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Determination of sorbic and benzoic acids as preservatives in some milk products by HPLC

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ANSTRACT

The aim of this study to determine sorbic and benzoic organic acids as preservatives in some dairy products. Therefore, 120 dairy product samples of feta cheese, processed cheese, milk powder and butter (30 for each) were collected from supermarkets and examined for the presence of sorbic and benzoic acids using high performance liquid chromatography. The results revealed that sorbic acid concentration in feta cheese samples ranged from 211.2 to 1330.4 ppm with mean values 631.42 ± 50.44. Of the toal, 3 samples (10%) were exceeding the permissible limits of NFSA decree 4/2020 and codex standard 192/2019 (1000 ppm). In addition, sorbic acid detected in the examined processed cheese samples with concentration ranged from 599.65 to 2884.43 ppm with mean value 1105.43 ± 92.75, and all positive samples werewithin the permissible limit set by NFSA decree 4/2020 and codex standard 192/2019(3000 ppm). While, sorbic acid is not detected in any milk powder and butter samples. On the other hand, benzoic acid was not detected in all examined samples. Because of the well-established hazardous consequences of these compounds by public health agencies, the findings underline the significance of more stringent monitoring of these preservatives

INTRODUCTION:

The Food and Agriculture Organization (FAO) defines a preservative as any substance added to food to prevent or delay the loss of nutrients dueto microbial, enzymatic or chemical changes in the food as part of extending the shelf life of the product so that, the milk remains sound and of hight nutritional value. Chemical preservatives, such as benzoic or sorbic acid or its salts are used in dairy products to inhibit the growth of yeast and mold and to effectively combat a varietyof bacteria during storage **(Kucukcetin et al. 2008)**.

Sorbic acid, a linear unsaturated fatty acid with a molecular weight of 112.13 g/mol and a molecular formula ($C5H7COOH$), is common-

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ly employed as a food preservative (E200) due to its lack of odor and flavor at the suitable concentration. It is available commercially in powder or granular form. The carboxyl group (COOH) in sorbic acid is very reactive, forming salts with calcium, sodium, and potassium. Potassium sorbate salt is available commercially in powder or granular form. In addition to sorbic acid, sodium, potassium, and calcium sorbic acid salts (E201, E202, and E203, respectively) may be utilized. Salts are often used because they have similar antimicrobial properties and are more soluble in water **(Russell and Gould, 2003; Silva and Lidon, 2016).**

Benzoic acid, a white (or colorless) solid organic molecule having the molecular formula C6H5COOH, has been employed as both a food preservative (E210) and an antioxidant. The structure consists of a benzene ring (C6H6) with a carboxyl substituent (−C(=O)OH). Benzoic acid is found naturally in many plants and acts as an intermediary in the biosynthesis of numerous secondary metabolites. Benzoates are employed as food preservatives, namely sodium benzoate (E 211), potassium benzoate (E 212), and calcium benzoate (E 213). Benzoic acid is a key precursor in the industrial synthesis of numerous other chemical compounds. Benzoates refer to benzoic acid salts and esters **(Chipley, 2020)** .

The toxicity of benzoates and sorbates to mammals is quite low. They are widely recognized to be fundamentally non-carcinogenic, but they can be transformed into potential mutagens (Piper and Piper 2017). The safety of these substances has been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). As a result, the Acceptable Daily Intake (ADI) is 0-5 mg/kg body weight/day for benzoic acid and benzoates and 0-25 mg/kg body weight/day for sorbic acid and sorbates **(WHO, 2016).**

Nonetheless, some studies have concluded that benzoates and sorbates pose health risks after demonstrating that exposure of tested mice to sorbate and/or benzoate resulted in general genomic injuries in almost all liver cell samples of pregnant rats and their fetuses when compared to the non-treated group. They stated that usage may induce DNA damage and promote micronuclei production. Therefore, they recommended that pregnant women avoid consuming commodities containing these chemicals **(Saatci et al. 2016).**

Lanthanide-sensitized luminescence, potentiometric, spectrophotometric, capillary zone electrophoresis, high performance thin layer chromatography, and LCMS/MS are some of the methods for determining benzoic and sorbic acids in food products. The most prevalent method employed in the food sector today is high performance liquid chromatography (HPLC) **(Lozana et al. 2007; Timofeeva et al. 2018).** The purpose of this study was to determine sorbic and benzoic acids in certain milk products using the HPLC method .

MATERIALS and METHODS

Sorbic and benzoic acids were determined according to **ISO 9231/2008.**

Reagents and Materials

Sorbic and benzoic acids certified reference material (99%), Glacial acetic acid and methanol HPLC grade were purchased from Sigma- Aldrich (El Masry Center company Potassium hexacyanoferrate (II), Zinc acetate, potassium dihydrogen phosphate (KH2PO4), potassium hydrogen phosphate, Sodium hydroxide were purchased from LOBA chem (El Tegara Eldowlya company).

Concentrated sulfuric acid was purchased from Elgomhoray company.

Instrumentation:

The samples were analyzed on HPLC (Ingos manufacturing Co. Ltd)equipped with a UV/visible detector, the separation was performed on theC18 column (250×4.6 mm id×5 µm particle diameter).

Calibrated automatic pipettes and analytical balance were used for the volume and weight measurement.

Water bath and pH meter used in sample preparation

Chromatographic condition:

Sorbic acid and benzoic acid were analyzed using a C18 column and mobile phase (10 volumes of methanol + 90 volumes of phosphate buffer). Prepare a phosphate buffer solution by dissolving 2.5 g of potassium dihydrogen phosphate (KH2PO4) and 2.5 g of potassium hydrogen phosphate trihydrate (K2HPO4·3H2O) in 1000 ml of water. Filter the solution. The injection volume was 20 µL, and the flow rate was maintained at 1.2 mL/min. The column temperature was maintained at 25 °C, with a UV wavelength of 227 for benzoic acid and 250 nm for sorbic acid. All samples and spikes were run in triplicate and the average results are reported .

Preparation of stock and working standard solution

50 mg of sorbic or benzoic acid CRM was weighted in 100 ml volumetricflask and diluted with methanol to the mark, the solution stored for 3wks in refrigerator. The working standard solution was prepared by pipette 5 ml of stock solution in 250 ml volumetric flask and completed with methanol deionized water (1:1),the result working solution contain 10 ppb

Sample and spike preparation

In 100 ml beaker, a mass of 3g of sample was weighted and dispersed by 10 ml distilled water and stirred by glass rod. 25 ml sodium hydroxide (0.1N) was added and then the beaker incubated in water bath at 70 ^{0}C for 15 minutes. After cooling the sample solution was adjusted to pH 8 ±1 by using sulfuric acid 0.5 M while mixed. Then, 2ml of potassium hexacyanoferrate (II) solution (10.6 g of potassium hexacyanoferrate (II) trihydrate/ 100 ml D.W.) and 2 ml of zinc acetate solution (21.9 g of zinc acetate dihydrate and 32 ml of acetic acid glacial/ 100 ml D.W.) were added, then mixedthe content and stand for about 15 minutes, after incubation the volume was completed to the mark (100 ml) by methanol and stand for 15minutes. The content was filtrated by watman filter paper and then passed through 0.45 micrometer nylon filter. The sample was injected into HPLC instrument. For spiking preparation of 2000, 1500, 1000, 500 and 250 ppm (2g, 1.5g, 1g, 0.5g and 0.25 g) for sorbic acid and 3000, 1500, 500 and 250 ppm (3 g, 1.5 g, 0.5 g and 0.25 g) for benzoic acid on kg of homemade manufactured cheese (free of the two preservatives). 3 g of prepared cheese was weighted and the previous steps for sample preparation were applied. Blank applied with cheese sample without any additives

RESULTS:

Figure 1. Typical HPLC chromatograms and retention time of sorbic acid.

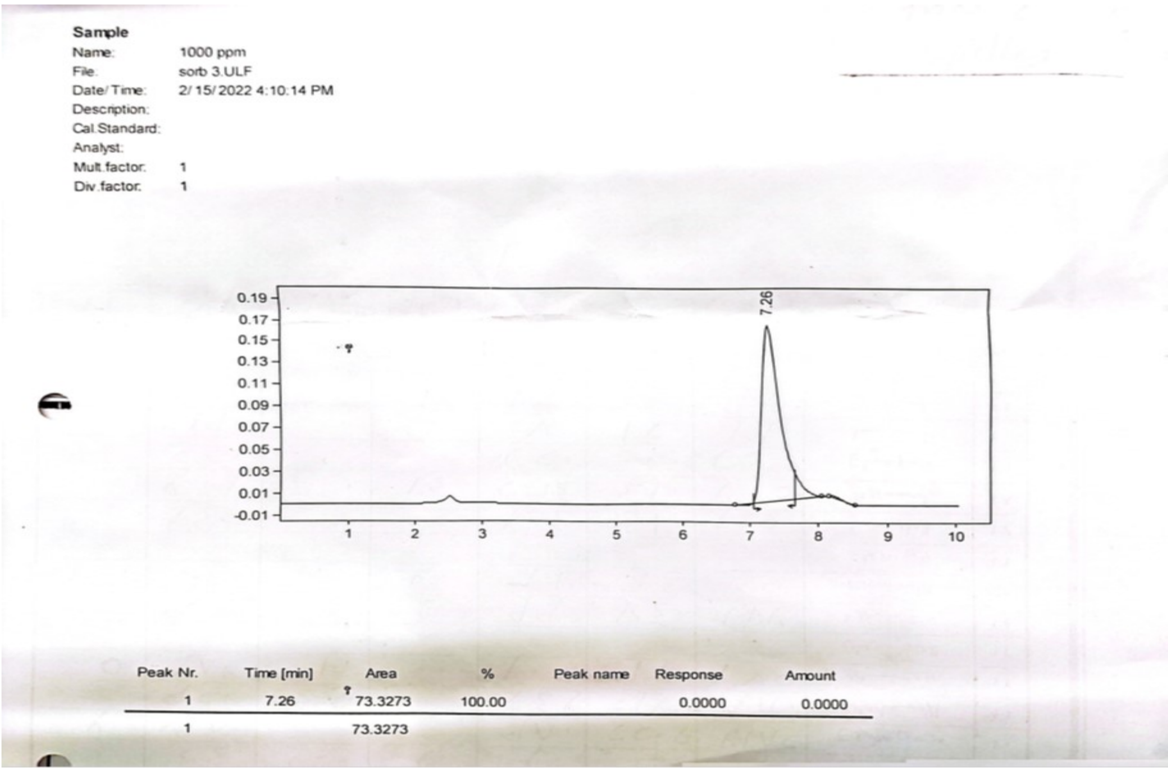

Figure 2 Typical HPLC chromatograms and retention time of benzoic acid.

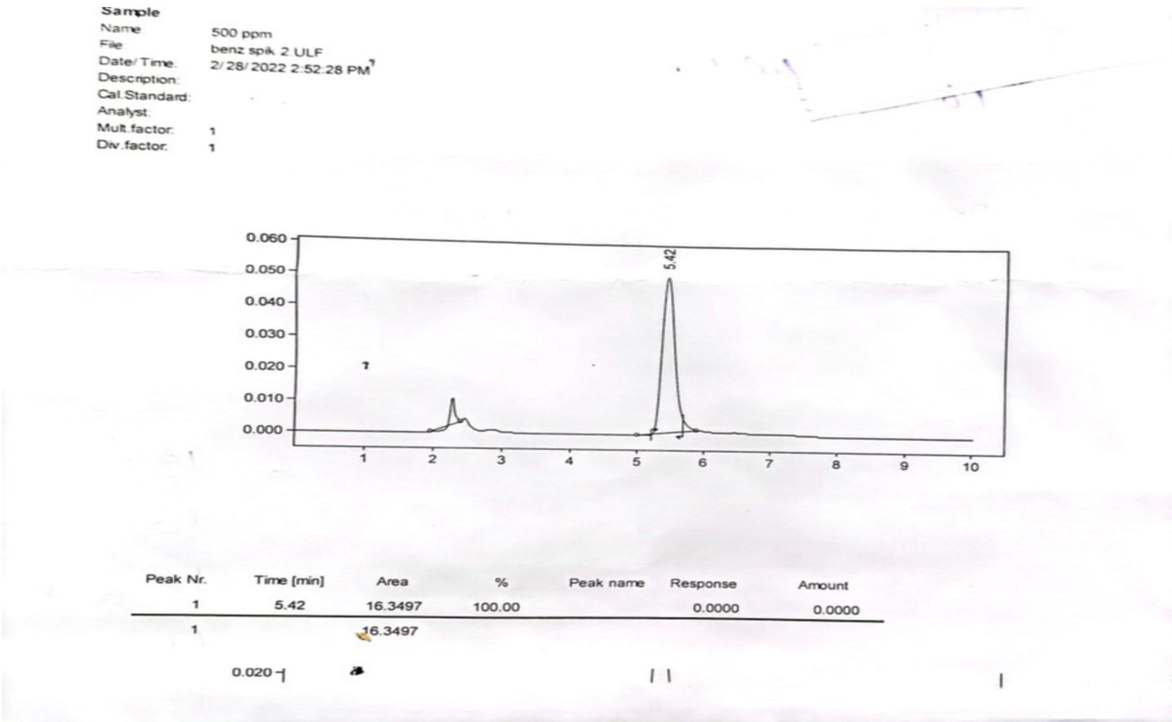

Table 1. Existing limits of sorbic and benzoic acids (Mean ± SE) expressed as ppm in examined dairy products samples (n=30 of each product)

Product type	Sorbic acid (ppm)			Benzoic acid (ppm)		
	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Processed cheese	599.65	2884.43	1105.43 ± 92.75	ND		
Feta cheese	211.2	1330.4	631.42 ± 50.44	ND		
Butter	ND			ND		
Milk powder	ND			ND		

N.B Sorbic acid permissible limit is 1000 ppm in feta cheese and 3000 ppm in processedcheese a cording to NFSA decree no 4/2020
ND: not detected sample

Figure 3. The mean concentration of sorbic and benzoic acids in different dairy products (ppm).

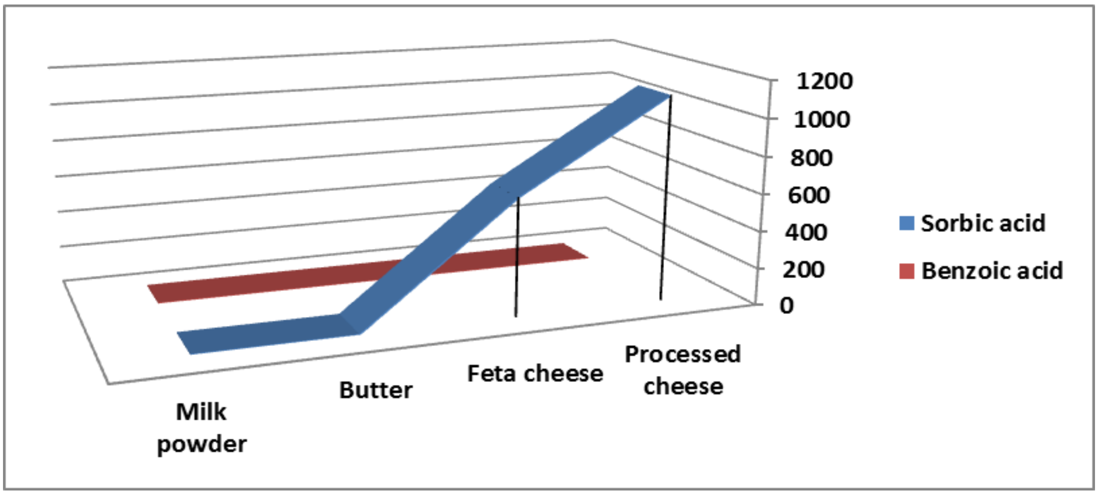

Table 2. Percentage of accepted and rejected samples based on sorbic and benzoic acids (NFSA, 2020) (n=30 of each product).

Product type	Sorbic acid				Benzoic acid			
	Rejected samples		Accepted samples		Rejected samples		Accepted samples	
	No	%	No	%	No	%	No	%
Processed cheese	0	0.0	30	100	0	0.0	30	100
Feta cheese	3	10	27	90	0	0.0	30	100
Butter	0	0.0	30	100	0	0.0	30	100
Milk powder	0	0.0	30	100	0	0.0	30	100

Sorbic acid permissible limit is 1000 ppm in feta cheese and 3000 ppm in processed cheese according to National Food Safety Authority (NFSA decree no 4/2020.)

DISCUSSION:

Benzoic and sorbic organic acids and its salts are commonly used as preservatives in food, pharmaceutical and cosmetic items. They are considered inhibitory materials to a wide range of bacteria, yeast and moulds (**Colmenero and Solana, 2008).** Some manufacturers using food preservatives to improve their hygienic production taking into consediration the regulating rules and permissible limits. A high-performanceliquid chromatography method was applied for the determination of the levels of benzoates and sorbates in examined dairy products samples (n=120)

The chromatographic analysis was carried out by an HPLC system from Ingos company (chromulan software) with LOQ 5mg/kg in fast duration period about 10 minutes. The detection using HPLC method was rapid and more accurate than other spectrophotometric method. Sorbicacid analytes separated in retention time 7.5 ± 0.5 minutes (figure 1).while, benzoic acid separated in retention time 5.3 ± 0.1 minutes (figure 2). The mean recovery of sorbic acid analysis was 81.7% while the mean recovery of benzoic acid analysis was109 %.

The results presented in table (1, 2) and figure (3) showed that sorbic acid concentration in the examined processed cheese samples ranged from 599.65 to 2884.43 ppm with mean values 1105.43 ± 92.75 ppm. All positive samples werewithin the permissible limit set by **NFSA decree 4/2020** and **Codex Standard 192/2019** (3000 ppm). The obtained results were higher than that recorded by **Koyuncu and Uylaser (2009)** who demonstrated that sorbic acid concentration ranged from 2.09 to 1133 ppm in examined cheese samples**. Küçükçetin et al. (2008)** summarized that sorbates ranged from 112.7-688.1 mg/kg**; Amirpour et al. (2015)** concluded that sorbic acid is not detected in the examined cheese samples and **Özdemir et al. (2020)** who found that levels of sorbic acid in the analyzed samples were between 21.3 mg/kg and 511.3 mg/kg, even though all the previous researchers results in in accordance with their country permissible limits which mostly 1000 ppm. On the other sides, the results in this study were lower than results reported by **Moawad and Ibrahim, (2023)** who revealed that 21%of the examined processed cheese samples are in disagreement with the lable. The actual concentration of additives or preservatives materials should be clearly stated on the label, as researches and regulatory authorities can detect any tampering that could affect consumer particularly for daily dietary usage **(Moawad and Ibrahim, 2023**).

In addition, the results obtained in table (1,2) and figure (3) showed that sorbic acid concentration in the examined feta cheese samples ranged from 211.2 to 1330.4 ppm with mean values 631.42 ± 50.44 ppm. Out of (30) positive feta cheese samples 3samples (10%) were exceeding the permisable limits set by **NFSA decree 4/2020** and **Codex Standard 192/2019** (1000 ppm). This results were higher than findings reported by **Gul and Dervisoglu, (2013)** who demonstrated that sorbic acid concentration ranged from 0 to 96.39 ppm in examined cheese samples**, Amirpour et al. (2015)** declared that sorbic acid is not detected in the examined cheese samples**, Salehi et al. (2017)** thus indicated that sorbates levels ranged from levels ranging 101.59 to 996.29 mg/kg and **Zamani et al. 2017** described that levels of sorbic acid in the analyzed samples were between 0 mg/kg and 247.36mg/kg, thus all the previous researchers results is in accordance with their country permissible limits mostly 1000 ppm. The Acceptable Daily Intake reports: 0-5 mg/kg body weight/day for benzoic acid and 0-25 mg/kg body weight/day for sorbic acid (E 200), potassium sorbate (E 202) and calcium sorbate (E 203) **(WHO, 2016)**. Some studies concluded that an increase in the dose above daily intake was found to be the reason for chronic urticarial and atopic dermatitis **(Bush and Taylor 2014**)

Conversely, the results in this study showed that sorbic acid is not detected in milk powder and butter samples. Our results of examined samples were in agreement with regulations set by **NFSA decree 4/2020** and **Codex Standard 192/2019** (free from any preservatives) for butter and (4000 ppm) for milk powder. The results were nearly close to results demonstrated by **Choi et al. (2007)** who concluded that sorbic acid is not detected in the examined samples.

In addition, data represented in table (1, 2) and figure (3) revealed that benzoic acid was not detected in all examined samples. The results were similar to results demonstrated by **Moawad and Ibrahim, (2023).** While lower than the recorded data by **Küçükçetin et al., (2008)** who revealed that benzoic acid concentration was ranged from 28.6 to 425.3 mg /kg and **Leth et al. (2010)** thus concluded that benzoic acid concentration ranged from 11 to 18 mg/ kg in examined dairy product samples, **Amirpour et al. (2015)** who reported that benzoic acid is detected in (67.5%) of the examined dairy products samples, also **Yerlikaya et al. (2021)** who studied that benzoic acid concentration in fresh kashar cheese samples ranged from 5.95 to 9.14 mg/kg. While in feta cheese ranged from 12.15 to 41.05 mg/kg. They added that benzoic acid concentration in ripened cheese samples ranged from 35.42 to 55.12 mg/kg.

There are several reasons why the Egyptian dairy industry uses sorbic acid and its salts instead of benzoic acid. First, sorbic acid and its salts have less harmful effects than benzoic acid and its salts because, like some fatty acids (such as butyric acid and caproic acid), they are rapidly metabolized in human and animal tissues **(Koyuncu and Uylaser, 2009).** Another reason is that benzoic acid and its salts require a highly acidic environment **(Chipley 2020)**

From the obtained results in the present study it could be concluded that the use of sorbic and benzoic acids or its salts by dairy companies should be supervised by the Egyptian authorities periodically to confirm are not exceed the permissible limit of addition and acceptable daily intake in dairy products. In addition, the examination of sorbic and benzoic acids should be inserted in the routine examination of dairy products. Application of good manufacture practices and better control of environmental conditions during storage and retail are warranted to reduce or eliminate food preservatives use in dairy products production to meet consumer demands for reduced and/or preservatives free food products

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