.014

Table 3. Sorbic acid and benzoic acid content in commercial type grape molasses samples

Sample	Sorbic Acid ($mg.kg^{-1}$)	Benzoic Acid ($mg.kg^{-1}$)
Grape molasses 1 (commercial type)	not detected	not detected
Grape molasses 2 (commercial type)	not detected	not detected
Grape molasses 3 (commercial type)	not detected	not detected
Grape molasses 4 (commercial type)	not detected	not detected
Grape molasses 5 (commercial type)	not detected	not detected
Grape molasses 5 (commercial type)	1969.84 ± 124.1	987.98 ± 63.23
Grape molasses 6 (commercial type)	not detected	not detected
Grape molasses 7 (commercial type)	348.87 ± 21.98	957.77 ± 61.30
Grape molasses 8 (commercial type)	not detected	not detected

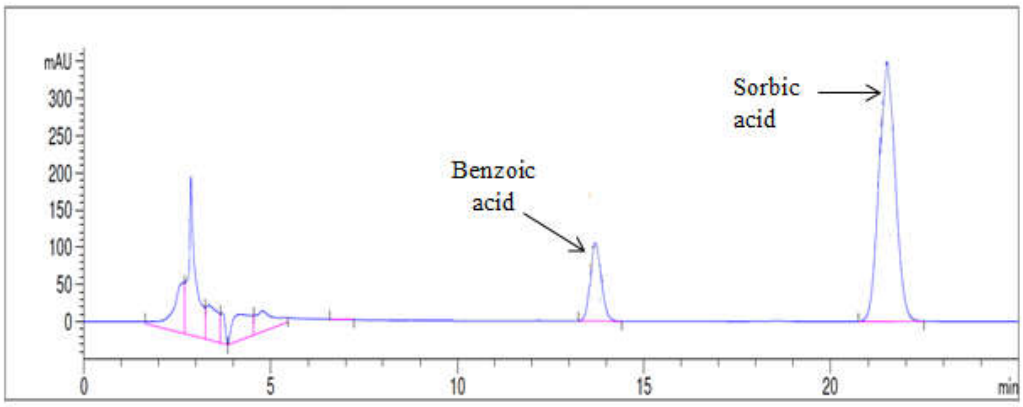


Fig. 1. Benzoic acid and sorbic acid chromatogram of grape molasses sample

Table 4. HMF content in commercial type grape molasses samples

Sample	HMF (mg.kg^{-1})
Grape molasses 1 (commercial type)	27.5 ± 3.6
Grape molasses 2 (commercial type)	25.6 ± 3.41
Grape molasses 3 (commercial type)	11.3 ± 1.5
Grape molasses 4 (commercial type)	18.8 ± 2.5
Grape molasses 5 (commercial type)	102.4 ± 13.5
Grape molasses 6 (commercial type)	6.8 ± 0.9
Grape molasses 7 (commercial type)	41.6 ± 5.5
Grape molasses 8 (commercial type)	12.2 ± 1.6

Table 5. HMF content in grape molasses samples produced by traditional method

Sample	HMF (mg.kg^{-1})
Grape molasses 9 (traditional type)	4865.7 ± 639.4
Grape molasses 10 (traditional type)	192.4 ± 25.3
Grape molasses 11 (traditional type)	57.9 ± 7.6
Grape molasses 12 (traditional type)	207.8 ± 27.3
Grape molasses 13 (traditional type)	484.6 ± 63.7
rape molasses 14 (traditional type)	440.3 ± 57.9
Grape molasses 15 (traditional type)	185.7 ± 24.4
Grape molasses 16 (traditional type)	355.5 ± 46.7

For eight traditional molasses sample, sorbic acid and benzoic acid were not detected. In Fig. 1, grape molasses sample for sorbic acid, and benzoic acid chromatogram were given.

In all of the commercial and traditional type molasses samples were detected HMF. In commercial type of grape molesses sample, it was found between 6.8-102.4 mg.kg^{-1} (Table 4). The chromatogram of molasses sample for HMF analysis were given in Fig. 2.

For traditional grape molasses, HMF amounts were calculated between, 58-4866 mg.kg^{-1} (Table 5). It was seen that the HMF contents of traditional molasses samples are higher than commercial types.

For the accuracy defined by developed method, known amount of componds were spiked to the molasses samples and analyzed by the HPLC method. Recovery % was determined on 10 analyzed samples for spiking levels. The results were found to be 91.5 for sorbic acid, 90.5 for benzoic acid and 85.9 for HMF with low level of RSD values at 0.884, 0.710 and 0.895 respectively. The high recovery values obtained show that the method is not affected by the matrix effects in molasses samples.

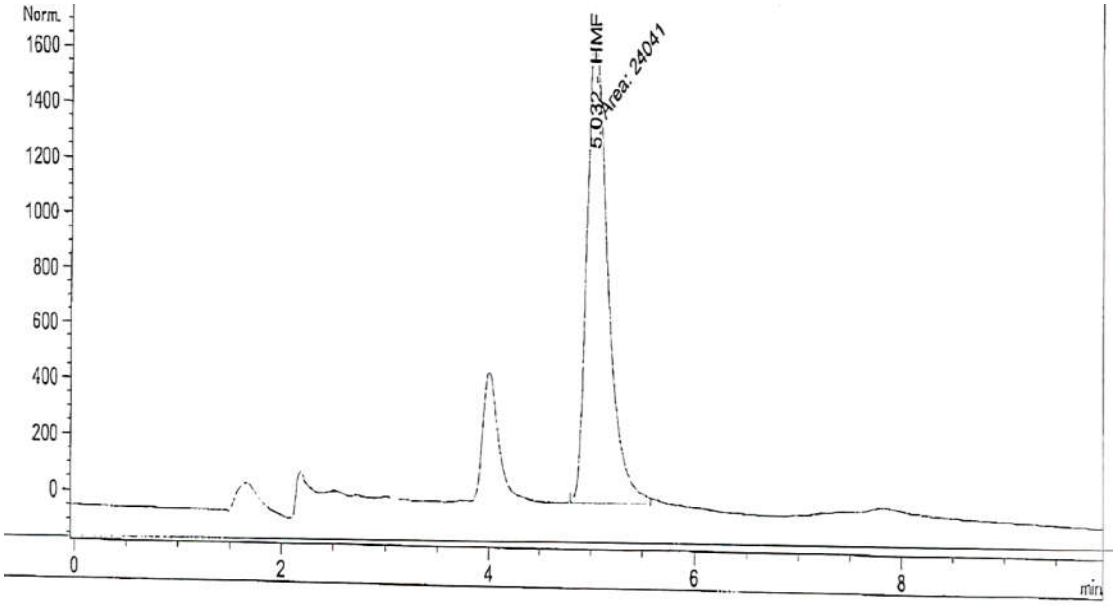


Fig. 2. HMF chromatogram of grape molasses sample

4. CONCLUSIONS

Sorbic acid and benzoic acids are widely used in food sample in Turkey. Therefore, the use of these compounds should be supervised by the Turkish public health authorities. In this study, the determination of sorbic acid and benzoic acid in 16 commercial and traditional molasses samples were done by HPLC method. Also evaluation of HMF content of 16 different traditional and commercial molasses samples could be achieved in this paper.

DISCLAIMER

Also there is no conflict of interest between the authors and producers of the products. The research was funded by personal efforts of the authors.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Carocho M, Barreiro MF, Morales P, Ferreira ICFR. Adding molecules to food, pros and cons: a review of synthetic and natural food additives. Compr Rev Food Sci Food Saf. 2014;13:377–399.
2. Qi P, Hong H, Liang X, Liu D. Food Control. 2009;20:414
3. Tfouni SAV, Toledo MCF. Determination of benzoic and sorbic acids in Brazilian food. Food Control. 2002;13(2):117-123.
4. Nair B. Int J Toxicol. 2001;20(3):23.
5. Soni MG, Carabin IG, Burdock GA. Food Chem Toxicol. 2005;43(7):985.
6. Theobald A, Müller A, Anklam E. Determination of 5-Hydroxymethylfurfural in vinegar samples by HPLC. Journal of Agricultural and Food Chemistry. 1998;46(5):1850–1854.
7. Özdemir A, Şanlı S, Sardoğan B, Sardoğan S. Determination of Sorbic Acid in Cheese Samples by Rapid HPLC-DAD Method. International Journal of Analytical Chemistry. 2020;1:1-4.
8. Codex Alimentarius Commission, Codex standard for honey, CODEX STAN 12-1981; Food and Agriculture Organization of the United Nations and the World Health Organization, Rome, Italy; 2001.
9. Mohsen KZ, Mirzaei S. Spectrophotometric resolution of ternary mixtures of pseudoephedrine hydrochloride,

dextromethorphan hydrobromide and sodium benzoate in syrups using wavelength selection by net analyte signals calculated with hybrid linear analysis. Anal Chim Acta. 2004;526(1):83-94.
10. Pernica M, Martinik J, Bosko R, Zustakova V, Benesova K, Belakova S. Determination of patulin and hydroxymethylfurfural in beverages by UPLC-PDA. World Mycotoxin Journal. 2021;14(1).
11. Rafaela Prata, Mateus Henrique Petrarca, Jos´e Teixeira Filho, Helena Teixeira Godoy, Simultaneous determination of furfural, 5-hydroxymethylfurfural and 4-hydroxy-2,5-dimethyl-3(2H)-furanone in baby foods available in the Brazilian market. Journal of Food Composition and Analysis. 2021;99:103874.
12. Saad B, Bari MF, Saleh MI, Ahmad K, Talib MKM. "Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatography," Journal of Chromatography A. 2005;1073(1-2):393–397,
13. Zor SD, Asçı B, Donmez OA, Kuçukkaraca DY. Simultaneous determination of potassium sorbate, sodium benzoate, quinoline yellow and sunset yellow in lemonades and lemon sauces by HPLC using experimental design. Journal of Chromatographic Science. 2016;54(6):952– 957,
14. Dong C, Mei Y, Chen L, Simultaneous determination of sorbic and benzoic acids in food dressing by headspace solid-phase microextraction and gas chromatography. Journal of Chromatography A. 2006;1117(1):109–114.
15. De Luca C, Passi S, Quattrucci E. Simultaneous determination of sorbic acid, benzoic acid and parabens in foods: a new gas chromatography-mass spectrometry technique adopted in a survey on Italian foods and beverages. Food Additives and Contaminants. 1995;12(1):1–7.
16. Fuselli F, Guarino C, La Mantia A, Longo L, Faberi A, Marianella RM. Multi-detection of preservatives in cheeses by liquid chromatography-tandem mass spectrometry. Journal of Chromatography B. 2012:906: 9–18.
17. Teixidó E, Moyano E, Javier Santos F, Teresa Galceran M. Liquid chromatography multi-stage mass spectrometry for the analysis of 5-hydroxymethylfurfural in foods. Journal of Chromatography A. 2008;1185(1):102-108.
18. Ferreira MPLVO, Mendes E, Brito P, Ferreira MA. Simultaneous determination of benzoic and sorbic acids in quince jam by HPLC. Food Research International. 2000;33(2):113–117.
19. "Ministry of agriculture and rural affair,"; 2005.
Available:http:// www.tarim.gov.tr.
20. Berg HE, Van Boekel, MAJS. Degradation of lactose during heating of milk. I. Reaction pathways. Netherlands Milk and Dairy Journal. 1994;48:157-175.
21. Morales FJ, Romero C, JimeÂnez-PeÂ rez S. Chromatographic determination of bound hydroxymethylfurfural as an index of milk protein glycosylation. Journal of Agricultural and Food Chemistry. 1997;45:1570-1573.
22. Kroh LW. Caramelisation in food and beverages. Food Chemistry. 1994;51:373-379.
23. ICH, ICH, Topic Q2A Validation of Analytical Procedures Methodology, Geneva, Switzerland, 2000.
24. Riley CM, Rosanske TW. Development and validation of analytical methods, Elsevier, New York, NY, USA; 1996.
25. Swartz ME, Krull IS. Analytical Method Development and Validation, Marcel Dekker Inc., New York, NY, USA; 1997.

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