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Structural and morphological factors influencing the quantification of resistant starch II in starches of different botanical origin

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Abstract

Seven cereal starches and 11 pea starches were analysed for total starch, amylose content, resistant starch type II (RS), total dietary fibre (TDF) and starch damage. The data from the cereal starches could be interpreted on basis of the already published polymorph type. In the pea starch series RS and TDF correlated with the amylose content for most starches. But one starch with 20.7% of amylose had an extraordinary high RS content of 16.8%. DSC thermograms, starch granule size distribution, scanning electron and light micrographs revealed a large fraction of small granules of high cristallinity and high α-amylase resistance. Another starch of 32% amylose had a very low RS content. The reason for this was a high fraction of large starch granules, some being damaged. This study shows that besides amylose content, granule morphology and size, its extent of crystallinity or damage have a strong impact on the amylase resistance.

Keywords: Resistant starch; Pea starch; Amylose; Damaged starch; Polymorph type

1. Introduction

The phenomenon of resistant starch was first described by Englyst, Wiggins, and Cummings (1982). The term 'resistant starch' referred to starch fractions not digestable by α-amylases in the human small intestine and fermentable by the colonic microflora to short chain fatty acids. According to Englyst and Macfarlane (1985) three different types of resistant starch can be classified:

Type I

Starch which is physically insulated from the access of the α-amylase by cell walls. This includes starch in incompletely ground cereal grains, leguminoses and seed. These starch granules are only accessable after microbial hydrolysis of the cell walls

Type II

This type encompassess semi-crystalline starches having a polymorph B- or C-type. These starches are only slowly or incompletely digested by α-amylases. Starch from green bananas, from potatoes or high-amylose wrinkled pea or maize starch belong to this type.

Type III

This type can be divided into two subtypes: IIIa containing crystalline amylopectin and type IIIb having a partially crystallized amylose network. These subtypes can be induced by repeated heating and cooling of starch.

In the last two decades, especially in the framework of the EURESTA program, several analytical procedures for quantification of resistant starch were developed and evaluated in collaborative studies (Berry, 1986; Björck, Nyman, Pedersen, Siljeström, Asp, & Eggum, 1986; Champ, Martin, & Gratas, 1999; Englyst, Kingsman, Hudson, & Cummings, 1996; Faisant, Planchot, Kozlowski, Pacouret, Colonna, & Champ, 1995; Goni, Garcia-Diz, Manas, & Saura-Calixto, 1996; Muir & O'Dea, 1993). However, none of these methods was accepted as standards. More recently, a method for measurement of resistant starch was reported (McCleary & Monaghan, 2002; McCleary, McNally, & Rossiter, 2002) and this method was subjected

Abbreviations AA, α-amylase; AMG, amyloglucosidase; AOAC, Association of Official Analytical Chemists; AACC, American Association of Cereal Chemists; ICC, International Association for Cereal Science and Technology.

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to interlaboratory evaluation under the auspices of AACC and AOAC International. On successful evaluation involving 39 laboratories, this method was accepted as AACC method 32–40 and AOAC International official method 2002.02.

Resistant starch is now considered to belong to the dietary fibre complex, as it has functional and nutritional properties in common with more traditional dietary fibres. As the enzymatic–gravimetric procedure for dietary fibre determination is not able to differentiate between fibre and resistant starch, an accurate and reliable analytical quantification of dietary fibre in the presence of resistant starch is not possible (Akerberg, Liljeberg, Granfeldt, Drews, & Björck, 1998; Gebhard, Dongowski, Huth, & Rabe, 2001). During the industrial-scale isolation of native starches resistant starch type I and especially type II fractions will lead to to high values for residual fibre in the accompanying process analysis. The objective of this study was to look at whether there are any correlations between total starch, amylose content, resistant starch and total dietary fibre in 18 starches from different sources.

In this study commercially available starches from different manufacturers were investigated as well as starches from different pea varieties, which were isolated on a pilot-scale. Special attention was paid to the influence of starch type, its botanical source and morphological characteristics of starch granules.

2. Experimental

2.1. Material

All the different starches analyzed, four from maize, two from wheat, one from rye, and 11 from pea, were given code numbers, quoted in curled brackets, for easy identification. Maize starches with medium amylose content {8,10} were obtained as commercial grade products from Cerestar Company (Krefeld, Germany). Two high amylose maize starches were supplied by Roquette Company (Lestrem, France), {39} and National Starch and Chemical Company (Bridgewater, New Jersey, USA), {4}. Two wheat starches were commercial products from Crespel and Deiters Company (Ibbenbühren, Germany) {9,29} and the rye starch (cv. Amado) {11} was supplied by the Institute for Cereal Processing (Bergholz-Rehbrücke, Germany). Six different pea starches {15,16,17,18,27,28} from different pea breeding lines were isolated on a pilot-scale at the BFEL, Detmold. The pea mutants were provided by the John Innes Centre, Norwich, UK. The starches were isolated from the seeded genotypes in a wet milling process according to a published procedure (Bergthaller, Themeier, & Lepa, 2001). The genetic background of the starches was as follows 15 *RRRbR*, 16 *rrRbRb*, 17 *RRrbrb*, 18 *rrrbrb*, 27 *RUG4* and 28 *RUG6*.

Morphological and biochemical data of the green-seeded pea genotypes were published previously (Bogracheva, Morris, Ring, & Hedley, 1998; Bogracheva et al., 1999; Hedley, Bogracheva, & Wang, 2002; Lloyd, Hedley, Bull, & Ring, 1996; Wang, Bogracheva, & Hedley, 1998). Two commercial pea starches {5,6} were supplied by Nutrio a/s (Braedstrup, Danmark). Pea starch {3} was supplied by the Institute for Food Processing, Technical University Berlin, Germany.

Two high amylose pea starches {20,2} were isolated from commercial wrinkled peas ({20} cv. Tristar, Amylose KG Stauderer and Co., Altenmarkt, Germany and {2} cv. Sprinter, Van Waveren-Pflanzenzucht GmbH, Rosdorf, Germany) according to the isolation procedure decribed by Bergthaller, Themeier, and Lepa (2001).

2.2. Analyses

Total starch content of the different starch samples was determined by two different procedures: The enzymic procedure was performed according to AACC method 76–13 (McCleary, Gibson, Solah, & Mugford, 1994; McCleary, Gibson, & Mugford, 1997) total starch assay procedure AA/AMG 11/01 (Megazyme International Ireland, Ltd, Bray, Ireland). The acid hydrolysis procedure was performed using the Ewers procedure (Analytical working party of the starch experts group, 1987; Kennedy, Stevenson, & White, 1989; Mitchell, 1990). The analyses were performed on duplicate samples.

Resistant starch was determined enzymatically according to AOAC International official method 2002.02 and AACC method 32–40 (McCleary & Monaghan, 2002) with resistant starch assay procedure RSTAR 11/02 (Megazyme International Ireland, Ltd, Bray, Ireland). The analyses were performed on duplicate samples. Starch damage was determined enzymatically according to AACC method 76–31 (Gibson, Kaldor, & McCleary, 1993) with starch damage assay kit (Megazyme International Ireland, Ltd, Bray, Ireland). The analyses were performed on duplicate samples. The amylose content of the different starch samples was determined enzymatically with the AM/AMP 7/98 assay kit. (Megazyme International Ireland, Ltd, Bray, Ireland). The assay is based on the publication by Yun and Matheson (1990). The analyses were performed on duplicate samples. Total dietary fibre of the different starch samples was determined according to AACC method 32–21 and 32–05 (Prosky, Asp, Furda, DeVries, Schweizer, & Harland, 1985; Prosky, Asp, Schweizer, DeVries, & Furda, 1988; Rabe, Seibel, Suckow, & Meuser, 1988) with the total dietary fibre assay procedure (Megazyme International Ireland, Ltd, Bray, Ireland). All analyses were performed on duplicate samples. Ash content was determined according to ICC standard method No. 105/2. Protein content was determined by Kjeldahl method ($N \times 6.25$) according to ICC standard method No. 104/1. The analyses were performed on duplicate samples. The particle size distribution of the starch samples was analyzed using a laser scattering particle sizer Mastersizer S (Malvern Instruments Ltd, Malvern,

UK). From the differential particle size distributions, which were given in volume fractions, the median was calculated as average value for particle size. Differential scanning calorimetry was performed using a Mettler Toledo DSC 821 (Mettler, Giessen, Germany).

The starch-water ratio in the suspension was 1:4 (w/w). The heating rate was 5 °C/min within the range from 25 to 95 °C. The specific enthalpy of gelatinization was determined by integration of the peak area. All measurements were performed by using aluminium cups with Al_2O_3 as reference. Scanning electron micrographs of pea starch granules were taken by using the Model XL 30, (FEI, Eindhoven, Netherland) in the high vacuum modus in combination with a SE-Detector. Samples were prepared by gold sputting with a Model MED 020, BAL-Tec (Lichtenstein). Further technical details are given in the micrographs. Pea starch granules were viewed using light microscopy with cross polarizer using an Olympus BX-41 microscope (Olympus, Hamburg, Germany) equipped with a polarization filter. The polymorphic composition (values with an error margin of $\pm 5\%$) of selected pea starches, determined from X-ray measurements, was kindly provided by the John Innes Centre, Norwich, England. Ball milling experiments with pea starch code {15} were performed with a Fritsch Pulverisette, Germany. 5 g of starch was milled for 10/15 min with three balls at range seven.

3. Results and discussion

3.1. Amylose and total starch content of starches from different sources

Several methods for starch determination in plant material have been used for years. Colorimetric methods, based on iodine-binding or formation of coloured products after chemical degradation, have been shown to be of limited reliability.

The enzymatic AA/AMG-procedure (McCleary et al., 1994, 1997) is now widely accepted as being reproducible and reliable and has become an AACC method (76–13). Alternatively starch was estimated by the polarimetric method of Ewers. (Analytical working party, 1987; Mitchell, 1990), although it has been shown that this method is not an acceptable satisfactory method for starch measurement (Kennedy, Stevenson, & White, 1989). (The Ewers method is currently the official EC method for the measurement of starch purity. For the EC starch regulation a minimum purity of 97% starch on dry substance basis is required).

In the present study 18 starches from different varieties of cereals and peas were analyzed with respect to starch and amylose content. All analyses were carried out including or omitting the DMSO treatment of the sample, as proposed by the manufacturer. Additionally starch was estimated according to the Ewers procedure.

Table 1
Amylose and total starch content of 18 different starches, determined by an enzymatic and a polarimetric method

Source	Code	Amylose[a] (%)	Total starch[b] AA/AMG (%)+ DMSO	Total starch[b] AA/AMG (%)− DMSO	Total starch[b] Polarimetry (%)
Maize	8	7.6	97.6	98.6	96.4
Maize	10	30.0	99.1	93.6	96.6
Maize	39	65.8	95.4	n.d.	82.9
Maize	4	66.5	99.0	86.9	80.3
Wheat	9	30.2	99.6	91.2	96.0
Wheat	29	33.7	95.6	94.5	97.8
Rye	11	31.1	99.5	94.3	96.6
Pea	27	30.1	92.0	91.7	97.6
Pea	15	31.8	94.5	95.1	97.8
Pea	6	32.5	99.8	93.2	96.7
Pea	18	47.6	94.4	90.8	88.1
Pea	20	62.5	95.4	88.1	85.6
Pea	16	62.5	95.5	89.5	85.3
Pea	5	62.9	98.1	87.7	84.8
Pea	2	70.2	91.7	n.d.	83.7
Pea	28	43.2	93.5	n.d.	92.6
Pea	17	20.7	93.6	n.d.	97.4
Pea	3	70.6	97.4	87.7	83.2

Results are expressed as averages of duplicates; n.d. not determined.
[a] Expressed as weight percentage (dm) of total starch.
[b] Expressed as weight percentage (dm) of sample.

Data of Table 1 clearly reveal that the accuracy of the enzymatic AA/AMG-procedure for total starch determination in samples from maize, wheat, rye and pea is dependent on the amylose content of the starch analyzed. With increasing amylose content ($>30\%$) the total starch values of DMSO pretreated samples were always higher than those from samples without pretreatment. This supports the results McCleary et al. published in 1997 (McCleary, Gibson, Solah, & Mugford, 1994; McCleary, Gibson, & Mugford, 1997) when showing that a prerequisite for a correct determination of total starch is a complete solubilization of the starch granules. This is accomplished by a DMSO pretreatment.

This is often necessary for medium or high amylose starches. According to the authors mentioned an insufficient solubilization of starch in the absence of DMSO is caused by a high proportion of enzyme resistant starch in the analyte. Comparison of total starch determined by polarimetry (Ewers procedure) with the enzymatic procedure including the DMSO pretreatment step showed that at least in high-amylose starches the polarimetric procedure leads to an underestimation of starch. This supports the assessment Kennedy et al. (1989) made on the Ewers procedure. Our results imply that the enzymatic AA/AMG procedure (AACC method 76-13) for total starch measurement must always include a DMSO pretreatment step for samples having an amylose content above 30% on a dry weight basis. Fig. 1 visualizes the influence the amylose fraction has on enzymatic and polarimetric total starch measurement.

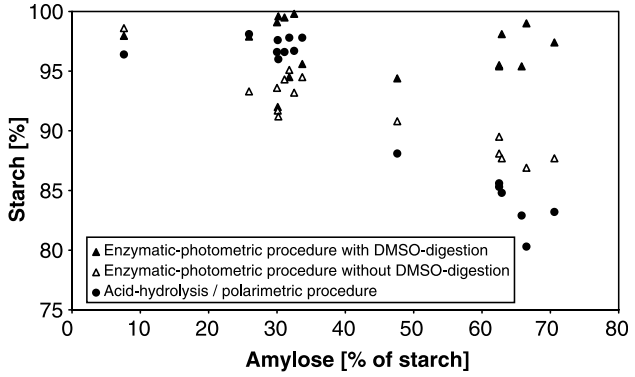

Fig. 1. Effect of amylose content on starch determination. Comparison of total starch data from polarimetric and enzymatic–photometric methods of determination.

3.2. Relationship between resistant starch and amylose and total dietary fibre content

According to the definition proposed by the American Association of Cereal Chemists (Prosky, Asp, Schweizer, DeVries, & Furda, 1988) dietary fibre is 'the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine'. Resistant starch, being that portion of starch, that is not broken down by human enzymes in the small intestine, is considered to be part of dietary fibre. In the present study the enzyme resistant starch (resistant starch Type II) proportion of 14 starches of different botanical origin was determined by means of the resistant starch assay procedure (McCleary, Gibson, Solah, & Mugford, 1994).

Additionally, the total dietary fibre content (TDF) of the starches was estimated by the total dietary fibre assay procedure (Rabe, Seibel, Suckow, & Meuser, 1988). Table 2 lists the values determined in starches having an amylose content up to 35%. The cereal starches investigated with an amylose content up to 34% had a low resistant starch fraction below 1% when determined with the procedure published by McCleary et al. (2002). This procedure has been proven to be the most reliable method for determination of resistant starch due to a very good correlation to in vivo values determined in ileostomy patients (Champ, Langkilde, Brouns, Kettlitz, & Le Ail-Collet, 2003; McCleary & Monaghan, 2002). The low resistant starch content of cereal starches measured are in line with what could be expected from their polymorph starch type. Cereal starches are 'A-type'-starches (Cairns, Bogracheva, Ring, Hedley, & Morris, 1997; Gernat, Radosta, Damaschun, & Schierbaum, 1990) always having a low resistant starch fraction of total starch. Maize, wheat, rye and pea starches with an amylose content lower than 34% contained only very low TDF below 2%. On the other hand pea starches with medium amylose (30–34%) content, however, showed a markedly higher RS content between 9 and 11.5% than cereal starches with a similar amylose content. Pea starches with medium amylose content are known to have a mixed polymorph C-type and concomitantly have a higher resistant starch proportion due to a higher degree of crystallinity of its starch (Bogracheva, Morris, Ring, & Hedley, 1998; Cairns et al., 1997). This was paralleled by a higher TDF fraction. Table 3 lists the values determined in starches having an amylose content above 60%. While the investigated pea starches had a RS content of around 18–19% and a TDF content between 11.6 and 12.6%, maize starches differed from pea starches by having much higher RS levels up to 54.4% and moderately higher TDF levels. Starch resistance to α-amylase has been shown to be proportional to increasing amount of B-type crystallites in starch (Hoover & Zhou, 2003). Starch {4} (Hylon VII™) contained 54.4% RS and 17.2% TDF, values that were in very good agreement with values obtained by others (Brown, McNaught, Andrews, & Morita, 2001; McCleary & Monaghan, 2002; Shi & Jeffcoat, 2001). Hylon VII starch has a pure B-type X-ray diffraction pattern (Shi & Jeffcoat, 2001), and so this starch is extremely resistant to digestion by α-amylase in comparison to medium amylose starches showing an A-type diffraction pattern. Wrinkled Pea starch {3} on the other hand shows also a 90% B-type polymorphism (Cairns et al., 1997), but contained only 18.4% RS and a moderate TDF value of 12.8%.

Table 2
Resistant starch and total dietary fibre in low and medium amylose starches

Source	Code	Amylose[a] (%)	Resistant starch[b] (%)	Total dietary fibre[b] (%)
Maize	8	7.6	0.5	1.5
Maize	10	30.0	0.7	0.4
Wheat	9	30.2	0.3	0.2
Wheat	29	33.7	0.2	1.0
Rye	11	31.1	0.2	0.3
Pea	15	31.8	11.2	2.7
Pea	28	34.2	9.3	5.7
Pea	27	30.1	11.4	1.6

Results are expressed as averages of duplicates.
[a] Expressed as weight percentage (dm) of total starch.
[b] Expressed as weight percentage (dm) of sample.

Table 3
Resistant starch and total dietary fibre in high amylose starches

Source	Code	Amylose[a] (%)	Resistant starch[b] (%)	Total dietary fibre[b] (%)
Pea	20	62.5	18.7	12.0
Pea	16	62.5	18.0	11.6
Pea	2	70.2	19.6	11.1
Pea	3	70.6	18.4	12.8
Maize	4	66.5	54.4	17.2
Maize	39	65.8	49.1	17.5

Results are expressed as averages of duplicates.
[a] Expressed as weight percentage (dm) of total starch.
[b] Expressed as weight percentage (dm) of sample.

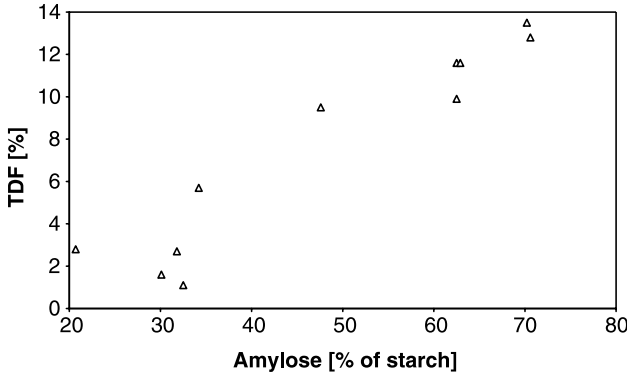

Fig. 2. Effect of amylose on total dietary fibre content of different pea starch varieties.

This indicates that at least in pea starches, a high amylose content and an identical polymorph type of the starches do not necessarily imply a high α-amylase resistance. Furthermore, it can be concluded from the data that the fraction of resistant starch that is detectable as TDF, is highly dependant on the morphological structure of the starch, despite a comparable amylose fraction. In Fig. 2 TDF amounts of 11 starches from different pea varieties have been plotted against their corresponding amylose amounts. The graph shows a more or less linear positive correlation between both analytical parameters. Significant TDF content above 5% was only found in pea starches with amylose contents higher than 34%, whereby the highest yield of 13.5% TDF was found in a pea starch with 70.2% amylose. Fig. 3 shows the positive correlation between the amylose content of eight different pea starches plotted against the corresponding RS contents.

A similar linear correlation between amylose and RS was found by for maize starches (Brown et al., 2001) but in contrast to maize starches, the slope of the graph for pea starches was lower and the highest level of resistant starch in a pea starch with 70.2% amylose was only 19.6%.

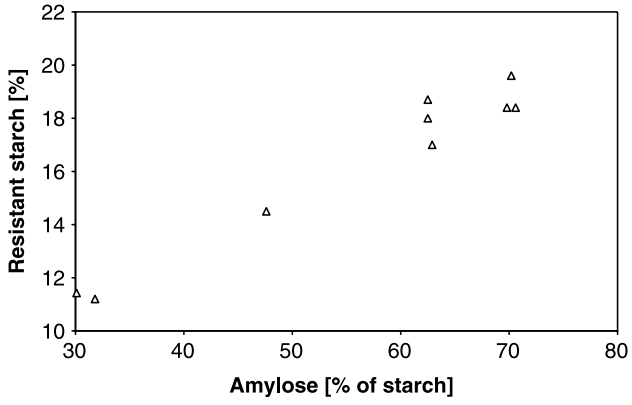

Fig. 3. Effect of amylose on resistant starch content of different pea starch varieties.

3.3. Role of morphological characteristics of starch granules on resistant starch content of pea starches

Within the study two starches were found from different pea varieties whose resistant starch data deviated markedly from the linear correlations shown in Figs. 2 and 3.

(1) Pea starch {17} had a rather low content of amylose of only 20.7% and an unexpectedly high content of RS of 16.8%, while other data like total starch content, TDF and polymorph type were comparable to those of other low amylose pea starches. Such a high amount of amylase resistant starch had previously only been observed in high-amylose pea starches.
(2) The TDF content of these samples was in the range expected from the correlation seen in Fig. 2.
(3) A very low RS value (3.6%) of pea starch {6} was found together with a medium amylose content of 33%,. This was the lowest we found in all of the pea starches. Other data, such as the total starch content, TDF and polymorph type were in good agreement with that obtained for other low amylose pea starches.

In order to explore the reasons for this deviant behaviour, the particle size distributions of the starch granules of the starches {6} and {17} were measured and compared to corresponding measurements on starches with medium amylose content {15,27} and high amylose content {3,5,16} following the correlations of Figs. 2 and 3 ('normal' starches). Differential scanning calorimetry thermograms were recorded and the enthalpy of gelatinization of the starches measured. In an additional experiment pea starch {15} was milled for 10 min and 15 min, respectively, to increase the fraction of damaged starch granules in the samples, thereby checking the influence that mechanically damaged starch granules had on those parameters.

Tables 4 and 5 list the experimental data from these measurements.

Table 4
Chemical analyses of deviant pea starches in comparison to 'normal' starches

Code	Total starch[a] (%)	Protein (%)[a]	Damaged starch[a] (%)	Amylose[b] (%)	Total dietary fibre[a] (%)	Resistant starch[a] (%)
6	99.8	0.3	3.7	32.5	1.1	3.6
15	94.5	0.2	<1	31.8	2.7	11.2
15/10′	94.5	0.2	10.9	31.8	2.7	6.9
15/15′	94.5	0.2	12.8	31.8	2.7	4.7
17	93.6	0.4	<1	20.7	2.8	16.8
27	92.0	0.2	<1	30.1	1.6	11.4
3	97.4	0.7	3.8	70.6	12.8	18.4
5	98.1	0.7	3.3	62.9	11.6	17.0
16	95.5	0.4	4.5	62.5	11.6	18.0

Results are expressed as averages of duplicates. Ash contents of starches were <0.3%.
[a] Expressed as weight percentage (dm) of sample.
[b] Expressed as weight percentage (dm) of total starch.

Table 5
Physicochemical analyses of deviant pea starches in comparison to 'normal' starches

Code	Median particle size (μm)	Enthalpy of gelatinization (J/g)	Temperature range of gelatinization (°C)			% Polymorphic composition	
			Initial	Peak	End	%A	%B
6	27.8	9.5	55.7	67.4	78.5	60	40
15	21.7	11.6	52.0	62.5	76.5	60	40
15/10′	n.d.	5.9	51.0	62.5	75.0	n.d.	
15/15′	n.d.	3.6	53.0	62.5	73.7	n.d.	
17	16.1	13.4	52.7	64.0	78.6	70	30
27	21.2	11.8	52.7	64.0	78.8	80	20

Results are expressed as averages of duplicate measurements; n.d. not determined.

Additionally polarized light micrographs and scanning electron micrographs (SEM) were taken to further characterize the morphology of the starch granules.

Figs. 4 to 7 show SEM images of starches {15,27,6,17} and Figs. 8–10 show the light microscopic images of the pea starches {6,15,17}.

The scanning electronic micrographs and the polarized micrographs of pea starch {17} show besides larger smooth and undamaged single starch granules many smaller single granules. Evaluation of the particle size distribution profile gave a median of the particle diameter of 16.1 μm. The damaged starch fraction is very low (below 1%), which is supported by the micrographs which showed hardly any damaged starch granules. The enthalpy of gelatinization is 13.45 J/g, the highest value measured within samples {6}–{27}. From this it may be concluded that the relatively high amount of α-amylase resistant starch within this sample {17} is explained by the presence of a large portion of small intact starch granules of higher crystallinity, which are resistant to amylolytic degradation.

By observing the proccess of α-amylase degradation of a sample of pea starch {17} at pH 6.0 and 37 °C under a light microscope we could verify that after 24 h most larger granules had vanished and many smaller granules remained undigested. This corresponds with results described by Hoover et al. (2003). The micrographs of pea starch {6} show morphologic details of the granules which underline the chemical and physicochemical data: There are less smaller single granules than in starch {17} and several of

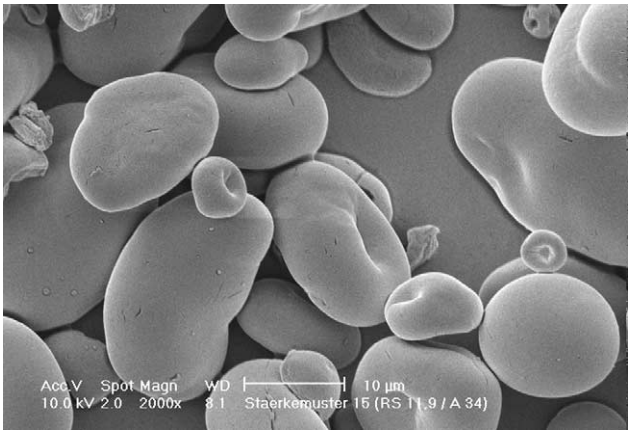

Fig. 4. Scanning electronic micrograph of 'normal' pea starch {15}.

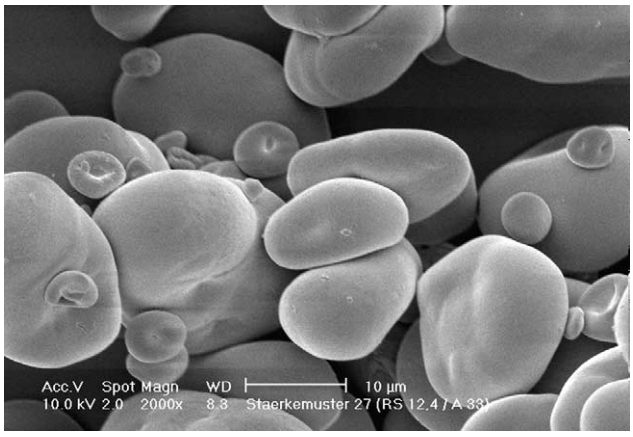

Fig. 5. Scanning electronic micrograph of 'normal' pea starch {27}.

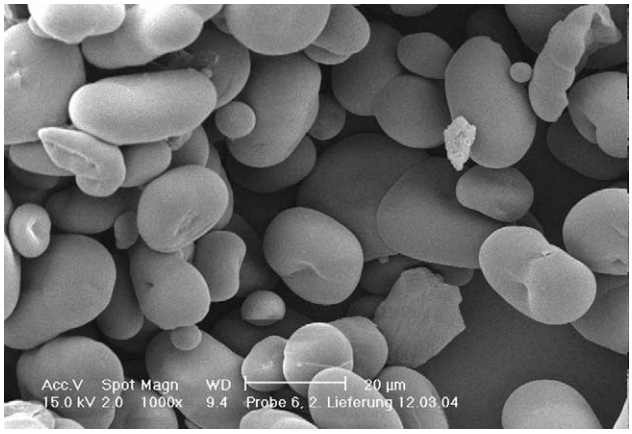

Fig. 6. Scanning electronic micrograph of pea starch {6} showing damaged starch granules.

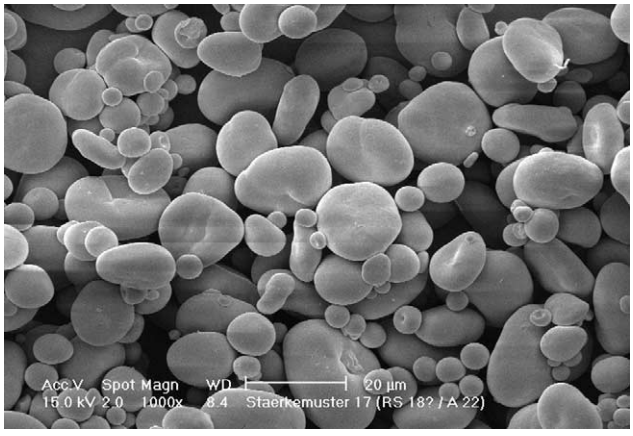

Fig. 7. Scanning electronic micrograph of pea starch {17} showing an increased small granule fraction.

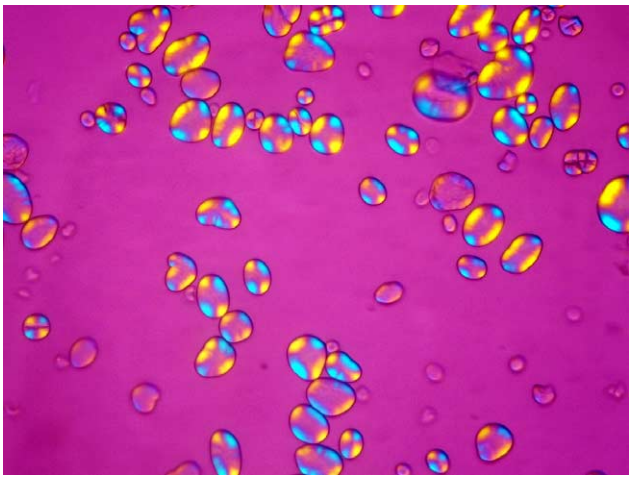

Fig. 8. Polarized micrograph of 'normal' pea starch {15}.

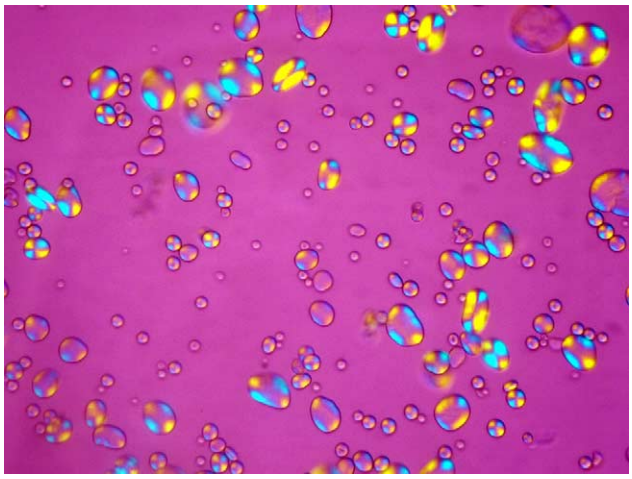

Fig. 10. Polarized micrograph of pea starch sample {17} showing an increased small granule fraction.

the larger granules show cracks or other forms of surface damage. The analytical values are supportive of these microscopic details: the median of the particle diameter was determined as 27.8 μm and the damaged starch fraction amounts to 3.7% and the enthalpy of gelatinization is 9.51 J/g. A 'normal' starch, {15 or 27}, has markedly different values, as Tables 4 and 5 indicate. The polarized light micrographs of {6} show larger granules with damages, which disturb the regular polarized light rotation patterns, seen with intact granules. The effect of mechanical damaging of the starch granules of pea starch {15} by milling gave the expected results: the damaged starch fraction increased, the resistant starch fraction decreased and the enthalpy of gelatinization fell drastically to a low value of 3.37 J/g. From these results we conclude that in contrast to pea starch {27}, for example, a higher fraction of larger granules and of damaged granules decreases the fraction of resistant starch in this sample markedly.The results of this study emphasize, that there are several factors which influence the α-amylase resistance of starch (Hoover et al., 2003). It could be shown that the strong linear correlation between amylose and resistant starch content of starch valid for maize starches (Brown, et al., 2001), cannot generally be found for pea starches from different varieties.

We unexpectedly found a very high amount of resistant starch in low-amylose pea starches with small starch granules. In contrast we also detected medium-amylose pea starches of the same polymorph type with large sized granules having a very low amount of resistant starch. We were also able to show that increasing resistant starch content is not strictly proportional to increasing TDF content. This underlines once again that the enzymatic–gravimetric method for estimation of total dietary fibre does not cover resistant starch reliably.

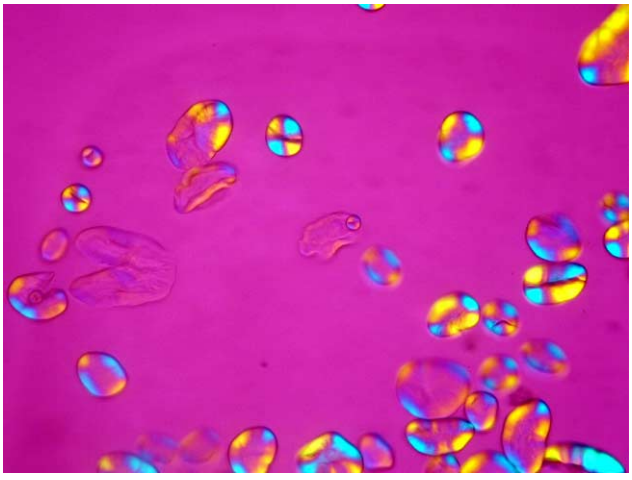

Fig. 9. Polarized micrograph of damaged pea starch {6} showing damaged starch granules.

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