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## A COMPARISON OF TWO ENZYMATIC METHODS FOR THE ANALYSIS OF DIETARY FIBER IN HIGH-FIBER PREPARATIONS

**Summary.** The aim of the study was to compare results of dietary fiber content determinations in seven high-fiber preparations produced from different waste products, using two enzymatic methods, most commonly applied in Poland, i.e. according to Asp and AOAC. The results of dietary fiber determinations obtained by the AOAC method were lower than those obtained according to Asp: by 5.4% for insoluble fiber, by 11.8% – for soluble fiber and by 7.5% for total dietary fiber. Additionally, a higher accuracy of determinations was shown, expressed in mean values of coefficients of variation, for AOAC method results. Differences in results of dietary fiber determinations indicate the need to unify the method of determination of this component for the purpose of certification and nutritional profiles.

**Key words:** high-fiber preparations, dietary fiber, determination, methods, comparison

### Introduction

In recent years interest in new sources of dietary fiber (DF) has been constantly growing, while on the other hand new methods of its determination are being developed. In view of the fact that different laboratories use different methods of determination for this component, the results concerning its contents may vary considerably.

The main category of methods proposed to analyse DF employs selective extraction of non-fiber materials and gravimetric determination of the residue as fiber. The most popular and recommended is the enzymatic digestion approach (RAVINDRAN and PALMER 1990, HASIK et AL. 1997).

Scientific communities associated with dietary fiber research within AOAC INTERNATIONAL have been the leaders in bringing consensus to the dietary fiber defini-

tion and method validation for over a quarter of a century. The consensus definition and subsequent methodology have served as the basis for regulations worldwide with regard to dietary fiber labelling and health claims (Official... 2004).

The enzymatic gravimetric method for the determination of total dietary fiber (TDF) content developed by AOAC (Official... 2000) is at present used as a reference method in many countries worldwide, especially the USA. It consists in the enzymatic digestion of protein and available carbohydrates from a fat-free sample and gravimetric determination of the residue, including protein and ash contents. Such an approach results in the need to perform a bigger number of replications, which increases the required time and cost of determinations. Moreover, the relatively high cost of reagents needs also to be taken into consideration.

In Poland an older enzymatic method according to Asp et al. (1983) is still being used much more frequently. This method does not require so many replications during determinations and makes it possible to use cheaper reagents, and for these reasons it is recommended by the respective Polish Standard.

The aim of the study was to compare results of determinations of dietary fiber content in high-fiber preparations, using the above mentioned enzymatic methods, i.e. according to Asp and AOAC.

## Materials and methods

Experimental material consisted of six high-fiber preparations by Microstructure in Warszawa, produced at the turn of 2004 and 2005, with mean particle size of approx. 100  $\mu\text{m}$ ; they were wheat bran (PS), cocoa husks (KA), sugar beet pulp (BC), apple pomace (JA), black chokeberry pomace (AR) and black currant pomace (CP). Additionally, analyses were performed on maize preparation (KU) – dry corncobs of mixed different maize cultivars, obtained from the Swadzim Agricultural Experimental Station of the Agricultural University of Poznań, ground using the same method as the other preparations in case of the above mentioned producer.

Contents of soluble dietary fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber (TDF), calculated from the sum of the above, were assayed using enzymatic methods: by ASP et AL. (1983), following the Polish Standard (PN-A-79011-15 1998) and by AOAC (Official... 2000), based on the methods of PROSKY et AL. (1984, 1985).

Determinations by the enzymatic method according to Asp were performed at the food analysis laboratory at the Department of Human Nutrition and Hygiene, the Agricultural University of Poznań, using the following enzymes: NOVOZYMES Termamyl 120L, MERCK 1.07185 Pepsin, SIGMA P-1750 Pancreatin 4xUSP.

In turn, the AOAC determinations were performed at the laboratory of the Department of Nutritional Value of Food, the National Food and Nutrition Institute in Warsaw, Poland, using analytical reagents: SIGMA TDF-100A Dietary Fiber, Total kit.

All determinations were performed using TECATOR FiberTec System E analyser, with TECATOR Filter-Crucibles 30 ml, Por. 2 (40-90  $\mu\text{m}$ ), after preliminary sample grinding in a TECATOR 1093-001 Cyclotec Sample Mill.

Detailed procedures for the above mentioned determinations were published earlier by other authors (BORYCKA and BORYCKI 1999, GRONOWSKA-SENGER et AL. 1992, HASIK et AL. 1997).

Analytical determinations were performed in at least four independent replications and the results were given as mean and SD in terms of dry matter of preparations. One-way analysis of variance (ANOVA) followed with Scheffe's test was used to determine the significance of mean differences, using the Statistica 6.0 computer program.

Coefficients of variation of results obtained for individual preparations and fiber fractions were applied as a measure of precision for the applied methods:

$$V (\%) = SD / \text{mean} \times 100.$$

## Results and discussion

Results of IDF, SDF and TDF content determinations in the analysed preparations obtained using both methods are presented in Table 1. Next to means from four replications, also standard deviations and coefficients of variation of results were given (in brackets). Different letter superscripts (a, b) were used to denote respective means of results obtained by different analytical methods, differing statistically at significance level  $p < 0.05$  (Scheffe's test).

The analysed preparations exhibited a high, but varied TDF content, the mean of which ranged from 49.7% (PS) to 83.2% (KU) according to Asp, at a much lower share of the soluble fraction in relation to the insoluble fraction.

A comparison of the results obtained using different methods showed distinct and in most cases statistically significant differences in dietary fiber content in the analysed preparations. Results of dietary fiber content determinations following the AOAC methodology were lower than those obtained according to Asp: on average by 5.4% for IDF, 11.8% for SDF and by 7.5% for TDF.

Additionally, a markedly higher precision of determinations was shown, as manifested by the mean values of coefficients of variation of results, in case of determinations obtained by the AOAC method at the National Food and Nutrition Institute in Warsaw.

On the basis of determination results for the seven analyzed preparations the following dependencies were established, presented by linear regression formulas:

$$\begin{aligned} \text{IDF (AOAC)} &= 0.827 \times \text{IDF (Asp)} + 7.834, \\ \text{SDF (AOAC)} &= 0.598 \times \text{SDF (Asp)} + 1.436, \\ \text{TDF (AOAC)} &= 0.974 \times \text{TDF (Asp)} - 3.603. \end{aligned}$$

In spite of the relatively high coefficients of linear correlation and significance levels (Table 1) obtained for the given dependencies, in view of the high differences in the presented results, especially for SDF determinations, the application of the above formulas may not be recommended for the estimation of dietary fiber determination results using the AOAC method on the basis of results obtained according to Asp.

Table 1. Results of IDF, SDF and TDF determinations using two enzymatic methods in high-fiber preparations (mean  $\pm$  SD, V (%) and difference (%), g/100 g; detailed description given in the text)  
 Tabela 1. Wyniki oznaczeń IDF, SDF i TDF dwoma metodami enzymatycznymi w preparatach wysokobłonnikowych (średnia  $\pm$  SD, V (%) oraz różnica (%), g/100 g; szczegółowy opis w tekście)

Method preparation	DF fraction								
	IDF			SDF			TDF		
	Asp	AOAC	percentage difference	Asp	AOAC	percentage difference	Asp	AOAC	percentage difference
PS	49.69 <sup>b</sup> $\pm$ 1.42 (2.85%)	44.85 <sup>a</sup> $\pm$ 0.12 (0.26%)	-9.7	3.47 <sup>b</sup> $\pm$ 0.28 (8.21%)	1.86 <sup>a</sup> $\pm$ 0.06 (2.98%)	-46.5	53.16 <sup>b</sup> $\pm$ 1.51 (2.85%)	46.70 <sup>a</sup> $\pm$ 0.16 (0.33%)	-12.1
KU	88.86 <sup>b</sup> $\pm$ 2.22 (2.49%)	79.84 <sup>a</sup> $\pm$ 0.22 (0.27%)	-10.2	0.84 $\pm$ 0.08 (9.88%)	0.58 $\pm$ 0.16 (27.45%)	-30.6	89.70 <sup>b</sup> $\pm$ 2.19 (2.44%)	80.42 <sup>a</sup> $\pm$ 0.27 (0.34%)	-10.3
KA	60.42 <sup>b</sup> $\pm$ 0.76 (1.25%)	54.37 <sup>a</sup> $\pm$ 0.11 (0.20%)	-10.0	11.21 <sup>b</sup> $\pm$ 0.75 (6.73%)	9.18 <sup>a</sup> $\pm$ 0.13 (1.42%)	-18.1	71.63 <sup>b</sup> $\pm$ 0.54 (0.75%)	63.55 <sup>a</sup> $\pm$ 0.10 (0.16%)	-11.3
BC	61.88 <sup>a</sup> $\pm$ 0.82 (1.33%)	70.90 <sup>b</sup> $\pm$ 0.25 (0.35%)	14.6	17.89 <sup>b</sup> $\pm$ 0.66 (3.66%)	9.67 <sup>a</sup> $\pm$ 0.41 (4.26%)	-45.9	79.76 $\pm$ 0.58 (0.72%)	80.57 $\pm$ 0.19 (0.23%)	1.0
JA	59.32 $\pm$ 2.19 (3.70%)	56.58 $\pm$ 0.07 (0.12%)	-4.6	10.19 $\pm$ 0.89 (8.75%)	10.76 $\pm$ 0.48 (4.46%)	5.6	69.51 $\pm$ 1.74 (2.51%)	67.34 $\pm$ 0.47 (0.70%)	-3.1
AR	83.24 <sup>b</sup> $\pm$ 0.59 (0.70%)	77.49 <sup>a</sup> $\pm$ 0.51 (0.66%)	-6.9	1.86 <sup>a</sup> $\pm$ 0.15 (8.22%)	3.15 <sup>b</sup> $\pm$ 0.32 (10.27%)	69.1	85.10 <sup>b</sup> $\pm$ 0.59 (0.70%)	80.63 <sup>a</sup> $\pm$ 0.83 (1.03%)	-5.3
CP	71.57 <sup>b</sup> $\pm$ 0.45 (0.63%)	63.71 <sup>a</sup> $\pm$ 0.34 (0.54%)	-11.0	8.51 $\pm$ 1.36 (15.99%)	7.10 $\pm$ 0.22 (3.04%)	-16.6	80.09 <sup>b</sup> $\pm$ 0.95 (1.19%)	70.81 <sup>a</sup> $\pm$ 0.54 (0.77%)	-11.6
Correlation coefficient	r = 0.90 (p < 0.01)		-	r = 0.88 (p < 0.01)		-	r = 0.95 (p < 0.001)		-
V (%) average	1.85 <sup>b</sup> $\pm$ 1.17	0.34 <sup>a</sup> $\pm$ 0.19	-	8.78 $\pm$ 3.75	7.70 $\pm$ 9.15	-	1.59 <sup>b</sup> $\pm$ 0.96	0.51 <sup>a</sup> $\pm$ 0.32	-
Percentage difference (average)	-	-	-5.4	-	-	-11.8	-	-	-7.5

It may not be excluded that such equations could be useful in case of homogenous groups of products, e.g. grain or bran of different cereals, different assortments of bakery products or different vegetables.

The presented results confirm analytical problems mentioned by BORYCKA and BORYCKI (1999) in the determination of dietary fiber, as well as the incompatibility of the applied methods.

Thus, the unification of methods to determine dietary fiber used nationally and their adaptation to world standards, proposed earlier by GRONOWSKA-SENGER et AL. (1992), seems advisable, with the implementation of the AOAC method as obligatory methodology in the determination of dietary fiber in food being the most reasonable.

## Conclusions

Statistically significant differences shown in the values of dietary fiber determination results were shown for the Asp and AOAC methods, with the results obtained using the AOAC method being significantly lower.

Differences in the results of dietary fiber determinations obtained using different methods indicate the need to unify the methodology of determining this component for the needs of food composition tables and nutritional certification.

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## PORÓWNANIE WYNIKÓW OZNACZEŃ BŁONNIKA POKARMOWEGO W PREPARATACH WYSOKOBŁONNIKOWYCH WYBRANYMI METODAMI ENZYMATYCZNYMI

**Streszczenie.** Celem pracy było porównanie wyników oznaczeń zawartości błonnika pokarmowego dwoma najpowszechniej stosowanymi metodami enzymatycznymi: Aspa oraz AOAC. Materiałem badawczym były preparaty wysokobłonnikowe otrzymane z surowców odpadowych. Wyniki oznaczeń błonnika otrzymane metodą AOAC były niższe od otrzymanych metodą Aspa, średnio o 5,4% dla IDF, 11,8% dla SDF oraz 7,5% dla TDF, co wskazuje na niekompatybilność wymienionych metod oraz potrzebę ujednoczenia metodyki.

**Słowa kluczowe:** preparaty wysokobłonnikowe, błonnik pokarmowy, oznaczanie, metody, porównanie

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