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ORIGINAL ARTICLE

Quantification of Calcium Propionate and Its Health Risk Assessment in Different Types of Bread

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KEYWORDS

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ABSTRACT: Bread is one of the most basic food products regarded as the leading and critical food consumed worldwide. Therefore, producers use an additive calcium propionate as a preservative to extend the shelf life and prevent microbial spoilage in different types of bread. This study aimed to investigate the calcium propionate levels in bread samples collected from different markets of Tabriz, which bread samples included three groups: toast, rusk, and bulky bread. The preservative level was measured by titration method, and then the percentage of consumption risk was estimated based on bread consumption per capita in Iran. Data was analyzed based on analysis of variance (ANOVA) and Duncan's multiple range test. The average concentration of calcium propionate in toast, rusk, and bulk was observed as 2152.75, 1729.57, and 1674.31 mg kg^{-1}, respectively; in total, the amount of this preservative in 55% of the samples was more than the allowed limit (1500 mg kg^{-1}), and no significant difference was observed in the three groups ($P > 0.05$). Additionally, based on data on the health risk assessment in this study, children are more threatened by chronic diseases than adults. Therefore, due to excessive use and possible side effects of chemical preservatives such as propionic acid, constantly monitoring it in food, especially bread, is needed.

INTRODUCTION

Bread is one of the essential food items regarded as the leading and critical food consumed worldwide [1, 2] and a crucial source of nutrients, micronutrients, dietary fiber, vitamins, and antioxidants [3]. One of the defects of bread is the staleness phenomenon- a set of complex physicochemical and sensory changes during storage- that is ultimately associated with reduced consumer acceptance [4]. Therefore, considering the importance and status of bread, which is one of the cost-effective and most essential foods used by humans, keeping it fresh and delaying staleness is one of the crucial issues of the baking industry and is also economically significant. There are several ways to delay staleness, including the use of additives. Additives include preservatives, proteins, carbohydrates, enzymes, fats, emulsifiers, and hydrocolloids (gums) [5].

Calcium propionate a preservative with little or no flavor, powdery or crystalline form, and high solubility in water that can be effective against mold or bacteria (without inhibiting yeasts) and is widely used in bread and bakery products [6-9]. Its antimicrobial activity against various organisms, including *Aspergillus flavus* [6], *Escherichia coli* O157:H7, *Salmonella Typhimurium* [10], and *Clostridia* [5] has been reported in different

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studies. Due to its remarkable antimicrobial properties, it can be used as a preservative in many industries, such as paper [11] and feed, and also to increase the shelf life of fruit and dairy products [1]. Based on the available evidence, it has no teratogenic activity or reproductive toxicity and is excreted in the urine, so there is no risk of accumulation in the human body, even at high doses [12]. However, the occurrence of side effects such as restlessness, hyperactivity, and sleep disorders in humans has been attributed to excessive consumption of this compound [13]. Moreover, hypophagia effects and decreased appetite have been reported in ruminants, broilers, and rats [14-16]. Iran's national standard and Codex Alimentarius has allowed the maximum use of 1500 mg kg^{-1} of this preservative in bulky bread products [17, 18], while the European Union allows its use of up to 3000 mg kg^{-1} [19]. Anyway, the decision to choose the permitted levels for the use of preservatives indicates the existence of concern about their excessive use.

Unfortunately, food additive monitoring is still not of noteworthy value in the food industry and is almost neglected; hence, the present study monitored and measured the amount of calcium propionate and evaluated its health risk in bread in Tabriz.

MATERIALS AND METHODS

In this research, the usual titration methods based on the description of national standard of Iran No.16437 were used for calcium propionate measurement. Furthermore, the spectrophotometric method was used to validate and confirm the test results based on the ISO 17025 standard [20].

Sample collection

Sampling was carried out randomly according to section 7 of National Standard of Iran No.2628 and with the maximum compliance of the necessary and suitable conditions from different parts of Tabriz [21]. Generally, 40 samples of bread (with a diameter of at least 10 cm) were collected and transported to the laboratory, which included 21 samples of rusk bread, 7 samples of toasted bread, and 12 samples of bulky bread.

Quantification of calcium propionate

Briefly, the amount of 400 mg of powdered homogenate was dissolved in 100 ml of distilled water, stirred with magnetic stirring (Iran, Kimia novin), and the solution filtered. Then 30 ml of 0.05 M disodium ethylene diamine tetra acetate (EDTA; Germany, Merck), 15 ml of sodium hydroxide (Germany, Merck), and 300 ml of hydroxy naphthol blue (Germany, Merck) reagent were added to the filtered solution. Finally, the titration continued with EDTA to the endpoint (The appearance of a blue color), each milliliter of EDTA is 9.3 mg of calcium propionate [13, 20-23].

Validation

After the initial preparation, the amount of calcium propionate absorption in the samples was estimated using a spectrophotometer (USA, Unico UV-2100) at 430 nm wavelength according to the standard method. Samples were evaluated based on the typical diagram obtained from adsorption of calcium propionate solutions at specified concentrations of 0.5, 1, 1.5, and 2 g kg^{-1}. They were determined using linear fitting of calcium propionate values in each sample [20, 21].

Risk assessment

The percentage of consumption risk was calculated after determining the amount of calcium propionate in bulk loaves of bread according to the country's per capita consumption. To estimate people's exposure to this substance was used the below equation:

Estimation of daily consumption (EDI) = (maximum concentration of propionate in the bread (mg kg^{-1})) × (The average per capita consumption of bulk bread in the country (gr)) (1)

The hazard quotient (HQ) method was used to investigate the risk of calcium propionate through the consumption of bulk bread in causing non-carcinogenic effects. The risk factor is the ratio between the exposure to the desired compound through the consumption of bulk bread and the allowable limits for calcium propionate, which was calculated using the following equation:

$$HQ = EDI / RFD \quad (2)$$

Reference Specific dose (RFD) was equivalent permissible daily exposure to calcium propionate in humans [24].

The values obtained were finally compared with national and international standards, then, the degree of deviation from the standards was determined.

Statistical analysis

All tests were performed in three replications, and statistical data were reported as their mean ± standard error. One-way analysis of variance test was used to compare the variance of the samples, and Duncan's multiple range test at 95% confidence level by SPSS20 was used to examine the significant difference between the means of the samples. LOD and LOQ calculations were performed using Excel software for tests analysis.

RESULTS AND DISCUSSION

Determining the quantity of the preservative

As mentioned above, calcium propionate preservative has been widely used in various food industries, especially bread and bakery products, due to its antimicrobial properties [6]. It is clear that food additives play a role in improving food quality, and not using them causes a decline in product quality and consumer frustration [25]. However, excessive use of these compounds has occasionally unpredictable consequences on human health; despite the many benefits of calcium propionate in food, some researchers reported side effects such as irritability, inattention, restlessness, visual hallucinations, hyperactivity, and sleep disturbance in children [13]. Some even claimed that calcium propionate in doses lower than the sub-inhibitory levels can stimulate the growth of microbial agents and damage food quality [26]. Therefore, it seems necessary to monitor and measure this additive compound to ensure its correct consumption in bread (as basic food in the household basket) and to assess the health risks caused by it, which the aim of the present study was the same. More precisely, calcium propionate levels in traditional and industrial bulky, rusk, and toasted bread loaves in Tabriz were quantified and compared with the permissible Iranian standard (1500 mg kg^{-1}). Finally, by calculating the person's exposure, health risks were assessed.

The average, minimum, and maximum concentrations of calcium propionate measured in the types of bread samples under study are given in Table 1. As seen, the highest concentration was related to rusk bread, and the lowest was to bulky. However, the differences among the three sample groups were not statistically significant (P> 0.05).

Table 1. Quantitative results of calcium propionate in examined breads.

Type of bread	No. sample	Calcium propionate concentration (mg kg^{-1})			Standard limit (mg kg^{-1})**
		Average ± SD*	Max	Min	
Rusk	21	1729.57±52.00^a	3775.90^a	1.25^a	
Toast	7	2152.75±64.00^a	3772.60^a	0.00^a	≤1500
bulky bread	12	1674.31±48.00^a	3764.1^a	0.00^a	

*SD: Standard Deviation; ** By Iran National Standard

Out of 21 samples of rusk bread, 13 samples (61%) were more, and eight samples (39%) were less than the allowed amount (1500 mg kg^{-1}) determined in standard No.3494 of the Iran National Standard Organization. The highest concentration of calcium propionate in rusk was 3775.9 mg kg^{-1}, and the lowest was 1.25 mg kg^{-1}. Out of seven toasted bread samples, three samples (33%) were higher, and four samples (67%) were lower than the allowed amount. The highest concentration of calcium propionate in toasted bread was 3772.6 mg kg^{-1}, and the lowest was 0.00 mg kg^{-1}. Out of 12 bulky bread samples, six samples (50%) were more than the standard acceptable limits, and six samples (50%) were less. The highest concentration of calcium propionate in bulky bread was 3764.1 mg kg^{-1} and the lowest was 0.00 mg kg^{-1}. Generally, out of the 40 bread samples examined, 22 samples (55 %) showed an amount exceeding the standard limit of the amount of calcium propionate. In a study, Iranian researchers reported much higher levels (3683-4752 mg kg^{-1}) than the permissible limit in all bread samples tested [27]. In another study, contrary to the results of the present study, the concentration of this preservative in the bread samples was reported between 196.5 to 1209 mg kg^{-1}, which was within the permitted range [28]. In addition, another study showed that the concentration of propionic acid in none of the samples of bread and bakery products did not exceed the permissible amounts [29]. Although researchers have reported unauthorized use of this preservative in seafood products [30].

Health risk assessment

The result of the calcium propionate levels calculation to the mean daily acceptable uptake compounds to determine the long-term risks of foods containing permitted calcium propionate additive for the consumer is shown in Tables 2 and 3.

Table 2. Risk and health indicators of calcium propionate in bread for adults.

Sample	Person's weight (kg)[1]	Daily consumption of bread (kg day^{-1})[2]	CP concentration (mg kg^{-1})[3]	CDI[4]	RFD[5]	HQ[6]
Rusk			1729.57	7.16		2.38
Toast	70	0.29	2152.75	8.91	3	2.97
Bulky bread			1674.31	6.93		2.31

[1]According to age; [2]Depending on their age and weight in Iran; [3]Calcium propionate in sampled bread; [4]Chronic daily index; [5]Reference Specific dose; [6]Health risk index.

Table 3. Risk and health indicators of calcium propionate in bread for children.

Sample	Person's weight (kg)[1]	Daily consumption of bread (kg day^{-1})[2]	CP concentration (mg kg^{-1})[3]	CDI[4]	RFD[5]	HQ[6]
Rusk		0.29	1729.57	33.43		0.8
Toast	15		2152.75	41.61	41.5	1.002
Bulk bread			1674.31	32.36		0.779

[1]According to age; [2]Depending on their age and weight in Iran; [3]Calcium propionate concentration in sampled bread; [4]Chronic daily index; [5]Reference Specific dose; [6]Health risk index.

Based on the calculations, the highest daily intake of calcium propionate in adults (2.97) and children (1.002) was assigned to toast samples. The lowest intestinal absorption of calcium propionate in adults (2.31) and children (0.779) belonged to bulk bread samples.

Generally, the health risk of HQ is higher than an unsafe range. The risk of exposure to the concentration of calcium propionate for adults in all types of bread was in the unsafe range, but the concentration of calcium propionate for children in toasted bread was in the unsafe range and the rest of the bread (rusk and bulky bread) was in the safe range. Generally, the reduction of vulnerable people's exposure to unauthorized concentrations of chemical preservatives by replacing chemical compounds with other antimicrobial methods, such as modified atmosphere, gamma radiation, biological protection, and natural antimicrobial compounds, to reduce their health risk rating in society is suggested [31-33].

CONCLUSIONS

In the present study, more than half of the investigated bread contained more than the recommended amount of preservatives; furthermore, the evaluation of the health risks of calcium propionate in consumers showed that children are significantly more susceptible to chronic diseases than adults through bread consumption. Therefore, considering that bread is one of the most significant products in the diet, needed to continuously examine the compounds used in these products and their effect on consumer health.

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Not applicable

Conflict of interest

Authors declare that they have no conflict of interest regarding the publication of this research article.

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This research was carried out in compliance with the ethical and safety standards in working with the devices in the laboratory, as well as preserving the information of the investigated companies.

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