Optimization and Validation of an HPLC-UV Method for Determination of Benzoic Acid and Sorbic Acid in Yogurt and Dried-Yogurt Products Using a Design of Experiment

Ala Yahya Sirhan

Department of Basic Science, Applied Science Private University, 11931 Amman, Jordan

Received August 22, 2017; Accepted January 22, 2018

ABSTRACT

A method for the determination and analysis of benzoic acid and sorbic acid in yogurt and dried-yogurt products has been developed. This method was based on the use of a simple solid-liquid extraction method, followed by the high-performance liquid chromatography with a UV detector (HPLC–UV), enhanced with the aid of response surface methodology and design of experiment (DOE). The method excludes the use of complicated procedures, time-consuming and labor-intensive pre-treatment processes. Separation of the benzoic acid and sorbic acid with higher selectivity and sensitivity, and within reasonable retention time was performed by using an isocratic mobile phase of acetate buffer (pH 5.6)-methanol 60:40 at a column temperature of 25 °C. Optimization of sample preparation and analytical conditions gave recoveries in the range of 81 to 111% at spike levels of 2–20 mg/L and the relative standard deviations (RSDs) was lower than 9% in all cases. The intra-day precision and inter-day precision results were in the range of 8.4–8.5% and 10.4–11.0%. Additionally, the limits of detection (LOD) were 0.66 and 0.51 mg/L and the limits of quantification (LOQ) were 1.3 and 1.0 mg/L for benzoic acid and sorbic acid, respectively.

Keywords: *HPLC; response surface methodology; design of the experiment; benzoic acid; sorbic acid; preservative*

ABSTRAK

Telah dikembangkan metode penentuan dan analisis asam benzoat dan asam sorbat dalam yogurt dan produk yogurt kering. Metode ini didasarkan pada penggunaan metode ekstraksi padat-cair sederhana, diikuti dengan kromatografi cair kinerja tinggi dengan detektor UV (HPLC-UV), dan ditingkatkan dengan bantuan metodologi permukaan respon dan desain percobaan (DOE). Metode ini tidak melibatkan penggunaan prosedur yang rumit, proses pra-perlakuan yang memakan waktu dan tenaga yang intensif. Pemisahan asam benzoat dan asam sorbat dengan selektivitas dan sensitivitas yang lebih tinggi, dan dalam waktu retensi yang wajar dilakukan dengan menggunakan fase gerak isokratik dari buffer asetat (pH 5,6) -metanol 60:40 pada suhu kolom 25 °C. Optimasi preparasi sampel dan kondisi analitik memberikan perolehan kembali dalam kisaran 81 hingga 111% pada tingkat pembubuhan 2–20 mg/L dan standar deviasi relatif (RSD) lebih rendah dari 9% dalam semua percobaan. Hasil presisi dalam sehari dan hasil presisi antar hari berada pada kisaran 8,4–8,5% dan 10,4–11,0%. Selain itu, batas deteksi (LOD) adalah 0,66 dan 0,51 mg/L dan batas kuantifikasi (LOQ) adalah 1,3 dan 1,0 mg/L masing-masing untuk asam benzoat dan asam sorbat.

Kata Kunci: *HPLC; metodologi permukaan respon; desain percobaan; asam benzoat; asam sorbat; pengawet*

INTRODUCTION

Yogurt is a fermented dairy product made by lactic acid fermentation of milk by mixed bacterial cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus.* Yogurt is very popular and consumed widely in different forms in the Middle East. Yogurt is manufactured under good manufacturing practices (GMP) and held under refrigeration during distribution and display in retail outlets. Yogurt should contain less than one yeast cell/g and should have an expects shelf life of 30 days [1-2]. Yeast cells that do not participate in the fermentation during yogurt production are the main cause of product spoilage. Referable to the inherent low pH of yogurt and the ability of yeasts to take in sugars, milk sugar and organic acids, the product acts as a selective environment for the development of yeasts. Yeast acts as contaminants from the processing equipment and to a smaller extent, from the fruit, sugar and honey used as additives in the product [1,3-4]. In some cases,

* Corresponding author.
Email address : sirhanala@yahoo.com

DOI: 10.22146/ijc.27675

resistance to preservatives may be an additional cause of their spread in the product [2].

According to the current legislation, benzoic acid, or its Na, K, Ca salts, (E210–213) is not permitted in yogurt manufacturing process. Nevertheless, benzoic acid is still found in trace amount in yogurt due to its use of during the production or naturally occurring as a natural by-product of microbial metabolism [5]. Despite the strict regulations and the good intention of the manufacturers, literature data showed that yogurt might contain benzoic acids as a preservative from the pre- and post-production sources [5]. The use of these antimicrobial agents has been linked to adverse effects such as metabolic acidosis, convulsions and hyperpnoea. These adverse effects were observed in experimental animals and humans that were given very high doses of benzoic acid. Some weak clastogenic activity was also noted by in vitro assays [6]. The progression of allergic reactions to the benzoate in humans, such as urticaria, non-immunological contact urticaria and asthma, has also been stated in the literature [7]. On the other hand, of benzoic acid, other studies have shown that sorbic acid has low cytotoxicity and illustrates the fact that it is metabolized rapidly by similar paths to those of fatty acids [8].

In general, sample preparation is often the most critical part of the analysis of preservatives and relies mostly on the physical and chemical properties of the products that are contaminated with preservatives. Manufactured products with high fat and protein contents, such as yogurt, require multi-steps treatment. Most methods currently applied in extracting preservatives use complicated, time-consuming and labor-intensive pre-treatment procedures. These procedures include extraction with a solvent or a mixture of organic solvents, followed by precipitating of the fat and protein content by adding potassium hexacyanoferrate trihydrate solutions (6% w/v) and zinc acetate solutions [9]. Furthermore, many of the reported methods the analyte is processed with multiple steps steam distillation before treating via solid-phase extraction cartridge [10].

Analytical methods for the determination of benzoic acid and sorbic acid have been developed. The most common methods for isolating benzoic acid and sorbic acid include gas chromatography (GC) [11], gas chromatography coupled with mass spectrometry (GC-MS) [12], high-performance liquid chromatography (HPLC) with a UV-Visible detector (UV) [9,13], and capillary electrophoresis [14]. Recently, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) [15] has been reported.

Here we report on a simplified solvent extraction procedure followed by HPLC separation of a mixture of benzoic acid and sorbic acid. The use of design of experiment (DOE) has increasingly been considered to be valuable supplements of high-performance liquid chromatography practices, as a large number of operating conditions can be controlled at the same time to achieve the desired separations [16].

The effect of the operating conditions on the separation of benzoic and sorbic acids was examined. Good separation conditions were documented with a limited number of experiments. The use of DOE with the aid of response surface methodology was applied to develop a fast separation method without affecting resolution between the two peaks and to provide a maximum peak area of benzoic acid and sorbic acid. Therefore, the DOE was built and the optimum conditions of mobile phase compositions with an optimum pH value were predicted. Central composite design (CCD) with twenty-six experimental points was performed randomly at all points. Experimental data were fitted to a quadratic polynomial model.

This study aims at developing a simple, reliable and affordable technique to test the presence of preservatives in yogurt. The main objective of this technique is the extraction of benzoic acid and sorbic acid in yogurt samples using a simple solid-liquid extraction method, followed by determining the concentration of the analyte using HPLC–UV with the aid of response surface methodology and DOE.

EXPERIMENTAL SECTION

Materials

Certified standard solutions of benzoic acid (99.6%) and sorbic acid (99.0%) were obtained from Acros Organics, Switzerland. Acetic acid (99.8%) was obtained from Fluka (Buchs, Switzerland). Sodium acetate anhydrous extra pure was purchased from SDFCL (India). Water (HPLC-grade) was purchased from VWR International (EC). Methanol (HPLC-grade) was purchased from Labscan (Dublin, Ireland). A nonsterile PTFE Syringe Filter with a disposable membrane filter (0.45 µm) was purchased from Whatman GmbH (Dassel, Germany).

Instrumentation

The HPLC analysis was performed using a Perkin-Elmer Series 200 (Llantrisant, UK), the system consisting of a pump with a quaternary configuration, a vacuum degasser, a column oven, a photodiode array detector and an autosampler equipped with a 200 µL sample loop. The chromatographic separation was performed with Brownlee Analytical, 5 µm C18 250 mm × 4.6 mm chromatographic column, which was purchased from PerkinElmer (Shelton, USA). The sample

Table 1. The effects of the pH and Buffer on the chromatographic peak areas for both preservatives benzoic acid and sorbic acid

Standard order	Run order	pH	Buffer	Peak area of benzoic acid	Peak area of sorbic acid
4	1	3.8	70	447434	818732
18	2	5.4	40	769887	1798297
3	3	3.8	60	752508	1658704
5	4	3.8	80	217131	2241
19	5	5.4	50	785986	2005387
20	6	5.4	60	787202	2041877
11	7	4.6	70	570826	1363249
14	8	5.0	50	743032	1788403
12	9	4.6	80	35168	733985
2	10	3.8	50	754610	1683712
21	11	5.4	70	694218	1907767
16	12	5.0	70	632522	1635508
24	13	5.6	50	752736	2045596
25	14	5.6	60	786757	2130046
26	15	5.6	70	725066	2043896
15	16	5.0	60	749761	1840111
6	17	4.6	40	755389	1829299
22	18	5.4	80	689758	1603057
23	19	5.6	40	669690	1790546
9	20	4.6	60	757071	1666760
7	21	4.6	50	785574	1844549
10	22	4.6	60	752508	1658704
17	23	5.0	80	415162	1234878
8	24	4.6	60	751990	1657594
13	25	5.0	40	746526	1833539
1	26	3.8	40	755866	1750042

extracts were analyzed isocratically using 60:40 acetate buffer (60 mM, pH 5.6)/methanol mixture as the mobile phase. The column was kept in a column oven at 25 °C at a flow rate of 1.0 mL/min to achieve the optimum resolution between benzoic acid and sorbic acid. The injection volume was maintained at 10 µL for both sample and standard solutions. The wavelength at 227 nm was applied for detection of both benzoic acid and sorbic acid as it gave maximum absorption for both benzoic acid and sorbic acid.

Procedure

Sample preparation

The benzoic acid and sorbic acids were extracted from the yogurt/dried yogurt samples using a solid-liquid extraction procedure. Five grams of well-homogenized samples were weighed and dissolved in 50 mL of extraction solution. The extraction solution was prepared by dissolving 8.2 g of sodium acetate in 1000 mL of distilled water. Subsequently, the pH was adjusted to 5.6 with acetic acid and mixed well with 500 mL of methanol. This mixture contains 67 mM of acetate buffer solution.

Sample recovery was performed with 5 g of the blank yogurt/dried yogurt samples with three different fortification levels; 0.5 mL of preservative mixed standards were spiked at 5, 10 and 20 mg/L of the standard mix. The spiked samples were left overnight at room temperature to allow the analytes to absorb into the matrix. The mixtures were then stirred for 3 min at high speed. After that, the mixture was filtered through a Whatman No.1 filter paper and passed through a 0.45 µm disposable membrane filter before HPLC analysis.

Preparation of standard stock solution, and calibration standard

The standard stock solution of preservative at 1000 mg/L was prepared with mobile phase. A series of standard solutions (1.9, 3.8, 7.5, 15, 30 and 60 mg/L) was prepared by diluting adequate volumes of the benzoic acid and sorbic acid stock solution with mobile phase.

Optimization procedure

The pH of the mobile phase and mobile phase ratio play a significant role in the chromatographic separations. The DOE and statistical analysis of the data were performed using Minitab® 17.0 software system. A CCD with twenty-six experimental points (Table 1) was performed randomly at all points for robustness study. Resolution (R_S) in addition to maximum peak was chosen as the response of the food preservatives.

Food samples

A total of 238 samples of yogurt and dried-yogurt samples were supplied to the Jordan food and drug administration in 2014 and analyzed. The samples were stored at 4 °C in a refrigerator. The samples were mixed at room temperature until a homogeneous solution was obtained. The samples were then stored in plastic bags at 4 °C in a refrigerator before analysis.

RESULT AND DISCUSSION

Optimization of HPLC Conditions

The chromatographic conditions were optimized using benzoic acid and sorbic acid standards based on conditions given in literature reports [17]. Variations in the ratio of buffer to methanol at a proportion of 20–80% in the mobile phase resulted a pH range between 3.8 and 5.6. Data were analyzed using Minitab® 17.0 software to maximize the peak area of benzoic acid and sorbic acid, to optimize the resolution between them and to decrease the retention time.

This work presents the results of experimental study design to determine the combined effect of pH and mobile phase composition on the reverse-phase liquid chromatographic behavior of benzoic acid and sorbic acid. The effect of the ratio of buffer to methanol from 20 to 80% was tested in proportion. The effect of pH at pH range from 3.8 to 5.6 was also tested. To provide a maximum peak area of benzoic acid and sorbic acid, to decrease retention time without affecting good separation resolution between the two peaks. The pH range from 3.8 to 5.6 was chosen due to the pK_a values of benzoic and sorbic acids are 4.2 and 4.8, respectively. The selected pH range gave good pH control of the mobile phase as well as good separations between benzoic and sorbic acids [18].

The DOE was applied to find out the best suitable ratio of buffer to methanol at a range pH from 3.8 to 5.6 which will give the best-resolved peak area of benzoic acid and sorbic acid. CCD with twenty-six experimental points (Table 1) was performed randomly at all points. Experimental data were fitted to a quadratic polynomial model. The prediction profilers provided in the response surface are shown in Fig. 1 and 2.

Fig. 1 and 2 indicate that a high peak area of selected analyte was achieved, since it gave 1 composite desirability when the pH adjusted to 5.6 using 60:40 (%, v/v) buffer/methanol solution and 0.93 of composite desirability when the pH adjusted to 3.8 using 40:60 (%, v/v) buffer/methanol solution. The latter solution was avoided due to the high ratio of methanol tends to elute peaks at the beginning of the chromatogram closed to the solvent peak. The solution with pH of 5.6 and 60:40 (%, v/v) buffer/methanol ratio resulted in 1 composite desirability and provided a better chromatographic resolution and increased the signal-to-noise ratio in a short elution time (within 5 min for analysis of benzoic acid and sorbic acid in food samples). Therefore, the solution with pH of 5.6 and 60:40 (%, v/v) buffer/methanol ratio were applied in this study. The retention time for benzoic and sorbic acids was

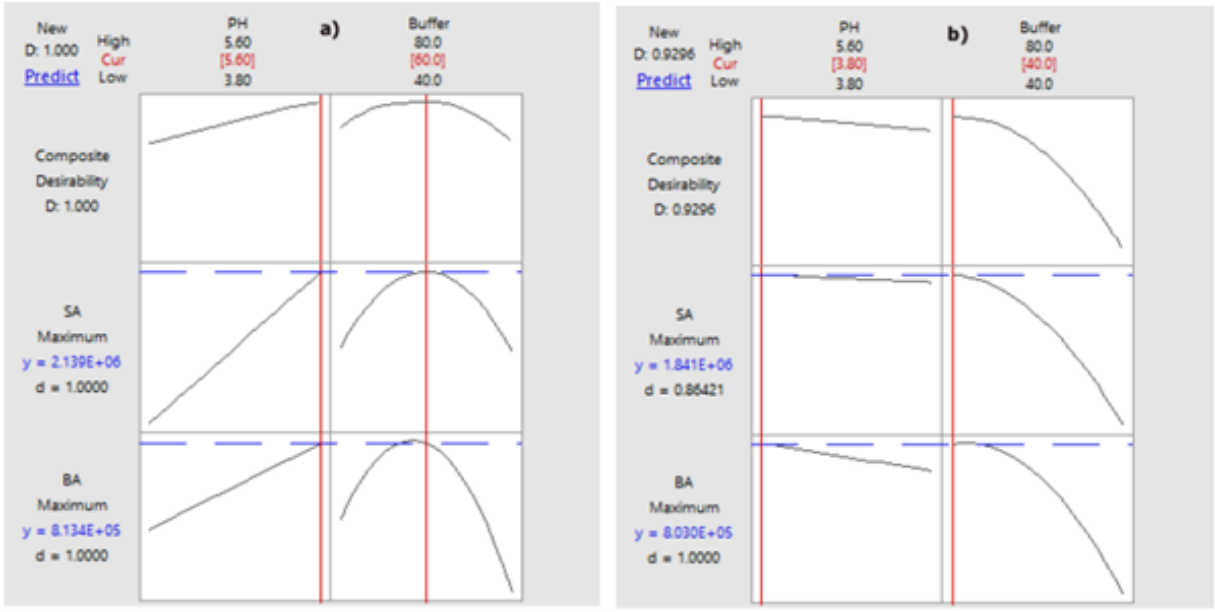

Fig 1. The Maximum Desirability Profiler displays optimal settings of pH 5.6 and 3.8 using 60:40 and 40:60 (%, v/v) buffer/methanol solution, respectively. It gave 1 and 0.93 composite desirability of maximum peak area (a) and (b), respectively

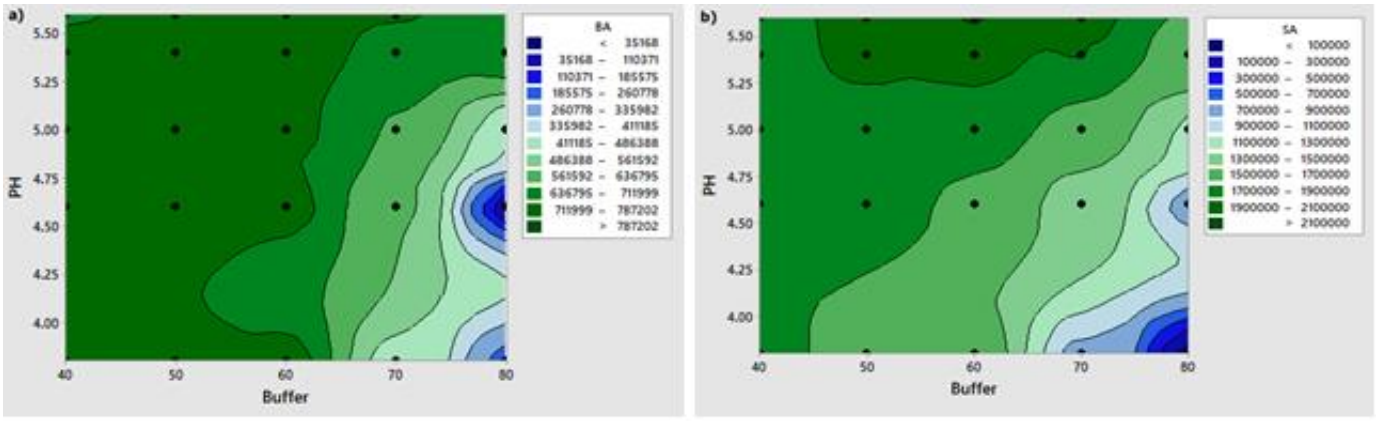

Fig 2. An overlay contour plot of both preservatives benzoic acid (a) and sorbic acid (b) peak area with 24 experimental points (the black dot point)

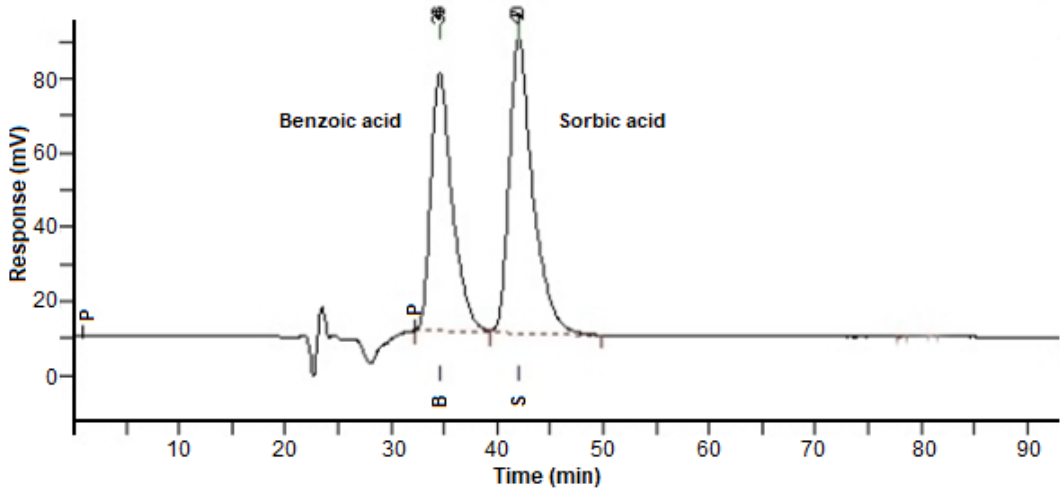

Fig 3. HPLC chromatogram of preservatives standard solutions containing 40 mg/L of benzoic acid with a retention time of 3.5 min and 25 mg/L of sorbic acid with a retention time of 4.2 min

3.5 min and 4.2, respectively (Fig. 3).

Column selection depends strongly on prior knowledge of the physicochemical properties of the analytes and the matrix [19]. The column type and its length were optimized by investigating various HPLC columns under the same chromatographic conditions to obtain the best chromatogram separation in the shortest analysis time.

Sample Pretreatment Optimization

In general, sample preparation is often the most important part in the analysis of preservatives and relies largely on the physiochemical properties of the products that are adulterated with preservatives. Products with high fat and protein contents require more treatment.

Most methods currently applied in extracting preservatives employs precipitating step, steam distillation multiple steps or solid-phase extractions clean up step. These further steps are complicated, time-consuming and labor-intensive pre-treatment procedures. Alternatively, these steps have been eliminated in the study due to consistent obtained chromatographic responses, thus reducing the sample treatment time and cost per analysis. Furthermore, the total extraction time for one sample is about 10 min, so this approach could be used in screening methods to achieve a fast and reliable way for the detection of the target preservatives in food.

In this study, the extraction efficiency of the yogurt and dried yogurt samples was optimized by testing the following pH of 3.6, 4.6 and 5.6 that had been spiked with 10 mg/L of the preservatives standards. The results are shown in Table 1 and all these pH values were then compared.

Table 2 indicates that a high extraction efficiency and good recovery of benzoic acid and sorbic acid were obtained when a pH of 5.6 was used. The extraction solution volume has excellent effects on the concentration factor. The increase of the extraction

solution volume increased the final volume obtained by extraction, leading to a decrease in the concentration of the target analyte in extraction solution, and so, concentration factor will decrease. Consequently, the optimal extraction solution volume should ensure both high concentration factor and enough volume for the subsequent analysis.

In this study, 5 g of blank yogurt samples were fortified with 10 mg/L of benzoic acid and sorbic acid standards. Then, the fortified extract was dissolved in 25 mL and 50 mL of the extraction solution. These extracts were then injected into the HPLC-UV instrument, analyzed, and their respective recoveries were compared using solvent standards (Fig. 4).

Fig. 4 shows that extraction of fortified sample extracted with 25 mL of the extraction solution resulted in high extraction efficiency and good recovery of benzoic and sorbic acids. Therefore, an external standard calibration can be applied in this procedure. Recoveries exceeding 80% were obtained for all the fortified extracts (Table 2). Therefore, clean up procedures can be avoided thus saving time, effort and expenses with greater accuracy.

Method Validation

The method was validated internally regarding linearity, accuracy, intra-day precision, inter-day precision, limit of detection (LOD), and limit of quantification (LOQ). The linearity was tested using standard solutions of preservatives in a concentration range from 2 to 60 mg/L. Table 3 shows good linear relationships between the concentration of the analyte and the peak area with correlation coefficients greater than 0.999 for all analytes. Calibrations with standard solutions were used for quantitation because moderate signal suppression was noticeable for both analytes. Furthermore, the ANOVA test did not give any significant difference at p = 0.05.

The accuracy was calculated by the determination of the recoveries of the preservatives from wet yogurt and dried yogurt samples spiked at 2, 10 and 20 mg/L of preservatives standards; the spiked samples were analyzed in triplicates (Table 2) and calculated according to the following Eq.1 [20]:

$$\text{Recovery}(\%) = \frac{\text{Recovered amount}\left(\frac{\text{mg}}{\text{L}}\right)}{\text{Added amount}\left(\frac{\text{mg}}{\text{L}}\right)} \times 100 \qquad (1)$$

The obtained recovery percentages ranged from 81 to 111%, with a relative standard deviation (RSD) less than 9 %. The recoveries for benzoic acid were slightly more significant than the sorbic acid.

The sensitivity was determined by estimating the limit of detection (LOD) and limit of quantification (LOQ). LODs and LOQs were calculated experimentally as the lowest concentration giving a response of three- and ten-times, respectively, the base-line noise given by the software, obtained from mycotoxin-free samples [20]. The LOD of benzoic acid and sorbic acid were 0.66, 0.51 mg/L and the LOQ were 1.3, and 1.0 mg/L for benzoic acid and sorbic acid, respectively (see the details in Table 3).

Intra-day precision was studied by calculating the relative standard deviation (RSD) of the peak area for five replicates of the same sample at a spiked level of 20 mg/L

Table 2. Mean of recoveries and RSDs (n = 5) of both preservatives benzoic acid and sorbic acid spiked into clean yogurt and dried-yogurt samples at three spiking levels using HPLC method

Preservative	Spiking level (mg/L)	Mean of recovery (%) ± RSD (%	
		Yogurt	Dried Yogurt
Benzoic acid	2	84.9 ± 6.5	83.0 ± 7.7
	10	85.7 ± 5.6	88.6 ± 8.8
	20	85.4 ± 7.6	108.1 ± 4.9
Sorbic acid	2	90.6 ± 2.8	86.0 ± 7.4
	10	93.1 ± 7.8	110.2 ± 5.0
	20	81.6 ± 4.7	96.5 ± 6.3

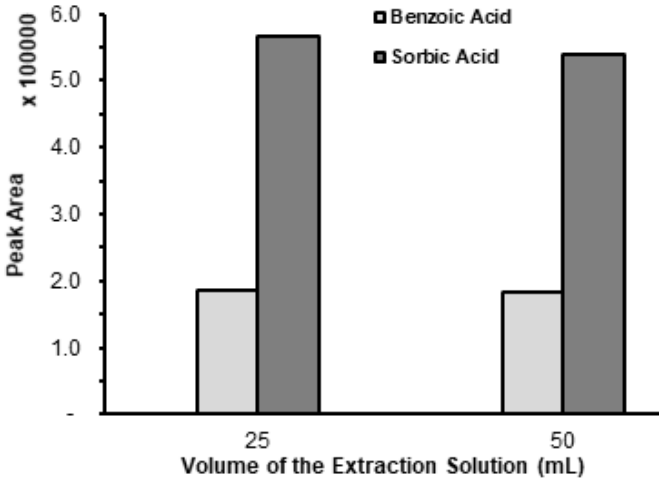

Fig 4. Effect of extraction solution volume on the peak area of benzoic acid and sorbic acid

Table 3. Linearity range, equation, r^2 value, RSD, LOD and LOQ of benzoic acid and sorbic acid

Preservative	Linearity range (mg/L)	Equation	r^2	LOD (mg/L)	LOQ (mg/L)
Benzoic acid	2–60	Y = (-24864.63) + (24270.60) X	0.9997	0.66	1.3
Sorbic acid	2–60	Y = (-59948.50) + (66780.09) X	0.9998	0.51	1.0

Table 4. The intra-day precision and inter-day precision of both preservatives benzoic acid and sorbic acid expressed as RSD% values

Preservative	Spiking level (mg/L)	Intra-day precision (n = 5)[a]	Inter-day precision (n = 15)[a]
Benzoic acid	20	8.5	10.4
Sorbic acid	20	8.4	11.0

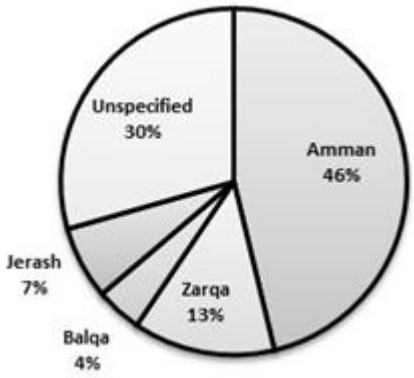

Fig 5. The analysis of the origin of the sample showed the vast majority of samples originated in the capital city Amman (46%), in comparison to a few samples from Zarqa (13%) and very few from other cities in Jordan of

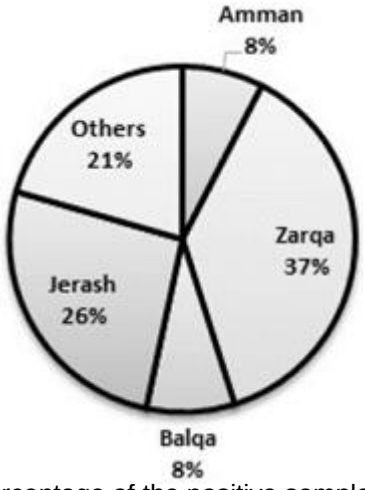

Fig 6. Percentage of the positive sample by area

benzoic and sorbic acids on the same day. For the inter-day precision, five replicates of the same sample at a spiked level of 20 mg/L of benzoic acid and sorbic acid were analyzed on three consecutive days. The intra-day precision and inter-day precision were calculated and tabulated in Table 4. The intra-day precision (n = 5) values were between 8.4 and 8.5%, while the inter-day variation (n = 15) values were between 10.4 and 11.0%. These values determined are lower than the acceptable maximum of 11%, confirming the good reproducibility and repeatability of this method.

Intra-day precision was calculated by assaying five replicates of the same sample at a spiked level of 20 mg/L of benzoic acid and sorbic acid on the same day. For the inter-day precision, five replicates of the same sample at a spiked level of 20 mg/L of benzoic acid and sorbic acid were analyzed on three consecutive days. The intra-day precision and inter-day precision were calculated and tabulated in Table 4. The intra-day precision (n = 5) values were between 8.4 and 8.5%, while the inter-day variation (n = 15) values were between 10.4 and 11.0%. These values determined are lower than the acceptable maximum of 11%, confirming the good reproducibility and repeatability of this method.

Considering the data obtained from the method validation; the current HPLC–UV analysis measured with the aid of response surface methodology, DOE and sample preparation procedures is considered as a selective, precise and robust method to determine sorbic and benzoic preservatives in yogurt samples.

Food Samples Analysis

The developed method was applied for the analysis of both preservatives benzoic acid and sorbic acid in 238 samples of commercial yogurt and dried-yogurt samples products supplied to the Jordanian food and drug administration. Benzoic acid is naturally present in milk and fermented dairy products up to 60 mg/L [9] unlike sorbic acid, which is not normally found in milk, and dairy products [21]. On the other hand, the current legislation does not permit the presence of benzoic acid, or its salts Na, K, Ca (E210-213) as well as sorbic acid, or its salts Na, K, Ca (E200-203) and all preservatives, at the point of addition (during production) in the milk manufacturing process [22]. Therefore, the upper limit that can be accepted as the original benzoic acid (negative sample) that has been found in the milk samples is 60 mg /L.

The analysis of the sample origin is shown in Fig. 5, the vast majority of samples originated in the capital city Amman (46%), in comparison to a few samples from Zarqa (13%) and very few from other cities in Jordan. This is expected due to the population demography.

Samples containing either sorbic acid or benzoic acid at a concentration above 60 mg/L as a preserver were shown in Fig. 6. A 36% of the positive sample with either sorbic or benzoic acid was manufactured in Zarqa city and 26% of the fortified sample were manufactured in Jerash city. The presence of the preservatives is due to Zarqa and Jerash are the manufacturing site of products, which is destined to Amman market and hence extra protection by the addition of preservatives to enable yogurt products

reaching safely to Amman during transportation, storage and displaying process.

The acceptable daily intake (ADI) of benzoic or sorbic acid (as benzoic or sorbic acids and their sodium, potassium and calcium salts) are 0–5 and 0–25 mg/kg body weight, respectively [23-24]. In Jordan, the use of benzoic or sorbic acid in the manufacturing process is prohibited in Milk and milk products [22]. On the other hand, it is permitted in some popular foods such as pickles [25] and soft drinks [26]. As a result of illegally adding preservatives to yogurt and their products, alongside the existence of these preservatives in other food, expose the consumer to relatively high concentrations levels of these preservatives which ultimately might lead to toxicity.

CONCLUSION

A simple, rapid, inexpensive and effective sample preparation method has been developed for the determination of benzoic acid and sorbic acid in yogurt and dried-yogurt product. The sensitivity of the HPLC–UV instrument could be significantly enhanced by optimizing the chromatographic conditions with the aid of response surface methodology and DOE. Extensive and expensive clean-up procedures could be replaced by adopting a simplified solvent extraction procedure followed by HPLC separation of a mixture of benzoic acid and sorbic acid. Separation of the benzoic acid and sorbic acid with higher selectivity and sensitivity and within reasonable retention time was performed. Excellent linearity, high recoveries, acceptable repeatability and reproducibility with lower LOQ values were achieved indicating the suitability of the proposed method for the determination of preservatives in yogurt and dried-yogurt product.

REFERENCES

[1] Davis, J.G., 1970, Laboratory control of yoghurt, *Dairy Ind. Int.*, 35 (3), 139–144.

[2] Green, M.D., and Ibe, S.N., 1986, Yeasts as primary contaminants in yoghurts produced commercially in Lagos, Nigeria, *J. Food Prot.*, 50 (3), 193–198.

[3] Suriyarachchi, V., and Fleet, G., 1981, Occurrence and growth of yeasts in yogurts, *Appl. Environ. Microbiol.*, 42 (4), 574–579.

[4] Fleet, G.H., and Mian, M.A., 1987, The occurrence and growth of yeasts in dairy products, *Int. J. Food Microbiol.*, 4 (2), 145–155.

[5] Mroueh, M., Issa, D., Khawand, J., Haraty, B., Malek, A., Kassaify, Z., and Toufeili, I., 2008, Levels of benzoic and sorbic acid preservatives in commercially produced yoghurt in Lebanon, *J. Food Agric. Environ.*, 6 (1), 62–66.

[6] Yildiz, A., Erdogan, S., Saydut, A. and Hamamci, C., 2012, High-performance liquid chromatography analysis and assessment of benzoic acid in yogurt, ayran, and cheese in Turkey, *Food Anal. Methods*, 5 (3), 591–595.

[7] Smolinske, S.C., 1992, "Benzoic Acid" in *CRC Handbook of Food, Drug, and Cosmetic Excipients*, CRC Press, Colorado, 41–47.

[8] Koyuncu, N., and Uylaser, V., 2009, Benzoic acid and sorbic acid levels in some dairy products consumed in Turkey, *Asian J. Chem.*, 21 (6), 4901–4908.

[9] Mihyar, G.F., Yousif, A.K., and Yamani, M.I., 1999, Determination of benzoic and sorbic acids in labaneh by high-performance liquid chromatography, *J. Food Compos. Anal.*, 12 (1), 53–61.

[10] Sofos, J.N., 2000, "Sorbic acid" in *Natural Food Antimicrobial Systems*, Eds. Naidu, A.S., CRC Press, California, 637–661.

[11] Iguchi, S., Yamamoto, M., and Yamamoto, T., 1964, Analysis of food preservatives by gas chromatography, *Eisei Kagaku*, 10 (1), 42–46.

[12] Ammen, E.W., and Al-Salhi, R., 2017, An application of Gas Chromatography-Mass Spectrometry (GC-MS) Fast Automated Scan/SIM Type (FASST) in determining the preservative levels in foods, *Am. J. Anal. Chem.*, 8 (5), 307–316.

[13] Dinç, Z.Ş., Aşçı, B., Aksu, D.Ö., and Yildirim, K.D., 2016, Simultaneous determination of potassium sorbate, sodium benzoate, quinoline yellow and sunset yellow in lemonades and lemon sauces by HPLC using experimental design, *J. Chromatogr. Sci.*, 54 (6), 952–957.

[14] Boyce, M.C., 2001, Determination of additives in food by capillary electrophoresis, *Electrophoresis*, 22 (8), 1447–1459.

[15] Jia, W., Ling, Y., Lin, Y., Chang, J., and Chu, X., 2014, Analysis of additives in dairy products by liquid chromatography coupled to quadrupole-orbitrap mass spectrometry, *J. Chromatogr. A*, 1336, 67–75.

[16] Sahu, P.K., Ramisetti, N.R., Cecchi, T., Swain, S., Patro, C.S., and Panda, J., 2018, An overview of experimental designs in HPLC method development and validation, *J. Pharm. Biomed. Anal.*, 147, 590–611.

[17] Saad, B., Bari, M.F., Saleh, M.I., Ahmad, K., and Talib, M.K.M., 2005, Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs

using high-performance liquid chromatography, *J. Chromatogr. A*, 1073 (1-2), 393–397.
[18] Heyrman, A.N., and Henry, R.A., 1999, Importance of controlling mobile phase pH in reversed phase HPLC, *Keystone Technical Bulletin*, 99-06, 1–7.
[19] Moldoveanu, S.C., and David, V., 2017, "Properties of Analytes and Matrices Determining HPLC Selection" in *Selection of the HPLC Method in Chemical Analysis*, Elsevier, Boston, 189–230.
[20] Shabir, G.A., 2003, Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization, *J. Chromatogr. A*, 987 (1-2), 57–66.
[21] Richardson, R.K., and Gray, I.K., 1981, The levels of natural benzoic acid in casein and whey by-products, *N. Z. J. Dairy Sci.*, 16, 179–186.
[22] Jordan Standards, 2002, Milk and milk products – Labaneh, 108/2002, *Jordan Standards and Metrology Organization (JSMO)*, Amman, Jordan.
[23] Smith, J., 1991, "Legislative Aspects" in *Food Preservatives*, Eds. Russell, N.J., and Gould, G.W., AVI, New York, 321–347.
[24] Stratford, M., and Eklund, T., 1991, "Organic Acids and Esters" in *Food Preservatives*, Eds. Russell, N.J., and Gould, G.W., AVI, New York, 48–84.
[25] Jordan Standards, 2008, Vegetables, fruits & derived products – Pickles, 73/2008, *Jordan Standards and Metrology Organization (JSMO)*, Amman, Jordan.
[26] Jordan Standards, 2012, Drinks and juices, 1761/2012, *Jordan Standards and Metrology Organization (JSMO)*, Amman, Jordan.