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Sodium Benzoate and Potassium Sorbate in Processed Meat Products Collected in Ho Chi Minh City, Vietnam

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***Abstract*— Sodium benzoate and potassium sorbates are the two typical preservatives widely used in Vietnam and other countries. The maximum level (ML) of sodium benzoate and potassium sorbate in processed meat products imposed by Ministry of Public Health is 1000 ppm. Although there are warnings about overusing of these preservatives that related to human health, many manufacturers do not follow the regulations. The aim of this study was to survey and consider the amounts and presence of these preservatives in processed meat products by using High-performance liquid chromatography (HPLC) method. 90 samples of brands and no brands including Vietnamese pork rolls, pâtés, hams, sausages, and fermented pork rolls that available at markets in Ho Chi Minh City were analysed for these two preservatives. There was a preference for using sodium benzoate in all samples. Moreover, Vietnamese pork roll samples had the highest percentage of samples with preservatives concentrations exceeding the ML. Among 90 samples, sodium benzoate was detected in 52.2% of samples and 17.8% of them exceeded the ML, while potassium sorbate was found in 24.4% of samples and only 2.2% of them exceeded the regulated amount. 46.4% of Vietnamese pork rolls, 12.5% of pâtés, and 9.1% of fermented pork rolls had sodium benzoate exceeded the ML, whilst ham and sausage samples contained the concentrations inside the safety limits. Furthermore, only one sample of Vietnamese pork rolls and one sample of sausages had potassium sorbate concentrations surpassing the ML with the level of 1,717.57 mg/kg and 1,814.00 mg/kg, respectively. Furthermore, branded samples showed a significantly different result in compared with no branded samples. Of branded samples, exceeding sodium benzoate level ML was detected in 10% of samples and none of the samples found surpassing potassium sorbate limit amount. Nevertheless, 27.5% and 5% of unbranded samples had sodium benzoate and potassium sorbate higher than the authorized amounts, respectively. New strategies should be established to monitor the food safety issues.**

***Keywords*— Processed meat products; sodium benzoate; potassium sorbate; HPLC**

I. INTRODUCTION

The consumption of processed meat products is more preferred by consumers in Vietnam, especially in Ho Chi Minh City. One problem associated with these products is its short shelf life due to a high level of nutrients, microbiological enzymatic or chemical changes occur during the storage period. These food products consist of a variety of microbes such as bacteria, yeasts, and molds which have been reported as the original agents of foodborne diseases or food spoilage [1]. As a consequence, the use of preservatives has become more outstanding in the food industry, especially in processed and convenience foodstuffs [2], [3]. The most commonly used preservatives are sodium benzoate and potassium sorbate [4], [5]

The consequence of food preservatives for consumers has always been a health safety concern [6]. Although sodium benzoate and potassium sorbate are generally recognized as safe (GRAS) but the increase of allergic reactions to benzoate in humans, such as urticaria, rash, migraine, hyperkalemia, non-immunological contact urticaria, metabolic acidosis, convulsions, hyperpnea, weak clastogenic activity and asthma has been reported in some studies [5]-[8], [20]. Potassium sorbate has a relatively low toxicity in humans. Moreover, if the concentration of sodium benzoate exceeds the regulated amounts, it may cause hyperactive behavior in children when combined with some artificial colors [9]. In human, some evidence of idiosyncratic intolerance to sorbate salts have been addressed [5]. In addition, potassium sorbate combined with ascorbic acid (vitamin C) is a chance for mutagenicity developed and DNA-damaging activity [10], while sodium benzoate causes carcinogenesis due to the cause of benzene, a carcinogenic agent. It may cause a danger to public health

if the concentrations of the two preservatives exceed the regulated amounts. Especially, children are the most sensitive group of these preservatives [11]. Therefore, it is necessary to determine the levels of preservatives in various types of processed meat products.

The analytical determination of two preservatives sodium benzoate and potassium sorbate is not only important for quality assurance purposes with regard to managers, and manufacturers in processed meat industry but also for consumer interest and protection. Furthermore, the information of levels of preservatives in processed meat products can support the additional approaches in the control of preservatives usage in processed meat industry.

This study was conducted to determine the concentration of the two preservatives, sodium benzoate and potassium sorbate, in several types of processed meat products, brand and non-brand ones, sold in supermarkets and roadside shops in Ho Chi Minh city by HPLC, the most common method used for determination of sodium benzoate and potassium sorbate.

II. Materials and Methods

A. Materials

A total of 90 samples of processed meat product of branded (declared labels) and unbranded (no declared labels), weighing from 100-250 g, were collected randomly in Ho Chi Minh City, Vietnam and chosen to be representative of what consumers could find easily in a market study. The samples were stored in a box during transportation at ambient temperature before arriving the laboratory. Then, these samples were placed in a refrigerator at 4 ^{o}C to ensure the quality of processed meat products.

B. Mobile Phase Preparation

The mobile phase consisting of 80% formate buffer with 20% HPLC-grade acetonitrile (J.T. Baker, USA) was prepared. The mobile phase was degassed for 10 min in an ultrasonic bath and then used for the sample dilution.

C. Preparation of Standard Solutions

Exactly 10.00 mg of either sodium benzoate and potassium sorbate (Supelco, USA) were dissolved in 10 ml HPLC-grade water (J.T. Baker, USA) to form stock solutions. Stock solutions were then diluted separately with the mobile phase to yield 10.0, 25.0, 50.0, 100.0 and 200.0 mg.L^{-1} of pure sodium benzoate and potassium sorbate solutions.

D. Sample Preparation

Each sample was blended with a sample homogenizer and 2.5 g of each sample were weighed into a 50 ml polypropylene centrifuge tube and 15 ml distilled water were added. The sample was shaken with a vortex mixer and then with an automatic shaker (Boeco, Germany) at 400 rpm for 10 min. After that, the centrifuge tube was put in an ultrasonic bath (VWR-Scientific, Germany) at ambient temperature for 10 min. Then 2.5 ml of $Zn(CH_3COO)_2$ 20% (Merck, Germany) and 2.5 ml of saturated Na_2HPO_4 were added to precipitate impurities and neutralize the mixture, respectively. The mixture was then shaken with a shaker for 10 min and centrifuged at 2,500xg for 10 min. After centrifugation, the supernatant was pipetted into a new 50 ml centrifuge tube. Next, 15 ml distilled water were added into the first test tube and vortexed for 30 s. $Zn(CH_3COO)_2$ 20% and Na_2HPO_4 were added, and the tube was shaken for 5 min and centrifuged for 10 min at 2500 x g. The supernatants from both the two time extractions were collected in the second tube and transferred to a 50 ml glass volumetric flask to the volume and filtered through a 0.2 μm nylon membrane filter (Alltech, USA).

Finally, sodium benzoate and potassium sorbate were separated by HPLC using C18 column and measured with an UV detector (Agilent 1100, USA) at the maximum absorption wavelength of 235 nm. This wavelength can detect sodium benzoate and potassium sorbate simultaneously with different retention time. The flow rate of the mobile phase was 1.0 ml.min^{-1} and the retention time was 8 min and 10 min for sodium benzoate and potassium sorbate, respectively. The total recording time for the chromatogram was 12.5 min. The injected volume was 20 μl. The peaks of sodium benzoate and potassium sorbate were recorded and identified by comparing the retention time and their spectrum.

E. Analysis of Sodium Benzoate and Potassium Sorbate Concentration

A standard curve was prepared using peak area and concentration of various standard solutions. The linear regression was applied for the quantification of sodium benzoate and potassium sorbate concentrations in the tested samples using Equation 1.

$$y = mx + b \quad (1)$$

With: y = peak area
x=sodium benzoate and potassium sorbate concentrations (ppm)
b = intercept of y

F. Reference Method

Sodium benzoate and potassium sorbate in processed meat samples were determined using the internal approach of Sub-Department of Animal Health of Ho Chi Minh City based on AOAC procedures 983.16 [12].

G. Method Validation

Of the matrices determined, a pork roll sample was composed of a more complex matrix. The pork roll sample was therefore selected as a representative product for the method validation of sodium benzoate and potassium sorbate determination in processed meat foods.

The limits of detection (LOD) and limits of quantification (LOQ) were determined from the calculation of LOD = 3σ and LOQ = 9σ for both sodium benzoate and potassium sorbate, where σ is the standard deviation of the responses. The value of σ was analyzed seven times with blank samples, determining the concentration of analytical background response and then calculating the standard deviation of the responses. The LOD values were found to be 30 and 30 mg.kg-1 and the LOQ values were found to be 90 and 90

mg.kg-1 for sodium benzoate and potassium sorbate, respectively.

Calibration curve of each preservative was established with five concentrations of 10, 25, 50, 100, and 200 $mg.kg^{-1}$. The linearity was achieved by using linear regression analysis of a five-point linear plot consisting of two replicates per point. In the HPLC method, the following equations are the standard curves of sodium benzoate and potassium sorbate given as: $y = 64.53125x + 105.78984$ and $y = 79.91594x + 106.10216$; respectively. The squared correlation coefficients were estimated with the value of $r^2 = 0.9998$ for sodium benzoate and $r^2 = 0.9999$ for potassium sorbate. In order to verify the accuracy and precision of the analytical procedure, the recovery studies for the HPLC method were performed with spiked pork roll samples at three fortification levels 500, 1000, 2000 $mg.kg^{-1}$ with six replicates, respectively. The samples were extracted and analysed six times on the same day according to the described method. The recovery was found as $80\% \leq H\% \leq 110\%$ for both sodium benzoate and potassium sorbate.

Precipitation of proteins and fat in pork roll samples was carried out by the addition of $Zn(CH_3COO)_2$, followed by centrifugation, filtration and temperature factor provided a suitable extract for chromatographic analysis and excellent recoveries. There were less interferences with sodium benzoate and potassium sorbate from background peaks.

For the repeatability test, the relative standard deviation (%RSD) values for sodium benzoate and potassium sorbate, ranging between 1.40-3.42 and 0.87-4.01 respectively, were attained using the corresponding peak area of 6 replicate analysis performed for pork roll samples (Table I).

TABLE I
ANALYTICAL CHARACTERISTICS of THE METHOD BASED ON PORK ROLL MATRIX

Analyte	LOD ($mg.kg^{-1}$)	LOQ ($mg.kg^{-1}$)	Recovery range (%)	%RSD	Linearity	Accreditation
						Yes/No
Sodium benzoate	30	90	80-110	< 8%	0.9998	Yes
Potassium sorbate	30	90	80-110	< 8%	0.9999	Yes

H. Statistical Analysis

The unit of sodium benzoate and potassium sorbate was used to measure is $mg.kg^{-1}$ (ppm). Chi-square test was carried out using Microsoft Excel 2010 and SPSS version 21, at a significance level of 5%. The results showed are the mean concentrations from duplicate analysis.

III. Results and Discussion

A. General Occurrence of Sodium Benzoate and Potassium Sorbate in all Processed Meat Samples

A total of 90 processed meat samples were analysed for the levels of sodium benzoate and potassium sorbate. The results of the preservative analysis in all the processed meat samples are given in Table II. 48 out of 90 samples were sodium benzoate positive. The concentrations ranged from 5.30-4,408.76 $mg.kg^{-1}$. There were 42 samples that had no sodium benzoate while 16 samples showed the preservative levels above the set standard. The percentage of samples with sodium benzoate (53.3%) was slightly higher than the percentage of those without sodium benzoate (46.7%).

TABLE II
CONCENTRATIONS of SODIUM BENZOATE and POTASSIUM SORBATE in PROCESSED MEAT SAMPLES

Preservatives	Number of tested samples	Range of concentration ($mg.kg^{-1}$)	Samples with preservatives		Samples with preservative concentrations exceeded the ML	
			n	Percentage (%)	n	Percentage (%)
Sodium benzoate	90	5.30-4,408.76	48	53.3	16	17.8
Potassium sorbate	90	5.55-1,814.00	22	24.4	2	2.2

n: number of samples.

Moreover, potassium sorbate was found in 22 samples of total 90 samples. The concentrations of potassium sorbate detected in the tested samples ranged from 5.55-1,814.00 $mg.kg^{-1}$. Only two samples (2.2%) of processed meat had potassium sorbate in a quantity above the maximum level set by the Vietnam's Ministry of Health [14]. In the study of Amirpour et al. [21], sodium benzoate is used more frequently than potassium sorbate, the mean concentrations of sodium benzoate and potassium sorbate were 46.7 ± 43.0 $mg.kg^{-1}$ and 79.2 ± 59.7 $mg.kg^{-1}$ in all tested samples, respectively.

The number of the samples with sodium benzoate concentrations exceeding the ML was one-third of the sodium benzoate positive samples, while the number of the samples with potassium sorbate concentration exceeding the ML was one-tenth of the potassium sorbate positive samples. Moreover, there were 13 of the total 90 samples containing both preservatives. The MLs of sodium benzoate and potassium sorbate are allowed to be used in processed meat products in Vietnam are as the same as those in other countries (1,000 $mg.kg^{-1}$). The two preservatives were identified in all processed meat products whose labels declared these ingredients, and were not identified in the products that the preservatives information was not declared. It can be seen that sodium benzoate was used more popular than potassium sorbate in all samples. At the concentrations of 20-700 $mg.kg^{-1}$ and 10-1,000 $mg.kg^{-1}$ of sodium benzoate and potassium sorbate, respectively, the growth of mold and yeast is inhibited depending on the variation of variety, strains of microorganisms, pH and other factors as well [7], [13].

B. Occurrence of Sodium Benzoate and Potassium Sorbate in Five Categories of Processed Meat Products

All the tested samples were grouped and divided into five categories including Vietnamese pork rolls, pâtés, hams, sausages and fermented pork rolls. The presence of the two preservatives in the tested samples is illustrated in Table III and Table IV.

The statistical analysis results indicated no significant difference between the amounts of potassium sorbate in the five categories of processed meat products ($p>0.05$). However, there was a significant difference among the

amounts of sodium benzoate in five categories of processed meat products ($p<0.05$; data not showed).

TABLE III
CONCENTRATIONS of SODIUM BENZOATE in FIVE TYPES of SAMPLES

Food category	Number of tested samples	Range of concentration ($mg.kg^{-1}$)	Samples with preservatives		Samples with preservative concentrations exceeded the ML	
			n	Percentage (%)	n	Percentage (%)
Vietnamese pork roll	28	13.98-4,408.76	24	85.7	13	46.4
Pâté	16	5.30-1,155.62	9	56.2	2	12.5
Ham	16	6.92-814.20	6	37.5	0	0
Sausage	19	5.33-328.66	7	36.8	0	0
Fermented pork roll	11	44.6-2,363.18	2	18.2	1	9.1

n: number of samples.

TABLE IV
CONCENTRATIONS of POTASSIUM SORBATE in FIVE TYPES of SAMPLES

Food category	Number of tested samples	Range of concentration ($mg.kg^{-1}$)	Samples with preservatives		Samples with preservative concentrations exceed the ML	
			n	Percentage (%)	n	Percentage (%)
Vietnamese pork roll	28	5.39-1,717.57	7	25	1	3.6
Pâté	16	nd	0	0	0	0
Ham	16	9.92-163.44	7	43.7	0	0
Sausage	19	5.55-1,814.00	4	21.1	1	5.3
Fermented pork roll	11	36.10-318.62	4	36.4	0	0

n: number of samples, nd: not detectable.

Benzoic acid and sorbic acid were found in some meat products such as Vietnamese pork rolls and fermented pork rolls in Quang Binh [15]. The ML of benzoic acid and sorbic acid in processed meat products is 0.1% as well. 35.7% of Vietnamese pork roll samples and 50% of fermented pork roll samples had the sodium benzoate levels surpassing the regulated levels. Moreover, 77.8% of Vietnamese pork roll samples and 36.4% of fermented pork roll samples had the concentrations of potassium sorbate exceeding the authorized amounts. It can be seen that the concentrations of potassium sorbate used in the five types of processed meat samples were much lower than those of sodium benzoate. Potassium sorbate was considered to be more effective than other benzoate salts [16]. When sodium benzoate and potassium sorbate were used in combination, only 0.05% of sodium benzoate and 0.05% of potassium sorbate can inhibit a wide range of bacteria, especially *L. monocytogenes* [17]. Moreover, if sodium benzoate and potassium sorbate are used in the same type of beverages, the regulated amounts are 150 $mg.L^{-1}$ and 250 $mg.L^{-1}$, respectively [19].

C. Occurrence of Sodium Benzoate and Potassium Sorbate in Branded and Unbranded Processed Meat Products

1) Occurrence of Sodium Benzoate and Potassium Sorbate in Branded Processed Meat Products

Out of the 50 branded samples analysed, 6 samples contained both sodium benzoate and potassium sorbate with the concentrations within the standard levels (Table V). 19 samples contained sodium benzoate in the range of 6.92 to 3,681.56 $mg.kg^{-1}$ including 4 Vietnamese pork roll and 1 pâté samples with the preservative concentrations exceeded the ML, while none of the samples contained potassium sorbate concentration exceeding the ML. The potassium sorbate positive samples had the concentrations ranging from 5.39 to 386.60 $mg.kg^{-1}$. The highest exceeding concentration of sodium benzoate was nearly four times higher than the ML.

TABLE V
SODIUM BENZOATE and POTASSIUM SORBATE USED in BRANDED MEAT SAMPLES

Preservatives	Number of tested samples	Range of concentration ($mg.kg^{-1}$)	Samples with preservatives		Samples with preservative concentrations exceeded the ML	
			N	Percentage (%)	n	Percentage (%)
Sodium benzoate	50	6.92-3,681.56	19	36	5	10
Potassium sorbate	50	5.39-386.60	10	20	0	0

n: number of samples

2) Occurrence of Sodium Benzoate and Potassium Sorbate in Unbranded Processed Meat Products

Among 40 unbranded samples analysed, 29 samples contained concentrations of sodium benzoate between 5.30 to 4,408.76 $mg.kg^{-1}$ and 12 samples contained concentrations of potassium sorbate between 5.55 to 1,814.00 $mg.kg^{-1}$ (Table VI).

TABLE VI
SODIUM BENZOATE and POTASSIUM SORBATE USED in SAMPLES of NO BRANDS

Preservatives	Number of tested samples	Range of concentration ($mg.kg^{-1}$)	Samples with preservatives		Samples with preservative concentrations exceeded the ML	
			n	Percentage (%)	n	Percentage (%)
Sodium benzoate	40	5.30-4,408.76	29	72.5	11	27.5
Potassium sorbate	40	5.55-1,814.00	12	30	2	5

n: number of samples

11 samples including 9 Vietnamese pork rolls, 1 fermented pork roll and 1 pâté samples contained sodium benzoate that exceeded the acceptable amounts, while only 1 Vietnamese pork roll and 1 sausage sample had potassium sorbate levels exceeding the ML. The concentrations of

sodium benzoate in these samples were four times higher than the ML, while the concentrations of potassium sorbate were twice as high as the ML. The number of samples contained both sodium benzoate and potassium sorbate were 7 samples. The statistical analysis results indicated no significant difference between the amounts of potassium sorbate in processed meat products of the branded and unbranded groups ($p>0.05$). However, there was a significant difference among the amounts of sodium benzoate in processed meat products of the two groups ($p<0.05$).

Generally, both sodium benzoate and potassium sorbate were used for the preservation of branded and unbranded processed meat products. Nonetheless, number of branded processed meat products had preservatives concentrations exceeded the ML was much lower in compared with unbranded products.

In the study of Pylypiw and Grether [18], food samples were categorized in two groups including products that enclosed the preservatives sodium benzoate and potassium sorbate on labels and products with "preservatives free" labels. Of declared label samples, sodium benzoate was determined in the the range of 330-350 $mg.kg^{-1}$ for soy sauce, 200-250 $mg.kg^{-1}$ for cola soda and 570 $mg.kg^{-1}$ for Chinese mustard. Potassium sorbate were found in apple juice, grape juice and apple cider with concentrations between 130-350 mg.kg-1, 230-450 $mg.kg^{-1}$ and 120-650 $mg.kg^{-1}$, respectively. In contrast, of samples that declared with "preservatives free" labels, sodium benzoate was found in cranberry juice with concentrations ranged from 12-38 $mg.kg^{-1}$, and potassium sorbate level were 410 $mg.kg^{-1}$ of strawberry cream cheese.

The study of Zor et al. [19] showed that the concentration of potassium sorbate ranged from 55.69 $mg.L^{-1}$ in lemon sauce I to 267.79 $mg.L^{-1}$ in lemonade I, while sodium benzoate concentrations ranged from 105.47 $mg.L^{-1}$ in lemon sauce I to 159.34 $mg.L^{-1}$ in lemonade I. Both results showed the amount of the two preservatives were higher than the regulated values.

Commonly, sodium benzoate and potassium sorbate are considered as GRAS with the acceptable levels of 0.1% in food. However, in some countries, these preservatives are not allowed to be used, in Switzerland, for instance [2]. High concentrations of these preservatives can cause skin rashes, flatulence and dyspepsia in a short period [15]. However, if these preservatives are consumed in long period, liver and kidney will be damaged. Sodium benzoate can destroy and switch off important parts of DNA, which causes Parkinson disease and neurodegenerative syndromes.

IV. Conclusions and Recommendations

Sodium benzoate and potassium sorbate with the concentrations that exceeded the permissible levels were evidently determined in processed meat products, both branded and unbranded ones. Vietnamese pork roll samples had the highest percentage of samples with sodium benzoate and potassium sorbate concentrations exceeding the ML. Almost the unbranded samples contained higher preservative concentrations exceeding the ML compared to the branded samples.

The sample size for detection was limited. Further study should be operated in a sizable scale to give a precise assessment about the use of food preservatives in processed meat products. Furthermore, it is essential to quantify some other preservatives, which commonly used in processed meat products such as potassium nitrate, sodium erythorbate, sodium triphosphate to notify the reality of preservatives usage. The companies and premises that had the products with preservative concentrations exceeding the ML should be frequently checked to ensure the food safety. Strict bans and financial punishment should be applied to these companies and premises.

It is suggested that the government and the Ministry of Public of Health should have more training courses for the use of food preservatives. However, food processors and manufacturers must have good knowledge and update continuously the knowledge about preservatives and side effects of them on human health. Consumers should buy products in the markets or supermarkets with preservatives on the labels disclosed obviously because most products are strictly audited by the Department of Animal Health.

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