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Original Article

Simultaneous Determination of Sodium Benzoate, Potassium Sorbate and Natamycin Content in Iranian Yoghurt Drink (Doogh) and the Associated Risk of Their Intake through Doogh Consumption

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Abstract

Background: Regarding the public health concerns over the use of food preservatives in yoghurt drink "Doogh", the aim of this study was the determination of sodium benzoate, potassium sorbate and natamycin in Doogh. Based on Iranian national standard, none of these preservatives are permitted to be used in Doogh.

Methods: A total of 39 Doogh samples were analyzed through RP- HPLC in order to quantify sodium benzoate, potassium sorbate and natamaycin simultaneously. Exposure to each preservative is estimated by mean and maximum concentrations as the residue levels. The per capita Doogh consumption was calculated by the published data from official reports for Doogh annual production in Iran.

Results: All samples were shown to contain sodium benzoate while natamaycin was detected in 10.25% of the samples and potassium sorbate was not detected in any of them. Sodium benzoate concentration extremely varied among the investigated samples ranged from 0.94 to 9.77 mg/l. Due to the result of the exposure estimation, no serious public health concern would exist regarding the mentioned preservatives.

Conclusions: The detection of sodium benzoate in all Doogh samples could indicate the natural production of benzoic acid in yoghurt. Sodium benzoate may be formed through the interaction of the added food grade salt to the Doogh formula which contains benzoic acid. The results of exposure estimation show the lack of health risk within the usage of preservatives in spite of the national regulatory agencies does not permit the preservative use.

Keywords: Sodium benzoate, Potassium sorbate, Natamaycin, Exposure estimation

Introduction

Preservatives are deliberately added to food products in order to stop or delay unwanted microbiological, enzymatic or chemical changes within the extension of the product shelf life. Sodium benzoate, potassium sorbate and natam-ycin are generally used to control mold, inhibit yeast growth and some bacteria (1). Sodium benzoate, C7H5NaO2, is added to a wide range of food products like soft drinks and natural fruit juice with acidic environment. Potassium sorbate, C6H7KO2, has a linear structure with two unsaturated bonds consumed in processed cheese, yogurt, beverage, processed

meat, cake and pastry (2). Natamycin, known as pimaricin, is a polyene macrolide antifungal drug, C33H47NO13. Natamycin is commonly used to delay mold growth in cheese (3).

The use of chemical preservatives in processed food products is well regulated by several safety organizations worldwide. In Iran, Food and Drug Organization (FDO) and Institute of Standards and Industrial Research of Iran issues production licenses for the registered processing units provided that they do not use chemical preser-vatives in their processed products, in this case the yoghurt drink "Doogh" (4, 5).

Doogh is the Iranian traditional fermented drink produced from diluted yoghurt in dairy manufacturing unites. Doogh is made up potable water, yogurt containing microbial culture of *Streptococcus thermophilus* and *Lactobacillus delbriuekii* sp. *bulgaricus* and food grade salt. Homemade Doogh, as a national drink, has a long history in Iran; moreover its industrial type has gained popularity in the recent decades. The annual production of this drink was 3000000 tons in 2010 for domestic consumption and export. Pleasant organoleptic properties as well as several health benefits positively influence the growth rate of Doogh production (6).

Doogh is susceptible to microbial putrefaction. Yeast, mould and some bacteria are identified as the spoilage microorganisms in Doogh because of its acidic pH.

Low microbial quality of raw milk, inadequate heat treatment of milk and improper sanitation conditions in yogurt manufacturing unit could result in high yeast count in the produced yogurt which adversely affects the contamination rate of the final product, Doogh. Water, salt, apparatus, environmental hygiene and packaging material could be regarded as alternative sources of yeast and mold growth which gives rise to such contaminations (7, 8). Cold storage is mandatory to restrict the growth and proliferation of the putrefactive flora. Despite the ban on preservative use in Doogh, some dairy manufacturers may use different preservatives illegally in order to solve the microbial problem. Particularly, the manufacturers use the preservatives in summer to solve the shortage of refrigeration facilities. Therefore, it ensures the reasonable shelf life for Doogh.

Iran confirms the recommendation of the Joint Food and Agriculture Organization/ World Hygiene Organization expert committee on food additives (JECFA) to evaluate the safety of food additives (9, 10).

The objective of this study was the simultaneous quantification of sodium benzoate, potassium sobate and natamycin in commercial bottled Doogh by RP-HPLC as a powerful instrument. Since no report has been previously published on this issue it would be helpful not only for quality assurance purposes in regulatory agencies but also for consumer protection. In addition, risk of exposure to sodium benzoate, potassium sorbate and natamycin measured was characterized in Doogh.

Materials and Methods

In a cross-sectional survey in summer 2012, when Doogh consumption was at its peak, 39 Doogh samples were collected on random basis from 18 different local dairy producers in Isfahan province, Iran. All samples were packaged in polyethylene terphetalate (PET) bottles, delivered and refrigerated in Ebnesina food Laboratory for experimental purposes.

A 10 ml of homogenized Doogh was accurately transferred into a 25-ml volumetric flask and diluted with mixture of buffer acetate 0.1 M (pH= 4.5): methanol (2:1, v/v). The solution was treated by ultra-sonication, centrifuged in 6000 rpm, both for about 10 min. Supernatant was passed through a PTFE 0.45 μm membranes filter (Capital HPLC specialist) and placed into HPLC vials. A 20 μL was injected by auto-sampler into the HPLC system.

The HPLC equipped with photodiode array detector (Waters, US), inlet vacuum degasser was applied to measure preservatives in accordance with the procedure described by Guarino et al. (11). Chromatograms were recorded at 227, 261 and 304 nm for sodium benzoate, potassium sorbate and natamycin, respectively. The data was processed in the Millennium 32 software. The separation was obtained by C18 column (250× 4.6 mm,

4 µm particle size) (Waters, US). The HPLC operational mode was in gradient. Effective quantification was performed in less than 16 min with the eluent flow rate 1 ml/ min.

The data for the per capita Doogh consumption was obtained from the latest report of Agri- Jahad Administration official announcement in 2010 to calculate the exposure estimation (6). The average concentration of each examined samples, containing the preservatives, was considered as the mean. The average amount of the investigated chemicals among the positive samples was taken into account as an alternative approach. For the worst case scenario, the maximum level of determined preservatives was used.

Collected data were analyzed using Statistical Package for Social Sciences (SPSS) version 16. Descriptive statistics and one way ANOVA test were used in the statistical analysis. *P*- value ≤ 0.05 was considered significant.

Results

The results of preservative content are shown in Table 1. Sodium benzoate (<10 mg/ l) was found in all the analyzed samples. Natamycin was found in the amount of 1.25 – 3.56 mg/ l which was more than the permissible level of Iranian standard. No amount of potassium sorbate was detected in the examined samples. There was no significant difference between the three sampling time regarding the mean of sodium benzoate concentration in the tested samples (*P*= 0.943).

Table 1: Content of sodium benzoate, potassium sorbate and natamycin (mg/ l) in Doogh samples

Preservative	July (n=13)a mean±SEM	August (n=13)a mean±SEM	September (n=13)a mean±SEM
Sodium benzoate	5.92± 0.65	5.26± 0.69	5.57±0.62
Potassium sorbate	nd[b]	nd	Nd
Natamycin	1.4±0.15	-	2.64±0.92

a) Number of Doogh sample analyzed
b) Non detectable

The exposure estimation of sodium benzoate and natamycin intake via Doogh consumption is expressed in Table 2. The Doogh consumption was derived from the nominal production of Doogh within the industrially annual production of 3000000 tons in 2010. The result shows even in the worst case condition, where all the utilized Doogh is contaminated with the highest concentration of the preservatives under investigation, the safety concern correspond to the utilized preservatives is negligible.

It should be mentioned that the daily Doogh consumption was considered in the amount of 109 ml for adult and children groups with body weight 60 and 10 kg, respectively (9, 10).

Table 2: Exposure estimation of sodium benzoate and natamycin through Doogh intake in Iranian community

Preservative	Theoretical estimated daily intake[b]		Estimated daily intake[b,1]		Worst case condition[b,2]	
	Adults	Children	Adults	Children	Adults	Children
Sodium benzoate[a]	0	0	10	61	19	114
Natamycin[a]	0	0	3.6	22	64	38

a) Acceptable daily intake is 0-5000 and 0-300 (**µ**g/ Kg bw/d) for sodium benzoate and natamycin, respectively
b) (**µ**g/ Kg bw/d)
1- The average concentration of each preservative was taken as the residue levels
2- The maximum concentration of each preservative was taken as the residue levels

Discussion

Several adverse health effects are associated with the uncontrolled use of chemical food preservatives. In a recent study the presence of sodium benzoate and/ or artificial colors in diet led to hyperactivity in children (12, 13). Some allergic reactions such as urticaria to benzoate have also been reported (14). It has been demonstrated that sodium benzoate and potassium sorbate could suppress some immune responses (15-16). However; natamycin appeared with a margin of safety greater than the aforementioned chemicals (17). Natamycin does not have any toxic, mutagenic, tetratogenic and allergenic effect (18).

According to FDO rules, the production license would be suspended if traces of above mentioned preservatives are detected in the products (5). No reliable information is accessible for the concentration of preservatives in Doogh. In spite of mentioned hazardous effect of preservatives on human health, their safe usage would make the products shelf life expanded by reduction the rate of common microbial spoilage (19-23). It is reported that the commercial Doogh could easily be contaminated by coliform and yeast at 3.2×10^2 and 3×10^4 CFU/ ml population range, respectively; while the corresponding standard limit is 10 and 100 CFU/ ml in Iran (4, 7). Hence it is justifiable that producers use the antimycotic agents such as sodium benzoate, potassium sorbet and natamycin to reduce the rejection rate of the product and ensure commercial the shelf life of Doogh (24, 25).

In this study, traces of sodium benzoate were detected in all Doogh samples. Doogh is composed of 59.3% water, 40% yoghurt and 0.7% potable salt. According to the previous studies, benzoic acid could be found in Yogurt as a natural organic compound. Evidences indicated the presence of benzoic acid in the cultured dairy products (26).Literature review showed the concentration of 32 mg/ kg benzoic acid in yogurt samples as a by-product of fermentation derived by lactic acid and hippuric acid (27). Moreover it was quantified the indigenous benzoic acid from 5.5 to14.7 mg/ kg in the yoghurt (28). In another study, the existence of sodium benzoate in all analyzed Doogh samples was reported based on the natural occurrence of sodium benzoate in Doogh (29). Since sodium benzoate was found in all samples, based on the finding of this study, it could be suggested that it is originated from naturally occurring through the mentioned bioprocess. Unfortunately no information has been reported on benzoic acid content in Iranian plain yoghurt.

Natamycin is an antibiotic produced by aerobic fermentation of *Streptomyces natalensis* and related species. This compound is applied in the cheese surface as a food additive to control the growth of yeasts and molds (10). For the same purposes it can be used in Doogh as well. Natamycin were detected in four Doogh samples of this study.

The acceptable daily intakes of sodium benzoate, potassium sorbate and natamycin are reported at 0-5, 0-25 and 0-0.3 mg/ kg bw, respectively (9, 10). Underlining the results of the present study, the acceptable daily intakes of the preservatives in the industrially produced Doogh does not convey any safety risk. Due to lack of comprehensive survey for national consumption of Doogh, it was not possible to study the intake of highest amount of consumption for preservatives through Doogh and it was missed in the evaluation of the risk. Moreover, it was not possible to measure the real added sodium benzoate by manufacturers since no reference exists for benzoic acid content in Iranian yoghurt.

Conclusion

The existence of sodium benzoate in all Doogh samples is inevitable based on the natural existence of benzoic acid in yoghurt and its interaction with added food grade salt to Doogh formula. Natamycin as a food preservative is introduced to Doogh in order to control microbial contamination. No potassium sorbate was detected in Doogh samples. The evaluation of exposure estimation for sodium benzoate, potassium sorbate and natamyicn showed the lack of health risk

within the usage of analyzed amounts for preservatives.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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