Resistant Starch in Dietary Fiber Values Measured by the AOAC Method in Different Cereals

M. E. SAMBUCETTI[1,2] and A. ZULETA [1]

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ABSTRACT

Total dietary fiber (TDF) was measured by the AOAC method 985.29 using 30 and 60 min of amyloglucosidase incubation. Resistant starch was determined in the TDF residues after gelatinization, dissolution with 2*M* KOH, and hydrolysis with amyloglucosidase. The cereals analyzed were: commercial samples of rice (white, parboiled, precooked, and brown parboiled); oat (rolled); maize (grits); and wheat (crackers with bran). Varietal samples of white rice with 1, 18, 24, and 30% amylose also were analyzed. The results showed that cereal starches have different resistances to hydrolysis. Oat and wheat starch were completely hydrolyzed after 30 min, while rice and maize starch remained ≈42 and 50%, respectively, of TDF values after 60 min. As expected in the varietal rices, when amylose content increases from 18 to 30%, TDF values were significantly higher because of resistant starch. After subtracting the starch content from the TDF values in white rice, results were similar to values obtained by a chemical method that included lignin. Therefore, it is suggested that corrections are necessary not only for protein and ash, but also for starch, especially on starchy foods.

Dietary fiber content is one of the interesting nutritive parameters in food. The enzymatic-gravimetric method of Prosky et al (1985), officially known as AOAC method 985.29 (AOAC 1990), has been adopted by government agencies in many countries for routine nutrient labeling analyses because it is simple and rather inexpensive. However, there is some concern that the values for total dietary fiber (TDF) obtained in some foods are higher than those obtained by other enzymatic-chemical methods (Mongeau and Brassard 1989, 1993, 1994).

The current AOAC method of dietary fiber determination includes undigested starch in its estimation (Englyst and Cummings 1988, Muir et al 1993, Vollendorf et al 1993, Marlett et al 1994). Several authors consider that analytically enzyme-resistant starch should be included in dietary fiber results because its physiological properties are similar to certain fiber polysaccharides (Theander et al 1994, Prosky et al 1985). However, the amount of this undigested starch would depend not only on the particular characteristics of a starch, such as amylose-to-amylopectin ratio, crystallinity, and gelatinization temperature, among others (Colonna et al 1992), but also on the sample treatment, such as grinding or cooking (Snow and O'Dea 1981). Therefore, the starch amount included in fiber results, might not reflect the true amount of resistant starch in human digestion (Englyst and Cummings 1988).

Starchy foods such as cereals might yield the highest results. We have noticed that rice is one of the cereals that shows larger differences among results from different methods. In this article, we describe an improvement to the AOAC method of starch hydrolysis that involves prolonging the incubation time with amyloglucosidase to obtain results closer to those obtained by enzymatic-chemical methods on rice, maize, oat, and wheat. Furthermore, we studied the effect of amylose concentration on the dietary fiber results of rice. Resistant starch was determined on the fiber residues as the total available starch for amyloglucosidase digestion after solubilization with 2*M* HOK (Champ 1992).

[1]Department of Food Sciences and Nutrition, School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina.
[2]Corresponding author. Fax: 54-1-964-8423.

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MATERIALS AND METHODS

Materials

Commercial samples of rice purchased for this study were all long grain and included white, parboiled white, precooked white, and brown parboiled. The commercial names (and suppliers) were: Gallo, Condor, and Máximo rices (from La Arrocera Argentina and Molinos Río de la Plata); Quaker brand rolled oats (Elaboradora Argentina de Cereales); maize grits (semiflint type from Arcor); wheat crackers enriched with bran (Granix and Terrabusi). These foods were purchased at local markets or supplied by the industry for analysis.

Rice varieties with different amylose content included: Hiyoku mochi (short grain), Itapé (medium grain), Blue belle (long grain), and Br409 (long grain), with 1, 18, 24, and 30 g of amylose /100 g, respectively. These samples were grown at the Department of Vegetal Production (Rice Program) of the School of Agricultural Sciences and Forestry, National University of La Plata, Argentina. They were provided by J. J. Marassi and A. Vidal. They were gathered, dried in an air-oven to 13% mc and milled with an experimental mill (universal type, Guidetti and Artioli). Samples were ground in a domestic grinder (Moulinex) and sieved through a 35-mesh (0.5-mm) screen.

Analysis

Samples were analyzed without any treatment, but at the same time, a portion of each sample was dried in an air-oven at 100°C until constant weight to express the results on dry weight basis.

TDF was measured according to the AOAC method 985.29 (AOAC 1990). Samples were gelatinized with a heat-stable α-amylase (pH 6, 100°C, 30 min) and then enzymatically digested sequentially with protease (pH 7.5, 60°C, 30 min) and amyloglucosidase (pH 6, 0°C, 30 min) to remove protein and starch. TDF was precipitated with ethanol, and after washing and drying, the residue was weighed. Results were corrected for protein and ash.

Each commercial sample was incubated with amyloglucosidase for 30 or 60 min; at least three determinations of each treatment were conducted. TDF in the rice varieties was measured using the AOAC method after 30 min of amyloglucosidase incubation. Each variety was analyzed in triplicate. The amylose content was determined according to ISO method 6647 (ISO 1987).

Starch in the TDF residues of the varietal rices and commercial cereals (after 30 and 60 min of incubation with amyloglucosidase, respectively) was determined as total starch by the method of Asp

et al as described by Champ (1992). The TDF residue was gelatinized with water (100°C, 20 min), treated with 2*M* KOH, and incubated with amyloglucosidase (pH 4.75, 60°C, 30 min). After centrifugation, free glucose was measured in the supernatant using glucose-oxidase and peroxidase reagent. On commercial samples, two replicates were made. On rice varieties, because the limited availability of the samples, only one determination was conducted on each of them.

The enzymes employed for TDF and starch determination were: α-amylase (Sigma A 3306); protease (Sigma P3910), and amyloglucosidase (Sigma A 9913). Enzyme activity was checked using a control kit (Sigma TDF-C10). Glucose was measured using GOD-POD test kit (Boehringer 100097).

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple comparison test.

RESULTS AND DISCUSSION

The effect of prolonging the incubation time with amyloglucosidase on TDF values is shown in Table I. TDF results obtained after 60 min were lower than those obtained after 30 min in rice and maize ($P < 0.01$, $P < 0.001$). On commercial rice samples, reductions between 11 and 17 % were achieved. Thus, 60 min of amyloglucosidase incubation removed the differences among the results of white, parboiled, and precooked rice. The latter had shown a TDF value higher than those of the other two ($P < 0.01$) after 30 min.

Because the starch in rice is very firmly associated with protein (Cone and Mechteldis 1990), which could prevent amyloglucosidase action, we also tried to improve the starch hydrolysis by prolonging the incubation with protease to 60 min. However ,the results were no different (results not shown).

The results obtained after 30 min of amyloglucosidase incubation were within the range of values determined with the AOAC method by other authors (Cardozo and Eitenmille 1988, Wang et al 1991). Differences between results may have been brought about by different varieties or processes.

The reduction in the TDF values for maize was 25% after 60 min of amyloglucosidase incubation, in spite of a recovery of 0% in the enzyme test with maize starch.

The results obtained on rolled oats and wheat bran crackers did not shown any difference between treatments. Oat TDF values are comparable with the values for rolled oat in the German food tables (Souci et al 1989) as measured by modified AOAC method, but they were lower than the value declared on the label, 9 g/100 g. We must point out that β-glucan recovery, measured by the enzyme test, was only 75%; β-glucanase activity in commercial amyloglucosidase has been observed by other authors (Theander et al 1994). The effect of rice amylose content on TDF values using the original method is shown in Figure 1. As amylose content rises, TDF values are higher. Significant differences ($P < 0.01$) were found among rices with 18, 24, and 30% amylose, showing a highly significant correlation ($r = 0.985$). Curiously, the Hiyoku mochi rice variety with 1% amylose tended to give higher TDF values than the Itapé variety with 18% amylose. This trend had already been observed with other samples. Total starch values determined on the TDF residues were ≈57% of TDF values and, as expected, they followed the same trend.

TDF determinations performed over two years are shown in Table II. After incubation with amyloglucosidase for 60 min, TDF of commercial samples of rice and maize still had a considerable amount of starch. For white rice, after subtracting this starch content from TDF we arrived at a result similar to the TDF value obtained by the chemical method (1.6 g/100 g) that includes lignin (Marlett et al 1989). Starch removal in brown parboiled rice was not complete, so the final result by subtracting it, is still high.

Particle size and different milling processes are important factors influencing TDF results (Mullin et al 1995). In a collaborative study by Prosky et al (1985), average values as low as 1.04 g /100 g, were obtained for rice, but it had been ground to a uniform size (0.35 mm). In this study, all foods were ground to a particle size of <0.50 mm, but the size was not uniform. Nevertheless, the results obtained by Prosky et al (1985) were highly variable (cv = 45.62%), with values between 0.35 and 2.18 g/100 g. Our results are very constant (cv = 3–5.5%), in spite of the changes in enzyme lots and samples over two years.

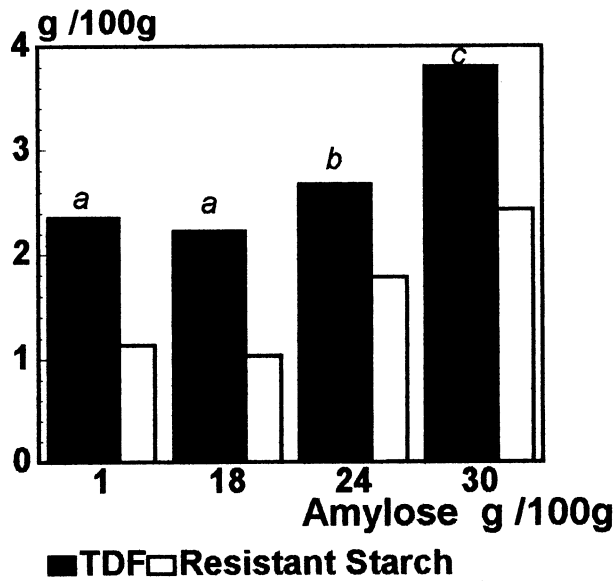

Fig. 1. Effect of rice amylose content on total dietary fiber (TDF) values measured using the AOAC method. Bars with different superscript letters are significantly different ($P < 0.01$).

TABLE I
Effect of Incubation Time with Amyloglucosidase on Total Dietary Fiber (TDF, g/100 g) Using the AOAC Method in Cereals[a]

Cereals	Time		Significance
	30 min	60 min	
Rice			
White	2.85 ± 0.03	2.54 ± 0.06	$P < 0.01$
Parboiled	2.90 ± 0.02	2.56 ± 0.04	$P < 0.01$
Precooked	3.15 ± 0.04	2.62 ± 0.01	$P < 0.001$
Brown parboiled	6.41 ± 0.04	5.46 ± 0.09	$P < 0.001$
Oats, rolled	6.19 ± 0.09	6.16 ± 0.06	ns[b]
Maize, grits	3.74 ± 0.01	2.80 ± 0.09	$P < 0.001$
Wheat crackers with bran	6.45 ± 0.14	6.41 ± 0.11	ns

[a] Values are expressed on a dry weight basis. Means (of three measurements) ± standard error mean.
[b] Not significant.

TABLE II
Influence of Resistant Starch (RS) on Total Dietary Fiber (TDF, g/100g) in Cereals[a]

Cereals	*n* =	TDF	CV%[b]	RS[c]	TDF – RS
Rice					
White	15	2.54 ± 0.08	3.1	1.06 ± 0.06	1.51
Parboiled	14	2.57 ± 0.13	5.0	0.98 ± 0.06	1.59
Precooked	8	2.56 ± 0.08	3.3	1.23 ± 0.05	1.33
Brown parboiled	16	5.37 ± 0.30	5.5	1.27 ± 0.06	4.14
Oats, rolled	7	6.16 ± 0.12	1.9	0.00	6.16
Maize, grits	9	2.84 ± 0.14	4.9	1.48 ± 0.05	1.36
Wheat crackers with bran	6	5.79 ± 0.19	3.4	0.00	5.79

[a] Values are expressed on a dry weight basis. Means ± standard deviation. TDF was measured after treatment with amyloglucosidase for 60 min.
[b] Coefficient of variation.
[c] Means of two measurements.

In wheat and oat TDF residues, no starch was found. This is in agreement with absence of change after 60 min of amyloglucosidase incubation (Table I) because the starch had been completely hydrolyzed after 30 min. In analyzing 38 foods, Mongeau and Brassard (1989) found close agreement between two enzymatic-gravimetric techniques: the rapid Health Protection Branch (HPB) and AOAC methods. However, among cereals, AOAC results for rice and maize were 57% greater than HPB values, while those for oats and wheat were similar using either method.

CONCLUSIONS

These results show different resistance to hydrolysis in the analysis conditions offered by starches from different cereals. Rice and maize starches are more resistant than oat and wheat starches. Consequently, the procedure for validating the efficacy of the enzyme treatment proposed by Prosky et al (1985) is not satisfactory, because the wheat and maize starches used in the test are not only from origins different from the test samples in many cases, but they are also free and have a very small particle size. Thus, it would be better to validate the results using suitable reference materials for the different food groups.

To avoid uncertain results involving the complexity of fiber, we consider that any vestige of starch must be eliminated. With that objective, Mongeau and Brassard (1994) working with legumes suggested the use of α-amylase from porcine pancreas between the heat-stable amylase and amyloglucosidase treatment. This has not yet been proved on cereals like rice and maize. Until then, we suggest as an alternative that for TDF results in starchy foods determined by the AOAC method 985.29, a correction be made for starch as well as for protein and ash.

Since resistant starch may have physiological properties similar to certain dietary fiber polysaccharides, the ideal situation would be to determine dietary fiber and resistant starch contents of food independently.

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