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Determination and validation of dietary fiber in food by the enzymatic gravimetric method

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Abstract

An adequate and validated method for the analysis of any nutrient is a fundamental step in achieving a good analytical result. The purpose of this study was to analyze dietary fibers (DF) and validate the results without the availability of a standard reference material (SRM). Ten samples of cracker biscuits in duplicate were analyzed using the enzymatic–gravimetric method. To validate, cracker biscuit was used as a matrix food and a fiber-rich product (47.63%) was used as a secondary reference standard (SRS). Three aspects were evaluated: (1) Precision—Samples from 10 assays in two lots of cracker biscuits and in the RS; (2) Accuracy—recovery test on biscuit plus three quantities of SR: 1.5, 2.5 and 5.0 g in 50 g of biscuit, (3) Linearity—recovery curve, through the correlation coefficient (R^2). Results obtained for coefficient of variation were 4.89 and 4.10% for biscuits and 1.37% for the RS, showing a good precision of approximately 20%. The accuracy values obtained were 4.10, 4.90 and 6.55 g/100 g, which represents recoveries of 101, 108 and 110%. These results indicate acceptable accuracy, since the replicates of the fortified samples showed analyte recovery within the acceptance range of 70–120%. The correlation coefficient (R^2) was 0.9999 indicating an excellent linearity in the method's response. The conclusion is that DF results obtained through the EGM method are reliable, considering the cracker biscuit sample parameters analyzed.

Keywords: Food analysis; Method validation; Dietary fiber; Food composition

1. Introduction

In recent years, the role of dietary fiber in the prevention of certain human diseases and in lowering blood cholesterol levels has received much attention in both the scientific and popular literature. In the past decade, research has documented connections between dietary fiber intake and decreased risks of chronic diseases (Connor, 1990; Dwyer, 1993; Grew, 1991; Truswell, 1993).

Due to the use of improper methods, the fiber data found on Brazilian tables are hardly reliable. Food composition tables are thus necessary that specify the soluble and insoluble fiber content of the various foods usually consumed by the Brazilian population. Such tables must be prepared using standardized analytical methods. Knowledge of the fiber content of foods customarily consumed by the Brazilian population is useful because of its relevance for any study on human nutrition. In order to ensure the quality of results obtained through experimental analyses, validation of the methodology employed is necessary. It is important as a way to verify and document the reliability of results obtained through a certain analytical method. Having a validated method or result means that we are always trying to improve, but documents proving such fact are essential, otherwise its significance level will be reduced (Leite, 1996). Interlaboratory studies may be utilized, as well as recovery with standard reference material or an internal standard. The parameters normally used in validations are accuracy, precision, selectivity, and range of linearity, detection limits and quantification limit (Funk, Dammann, & Donnevert, 1995).

Selection criteria for the appropriate validation procedure will depend upon laboratory conditions, the method used, the type of sample under analysis, the component being measured, and the availability of cer-

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tified reference material (Stewart & Stewart, 1995). This last item presents certain limitations such as the high cost and little availability of some matrices and analytes (Funk et al., 1995). Certified standards for proteins, lipids and other organic molecules show alteration problems; it is difficult to ensure the initial concentration of these nutrients for long periods because they are hardly stable (Tanner, Wolf, & Horwitz, 1995).

Interlaboratory studies are high-level procedures for validating methods (Parker, 1991), and their objective is not to determine statistical differences among results from various laboratories; instead, they aim at determining the acceptable variability of the analytical results obtained by the laboratories (Horwitz, 1982).

It is important that the method be validated at the laboratory or be carried out by the analysts involved using equipment and reagents from such laboratory. Today, the scientific community is highly concerned about the quality of analytical data in food analysis. That is essential for the creation of reliable food composition tables. As regards Nutritional Labeling, the enzymatic–gravimetric method (AOAC) is recommended for total fiber analysis with the purpose of stating the dietary fiber content on food labels. This resolution advocates that difference, 100 (humidity, ashes, protein, lipids and dietary fiber) should calculate carbohydrate contents.

In view of the relevance of controlling the quality of the dietary fiber data obtained, this study aims at introducing in the State Reference Laboratory, for food analysis, an adequate dietary fiber analysis method and carrying out its validation so as to achieve reliable results and meet the International standard, as well as to contribute to the preparation of the Brazilian food composition table.

2. Materials and methods

2.1. Materials

The sample (a cracker biscuit, from two lots was used as matrix and fiber-rich product (47.63%), pill commercialized locally, was employed as a secondary reference standard for adding fiber to the biscuit.

2.2. Methods

2.2.1. Fiber preparation

Samples were processed with a mill until all passed through a 35-mesh screen size.

2.2.2. Chemical composition

Two aliquots from original fiber samples were analyzed for moisture and fat contents (AOAC, 1995), while the remaining samples were defatted for 8 h using hexane as solvent (5 ml/g sample). Two separate samples, taken from deffated and dry fiber sources, were analyzed in duplicate for protein and ash (AOAC, 1995).

2.2.3. Dietary fiber determination

Duplicate fat free dry samples were analyzed for soluble and insoluble fiber using the method of Lee, Prosky, and Devries (1992). The method includes enzymatic hydrolysis with α-Amylase, Protease and Amyloglucosidase, using the MES–TRIS buffer.

The sample was previously dried and was fat or carbohydrate free. The triplicate approximately 1 g of a sample were suspended in 40 ml MES–TRIS buffer, submitted to enzymatic hydrolysis sequence: 50 μl of thermo resistant α-amylase, in water bath for 35 min, and 100 μl of protease in water bath at 60 °C ± 1 °C for 30 min. After that, the pH was corrected to a range of 4.0–4.7, and 300 μl de amiloglicosidade, in water bath at 60 °C ± 1 °C for 30 min, was added. After the soluble fiber precipitation with ethanol (95% v/v) at 60 °C. The sample was filtered in fritted (sintered) glass crucibles, (gooch type) and glass wool as filtration agent. The crucibles containing the residue were dried at 105 °C oven, after that cooled in a dessicator and weighted.

2.2.4. Accuracy

In order to determine the accuracy of the dietary fiber method, a recovery test was performed using the cracker biscuit as a matrix (ingredients: wheat flour, hydrogenated vegetable fat, refined salt, malt extract, inverted sugar, baking powder—contains gluten). Firstly, the sample is analyzed in order to determine the analyte level. The amount of analyte having been determined, many additions of half, twice and the same quantity present are made to portions of the sample (Horwitz, Kamps, & Boyer, 1980).

2.2.5. Recovery

In order to perform the dietary fiber recovery test, fiber was added to the cracker biscuit in three concentrations, different from the product's fiber concentration. In this study, three quantities of the secondary reference standard (SRS) were added: 1.5, 2.5 and 5.0–50 g, corresponding to the expected theoretical dietary fiber values of 3.72, 4.55 and 6.51 g/100 g. Aspects that may affect the variability are the instrument, the techniques and the method (Holden, Schubert, Wolf, & Beecher, 1987; Smith & Evans, 1994). This type of variability among results is related to random, non-predictable errors, which occur by chance and tend to decrease after several repetitions (Taylor, 1987).

The total dietary fiber content of the cracker biscuit (matrix) was thus previously analyzed in 10 assays performed in duplicate in order to determine the initial

content of such nutrient. Some samples can serve as substrates in the recovery study so as to ensure that minimal quantities of material added is recovered in satisfactory quantity (Horwitz et al., 1980). Since there were no certified reference standards available for this study, an SRS was employed due to its high fiber content (47.63%).

The SRS contains the following ingredients: oat fiber, beet root fiber; vehicles: oyster shell, starch and stearic acid. The SRS was previously analyzed in 10 assays performed in duplicate so as to determine its exact fiber content. The dietary fiber recovery percentage was calculated as follows:

$$\%\ \text{Recovery} = \frac{\text{value obtained by analysis}}{\text{expected value}} \times 100$$

2.2.6. Precision

In order to evaluate precision, the analytical procedure for dietary fiber determination (10 assays performed in duplicate, $n = 20$) was applied to three different samples: two lots of cracker biscuits (1–2) and the secondary reference standard. The mean, the standard deviation and the coefficient of variation were calculated in order to determine the method's precision level.

$$\%\ \text{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{mean}} \times 100$$

2.2.7. Linearity

The correlation coefficient of the linear regression curve was adopted as a parameter to determine linearity.

2.2.8. Analytical quality control

The method of dietary fiber analysis adopted in this study has been subject to International Analytical Quality Control assessment: the FAPAS—Food Analysis Performance Assessment Scheme by England's C.S.L. Food Science Laboratory. Eighty laboratories from different countries have taken part in controlling the dietary fiber analysis of breadcrumbs. This laboratory keeps all data for quality control purpose, like the Interlabolatory Analysis.

2.2.9. Statistical analysis

Summary measures and tables obtained through an electronic spreadsheet were used for data analysis and for drawing the regression curve.

3. Results and discussion

Table 1 shows that the recoveries of fiber found in the biscuit were 101, 108 and 110% for samples A, B and C, with coefficients of variation of 0.44, 1.44 and 0.73%, respectively. The method's accuracy is good, since the replicates of the fortified samples showed analyte recovery within the tolerance range of 70–120% (Parker, 1991).

There is a tendency today to accept with reservations data obtained by laboratories that do not follow quality control programs (Holden et al., 1987). Globalization and technological developments have given rise to an increase in the variety of new industrialized food products in the consumer market, which foods contain several different ingredients. In order to meet this demand, a change is necessary in the methodological procedures used for analyzing such products. New food analysis methods are thus introduced. However, if laboratories do not follow programs for controlling the quality of such methods, the data obtained through analyses will not be sufficiently reliable.

The chief differences found among the known enzymatic–gravimetric methods regard analysis conditions such as the enzymes employed and the time and temperature of the reaction Mattos (1997). Lee et al. (1992) introduced changes in AOAC's enzymatic–gravimetric method with the purpose of reducing analysis time and improving the method's precision. The phosphate buffer was replaced by the MES/TRIS buffer 2(N-morpholino) ethanesulphonic acid/tris hydroxymethyl amino-methane. In this case, one pH adjustment stage was eliminated and the amylase volume was reduced by half.

The parameters studied in the validation of the dietary fiber method were accuracy, precision and linearity. Determination of the accuracy of the method to be employed is fundamental in controlling analytical quality and may be carried out in several ways: using recov-

Table 1
Fiber determination method recovery

Cracker biscuits	Expected value	Obtained value	Recovery (%)	Standard deviation	% Coefficient of variation
Biscuit A[a]	6.51	6.55	101	0.03	0.44
Biscuit B[a]	4.55	4.90	108	0.07	1.44
Biscuit C[a]	3.72	4.10	110	0.03	0.73

[a] $n = 6$. A—addition of 5.0 g of reference standard to cracker biscuit 1 (50 g). B—addition of 2.5 g of reference standard to cracker biscuit 1 (50 g). C—addition of 1.5 g of reference standard to cracker biscuit 1 (50 g).

Table 2
Precision of the total dietary fiber determination method

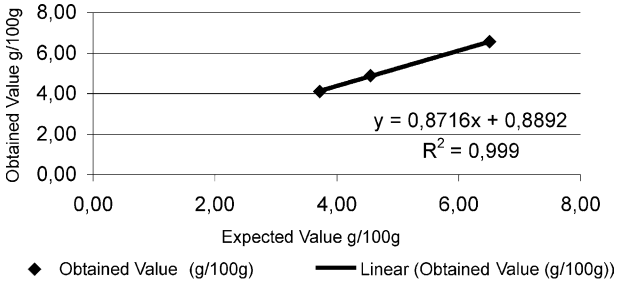
Samples	Standard deviation	% Coefficient of variation	Mean	Absolute error	Relative error
Fiber-rich product	0.65	1.37	47.63***	0.13	0.003
Biscuit 1[a]	0.12	4.89	2.39**	0.02	0.01
Biscuit 2[a]	0.13	4.10	3.17	0.03	0.009

[a] $n = 20$, that is, 10 assays performed in duplicate.
** Mean value for the biscuit used as a matrix in the recovery of the total dietary fiber method.
*** Mean value for the sample used as a secondary standard to add fiber to the biscuit.

Table 3
International analytical quality control: FAPAS

Bread crumbs	FAPAS fiber (g/100 g)	[a]Fiber (g/100 g)	[a]Scores	[a]%
1	9.89	9.48	−0.5	95.85
2	10.10	9.74	−0.4	96.00

[a] Experimental analysis.



Graph 1. Linearity range for the fiber determination method.

ery assays, certified reference material and internal standards (Funk et al., 1995; Long & Winefordmer, 1983).

In this study, a recovery test was applied to the biscuit plus three quantities of the (fiber-rich) secondary reference standard: half, twice and the same quantity of fiber contained in the cracker biscuit sample (matrix).

The absolute error (Ae) is the uncertainty of a measurement with the established reliability (50, 95 and 99%). The absolute error represents the accuracy and informs the approximation to the most probable value of a set of measurements (Parker, 1991).

Precision is a measure of the level of dispersion of the many individual values around the most probable value. It is also indicated by the relative error, which corresponds to the values' coefficient of variation.

Table 2 shows the parameters used for analyzing the precision of the dietary fiber determination method, whose coefficients of variation for the respective samples were 1.37, 4.98 and 4.10%. These values indicate good precision—since precision refers to the collection of values with minimal variation among each other—and fall within the maximum acceptable limit for scientific work, which is 20%. The smaller the coefficient of variation, the lower the dispersion from the mean and, therefore, the greater the precision (Parker, 1991). The coefficient of variation informs whether the repeatability is satisfactory or not. It may be said that the method in question is repeatable, i.e., there is similarity between independent analytical determinations performed by the same analyst using the same equipment and the same method, which corresponds to Interlaboratory precision.

The search for linearity relates to obtaining results in direct proportion to the concentrations of the substances under study. This study required the creation of a calibration curve with the concentration on the x-axis and the response on the y-axis (Flávio Leite, 1996).

Linearity was observed for the dietary fiber determination method in the range of concentrations of 1.39–4.33 g/100 g of added fiber, corresponding to the expected theoretical values for dietary fiber: 3.72, 4.55 and 6.51 g/100 g, since the biscuit already had a natural fraction of fiber. The values obtained by laboratory analysis were 4.10, 4.90 and 6.55 g/100 g.

As can be observed on Graph 1, satisfactory linearity was reached for the dietary fiber determination method. The correlation coefficient (R^2) is high (0.9999), which indicates a good linear relation in the method's response Wang and Li (1995). The graphical representation provides a good indication of the calibration curve's linearity. The linearity of an analytical method's calibration curve represents the method's ability to generate results proportional to analyte concentration within a certain range.

Table 3 shows that the quality control results obtained for bread crumb dietary fiber analysis during this study with AOAC's enzymatic–gravimetric method are fairly close to the FAPAS and show satisfactory scores and good recovery (95.85 and 96.00%), indicating acceptable variability in terms of analytical quality control. Recovery must be between 95% and 100% in relation to the similar food from literature data, that is, use of the same type of sample by other laboratories. The results of these studies demonstrated that the method in question shows a satisfactory score, i.e. that the results obtained are within the acceptable range of variability. This study shows that the method has

reproducibility, since there was similarity between analytical determinations carried out by different laboratories, which corresponds to Interlaboratory precision. Besides that, if there is no standard reference material, one can achieve good data quality using a secondary reference standard. Therefore, it is possible to observe the legislation in effect as well as to contribute to the preparation of the Brazilian food composition table with fiber data obtained using the method in question.

4. Conclusions

Dietary fiber results obtained through the enzymatic–gravimetric method are reliable, considering the accuracy, precision and linearity obtained for cracker biscuits.

The fiber values found in the analytical quality control of the FAPAS demonstrated that the method in question shows a satisfactory score, i.e., the values are within the acceptable range of variability.

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