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ORIGINAL RESEARCH PAPER

DEVELOPMENT OF A NOVEL REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF SODIUM DITHIONITE IN BREAD PRODUCTS

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Abstract: Bread is a valuable food in Iranian families that is used as a source of energy, protein, minerals, and vitamins. Unfortunately, most bakeries process bread artificially to speed up the production process and inevitably resort to the use of harmful chemicals such as baking soda, and SDT (sodium hydrosulfite). In this study, the types of bread produced in Ilam City in terms of unauthorized use of SDT were measured. The research site is all active bakeries in Ilam city. Statistical analysis between different groups were determined using t-tests and ANOVA (SPSS-18). Data were expressed as mean ± SD. For all tests, P values of less than 0.05 were considered significant. Each test was repeated three times. In this study, 80 bakeries were surveyed, of which 22 bakeries (27.5 %) were Lavash, 26 bakeries (32.5 %) were Barbari and 32 bakeries (40 %) were Sangak. The average SDT consumption in all bakeries was 20.62 and 36.3 %. The amount of SDT consumption was significantly higher in Lavash bread than in Barbari and Sangak bread. The lowest SDT consumption was reported in Sangak. The results showed that 95.5 % of Lavash bakeries did not meet the national standards in the use of SDT and this amount was reported 73.1 % and 34.4 % of Barbary and Sangak bakeries, respectively. In Lavash bakeries 4.5 %, Barbari 26.9 and Sangak 65.6 %, the SDT consumption was significantly in line with national standards (56.3 %). The results showed that the use of SDT has been done with different amounts depending on the type of bread in bakeries in Ilam city so the most use is related to Lavash bread and then Barbari and Sangak.

Keywords: *bread, chemicals agents, food, harmful, Ilam, sodium dithionite*

INTRODUCTION

Food is an essential substance for survival and can be of plant or animal origin. Food can contain fat, carbohydrates, vitamins, protein, and minerals [1]. Bread is the main food that is popular around the world [2].
The presence of a large number of useful and essential substances in bread has made this the most important cereal to be recognized as a basic food and one of the main sources of the food pattern in Iran. Fiber, protein, fat and carbohydrates are the main components of bread. Therefore, most of the body's needs can be met by daily consumption of this food [2]. Bread provides a major part of energy, protein, minerals such as iron and calcium and some vitamins such as thiamin 1 and niacin 2 that we need on a daily basis, compared to other food items such as meat and milk. It has the highest nutritional value [2]. Yeast plays an important role in the quality of bread and can convert fermentable sugars into carbon dioxide and ethanol [3]. Because fermentation is a time-consuming process, bakeries today use sodium dithionite (SDT) ($Na_2S_2O_4$) (DOT No. UN 2005 and CAS Number: 7775-14-6), to reduce fermentation time [4]. SDT is a white crystalline powder with a sulfur odor that has many industrial uses and can inhibit antioxidants in the body and cause high blood pressure, gastrointestinal cancer, and skin and respiratory disorders [5]. After the SDT enters the digestive system, it causes villi in the stomach and intestines, and during the time when the oxidants are destroyed, it causes the cancer of the digestive tract to accelerate. So, SDT determination is very important, and developing efficient analytical methods is highly required. Several methods have been demonstrated for SDT determination including Iodometric [6], voltametric sensors [7], and density functional theory (DFT) [8]. In food samples, the SDT active principle has been determined by spectrophotometer [9], gas-diffusion flow injection analysis utilizing spectrophotometric *p*H detection [10], and high-performance liquid chromatography methods [11]. This study aimed to establish a simple, sensitive, rapid, cost-effective, reliable, and specific HPLC assay for the quantitation of SDT concentrations in bread.

MATERIALS AND METHODS

Sample collection

To estimate the amount of SDT remaining in the bread sample of Ilam city, Ilam province, among 850 bakeries, 80 active bakeries in different places were considered at the time of research, and from each sample, 3 samples of different doughs based on the standard sampling method of Iranian industrial research selected.

Sample preparation

For the SDT quantification test by liquid chromatography, 10 g of dough samples were weighed accurately into a 250 mL beaker and 100 mL of deionized water was added. The mixture remained for 2 hours before filtering. For the HPLC assay, the filtrate was further diluted 10 times with a mobile phase before injection [12]. 50 mL of 10 % (v/v) ethanol was added to a 250 mL calibrated flask (degassed with nitrogen for 5 min).

Then 25 mL of dough solution was pipetted and KOH (10 mL, 2.5 M) was added. Then, sulphuric acid in 10 % ethanol (6.25 mL, 2 M) is added to the mixture to neutralize the KOH. Then, 25 mL of the sulphuric acid in 10 % ethanol is added and the flask is filled up with the solution of 10 % (v/v) ethanol. This final solution has to be injected immediately into the HPLC system. Standard solutions were always prepared freshly from the stock solution at the same preparation steps [13].

Measurement by HPLC

Quantitative determination of SDT in bread samples was employed using High-performance liquid chromatography (HPLC) (Smart line; Knauer, Germany) equipped with an ultraviolet detector (Well chrome, K-2600; Knauer) and a C18 column (Nucleosil H.P.; 25 cm * 0.46 cm internal diameter, 100 Å pore size, particle size 3 µm, Knauer) using gradient elution was utilized. A mixture of 0.1 % formic acid in water (B) was maintained at a range from 5 - 70 % and solvent methanol (A) was the successively mobile phase. The flow rate was 1.0 $mL \cdot min^{-1}$ and the presence of SDT was monitored at 560 nm. Linearity was performed by using a series of standard solutions in the range of 25.0 - 250.0 $\mu g \cdot mL^{-1}$. Precision was expressed as the relative standard deviation percent (% RSD) of replicate samples of each concentration. HPLC method specificity was obtained by comparing the sample chromatograms with the standard and standard spiked samples. SDT retention times of sample and standard spiked-sample might equal that of the SDT standard [12].

Statistical analysis

At least, each test was repeated three times. Statistical analyses between different groups were determined using t-tests and ANOVA (SPSS-18). Data were expressed as mean ± SD. For all tests, P values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Determining and comparing the average concentration of SDT

In this study, 80 bakeries in Ilam were surveyed, of which 22 bakeries (27.5 %) were Lavash, 26 bakeries (32.5 %) were Barbari, and 32 bakeries (40 %) were Sangak (Table 1). The average SDT consumption in all bakeries was 20.62 and 36.3 %, *i.e.* 29 bakeries; the SDT consumption in them followed national standards.

Table 1. *Determining and comparing the average concentration of SDT in different bread doughs*

Type of bread	Number	Mean±SD	P-Value
Lavash	22	44.54±28.44	0.001
Sangak	32	8.74±7.18	0.11
Barbari	26	17.11±16.92	0.06

The amount of SDT consumed was significantly higher in Lavash bread than in Barbari bread and Sangak bread. Barbary bread came in second. The lowest consumption of SDT was reported in Sangak bread, which was significantly different.

Comparison of the use of SDT in different bread types

Table 2. *Comparison of the use of SDT in different bread doughs with national standards*

Type of bread	National Standards N [%]		P-Value
	≥10	< 10	
Lavash	21 (95.5 %)	1 (4.5 %)	0.001
Sangak	19 (73.1 %)	7 (26.9 %)	0.12
Barbari	11 (34.4 %)	21 (65.6 %)	0.05

The results showed that 95.5 % of Lavash bakeries did not meet the national standards in the use of SDT and this rate was reported in 73.1 % and 34.4 % of Barbary and Sangak bakeries, respectively. In Lavash bakeries 4.5 %, Barbari 26.9 and Sangak 65.6 %, the consumption of SDT significantly complied with national standards (Table 2).

SDT use in comparison with international standards

Table 3. *Comparison of the use of SDT in different bread doughs with international standards*

Type of bread	International standards [%]		P-Value
	> 0	0	
Lavash	22 (100 %)	0	0.001
Sangak	21 (80.8 %)	5 (19.2 %)	0.05
Barbari	18 (56.3 %)	14 (43.8 %)	0.12

The results showed that 100 % of Lavash bakeries did not comply with international standards in the use of SDT and this amount was reported in 80.8 % and 56.3 % of Barbary and Sangak bakeries, respectively. In Barbary bakeries (19.2 %) and Sangak bakeries, 43.8 %, the consumption of SDT significantly complied with international standards (Table 3).

Table 4. *Average consumption of SDT consumption according to national standards*

National standard	Number	Mean±SD	P-Value
< 10	29	2.24±3.32	0.001
≥ 10	51	31.07±24.38	

The average consumption of SDT according to national standards was 2.24 and non-compliance with national standards and 31.07, which was not significantly in line with national standards (Table 4).
SDT consumption was reported according to national standards (36.3 %) and according to international standards (23.8 %) (Table 5).

Table 5. *SDT consumption according to national and international standards*

National standard	Frequency [%]	International Standard	Frequency [%]
< 10	29 (36.3)	0	19 (23.8)
≥10	51 (63.8)	31.07±24.38	61 (76.3)

Today, antioxidants, emulsifiers, flavor enhancers, thickeners, flavorings, sweeteners, and synthetic dyes are groups of additives that are added to food for a variety of reasons [14, 15].

The results of this study showed that the concentration of SDT in bread has not been researched internationally because in other countries the use of this product in food processing is prohibited. In this study, 80 bakeries in Ilam were surveyed, of which 22 bakeries (27.5 %) were Lavash, 26 bakeries (32.5 %) were Barbari, and 32 bakeries (40%) were Sangak. The average SDT consumption in all bakeries was 20.62 and 36.3 %, *i.e.* 29 bakeries; the SDT consumption in them was under national and international standards. In a study, in different months of sampling, the concentration of dithionite was zero mg/kg, which complied with the national standard in this field. Also, in the second study, the results showed that the amount of SDT in the produced plant is 90% of the set standard limit [16]. However, it has been reported that the concentration of SDT in the crop produced was high in most workshops [17]. In measuring SDT in sugar and sugarcane reported that the SDT level was from less than 1.4 to 13.24 $mg \cdot L^{-1}$ [18]. The amount of SDT consumed was significantly higher in Lavash bread than in Barbari bread and Sangak bread. Barbary bread came in second. The lowest consumption of SDT was reported in Sangak bread, which was significantly different. In the study, the amount of SDT used in the preparation of candy was more than 80 $mg \cdot L^{-1}$. Of course, it is worth noting that due to the considerable heat pressure on the amount of SDT, it cannot be said with certainty that bakeries that have a small amount of SDT in their sample have not used this chemical compound [17]. The results showed that 95.5 % of Lavash bakeries did not meet the national standards in the use of SDT and this rate was reported in 73.1 % and 34.4 % of Barbary and Sangak bakeries, respectively. In Lavash bakeries 4.5 %, Barbari 26.9 and Sangak 65.6 %, the consumption of SDT significantly complied with national standards. The results showed that 100 % of Lavash bakeries did not comply with international standards in the use of SDT and this rate was reported in 80.8 % and 56.3 % in Barbari and Sangak bakeries, respectively. In Barbary bakeries (19.2 %) and Sangak bakeries 43.8 %, the consumption of SDT significantly complied with international standards. The average consumption of SDT based on national standards was 2.24 and non-compliance with national standards was reported at 31.07, which did not significantly correspond to national and international standards. The results showed that 36.3 % of the bakeries studied did not comply with national standards and 63.7 % did not comply with national standards. SDT consumption was reported according to national standards (36.3 %) and according to international standards (23.8 %). In the study, regarding the evaluation of residual sodium hydrosulfite in 17 samples of sugars produced, they showed that in 6 % of the samples collected, the amount of SDT was more than 10 ppm [18]. WHO has defined food hygiene as all the conditions and measures taken during the production, processing, storage, distribution and preparation of food to ensure its safety, health, and suitability for human food contamination. Chemicals and toxic substances are very

important and food quality control laboratories must investigate food safety because the health and safety of food is the main goal of public health [19, 20].

CONCLUSIONS

The results of our study showed that in the bakeries of Ilam city, the use of SDT has been done with different amounts depending on the type of bread so the most use is related to Lavash bread and then Barbari and Sangak. The amount of SDT used in bakeries in 36.3 % of the cases was under national standards and 23.8 % were following international standards. Due to the consumption of its harmful substances in unauthorized consumption for the health of the community and its consumption in certain devices, the need for more monitoring by the relevant regulatory institutions and health authorities and food monitoring bodies is recommended in this field.

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