"Science Stays True Here"
Biological and Chemical Research (ISSN 2312-0088), Vol.6, 8-13 | Science Signpost Publishing

Determination of Benzoic Acid in Cranberry (*Vaccinium Macrocarpon* Ait) by Hplc with Using Different Extraction Methods

Busra Nagihan OZTURK[1], Serap Ayaz SEYHAN[2], Dilek Bilgic ALKAYA[2]

1. Department of Analytical Chemistry, Marmara University Institute of Health Sciences, Istanbul, Turkey.

2. Department of Analytical Chemistry, Marmara University Faculty of Pharmacy, Istanbul, Turkey.

Received: September 17, 2018 / Accepted: October 15, 2018 / Published: January 25, 2019

Abstract: Benzoic acid (C_6H_5COOH) is widely used in the food industry as a preservative in acid foods, under code number E210, owing to its antimicrobial activity against various bacteria, yeasts and fungi involved in food poisoning and food spoilage. It is a safe preservative used to protect taste, odor, appearance, structure of food during storage, preparation, packaging, transport and storage of food. Benzoic acid can be obtained synthetically or it can be obtained naturally from some foods such as fruits, vegetables, spices, nuts, milk and dairy products. However, their excessive use could lead to metabolic acidosis, convulsions, and hyperpnoea in humans. In this study, it was aimed to obtain natural benzoic acid at the highest level in cranberry (*Vaccinium macrocarpon* Ait) by trying different extraction methods such as ultrasonic extraction, orbital shaker at different durations. . Chromatographic conditions; mobile phase: 0.1 M acetate buffer/ MeOH. Column: Kromasil C18 (5µm, 4.6 ×250 mm i.d.), Column Temperature: 25 ^{0}C, Detector: DAD, Wavelenght λ: 230 nm, Flow Rate: 1 mL/min., Injection Volume: 20µL. The best result was obtained from 15 minutes of ultrasonic extraction as 30.8 ppm (mg/kg) by using methanol: water (70:30) solution. According to this study 15 minutes of ultrasonic extraction gave better results than the 2 hour of orbital shaker and it showed that benzoic acid's structure was damaged by over 15 minutes of ultrasonic extraction.

Key words: Benzoic acid, Crannberry (*Vaccinium macrocarpon* Ait), Extraction, HPLC, Determination

1. Introduction

At the present day, many of the foods which we consume are supplemented with food additives to extend their shelf life. Food additives are substances which are used to protect taste, odor, appearance, structure and

Corresponding author: Busra Nagihan OZTURK, Department of Analytical Chemistry, Marmara University Institute of Health Sciences, Istanbul, Turkey, busra.n.ozturk@hotmail.com

other properties of food during storage, preparation, packaging, transport and storage of food, which are not consumed as food alone or as food raw and auxiliary substances [1]. The most known and reliable one of them is benzoic acid. Benzoic acid (C6H5COOH) is widely used in the food industry as a preservative in acid foods, under code number E210, owing to its antimicrobial activity against various bacteria, yeasts and fungi involved in food poisoning and food spoilage. Benzoic acid can be obtained synthetically or it can be obtained naturally from some foods such as fruits, vegetables, spices, nuts, milk and dairy products [2]. However, their excessive use could lead to metabolic acidosis, convulsions, and hyperpnoea in humans [3]. The cultivated cranberry, Vaccinium macrocarpon Ait., is a member of the Ericaceae family, evergreen, creeping shrubs native to cool temperate, acidic soils and peat wetlands of the northeastern US and southern Canada. Cranberry contains high levels of phytochemicals which have health promoting properties. Organic acids such as quinic, citric and malic acids, and small amounts of benzoic and glucuronic acids are important components in cranberry fruits and contribute to their characteristic flavor. The primary purpose of this study is to encourage the use of extracts derived from nutrients containing benzoic acid naturally in the structure instead of synthetic benzoic acids used to extend the shelf life of foods. In this study, it was aimed to obtain natural benzoic acid at the highest level in cranberry (*Vaccinium macrocarpon* Ait) by trying different extraction methods such as ultrasonic extraction, orbital shaker at different durations. After extraction procedure, amount of benzoic acid was determined by using high performance liquid chromatography (HPLC-DAD). Obtained results have been examined.

2. Materials and Methods

2.1. Material

The cranberry, which had been analyzed in this study, was obtained from a spice seller in Istanbul.

2.2. Chemicals

Acetic acid (CH3COOH), 5 M Sodium Hydroxide (NaOH), Methanol (CH3OH), Water (Deionized or HPLC purity), Benzoic asid standard.The chemicals had been used in the analyzes are analytical or HPLC-purity and imported by Merck (Darmstad, Germany) and Sigma-Aldrich (St. Louis, USA).

2.3. Apparatus

Ultrasonic bath, Orbital shaker, HPLC- DAD (Shımadzu series)

2.4. Chromatographic Conditions:

Mobile phase: 0.1 M acetate buffer/MeOH, column: Kromasil C18 (5µm, 4.6×250 mm i.d.), column temperature:

25 ^{0}C, detector: Diode Array Dedector (DAD), wavelenght λ: 230 nm, flow rate: 1 mL/min, injection volume:20µL

2.5. Preperation of 0.1 M acetate buffer pH 4.74 and Sample

5.7 ml of concentrated acetic acid is diluted with 900 ml of water. The pH is adjusted to 4.74 with 5 M NaOH.

This solution is supplemented with water to 1000 ml. It is filtered through a membrane filter.

5 g cranberry (*Vaccinium macrocarpon* Ait) was weighed into a 100 mL flask. 30 mL distilled water was added.

The flask contents were thoroughly mixed. Flask was fulfilled with methanol to 100 mL. [4]

2.6. Extraction of Sample

All of samples were extracted with methanol: water (70:30) solution. Three different samples were prepared for ultrasonic extraction and samples were extracted during 5, 15 and 30 minutes at room temperature. The other three different samples were prepared for orbital shaker and samples were extracted during 30, 60 and 120 minutes. The last sample was extracted during 30 seconds by shaking with hand. After extraction, the samples were firstly filtered through a filter paper and then some of filtrates were filtered through a membrane filter. Finally, the filtrates were put into vials for HPLC analysis.

Table 1

	Results of different extraction methods					
	Retention Time (seconds) (Benzoic acid of control standard)	**Retention Time (seconds) (Benzoic acid of sample)**	**Extraction Time (minutes)**	**Concentratins of Benzoic Acid (ppm)**	**LOD (ppm)**	**LOQ (ppm)**
Shaking with Hand		7.564	0.5	6.3		
		7.577	5	7..4		
By Ultrasonic Bath	7.596	7.589	15	30.8	5.2	5.6
		7.576	30	19.6		
		7.576	30	23.0		
By Orbital Shaking		7.572	60	27.6		
		7.572	120	28.1		

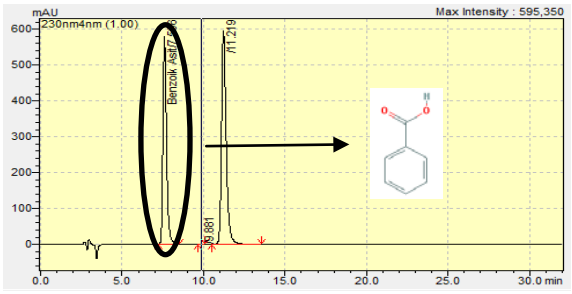

Fig. 1: Control Standard of Benzoic Acid

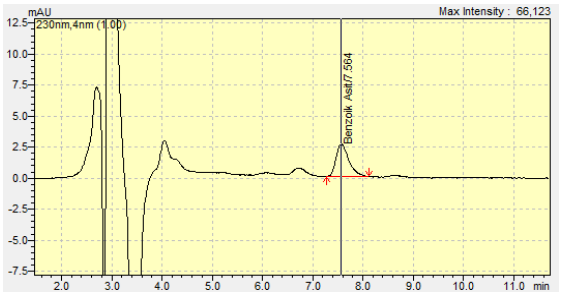

Fig. 2: 0.5 minutes of Shaking with Hand

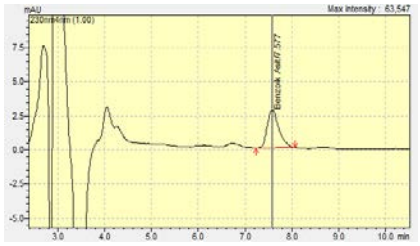

Fig. 3: 5 minutes of Ultrasonic Extraction

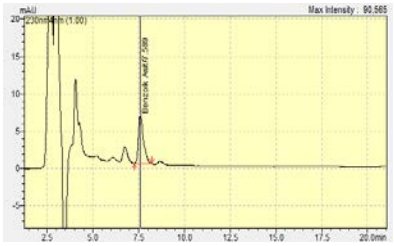

Fig. 4: 15 minutes of Ultrasonic Extraction

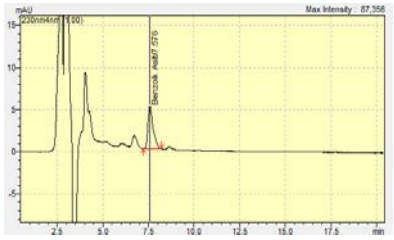

Fig. 5: 30 minutes of Ultrasonic Extraction

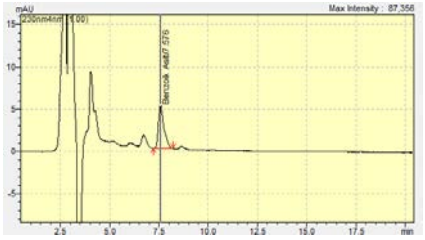

Fig. 6: 30 minutes of Orbital Shaking

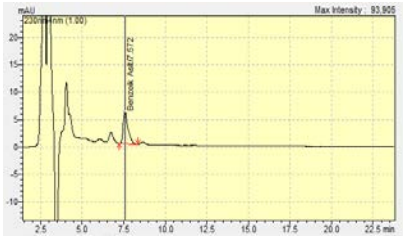

Fig. 7: 60 minutes of Orbital Shaking

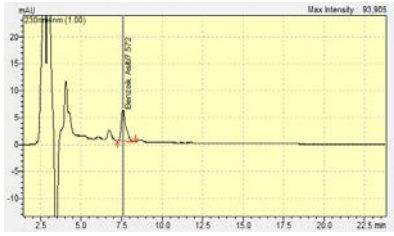

Fig. 8: 120 minutes of Orbital Shaking

3. Results and Discussion

In this study, amount of benzoic acid in cranberry was determined by HPLC-DAD. Shaking with hand, ultrasonic bath and orbital shaking were used as extraction methods. Different durations were applied in extractions.

The results of the analysis were found respectively as; 6.3 ppm just by shaking with hand during 0,5 minutes; 7.4 ppm, 30.8 ppm, and 19.6 ppm by using ultrasonic extraction during 5, 15, and 30 minutes; 23.0 ppm, 27.6 ppm and 28.1 ppm by using orbital shaker during 30, 60 and 120 minutes.

It has been found that the maximum results are obtained in the ultrasonic extraction. Minimum results are obtained in the shaking with hand.

The control standard and sample chromatograms were compared and the retention times matched with each other.

The cranberry contains a small amount of benzoic acid so extraction processing can be difficult. These results suggested that the ultrasonic bath system was simple and rapid determination method of benzoic acid in cranberry.

There are several of study about this subject. One of this, for organic acid identification and quantification, about 3 g of fruit were weighed and crushed in 30 ml distilled water in a blender for 1 min and heated in 90 °C water bath for 10 min. After filtration through filter paper, aqueous extracts were collected for HPLC analysis. A Waters Atlantis® 250 × 4.6 mm C18, 5 μm LC column was used and organic acids were detected at 210–230 nm in PDA detector. Benzoic acid was detected in extremely low concentrations at the first sampling date and increased slowly through Jul, with concentrations less than 0.01 mg/g fruit in all cultivars. From August onwards, benzoic acid accumulated in all cultivars, along with fruit development, and continued to increase from middle September to October during fruits final ripening [5].

In another study, Sep-Pak C cartridge ad hydrolysis methods were used for extraction. An Eclipse XDR-C reversed-phase column (150 mm x 4.6 mm, 5 mm; Waters) was used and the flavonoids and phenolic acids were detected at both 280 nm and 360 nm in DAD detector. Gradient elution program using methanol–acetic acid–water as solvent was chosen. Benzoic acid is the major phenolic acid detected in both studied cranberry juices. The canned cranberry juice contains about 34 mg/ l of benzoic acid, and freshly squeezed juice contains more than 41 mg/l. This amount is in the range that used in the preservation of most perishable foods [6].

In this study; extraction was not performed at high temperature because high temperature is thought to be able to damage the analyte structure. On the other hand, the gradient programme may causes fluctuations in the chromatogram, which can lead to loss of concentration so isacratic programe was used to obtain better peaks.

4. Conclusion

According to this study 15 minutes of ultrasonic extraction was given the best results and it showed that benzoic acid’s structure was damaged by over 15 minutes of ultrasonic extraction. On the other hand, it was determined that the over 60 minutes of orbital shaking does not give meaningful results. This study itself is easy to perform. The same process principles can be used also to other foodstuffs containing natural or added benzoic acid. This study has shown that low concentrations of benzoic acid contained in cranberry can be

obtained in a better technique as ultrasonic extraction.

Acknowledgments

The authors would like to thank Formul Laboratory that provided facilities for this study and laboratory team for their valuable technical assistance.

References

[1] Boğa, A., and Binokay, S. (2010). Gıda Katkı Maddeleri ve Sağlığımıza Etkileri. Arşiv Kaynak Tarama Dergisi 19.

[2] Sieber, R., Bütikofer, U., and Bosset, J.. (1995). Benzoic acid as a natural compound in cultured dairy products and cheese. International Dairy Journal 5, 227-246.

[3] Fujiyoshi, T., Ikami, T., Kikukawa, K., Kobayashi, M., Takai, R., Kozaki, D., and Yamamoto, A. (2018). Direct quantitation of the preservatives benzoic and sorbic acid in processed foods using derivative spectrophotometry combined with micro dialysis. Food Chemistry 240, 386-390.

[4] NMKL 124 Benzoic acid, sorbic acid and p-hdroxybenzoic acid esters. Liquid chromatographic determination in foods.

[5] Wang, Y., Johnson-Cicalese, J., Singh, A.P., and Vorsa, N. (2017). Characterization and quantification of flavonoids and organic acids over fruit development in American cranberry (Vaccinium macrocarpon) cultivars using HPLC and APCI-MS/MS. Plant Science 262, 91-102.

[6] Chen, H., Zuo, Y., and Deng, Y. (2001). Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. In Journal of Chromatography A, pp. 387-395.