

SHORT COMMUNICATION

Elimination of resistant starch type II within the framework of total starch and dietary fibre analysis by microwave irradiation

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Abstract

Introduction The presence of resistant starch in samples containing non-starch polysaccharides has always been a challenge to enzymatic total starch and total fibre analysis. **Objective and methods** Based on microwave-induced pressure disintegration technique the Association of Official Analytical Chemists methods for the determination of total starch (AOAC 996.11) and total dietary fibre (AOAC 991.43) have been modified to completely eliminate undesirable resistant starch fractions with respect to digestion procedures using thermostable α -amylase and amyloglucosidase. **Results** Microwave treatment of high-amylose starch samples resulted in excellent total starch recovery in the Association of Official Analytical Chemists standard method no. 996.11. After integration of microwave disintegration technique into the total dietary fibre method AOAC 911.43 irradiation experiments with different model mixtures consisting of non-starch polysaccharides components and high-amylose starch fractions resulted in the complete elimination of undesirable resistant starch fractions. **Conclusion** Therefore the microwave technique can be a very efficient means for the elimination of resistant starch and provides more realistic values in analytical total dietary fibre procedures with respect to samples containing critical enzyme resistant starches.

Introduction

Since resistant starch (RS) has been recognized as an important part of the dietary fibre complex its presence in samples containing traditional fibre components has always been a great challenge to total dietary fibre (TDF) analysis (McCleary, 2003; DeVries & Rader, 2005). RS is defined as that fraction of starch not being digested by hydrolytic enzymes in the small intestine. Similarly the term 'dietary fibre' encompasses several plant-derived food components (cellulose, pectin, glucans, etc.) possessing common characteristics resisting attack by human enzymes in the alimentary tract (Delcour & Erlingen, 1996; Champ *et al.*, 2003). However, although there are several methods for the separate determination of RS (Englyst *et al.*, 1992; Sajilata *et al.*, 2006) unfortunately in the Association of Official Analytical Chemists (AOAC) standard method no. 991.43 for the determination of TDF components only parts of RS fractions can be

detected, depending on its structure and polymorph type corresponding to its origin (Themeier *et al.*, 2005).

This can be attributed to the unfavourable thermal digestion conditions in the enzymatic-gravimetric TDF procedure in which thermostable α -amylase at 95 °C and amyloglucosidase (AA/AMG procedure) are used. On the one hand this digestion temperature does not result in complete disintegration of RS so that all the starch present in the sample can be removed. On the other hand this temperature does not correspond to physiological conditions so that it is not possible to recover the complete RS fractions together with other non-starch polysaccharides (NSP). In the meantime several solutions for this analytical challenge have been proposed. One modification of the TDF methods has been developed using more physiological-like digestion conditions (37 °C) with the aim of recovering the complete RS fraction in the enzymatic gravimetric TDF procedure (McCleary, 2007). Another variation is based on

the idea of removing the complete RS fraction in the TDF method AOAC 991.43 using the solvent dimethyl sulphoxide (DMSO), a procedure which is also strongly recommended in the enzymatic procedure for quantifying total starch (AOAC 996.11) in connection with samples containing considerable fractions of RS (McCleary & Rossiter, 2004). However, a strong disadvantage of applying DMSO in the TDF procedure 991.43 requiring open filtration steps is the risk of health impairment such as skin irritation resulting from decomposition products due to the chemical instability of DMSO at 95 °C. A new and elegant methodological attempt at solving this problem without chemicals could be the use of the microwave technique to disintegrate RS fractions in enzymatic methods based on *in vitro* AA/AMG digestion steps. Unlike conventional heating at 95 °C for gelatinizing starch fractions in which only partial penetration of heat irradiation into organic materials is achieved (surface hot, inside cold), microwave heating results in a more effective heat penetration into the inner highly crystalline domains of the RS, so that an improved digestion can be enabled in all enzymatic procedures which are based on thermostable α -amylase and amyloglucosidase.

The main objective of this work was to investigate the suitability of a microwave-induced pressure disintegration of RS in analytical procedures for the determination of TDF based on thermostable α -amylase and amyloglucosidase digestion. The fundamental possibility of a microwave assisted elimination of RS should be proved using the standard method for total starch determination AOAC 996.11 by analysing high-amylose starch samples. Furthermore, the integration of a microwave pre-treatment step into the analytical procedure of the AOAC method 991.43 for the determination of TDF should be investigated. For this purpose, mixtures consisting of high-amylose maize starch (RS Type II) as a standard and different traditional dietary fibre components were analysed for TDF recovery after microwave irradiation with the aim of recovering only the NSP components.

Materials and methods

Dietary fibre sources

A low-amylose maize starch, product no. 03402, was donated by Cerestar Deutschland GmbH (Krefeld, Germany), a high-amylose maize starch, product no. 39, was supplied by Roquette Freres (Lestrem, France), Amylogel 03003, an unmodified high-amylose food starch based on American corn (including 70% amylose) was obtained from Cargill Inc. (Food & Pharma Specialities, Minneapolis, MN, USA).

A high-amylose wrinkled pea starch was isolated from pea variety Tristar and a pea starch, 16 (Themeier *et al.*, 2005), based on a pea mutant provided by the John Innes Centre (Norwich, UK), was isolated in a pilot-scale process at the Max Rubner-Institut MRI (Federal Research Institute of Nutrition and Food) at Detmold, Germany. Cellulose (Sigma-cell Type 101, S-6790) and apple pectin (P8471) were provided by Sigma-Aldrich GmbH (Taufkirchen, Germany). Wheat arabinoxylans from wheat (medium viscosity) and β -glucans from barley (medium viscosity) were supplied by Megazyme (Megazyme International, Bray, Ireland).

Standard methods

Crude protein was determined by the Kjeldahl method ($N \times 6.25$) according to the ICC standard method no. 104/1, ash was determined according to ICC standard method no. 105/2 and lipid content according to ICC standard method no. 136 (Standard Methods, 2001). Amylose content was determined according to the AOAC standard method no. 2002.02 [Association of Official Analytical Chemists (AOAC), 2003] using K-AMYL assay kit from Megazyme (Megazyme International). RS was detected enzymatically according to AOAC standard method no. 2002.02 using assay kit K-RSTAR from Megazyme. Total starch was determined according to the AOAC standard method no. 996.11 (AOAC, 2003) using assay kit K-TSTA from Megazyme. Both assay variants, standard procedure A without DMSO sample pre-treatment (AA/AMG procedure) and modified procedure B for samples containing RS (DMSO/AA/AMG procedure) were performed. The TDF content of samples was measured according to the AOAC standard method no. 991.43 (AOAC, 2003) using assay kit K-TDFR from Megazyme. Modified analysis of TDF using DMSO for starch solubilization was performed according to a scheme proposed by McCleary for introduction of DMSO into the AOAC method 991.43 (McCleary & Rossiter, 2004). All analyses according to standard methods quoted above were conducted in duplicate.

Microwave experiments

The microwave-irradiation experiments were carried out in a microwave oven designed for use in chemical laboratories. A commercially available Teflon vessel (as a pressure reactor) with burst disc and screw cap was used to accommodate the different reaction tubes in which the real digestion and analyses were performed. This experimental setup was chosen to assure minimal hazard for the laboratory personnel performing the analyses. The following microwave

equipment was manufactured and supplied by Landgraf Laborsysteme GmbH (Langenhagen, Germany):

- Laboratory microwave oven Type 3100 with flow cooler, maximum irradiation efficiency (power) 600 W, irradiation frequency 2450 MHz,
- 100 mL Teflon microwave vessel (pressure reactor) with screw cap (Type 7011/15) including burst disc,
- for total starch determination: open glass tubes (16 mm × 120 mm),
- for TDF determination: open round bottomed Teflon tubes, 70 mL total volume, length 116 mm, inner diameter 29 mm, outer diameter 35 mm, with screw cap.

Incorporation of microwave technique into AOAC method 996.11 for analysing total starch

Hundred milligrams of starch samples were accurately weighed out into glass tubes (16 mm × 120 mm) and, after wetting with 0.2 mL aqueous ethanol (80% v/v), 4 mL H₂O were added to completely disintegrate the starch granules during microwave heating. This starch water ratio resulted in optimal heat penetration effects, which led to complete disintegration of the starch fractions. After stirring the tubes on a vortex mixer until there were no more lumps, a magnetic stirring rod was added and three *open* tubes were placed into the Teflon vessel containing 20 mL of water. This water facilitated the transfer of microwave radiation energy to the analytical samples. The mixtures in the sealed teflon vessels were irradiated for 5 min at maximum power of 600 W, stirred outside the microwave oven on a magnetic stirrer for 5 min, and irradiated for another 5 min. After cooling the reactor for 15 min to relieve pressure within the Teflon vessel, the cap was removed. All subsequent steps were carried out in the glass tubes according to the standard procedure of the AOAC method no. 996.11 for total starch determination. All total starch values are given as means of triplicate measurements.

Incorporation of microwave technique into AOAC method 911.43 for analysing TDF

The modified analysis of TDF using microwave technique for starch solubilization was performed according to the following scheme:

One gram sample dry matter was accurately weighed out into a Teflon tube and the *open* tube was placed into the vessel. After addition of 40 mL MES-Tris buffer pH 8.2 the mixture was stirred until the slurry was free of lumps. Thereafter the vessel was capped and irradiated at maximum

power (600 W) for 3 min, stirred outside the microwave oven for 5 min before restarting microwaving for another 3 min. After cooling for 15 min, the vessel was unsealed and the Teflon tube taken out. Subsequently, the enzymatic digestion was performed in the Teflon tube according to the standard method AOAC 991.43. After the enzymatic digestion steps the mixture in the Teflon tube was poured into a glass beaker using ethanol water mixtures corresponding to the required precipitation concentrations in accordance with the standard method. All analyses are given as means of duplicate measurements in accordance with the standard AOAC procedure.

Results and conclusion

Incorporation of microwave technique into AOAC method 996.11 for analysing total starch

The total starch data of the high-amylose maize starch Amylogel 03003 as a model substance in Figure 1 demonstrate the suitability of using the microwave technique to disintegrate RS.

It is known that the AOAC method no. 996.11 in connection with high-amylose starches containing RS type II provides too low total starch contents (Themeier *et al.*, 2005). Total starch determination according to the standard procedure AOAC 996.11 of the Amylogel 030003 sample resulted only in about 85% starch recovery (see Figure 1). Applying microwave irradiation under an optimum starch–water ratio of 1:40 w/w led to an increase of total starch recovery of approximately 98% after already 350 s. This indicates that the penetration of microwaves into crystalline structural elements of RS is able to disintegrate the crystalline enzyme resistant domains of high-amylose starches. Microwave energy is absorbed by water molecules giving origin to local heating of the inner crystalline regions of the granules. Under the influence of increasing

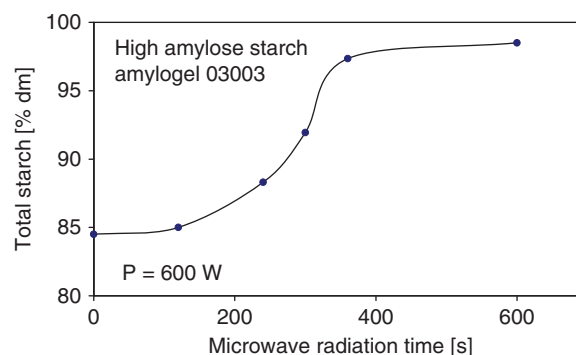


Figure 1 Effect of radiation time on total starch recovery in high-amylose maize starch.

Table 1 Incorporation of microwave technique into AOAC method 996.11 for analysing total starch in comparison with standard procedure and DMSO treatment

	Low-amylose maize starch 03402	High-amylose maize starch 39	High-amylose maize starch Amylogel	High-amylose pea starch 16	High-amylose pea starch Tristar	Cellulose as control
Protein (% d.m.)	0.38	0.75	0.15	0.43	0.5	–
Ash (% d.m.)	0.07	0.09	0.79	0.12	0.16	–
Lipid (% d.m.)	0.14	0.50	0.50	0.49	0.21	–
Amylose (% total starch)	26.9	65.8	~70 ¹	62.5	62.5	–
Resistant starch (% d.m.)	0.6	49.1	47.0	18.0	18.7	–
Total starch ² AA/AMG (% d.m.)	91.8	87.2	84.5	89.5	88.1	–
Total starch ³ DMSO/AA/AMG (% d.m.)	94.4	95.4	94.7	95.5	95.4	–
Total starch ⁴ microwave/AA/AMG (% d.m.)	94.6	96.4	98.5	96.3	92.3	0.1

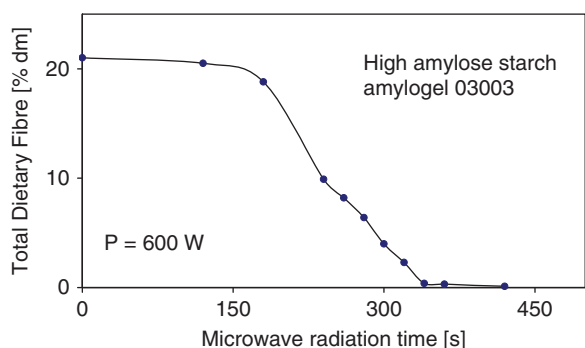
¹Nominal value according to company information.

²Standard method according to AOAC 996.11 without DMSO pre-treatment, Results expressed as averages of duplicates.

³Standard method according to AOAC 996.11 with DMSO pre-treatment for samples containing RS, results expressed as averages of duplicates.

⁴Modified total starch procedure using microwave radiation for elimination of resistant starch; $P = 600$ W, $t = 2 \times 5$ min, results expressed as averages of triplicates.

AOAC, Association of Official Analytical Chemists; DMSO, dimethyl sulphoxide; d.m., dry matter ; RS, resistant starch.

**Figure 2** Effect of radiation time on total dietary fibre recovery in high-amylose maize starch.

temperature and pressure the highly ordered regions are destroyed and melting starts. This effect of microwave treatment on the dissolution of RS can therefore completely replace the hot DMSO treatment of high-amylose starches in the AOAC method 996.11. To verify this effect Table 1 shows total starch data of different RS Type II sources with respect to their amylose content and crystal polymorph type after microwave-induced pressure disintegration.

The microwave technique eliminated more RS and led to higher values for total starch content in comparison with the DMSO procedure.

Incorporation of microwave technique into AOAC method 911.43 for analysing TDF

A first comparative evaluation for the successful incorporation of the microwave-induced pressure disintegration of RS

into dietary fibre analysis is presented in Figure 2 and Tables 2 and 3.

After 340 s of microwave heating of Amylogel 03003 those partial amounts of RS, which had been detected as TDF with the AOAC method 911.43, were no longer detectable (Figure 2). Without microwaving about 21% TDF was found for this RS source. This proves that the microwave technique as a fundamental possibility for the elimination of RS can be technically integrated into TDF method AOAC 911.43. Table 2 presents comparative data of TDF measurements of various starches and NSP achieved with the AOAC 991.43 standard method, the DMSO-modified procedure and the microwave technique at different energy levels, respectively.

Determination of TDF in high-amylose starches resulted in relatively high TDF contents when the AOAC 991.43 standard procedure was used. DMSO pre-treatment of samples led to significantly reduced TDF values in the range of 4–7% of dry matter. Microwave treatment at an energy level of 480 W for 2×3 min reduced the TDF in maize starches to very small values. The TDF content of high-amylose pea starch 16 remained high. This can be explained by the known stability of the crystalline C-type structure of leguminose starches like pea starch 16. When irradiating this starch with 600 W for 2×3 or even 2×5 min the TDF value drops to 1.1% of dry matter or is even no longer detectable, respectively. Such low TDF values can also be found in low-amylose cereal starches (Themeier *et al.*, 2005). With the exception of pectin other NSP are neither influenced by DMSO nor by microwave treatment. Recovery of pectin after microwave heating was around 50–80%, depending on

Table 2 Incorporation of the microwave technique into AOAC method 911.43 for analysing TDF in comparison with the standard procedure and the DMSO-modified TDF procedure in different starch and non-starch polysaccharides

Polysaccharide component	Standard procedure ¹ (% d.m.)	DMSO procedure ² (% d.m.)	Microwave procedure ³	Microwave procedure ³
			<i>P</i> = 480 W <i>T</i> = 2 × 3 min (% d.m.)	<i>P</i> = 600 W <i>T</i> = 2 × 3 min (% d.m.)
Low-amylose maize starch 03402	0.9	n.d.	0.7	0.5
High-amylose maize starch Amylogel 03003	21.0	4.5	1.52	0.31
High-amylose pea starch 16	11.6	6.9	12.0	1.1
Cellulose	99.3	97.3	99.9	100.1
Arabinoxylan	96.9	102.6	96.4	98.9
β-Glucan	95.4	97.9	96.6	98.8
Pectin	91.6	124.1	78.6	55.5

¹TDF standard method according to AOAC 991.43.

²Proposed scheme by McCleary and Rossiter (2004) for introduction of DMSO into TDF procedure.

³Microwave TDF procedure using different power settings.

All TDF data expressed as averages of duplicates related to d.m.

AOAC, Association of Official Analytical Chemists; DMSO, dimethyl sulphoxide; d.m., dry matter; n.d., not determined; TDF, total dietary fibre.

Table 3 Incorporation of microwave technique into AOAC method 911.43 for analysing TDF in comparison with standard procedure and DMSO-modified TDF procedure in model mixtures containing RS Type II fractions

DF model mixture mass ratio d.m. Starch/NSP = 10:1	Theoretical content ¹ including detectable RS (% d.m.)	Theoretical content ² without RS (% d.m.)	Standard procedure ³ (% d.m.)	DMSO procedure ⁴ (% d.m.)	Microwave procedure ⁵ <i>P</i> = 480 W <i>t</i> = 2 × 3 min (% d.m.)	Microwave procedure ⁵ <i>P</i> = 600 W <i>t</i> = 2 × 3 min (% d.m.)
	Regular starch ⁶ /cellulose	9.8	9.8	10.5	n.d.	10.9
Regular starch ⁶ /arabinoxylan	9.6	9.6	11.9	n.d.	10.0	9.8
Regular starch ⁶ /β-Glucan	9.5	9.5	11.9	n.d.	10.3	9.8
Regular starch ⁶ /pectin	9.1	9.1	9.5	n.d.	8.7	8.1
RS starch ⁷ /cellulose	28.1	9.9	27.4	9.8	23.3	10.5
RS starch ⁷ /arabinoxylan	27.9	9.7	23.9	10.7	23.8	9.5
RS starch ⁷ /β-glucan	27.8	9.6	25.2	10.3	23.8	9.7
RS starch ⁷ /pectin	27.4	9.2	25.3	14.8	11.2	9.3
Cellulose/pectin	98.6	98.6	99.1	110.8	104.8	104.6

¹Theoretical amount of detectable TDF calculated on the basis of analyzed TDF data (AOAC 991.43) from the single components.

²Theoretical amount of detectable TDF calculated on the basis of analyzed TDF data from the single components – the high-amylose TDF data were corrected on the basis of analyzed total starch data

³TDF standard method according to AOAC 991.43.

⁴Proposed scheme by McCleary & Rossiter (2004) for introduction of DMSO into TDF procedure.

⁵Microwave TDF procedure using different radiation power settings.

⁶Low-amylose maize starch no. 03402.

⁷High-amylose maize starch amylogel 03003.

All TDF data expressed as averages of duplicates related to d.m.

AOAC, Association of Official Analytical Chemists; DMSO, dimethyl sulphoxide; d.m., dry matter; n.d., not determined; RS, resistant starch; TDF, total dietary fibre.

microwave impact causing partial degradation. Thermal instability of some pectin preparations is a known phenomenon (Diaz *et al.*, 2007). Therefore, in further experiments the dependence of the stability of native pectin subjected to

microwave irradiation was checked. Control measurements of TDF of freeze-dried apple peels using AOAC 991.43 gave a TDF content of 17% of dry matter while microwave assisted determination for 360 s at an energy level of 600 W revealed

a TDF content of around 15% of dry matter (data not shown). This points to a greater stability of native pectin within its network of polymer chains linked by galacturonic acid and rhamnose residues than of isolated pectin preparations of technical quality. Amazingly the DMSO pre-treatment increased the pectin recovery to nearly 125%.

In order to model real food samples and simulate matrix effects, binary mixtures consisting of a high fraction of starch and a low fraction of NSP, were analysed for their TDF using standard and microwave-assisted methods (Table 3). Each mixture contained 1.0 g of starch and 0.1 g of NSP on a dry matter basis. Calculated TDF values based on known TDF values of the individual components.

Mixtures of low-amylose starches and NSP showed only slightly higher TDF contents than the calculated ones as measured by the AOAC 991.43 procedure. In mixtures containing high-amylose starches values were found deviating from the calculated ones due to the RS fractions. This may originate from matrix effects of NSP on the gelatinization and amylolysis of the starches. In contrast, the microwave-assisted determination at an energy level of 600 W provided TDF values that conformed very well to the theoretical ones without RS. Four hundred and eighty watts was insufficient to dissolve RS completely (Table 3). Pre-treatment with DMSO yielded TDF values for high-amylose maize starch containing binary mixtures that were distinctly higher than calculated. This shows that DMSO is, at least in high-amylose maize starch containing model mixtures and possibly in real samples, not always a proper solvent for complete dissolution of RS. The results of these experiments with model mixtures of fibres clearly show that microwave heating of RS containing cereal starches and mixtures with other fibres can be a very efficient means for eliminating the RS fraction. Further investigations for the optimization of the microwave technique are necessary in particular with respect to the structure–functionality relationships of RS and NSP dietary fibre components in complex food systems.

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