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Review Article

Comparative Assessment of National and International Standards for Benzoic Acid and Its Derivatives in Different Types of Iranian Foods: A Systematic Review

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Abstract

Background: The use of benzoic acid (BA) and its derivatives as food preservatives raises significant food safety concerns, as they may have potential adverse health effects.

Method: In this systematic review, BA content was examined in different types of Iranian food with classification of dairy, acidic, beverage, and bakery products. Furthermore, the levels of this preservative compared with Iranian National Standards Organization (INSO) and the Codex Alimentarius Commission (CAC) value. Databases like PubMed, Scopus, and Web of Science were used to extract published studies until Feb 2024.

Results: The limit defined by INSO was stricter than the CAC regarding BA in food. Consequently, the results demonstrated a relatively high level of impermissibility of BA with the INSO limit. However, the food products within the permitted range of BA content included a more significant portion according to the CAC limit. The majority of BA levels found in various Iranian studies exceeded the permissible limits set by the INSO, raising health concerns.

Conclusion: The study's findings suggest that Iranian food authorities should regularly monitor and analyse food products for BA and its derivatives to mitigate potential future health risks and ensure food safety.

Keywords: Benzoic acid; Food; Permissible level; Iran

Introduction

Preservatives can be considered as one of the most important parts of the daily diet. These compounds show antimicrobial effects against microorganisms. However, their usage has been restricted by health organizations due to food safety issues (1). Generally, food additives are only allowed if considered harmless, and their names should indicate on the food packaging (1-3). BA and its derivatives have attracted the attention of researchers due to their positive influences as food preservatives and the capability of microorganism growth inhibition in acidic pH. However, these groups of preservatives can cause health risks for consumers (2). Excessive consumption of BA can

be harmful to consumers, potentially causing various adverse effects such as difficulty breathing, irregular metabolism, seizures, and restlessness in lab animals. In some individuals, high levels of BA can also cause allergic reactions, irritation to the eyes, skin, lungs, and digestive tract (1,2,3).

Choosing the appropriate antimicrobial agent is related to several factors, including the range of antimicrobial activity, chemical properties, food composition, type of processing and storage conditions (4). Improper use of preservatives in various foods can endanger the health of consumers. Therefore, it is important to pay attention to permissible limit and concentration ranges regarding consumption of these compounds, determined by health legislators (4).

In Iran, a non-carcinogenic risk in some samples was found such as mayonnaise, salad and carbonated soft drinks for BA (5). In addition, low level of BA in tomato paste and juice may effect on sensitive people (6). Carbonated drinks significantly contribute in BA transfer to consumers in New Zealand and South Korea (7,8). Although the addition of BA is not allowed, this compound has been found in some products analysed in Iranian studies (6), which shows failure to follow the relevant standards and laws (1,9). According to the results of such studies, it is highlighted the need to assess the value of BA and its derivatives regarding to compliance or non-compliance with the food national and international standards. Thus, this first systematic review of literature aimed to evaluate and compare the BA and its derivatives with INSO and CAC limit in different Iranian foodstuffs.

Methods

Literature Search

The search was conducted based on the guideline for systematic review (PRISMA checklist 2020). Databases like PubMed, Scopus, and Web of Science were used to extract published studies until February, 2024 that evaluated the articles with the keywords "benzoic acid", "benzoate", "food", and "Iran". In addition to the above keywords, the searching of other words such as milk and dairy, ketchup, mayonnaise, pickles, lemon juice, tomato paste, jam, drink, soft drink, bread, and cake were separately examined. It did not perform any restrictions for date. The paper in the form of conference abstracts, letters to the editor and editorials excluded from the investigation. It was checked the reference lists of studies in order to retrieve additional publications. The full text of chosen study investigated completely (Fig.1).

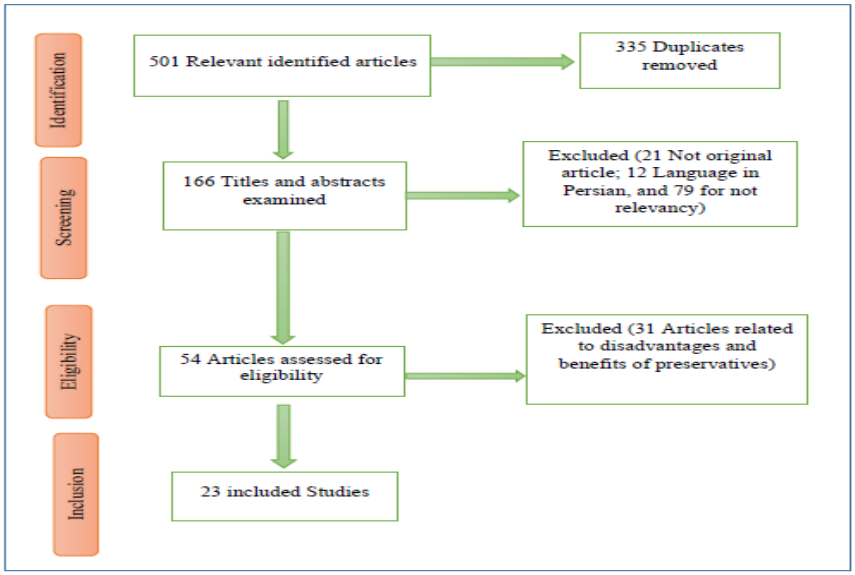

Fig. 1: Flow chart detailing the review process

The included criteria as well as different examined and classified food types are presented in Tables 1 to 3. The range of detected concentrations and its mean amount were presented in several units, which all converted to the same unit of mg/ kg (ppm) in this paper.

Table 1: The amount of BA and its derivatives (ppm) in dairy products studied in Iran

Concentration Range	Sample Size	Type of Sample	City	Reference
ND[1]-135.8	10	Doogh	Shiraz	(62)
0.9-9.8	39	Doogh	Isfahan	(1)
18.3-2345.16	27	Doogh	Tehran	(63)
2.4-40.0	10	Doogh	Tehran	(64)
NM[2]	3 1 1 1 1 1 1	Doogh Yogurt Vegetable Yo-ghurt Creamy cheese Walnut cheese Feta cheese Flavored milk	Tehran	(20)
14.7-30	130	Doogh	Tehran	(4)
18.4-91.2 16.7-84.1 11.9-25.6 15.4-47.6 30-14.9	40 40 40 40 40	UF-Feta Cheese Lighvan cheese lactic cheese Yogurt Doogh	Tehran	(37)
143.5-448.8	15	Doogh	Tabriz	(35)
8.9-28.0	15	UHT milk	Tabriz	(6)
3.0-5.6 1.5-5.0	36 24	Doogh Yoghurt	Isfahan	(27)
ND[1]-58.188 1.735-125.771	19 5	Yogurt Cheese	Tehran	(9)
ND[1]-534.26 ND[1] ND[1]	40 45 45	UF cheese Yogurt Doogh	Hamedan	(21)

[1] Not Detected [2] Not Mentioned

Table 2: The amount of BA and its derivatives (ppm) in acidic food studied in Iran limit

Concentration Range	Sample Size	Type of Sample	City	Reference
71.3-395.0	7	Pickled cucumber	Shiraz	(62)
1.5-4.4 1.5-4.0 180-400	33 25 7	Canned pickled Cucumber canned pickles Bulk pickled cucumber	Markazi	(33)
ND[1] - 38.7	15	Ketchup	Tabriz	(6)
<1.9 ND[1]-73.2 <1.9	40 40 40	Pickled cucumber Tomato paste Sour cherry jam	Tehran	(37)
ND[1]-27.7	15	Tomato paste	Tabriz	(35)
178.8-639.9 ND[1] – 259.4 ND[1]-8.7 ND[1]	3 2 4 3	Mayonnaise Ketchup Tomato paste Lemon juice	Karaj	(38)
6.70-36.10 65.40-85.20 47.60-96.30 330.01-660.02 330.00-660.00	10 6 16 16 16	Canned pickles Pickled cucumber Lemon juice Mayonnaise Salad dressing	Tabriz	(41)
161.68-296.2 ND[1]	15 15	Mayonnaise Tomato paste	Kashan	(5)
NM[2]	26	Olivieh salad	Mashhad	(43)
ND[1]-1896	45	Tomato paste	Qazvin	(39)
NM[2]	60	Pickled cucumber	Tehran	(34)
ND[1]-673	49	Mayonnaise and salad dressing	Urumia	(2)

[1] Not Detected [2] Not Mentioned

Table 3: The amount of BA and its derivatives (ppm) in beverage products studied in Iran

Concentration Range	Sample Size	Type of Sample	City	Reference
NM[1]	5 5 5 5 5	Carbonated sour cherry Carbonated apple Carbonated Cola Carbonated multi-fruit Beverage Carbonated orange	Tehran	(65)
3.5-1520.0	15	Soft drinks	Tabriz	(6)
125-83.2 ND[2]-288.5	40 40	Carbonated soft drink Fruit juice	Tehran	(37)
ND[2]-95.5	15	Fruit juice	Tabriz	(35)
ND[2]-304.29	105	Sports drinks	Tehran	(66)
34.7-359.9 ND[2]	2 2	Soft drinks Fruit juice	Karaj	(38)
ND[2]-58.88	30	Orange juice	Tehran	(3)
44.00-77.01 44.03-72.01 65.90-86.02 42.00-64.10 14.70-58.02 30.40-58.20 16.01-18.70	5 5 6 4 4 4 4	Carbonated Cola Carbonated orange flavored Carbonated lemonade Flavored orange juice Peach juice Pineapple juice Mango juice	Hamedan	(41)
2.12-130	19	Carbonated soft drink	Kashan	(5)

[1] Not Mentioned [2] Not Detected

Results

After searching the databases, 501 relevant articles were downloaded. We removed 335 duplicates, and 166 abstracts as well as titles were enrolled (Fig.1). After examining the full texts and reference lists, 54 eligible studies were chosen for the current review. At this stage, 31 studies for advantages and disadvantages of preservatives were identified and removed. Finally, it was found 23 articles reported the amount of BA and its derivatives in different types of food in Iran. Comparison of the reported amounts of BA and its derivatives in 4 food classifications with the INSO and CAC limits was presented in Fig.2. The highest degree of impermissibility has been observed in studies related to beverage, followed by acidic products and dairy products. Moreover, a clear difference was observed between the studies that showed non-compliance with INSO and CAC values.

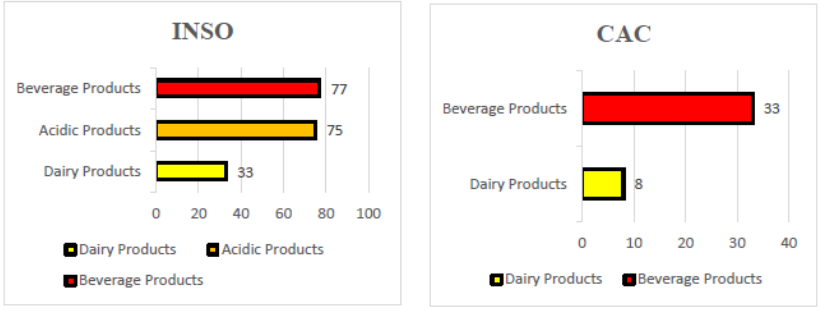

Fig. 2: The percentage of studies reporting the impermissibility of different products due to the presence of BA regarding with permissible limit in INSO and CAC

Discussion

In current study, twenty-three papers were reviewed from different cities of Iran. It was extracted the information related to the amount of BA in different food groups, including milk and dairy, acidic, beverages, and bakery products. After extraction the values of BA, it was compared with INSO and CAC limit. The permissible values declared by INSO were lower and stricter than the CAC.

The amount of BA was identified in most of 355 samples of examined Doogh (Table 1). This dairy based beverage is an indigenous, and fermented drink generally produced by mixing water, salt, yogurt, local herbs and acidified by fermentation process. Totally, BA was present in most of the examined dairy samples. Addition of preservatives to different types of yoghurt, and Doogh is forbidden based on the INSO rule (10-13). The existence of BA has also been reported in Cheese (9). According to INSO rule, it is allowed to add starter, calcium chloride, enzymes, and protein powders to cheese (14). Additionally, INSO has declared that adding any preservative to cream cheese is not allowed (15). The presence of BA in most foods of animal origin is usually minimal unless it adds as a food preservative. However, milk and especially fermented dairy products may naturally contain high amounts of natural BA (1). It was mentioned some reactions like enzymatic-microbial conversion pathway of hippuric acid to BA by lactic acid bacteria, *Escherichia coli* and *Pseudomonas*, decomposition of phenylalanine and its combination with beta-phenylpropionic acid and cinnamic acid, or autoxidation of benzaldehyde by specific strains of lactic acid bacteria during ripening period (16).

Additionally, another reason for the existence of BA can be related to presence of this compound in plant essential oil used as flavouring agent in Doogh (9). The concentration of BA on natural basis usually does not exceed the average values of 30 to 50 ppm in food. In another study, researchers mentioned that fermented dairy products such as yogurt, sour milk with *bifidobacteria*, or kefir usually contain 20 ppm of BA (17,18). Therefore, most of reported values can be considered permissible (Table 1).

In most cheese samples, pH was not in effective range for adding BA as a preservative (9). According to the studies conducted on the investigation of BA in sterile and flavored milk, the preservative was present in all samples that is not allowed base on INSO limit (19). However, the detection of small amounts of benzoate compounds in milk can be ignorable (20).

The range of BA in 669 different samples of dairy products in Iran was between "not detected" to 2345 ppm (Table 1). The highest concentration of preservative was related to Doogh distributed in the city of Tehran. Additionally, the highest average concentration in dairy products was reported 366.73 ppm, estimated in the city of Hamedan for UF feta cheese (21). In studies of other countries, researchers have reported concentrations of BA up to 18.8 ppm and 24 ppm in fermented cow milk in Germany and Lithuania, respectively. At those studies, the presence of BA has attributed to the activity of lactic acid bacteria (22,23). Among 100 cheese samples collected from Italian market and food processing plants, BA was detected in 18% of the samples at the levels between 11.3 and 28.7 ppm (24).

Totally, the value of benzoate compounds was inconsistent with INSO limit in 30% of examined dairy products (Table 1). However, CAC has considered the maximum permissible level for fermented dairy products in 300 ppm (25). Therefore, some of these cases are not permitted according to the INSO limit. In other hands, they were acceptable according to the CAC limit. About 7% of the studies showed the impermissibility of the presence and concentration of BA in dairy products based on CAC limit. It is not provided any document for the permitted range of BA in some products in CAC.

The concentration of BA increases sharply during fermentation and the beginning of the storage period. Afterwards, this compound decreases at the end of the storage period in fermented milk.

Therefore, the sampling time can also be considered as one of the effective factors in the amount of BA in the fermented dairy product (26). The content of BA in milk can be subjected to various factors. This depends on the effect of animal feeding, storage conditions, production situations, hippuric acid content in raw milk, sample size, type of lactic acid starter bacteria, processing method and milking season (1,26). For example, some researchers have attributed the high level of BA in Doogh samples available in the market for high temperature of summer season and non-observance of health principles (1,4). Additionally, the existence of sodium benzoate in food and veterinary medicine can be based on the conversion of hippuric acid to BA by lactic acid bacteria (27). However, benzoate does not remain in the body. It excreted mainly as hippurate through the urine. Still, concerns regarding its use are significant due to the probable adverse effects of high consumption of dairy product in the long term (28).

As acidic foods, it was included ketchup, mayonnaise and salad, pickles; lemon juice, tomato paste, jam, and olivieh salad, which often have a pH of 4.6 or less (Table 2). While adding any preservative to ketchup is prohibited according to the INSO limit, this additive was found in some of this type of product (29). However, the concentration of BA in some samples of ketchup and paste was lower than the range of antimicrobial use influenced by various factors such as contaminated raw materials, processing and storage conditions (6). For mayonnaise, the maximum amount of BA and its salts alone or mixed for use is 750 ppm according to INSO limit. This amount was observed in all samples (30). However, in the case of various types of sauces and mayonnaise, the CAC has declared 1000 ppm as permitted level (25). Moreover, several studies have investigated the amount of BA in pickle product and it was present in most pickled cucumber samples. While, adding preservatives, flavouring, and colouring agents to pickled cucumbers is not allowed based on the INSO limit. However, it is allowed to use sorbic acid at this type of product based on the INSO rule (31,32). The amount of measured BA was low and negligible in mixed pickles (33,34). Detecting the lower amounts of BA compound than the antimicrobial range can be the indicator of the possibility of transferring this additive from the contaminated air, rain, soil, and water during field planting. In general, the BA content in the samples may be affected by various factors, such as contaminated raw materials or inappropriate condition in processing and storage stage (35). In return, CAC has allowed the addition of benzoate and sorbate preservatives (alone or together) at the rate of 1000 ppm in pickled cucumbers (25). The amount of benzoate compounds was higher in bulk pickled cucumbers than industrialized packaged ones that required more attention (33,34).

The presence of benzoate compounds was investigated in 119 samples of tomato paste (Table 2). Some properties of tomato paste, such as low pH (3.5-4.7) and low water activity can prevent microorganisms' growth without adding preservatives. However, manufacturers may illegally use preservatives to cover up defects such as improper processing, rotten ingredients, insufficient pasteurization, or unsanitary packaging. The INSO prohibits the addition of any preservative to tomato paste, which is consistent with the CAC rule (25,36). However, this preservative was observed in several samples (35,37-39).

In the studies related to investigation of lemon juice, there was no report on the presence of BA and its derivatives. The absence of these preservatives in these products is accordance with INSO limit (40). Information about the amount of permitted value for BA in lemon juice was not mentioned in CAC. However, BA can naturally form in lemon juice (41). The presence of BA was not observed in sour cherry jam that corresponds with INSO limit (37,42). However, CAC allows the usage of preservative in jam up to 1000 ppm (25). Olivieh salad is a typical ready-to-eat food that is consumed without secondary processing. This product contains cooked chicken meat, potato, sour cucumber, green bean, salt and spices (43). Adding BA and its derivatives alone or mixed with sorbate compounds is allowed at a rate of 150 ppm

in Olivieh salad due to INSO act because the refrigeration is the only approach to keep and preserve the safety of this product (44). It was reported the existence of BA in some samples of Olivieh in only one study in Iran that exceeded the the permissible limit of INSO (43). The range of BA and its derivatives reported in 508 samples with acidic basis was ND-1896 mg/kg in Iran (Table 2). The highest concentration was related to tomato paste in Qazvin city with the mean amount of 990.62 ppm (39). Some studies demonstrated different amount for BA in acidic products in other countries. For example, 23% of 63 samples of ketchup analysed in Turkey had the higher amount of this additive than the European permitted level. The results of this study are almost as same as the total evaluation in Iranian studies (45). The results of the present study are more than some studies in other countries, and some are mentioned below. For example, 36.4% of ketchup samples in Austria contained BA, with an average of 788.7 mg/kg (46). Besides, the analysis of hot and plain ketchup samples supplied to the Spanish market showed that these products contained BA with average levels of 255 and 485 mg/kg, respectively (47). In Denmark, BA was detected in 3 out of 9 sauces (pepper sauce/ketchup/grill sauce) with an average amount of 3.3 mg/kg (48). In another study performed in Malaysia on 67 samples including soft drinks, canned fruits and vegetables, jams, jellies, and sauces, it was shown that only 16.4% of the samples contained detectable amounts of BA. Still, all of them exceeded the Malaysian legal limit (49). In Portugal, it was examined 11 jam samples and reported that all samples had detectable amounts of BA, as well as 7 samples contained amounts exceeding the legal limit (50).

In the current study, about 75% of the reports has shown the impermissibility of BA and its derivatives in acidic food according to the INSO limit. However, the limit of these preservatives was in accordance with CAC limit in all studies. The permissible range of the CAC is not characterized for 6 acidic items of surveyed investigates. It was suggested to apply pasteurization process, high pressure sterilization or ultrasound for vegetables in order to prevent to use the preservatives (47,48).

Nine articles reported the amount of BA in 325 of Iranian beverage products listed in Table 3. The value of these preservatives showed the incompatibility with INSO limit in carbonated soft and sports drinks. The amount of BA is defined 150 ppm in order to add in carbonated soft, sports, and energy drinks in INSO (51,52) as well as 250 ppm for such drinks in CAC (25).

It was identified BA in fruit juice and non-carbonated drink (3,6,35,37-38,41). The use of preservatives in these products is not allowed based on the INSO (53). Benzoate compound was found in all samples of carbonated juices that is illegal based on the INSO (54). However, CAC has pronounced utilizing 1000 ppm of BA as allowable limit (25).

It is essential to consider to this point that the high acidic circumstance (pH 3-4) of fruit juice is one of the preventive factors against microorganisms. In fruit juice, the recognizable level of benzene as carcinogenic compounds was detailed due to existence of BA (55).

The range of BA is between non detectable and 520 ppm in beverage products in Iran (Table 3). The highest concentration was related to soft drinks in Tabriz city with the mean amount of 631.66 ppm (6).

The results of the present study appear with higher or lower values than some investigation of other countries for beverages. In a study of 300 samples of different types of food from New Zealand markets, it was found that 98% of the samples contained BA below legal limits. Unauthorized levels can be due to the natural occurrence of BA in other samples (cheese and fruit cakes) (7). In Hong Kong, 17% out of 211 samples of non-alcoholic beverages had detectable levels of BA that were all beneath legal restrictions (56). In Portugal, 48 samples of beverages were examined. About 50% of soft drinks contained BA, and 26% exceeded the maximum levels allowed by European and Portuguese laws (57). In an Indian study for comparison of the amount of BA in ketchup and pepper sauce with brand and unbranded samples, all

labelled samples contained BA below the permissible limit. In contrast, 59% of ketchup and 100% of not labelled red pepper sauce samples had more than the permissible levels (58). The results of two previous studies showed higher amounts of BA in drink than in current review study. A significant contribution was mentioned for the presence of BA in food products like non-alcoholic carbonated drinks as the primary sources of BA intake (7,48). On the contrary, it was estimated the role of BA in these drinks from 6 to 10% in Austria (46).

According to the studies reviewed in Iran, the highest percentage of illegal presence of BA in soft drinks was 50. Beverages showed the highest rates of non-compliance with regulatory limits (Fig. 2). This situation could be related to the nature of beverages that have a low pH level that is appropriate for influence of BA against bacterial growth. In general, it was about 77% of the studies for the impermissibility of some of these products according to INSO and CAC. About 33% of the analysed products in the studies had quantities of BA in non-allowable value. Unfortunately, the permissible amount of BA was not determined for some products in CAC. A wide range of foods may not contain BA in daily utilization. Some individuals ordinarily consume a particular product with specific brand according to their preferences. At this situation, the selected commercial products can be a continually exposure route with BA and probable adverse effect on health (26). Hence, it is recommended to reduce the consumption of non-alcoholic beverages in order to control excessive preservative intake.

BA and its derivatives are also used as preservative agents in bakery products. Addition of these compounds is not permitted in INSO and CAC (25,59). However, BA and its derivatives was found in all 15 samples of cookie investigated in Tabriz in the range of 12-54.7 ppm (35). It is necessary to pay attention to this point that BA and its derivatives was identified in cookies in low amount that could be due to benzoyl peroxide, added to baking flour as a bleaching agent (35). In Turkey, it was found BA in the range of 8 to 41 ppm in 11 samples of cookies and tiramisu. This limit did not comply with the Turkish standard that states the non-use of preservatives in these products. High level of BA was identified in one tiramisu sample with the amount of 231 ppm (60). Higher range of BA addition in Turkey is comparable with the results of current study (12-54.7 ppm).

Totally, overuse of preservative such as antimalarial agents in agriculture, veterinary and food accelerates the development of resistant bacteria, reducing the effectiveness of antimicrobial agents in treating human and animal infections and plant diseases. In fact, while preservatives and antimicrobial agents in food production prevent disease, enhance growth of agriculture, animal, and increases the shelf life of food, their overuse poses significant risks to human health, the environment, and sustainable agriculture (61).

This study has some limitations. First, the permitted levels of BA in some food products were not clearly defined in INSO and CAC regulations. Additionally, while a relatively large number of samples were analyzed, the findings may not represent all food products in Iran, which is another limitation. The results could also vary over time and across different locations and cities. However, despite these limitations, the study provides valuable insights into the current status of BA and its derivatives in various food products in Iran.

Conclusion

Some positive effect of BA and its derivatives are attributed to the prevention of microbial spoilage and extending the shelf life of food. However, these preservatives can endanger consumers' health while they add to foods more than the permissible limit. This first systematic review demonstrated the existence of BA and its derivatives in different Iranian foodstuff in unacceptable level compared to national standard. However, some food samples comply with the permissible limit of CAC. The presence of some food items with a higher than acceptable level of BA is a cautious for the health organization in Iran. Hence, there is a need for assessment of BA and its derivatives on regular basis in Iranian

foods. This action can be effective for prevention of probable health issues. Besides the need for a regular assessment of BA and its derivatives in Iranian food, some other suggestions to control BA usage in food could be using natural preservatives by food manufacturer. Furthermore, raising awareness about the dangers of BA and its potential health risks can help consumers make informed decisions about their food choices. For this purpose, mandatory labeling and product transparency can help consumers identify products with food preservatives.

Journalism Ethics 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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Conflict of Interest

The authors declare that there is no conflict of interests.

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