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Validation of a Fast Sample Preparation Procedure for Quantification of Sodium in Bread by Flame Photometry

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Abstract Reduction of salt intake through bread requires reliable monitorization when the control of a large number of samples is in order. The use of flame photometry is attractive; however, the usual dry-ash sample pretreatment is fastidious. Direct dissolution of bread sodium in water is a good alternative. A good correlation between results from these two sample preparation methods was obtained for sodium quantification in nine different types of bread. The proposed method was applied to the quantification of sodium in 48 bread samples of randomly chosen from local market to evaluate compliance with legislation requirements (lower than 550 mg sodium per 100 g of bread—Lei no. 75/2009).

Keywords Bread · Sodium · Salt restriction · Hypertension · Flame photometry · Validation

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Introduction

Bread is an inexpensive source of energy: it contains carbohydrates, lipids, and proteins, and it is important as a source of essential vitamins of the B complex and of vitamin E, minerals, and trace elements (Fernández 2003).

Salt is added to bread to provide flavor and increase shelf life, and because of its effect on the breadmaking process. Salt has an influence on the rheological properties of dough: it inhibits the hydration of gluten; the gluten shrinks, the dough does not collapse, and gas retention is improved. Higher concentrations of salt inhibit enzymatic reactions and also inhibit the fermentation activity of yeast. In general, the proportion of salt used is 1–2% (Fernández 2003). Thus, the salt content present in bread is of great concerns for food processing (Chen et al. 2005).

Food and Nutrition Board has set an estimated minimum requirement for healthy person for the three minerals (sodium, chloride, and potassium) that are major electrolytes in body fluids. The Estimated Minimum Requirement for sodium is 500 mg/day for healthy person over age 18. The body can adjust to a rather wide range of dietary sodium by mechanism designer to conserve or excrete this mineral. To cover wide variations in individual patterns of physical activity and climate which influence relative losses in perspiration, the Upper Limit for sodium in adults has been set at 2,400 mg/day or less. Higher intakes carry no health benefits and may actually be detrimental (Schlenker 2003). Salt restriction is recommended to prevent and to treat hypertension (Korhonen et al. 2000; He and Macgregor 2007).

There is a growing need for quantitative data on sodium content in general, and in bread, in particular, in order to update sodium Recommended Daily Allowance and harmonize food composition data across Europe (Castanheira et al. 2009; WHO 2006; Egan et al. 2007). Recently, Portuguese legislation established 550 mg of sodium per 100 g of bread as the maximum level allowed (Lei no. 75/2009. Diário da República, 1.[a] série-No. 155–12 de Agosto de 2009). Thus, rapid and reliable methods are needed to control a large number of samples.

The classic method for the determination of salinity in foods is Mohr's titration method. In this method, the calculation is based on the concentration of chloride ion titrated with silver nitrate solution (Chen et al. 2005). Disadvantages of Mohr's method include the de-coloration pretreatment necessary for strongly colored foods, side reactions of silver ion with anions, and the adjustment to nearly neutral condition before titration. Thus, direct determination of sodium ion provides alternatives to overcome these disadvantages (Skoog et al. 2000).

Ion selective electrode method (Rojas and López 2003), atomic absorption spectrophotometry (Villanneva et al. 2000), inductively coupled plasma-atomic emission spectrometry (Fingerová and Koplík 1999), inductively coupled plasma-mass spectrometry (Villanneva et al. 2000; Fernández-Turiel et al. 2000), and flame photometry (Chen et al. 2005; Castanheira et al. 2009; Folarin et al. 2001; Joossens et al. 1994) are methods for direct detection of sodium ion concentration. Among them, flame photometry is a simple and relatively inexpensive method, and applications of this method in determination of sodium ion concentrations in bread have been reported (Castanheira et al. 2009). This method has the advantage of allowing the direct determination of the sodium ion; however, the sample pretreatment described for bread analysis is time-consuming since test portions were dry-ashed in a muffle oven (Castanheira et al. 2009; Joossens et al. 1994). Other sample pretreatment procedures can be used, namely wet digestion in a heating block, or on a hot plate and microwave digestion (Julshamn et al. 2005; Ehling et al. 2010). The last is a rapid technique, but it presents also some limitations, since the sample number is limited for each digestion, the digestion vessels are expensive and lifetime-limited (each vessel can only be used for approximately 100 digestions), and the cleaning of the digestion vessels is more complex than for glassware (Sun et al. 2000).

The objective of the present study was to validate, in terms of precision and accuracy, a faster sample pretreatment procedure that can be applied to quantification of sodium in different types of bread, based on direct dissolution of bread sodium and flame photometry quantification. Comparison with dry-ash sample pretreatment method was performed. The proposed method was applied to analysis of bread samples from local market to evaluate compliance with legislation requirements.

Experimental

Chemicals and Samples

All reagents used were of analytical grade purity. Standard solution of sodium (1,000 mg/L) was supplied by JenWay, England. Calibration curves were constructed using 0.2, 2.5, 5.0, 7.5, and 10 μg/ml standards. The solutions were stored in a refrigerator. A Certified Reference Material EnviroMAT ES-H-1 CRM, purchased from SCP Science (France), was used.

To avoid contamination of the samples, all PTFE materials (Teflon vessels, pipettes, micropipette tips) were immersed in freshly prepared 15% (*v*/*v*) proanalysis HNO_3 (Merck) during 24 h, then rinsed thoroughly with doubly deionized water, and dried in a dust-free area before use.

Instrumentation

A flame photometer (Model PFP7, JenWay, England) with filters for lithium, sodium, and potassium was used. Butane gas and air were supplied as the source of flame. The flow rate of fuel was adjusted to get a maximum sensitivity. A standard curve with sodium concentrations between 0.2 and 10 μg/ml was established daily, and the signal of 5 μg/ml was checked occasionally during the analysis. Other apparatus used were a Seralpur PRO 90 CN and Seradest LFM 20 Water Purification System, a Heidolph REAX 2000 vortex, a Kern ALS 120–4 balance (Ziegelei), an Ultra Turrax blender T25 (Sotel), a Heraeus stove D-6450 model, a Centrifuge 5810 R Eppendorf, and a Thermolyne 4800 muffle furnace. Dry matter was evaluated using Scaltec instruments (Scaltec Instruments GmbH, Heiligenstadt).

Sampling

Bread samples used for validation studies included nine types of bread made from wheat, rye, or corn flour purchased from a bakery in Porto, Portugal. These bread samples were prepared with different types of flour (according to French designation), and presented the characteristics described in Table 1. The flour type numbers indicate the ash content (in milligrams) per 10 g flour. Type 55 is the standard, hard-wheat white flour for baking. Types 65 and 70 are strong bread flours of increasing darkness, and type 150 and 170 are wholemeal flours (Portaria no. 425/98. Diário da República, 1.[a] série B, N.[o] 170–25 de Julho de 1998).

Bread samples used for application of the validated method (based on direct dissolution of bread sodium in water) included 48 bread samples randomly acquired in different bakeries of Porto, including 18 samples of white wheat bread (including special white bread, white bread from 6 different bakers), 9 samples of whole wheat bread (from 3 different

Table 1 Characterization of the nine types of bread samples (n=3) used for method validation

Sample code	Type of bread	Flour type (ash, %)	Moisture content, %
1	Mixture	Mixture 80:20 of type 65 wheat flour and type 70 rye flour (~0.7% ash)	29.0
2	Special white bread	Type 55 or 65 wheat flour (~0.55% ash)	36.7
3	White bread	Type 65 wheat flour (~0.65% ash)	29.4
4	Water bread	Mixture 70:25:5 of type 65 wheat flour, type 80 wheat flour and type 70 rye flour (~0.7% ash)	41.6
5	Rye bread	Mixture 50:50 of type 65 rye flour and type 170 rye flour (~1.2% ash)	30.2
6	Whole wheat bread	Mixture 50:50 of type 150 wheat flour and type 65 wheat flour (>1.1% ash)	32.4
7	Corn bread	Mixture 65:25:10 of type 150 corn flour, type 170 rye flour and type 65 wheat flour (>1.50% ash)	40.9
8	Regional wheat bread	Mixture 80:20 of type 150 wheat flour and type 150 corn flour (>1.50% ash)	41.4
9	White bread without salt addition	Type 65 wheat flour (~0.65% ash)	28.0

bakers), 9 samples of rye bread (from 3 different bakers), and 12 samples of mixture bread (including mixture, water bread, corn bread, and regional wheat bread from 4 different bakers).

Sample Preparation

All bread samples were stored in a plastic bag in a refrigerator (4 °C) until used. Each bread was homogenized, a portion of 2 g was sampled and mixed with double deionized water (20 ml) using a Ultra Turrax blender, the volume was completed up to 40 ml and after 30 min was centrifuged (4,000 rpm, 15 min), and 1.00 ml of supernatant was diluted up to 40 ml. Comparison of results obtained by direct dissolution of bread sodium in water was performed with analyses carried out by dry-ash sample pretreatment method as proposed by other authors (Castanheira et al. 2009; Joossens et al. 1994). Thus, after predrying in an oven (60 °C, 24 h), the product was triturated and homogenized in a kitchen grinder. Test portions (4 g) were weighed and dry-ashed. Sodium content was determined by flame photometry in hydrochloric solution of the ashes.

Method Validation

For the evaluation of the instrumental precision, intensity emitted were determined in the same bread extract 20 times under the established instrumental conditions.

Linearity was observed in the working ranges (in microgram per milliliter) from 0.2 to 10.0. To calculate the detection limit of the instrumental method, 20 determinations were carried out on deionized water, and the value was calculated as $3s/m$, where s is the standard deviation of the blank measurements and m is the slope in the calibration curve. The limit of quantification was calculated as $10s/m$.

Because reference materials for sodium are not available for bread matrix, a EnviroMAT reference material was tested, and the method of standards addition was also applied for accuracy evaluation. Four different concentrations (between 2.0 and 8.0 μg/ml) of standard solutions of sodium were added to different aliquots of bread samples (six replicates for each concentration) and submitted to the extraction procedure, and the analyte recoveries were calculated.

Repeatability of the extraction procedure was evaluated by the coefficient of variation using six aliquots of each bread type that were submitted to extraction by direct dissolution of bread sodium in water and by bread dry-ash pretreatment.

Statistical Analysis

Data are presented as the mean±standard deviation. Analysis of variance (t test) at 5% significance level was

Table 2 Analytical figures of merit found for the validated method to quantify sodium in bread samples

Linearity, μg/ml	Detection limit, μg/ml	Quantification limit, μg/ml	Precision (CV%) instrumental procedure	Certified Reference Material	
				Certified value, μg/ml	Measured value, μg/ml
0.2–10.0	0.2	0.66	3.3% (n=20)	43.3 (42.6–44)[a]	43.1±0.3 (n=6)

[a] Minimum and maximum values are indicated in parentheses. n=number of assays

Table 3 Concentration of sodium in various types of bread using direct water dissolution and dry-ash pretreatments

Sample code	Type of bread	Direct water dissolution		Dry-ash pretreatment	
		Conc. ± SD (mg/100 g bread dry matter)	CV% (n=6)	Conc. ± SD (mg/100 g bread dry matter)	CV% (n=6)
1	Mixture	526±3	0.6	518±11	2.2
2	Special white bread	541±13	3.0	527±7	1.3
3	White bread	520±4	0.7	510±10	2.0
4	Water bread	416±17	4.2	424±16	3.7
5	Rye bread	655±10	1.5	578±7	1.3
6	Whole wheat bread	513±32	6.3	532±39	7.4
7	Corn bread	599±8	1.3	604±33	5.4
8	Regional wheat bread	642±43	6.7	581±9	1.6
9	White bread without salt	58.0±2.1	3.6	59.6±3.1	5.2

Coefficients of variation for each method are expressed as percentage

used to compare the results obtained between the proposed method (direct dissolution of bread sodium in water) and dry-ash sample pretreatment method. Statistical analyses were carried out with SPSS (version 17, Chicago, USA).

Results and Discussion

Method Validation

The parameters determined to evaluate the performance of the method for sodium quantification in bread samples are summarized in Table 2. The linearity between the concentration of sodium and the photometric signal was maintained over the concentration range of 0.2–10.0 µg/ml. The mean regression equation for concentrations of sodium versus photometry signal was $y=0.991x+0.436$. The correlation coefficient for standard curve exceeded 0.999. The limit of detection was 0.2 µg/ml, and the limit of quantification was 0.66 µg/ml. These values show that the method is precise and sensitive, enabling the quantification of low levels of sodium.

The instrumental precision was 3.3%. Additionally, the results obtained for sodium by applying the Certified Reference Material (ES-H-1 CRM) showed that there was good agreement between the certified values and the concentration found, indicating that the method is accurate for sodium quantification.

Concentration of sodium in nine types of bread was performed by direct dissolution of bread sodium in water (WD) and by dry-ash pretreatments (DA), and the results expressed as milligram of sodium per 100 g of bread dry matter are presented in Table 3. No significant statistical difference between the two methods was observed for each type of bread, at the 5% significance level, from paired t test, except for rye bread. As observed by other authors (Koh et al. 1999), there is a possibility that some elements of interest such as sodium escape during the dry-ashing process.

A good correlation between the two methods viz. WD and DA was obtained for sodium quantification (expressed as milligram of sodium per 100 g of bread dry matter) as apparent from the linear relationship ($y=ax+b$) between the two methods (WD and DA) established by the regression equation $WD=1.07DA-15.7$; $r=0.951$.

To guarantee the repeatability of the extraction procedure, six aliquots of each bread type were submitted to extraction and quantification, and the coefficient of variation of the results was evaluated. As is shown in Table 3, the coefficient of variation was lower than 6.7% for direct

Table 4 Results obtained for sodium quantification in various types of bread by direct dissolution of bread sodium in water

Type of bread	Number of bakers	Minimum and maximum sodium levels (mg/100 g of bread)	Mean concentration±SD (mg/100 g of bread)	CV (%)
White wheat bread (n=18)	6	280–581	465±109	23.4
Whole wheat bread (n=9)	3	327–608	506±122	24.0
Mixture bread (n=12)	4	331–460	403±50	12.5
Rye bread (n=9)	3	453–586	521±71	13.6

dissolution of bread sodium in water and lower than 7.4% for dry-ash pretreatment.

Recovery studies were carried out to determine the accuracy of the method. Samples were analyzed before and after the addition of known amounts of sodium (2.0, 4.0, 6.0, and 8.0 μm/ml), and it was found that recoveries ranged between 90.0% and 96.8%. These results confirmed that although the matrix composition is complex and presented different mineral content, it does not cause interference effects. A direct water dissolution and centrifugation procedure in a closed vessel were effective for the simplification of the bread matrices to obtain the necessary dissolution of sodium for its reliable quantification. The results showed that there was no contamination or losses during the pretreatment procedure for sodium extraction.

Analysis of Bread Samples Randomly Acquired

The proposed method was applied to the quantification of sodium in 48 bread samples randomly chosen from local market. The levels (mean values, milligram per 100 g of bread as expressed in legislation) found are presented in Table 4, as well, as the relative standard deviations and the respective ranges. The mean sodium content for white wheat bread (the most consumed type of bread) was 465±109 mg per 100 g of bread. For whole wheat bread, mixture bread, and rye bread, the mean values were 506±122, 403±50, and 521±71 mg/100 g of bread, respectively. In addition, the high dispersion of values indicates great variability in sodium content among bread samples. This variability is most likely due to variable amounts of salt added by industry, driven by consumer preferences. As we can observe some bread samples from the selected types analyzed presented sodium content above the legislated level (550 mg/100 g of bread) (Lei no. 75/2009. Diário da República, 1.ª série-N.º 155–12 de Agosto de 2009). However, these results are in accordance with those found by other authors (Castanheira et al. 2009; Vieira et al. 2007).

Conclusions

The principal challenge of the present work is a substantial reduction of time necessary for sample pretreatment. Direct dissolution in water is a good alternative for the routine analyses of sodium in bread to achieve accurate and precise results in a short period of time. In spite of the complexity of bread matrix, the process was economic, time-saving, and easy to carry out when compared with dry-ash pretreatment.

The proposed faster sample preparation procedure for quantification of sodium by flame photometry in different types of bread can help regulatory entities to ensure that commercialized bread meet the requirements of recent legislation (lower than 550 mg/100 g of bread) and health claims (such as *low sodium content*).

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