ELSEVIER

PII: S0308-8146(97)00025-3

Food Chemistry, Vol. 60, No. 4, pp. 527–532, 1997
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0308-8146/97 $17.00+0.00

An approach to the influence of nutrients and other food constituents on resistant starch formation

A. Escarpa,[a] M. C. González,[a] M. D. Morales[a] & F. Saura-Calixto[b]

[a]*Departamento de Química Analítica, Facultad de Ciencias, Universidad de Alcalá de Henares, 28871 - Alcalá de Henares, Madrid, Spain*
[b]*Departamento de Metabolisimo y Nutrición. Instituto del Frío. CSIC, Madrid, Spain*

(Received 17 November 1995; revised version received 17 December 1996; accepted 17 December 1996)

The influence of nutrients and other food constituents, such as dietary fibre components, catechin and phytic acid, on resistant starch (RS) formation was systematically investigated. This investigation was carried out under standardized gelatinization conditions by using a high pressure autoclave (HPA). Except for insoluble dietary fibre constituents (cellulose and lignin), all the tested food ingredients reduced the formation of RS. Calcium ions, potassium ions and catechin showed the highest reduction of RS formation, while the nutrients studied (albumin, olive oil and sucrose) as well as phytic acid affected it to a lesser extent. These results were not significantly changed by varying amounts of the studied dietary components. © 1997 Elsevier Science Ltd

INTRODUCTION

EURESTA (European Flair Concerted Action on Resistant Starch) defines enzyme resistant starch (RS) as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Euresta, 1992). RS has been categorized into three main types: type I, or physically trapped (i.e. partly milled grains and seeds); type II, or resistant starchgranules (i.e. the native crystalline starch granules in raw potatoes and green bananas); and type III, retrograded starch (recrystallized starch after gelatinization and cooling or storage of foods) (Englyst *et. al.*, 1992). Additionally, chemically modified starch fragments produced by heat treatments, non-digestible starch-nutrient complexes, and undigested starch resulting from the action of enzyme inhibitors and antinutrients may contribute to the RS content of foods (Saura-Calixto & Abia, 1991). In order to form RS type III from native starch granules (raw starch), the starch has to be gelatinized and retrograded afterwards. During the gelatinization process, the starch granule is gradually and irreversibly destroyed. The starch paste or gel obtained after gelatinization is not stable. Structural transformations occur during storage. Both starch-constituent polymers (amylose and amylopectin) are involved in these transformations, which are collectively described by the term retrogradation.

The influence of nutrients such as sugars, proteins and lipids on RS formation has been widely studied by different authors. The influence of native protein has been studied (Slack *et. al.*, 1979; Van Twisk, 1979; Holm *et. al*, 1985; Chandrashekar & Kirleis, 1988); the influence of lipids has been studied (Mercier, 1980; Eliasson, 1985; Eliasson *et. al.*, 1988; Eerlingen, 1994) and the influence of sugars has been investigated (Buck & Walker, 1988; I'Anson *et. al*, 1990; Nohyama & Nishinari, 1991; Eerlingen, 1994). However, in these studies, the autoclaving process was carried out without control of sample temperature. It is well known that gelatinization temperature has an important influence on RS yields (Berry, 1986; Sievert & Pomeranz, 1989). Therefore, control of the sample temperature and heating curve during RS formation is essential.

On the other hand, there is little information on the influence of other food constituents such as micronutrients, dietary fibre, flavonoids and phytic acid on RS formation.

The aim of this work was to carry out a systematic study of the influence of nutrients and other food constituents on RS formation under standardized gelatinization and retrogradation conditions.

The gelatinization step is controlled by using a high pressure autoclave (HPA) with a continuous control of pressure, stirring and sample temperature (Escarpa *et. al.*, 1996), which increases the RS yields as reported in the literature (Berry, 1986; Sievert & Pomeranz, 1989; Eerlingen, 1994).

MATERIALS AND METHODS

Dietary components

The nutrients used were: bovine serum albumin (Merck), olive oil with a content of 97% of total triglycerides purchased in a local supermarket, and sucrose (Oesceder). The dietary fibre components were lignin (ENCESA, Spain), and cellulose (S-3755) and citrus pectin (P-9135) from Sigma. Guar gum (G-9752), phytic acid (P-3168) and catechin (C-1251) were also obtained from Sigma. Anhydrous calcium chloride ($CaCl_2$) (fused granular about 0.5–2.0 mm) as well as anhydrous potassium nitrate (KNO_3) were from Merck.

Enzymes

Pancreatic α-amylase (Sigma Chemical Co. St. Louis, Mo), pepsin (Merck) and amyloglucosidase (Boehringer Mannheim) were used in the analysis of RS. For glucose determination, an enzymatic kit (Peridochrom Glucose. GOD- PAP, Boehringer Mannhein) was used.

Standards

Potato starch (S-4251) from Sigma was used as standard for RS formation, and D (+) Glucose (Merck) as spectrophotometric standard.

Reagents

All reagents were of analytical grade.

Apparatus

A high pressure autoclave (HPA) (Berghof, Eningen, German; Cat.No. 537263), equipped with a pressure vessel (0–160 bars) with vacuum line (PTFE) and thermocouple, (DIN-434710), heating mantle with magnetic stirring and thermosensor, and two temperature control systems and stirring rate, control was used as the gelatinization system. The HPA provided a continuous control of the heating conditions and stirring speed necessary to obtain homogeneous gels.

A UV-Visible Spectrophotometer (Perkin-Elmer Lamda 5) was used to measure free glucose from starch hydrolysis.

For pH measurements, a MicropH 2000 Crison pH meter with a glass/reference electrode was used.

METHODS

RS formation procedure

Potato starch and mixtures of potato starch/dietary constituent were autoclaved for gelatinization under previously standardized conditions (starch/water ratio of 2 g 40 ml^{-1}). Initial conditions of pressure and stirring rate were 2 bars (N_2) and 1300 rpm, respectively. Gelatinization was achieved after 20 min at 123 ± 7°C. Gelatinized samples were allowed to cool down to room temperature (cooling rate approx. 4°C/min) and frozen at –20°C. After 12 h the samples were defrosted (defrosting rate approx. 0.4°C/min) and vacuum-dried at 40°C for 12 h. Finally, samples were ground to a particle size ≤ 1 mm.

RS determination procedure

A Berry-modified method for RS determination was used (Goñi *et. al.*, 1996). In this method, RS is considered as the residual starch after incubation of samples with protease and α- amylase. The sample (100 mg) was preincubated with pepsin (40°C, 1 h, pH 1.5) and then purified α-amylase (40 mg ml^{-1}) for starch hydrolysis (37°C, 16 h, pH 6.9) was added. After amylolysis, the sample was centrifuged and the residue (isolated RS) repeatedly washed with water. The final residue was dispersed in water before adding KOH to a final concentration of 2M (30 min, room temperature with constant shaking).

pH was adjusted to 4.75 in buffer acetic acid/sodium acetate and the suspension incubated with amyloglucosidase (30 min at 60°C). Free glucose was measured in the supernatant using the enzymatic kit (glucose oxidase/peroxidase). All the treatmens were carried out in the same centrifuge tube.

Calculations

RS content was calculated as the product of free glucose (FG) from resistant starch hydrolysis with amyloglucosidase and a correction factor glucose-polysaccharide of 0.9 as follows:

$$RS = FG \times 0.9$$

(expressed in g 100 g^{-1} starch dry matter, DM)

DM content for the samples was determined by drying at 110°C to a constant weight.

The results correspond to the average of three gelatinization treatments, analyzed in triplicate.

Statistical analysis

A one-way analysis of variance ($P \leq 0.05$) was performed to evaluate the influence of dietary components on RS formation.

RESULTS AND DISCUSSION

Water content influence

Interactions between starch and other food constituents are governed by the mobility of the amorphous phase of each particular system. Water, acting as a plasticizer,

depresses the glass transition temperature (Tg) and thereby alters the kinetics of state transformations (e.g. gelatinization, retrogradation) and reactivity of starch (Biliaderis, 1991). The literature reveals that the yields of RS in different starches formed in heat-moisture treatments are closely related with the water content (Berry, 1986; Sievert & Pomeranz, 1989).

In this work, samples containing potato starch and different amounts of the studied dietary constituent were autoclaved keeping a mass:volume ratio of 2 g 40 ml^{-1}. To check the possible influence of water content on the yield of RS, control assays with the corresponding amounts of potato starch but without the dietary constituent were also performed. Tables 1 and 2 show the RS yields obtained in autoclaved and retrograded samples including control assays.The impact of water content is discussed in the *Stastistical Analysis* section.

Influence of nutrient content on the formation of RS type III

To study the influence of protein on RS formation, bovine serum albumin was chosen. The presence of this protein decreased the yield of RS irrespective of the amount added, although a linear relationship was not observed.

Chandrashekar and Kirleis, (1988) also studied the influence of protein on starch gelatinization in sorghum. These authors concluded that protein bodies around the starch granule provide a rigid cover and full gelatinization of the starch granule can take place only when this barrier is removed. Other studies on starch availability in wheat products indicated that a considerable fraction of the starch is encapsulated in a protein matrix (Holm *et. al.*, 1985). These structures have also been reported for starches of different botanical origin (Slack *et. al.*, 1979; Van Twisk, 1979).

To our knowledge, there is no report in the literature about the influence of added protein on RS formation. Starch-protein interactions could also be expected with added protein. During starch retrogradation, hydrogen bonds are formed between amylose chains and, similarly, protein could be bound to starch during the retrogradation process. Nevertheless, the results obtained suggest that these interactions could be excluded under the assayed gelatinization and/or retrogradation conditions since the addition of albumin resulted in a decrease of the RS yields.

Lipid influence was studied using olive oil. As can be observed in Table 1, the presence of the olive oil decreased the yields of RS. This is in agreement with the results reported by Eliasson *et. al.*, (1988) who also found that the addition of an excess of lipids to autoclaved high-amylose maize reduced the RS yields. Amylose crystallization (RS formation) is competitively affected by amylose complexation with monoglycerides. These structures have been studied in the literature using differential scanning calorimetry (DSC) (Eliasson, 1985; Eliasson *et. al.*, 1988). Studies carried out by Eerlingen *et. al.*, (1994*a*) using DSC and X-ray diffraction analysis also indicated the presence of amylose-monoglyceride complexes. Mercier (1980) found

Table 1. Resistant starch (RS) yields in autoclaved and retrograded mixtures of starch/nutrients (% of total starch, dry matter)

Starch (g 40 ml^{-1})	Constituent (g 40 ml^{-1})	RS yields					
		Albumin	Oil	Sucrose	Potassium	Calcium	Control[a]
2.00	0.00	18.2±0.2	18.2±0.2	18.2±0.2	18.2±0.2	18.2±0.2	18.2±0.2
1.90	0.10	9.2±2.1	11.3±1.6	14.0±1.8	10.8±2.3	4.6±0.8	14.9±0.3
1.80	0.20	12.7±0.8	13.6±0.6	11.8±1.7	8.46±1.1	3.8±0.6	15.3±0.2
1.70	0.30	10.8±2.5	12.7±1.6	13.6±1.3	7.7±0.5	ND[b]	17.1±0.6
1.60	0.40	11.7±1.0	11.9±1.6	12.2±0.6	5.6±0.4	ND[b]	17.2±0.5

Mean values of three gelatinization treatments ± standard deviation.
[a]Values of starch with no added constituent.
[b]ND - Not determined.

Table 2. Resistant starch (RS) yields in autoclaved and retrograded mixtures of starch/other dietary constituents. (% of total starch, dry matter)

Starch (g 40 ml^{-1})	Constituent (g 40 ml^{-1})	RS yields						
		Cellulose	Lignin	Pectins	Gum	Catechin	Phytic Acid	Control[a]
2.00	0.00	18.2±0.2	18.2±0.2	18.2±0.2	18.2±0.2	18.2±0.2	18.2±0.2	18.2±0.2
1.90	0.10	14.9±2.3	14.1±0.4	15.2±1.8	12.8±2.0	12.1±1.6	12.8±1.8	14.9±0.2
1.80	0.20	14.7±2.5	ND	13.3±0.8	12.8±1.1	6.7±0.8	9.6±1.8	15.3±0.3
1.70	0.30	13.0±1.9	ND	12.0±1.5	ND[b]	5.2±0.5	11.7±1.2	17.1±0.6
1.60	0.40	11.7±0.6	ND	10.5±1.5	ND[b]	5.0±0.5	9.3±0.1	17.2±0.5

Mean values of three gelatinization treatments ± standard deviation.
[a]Values of starch with no added constituent.
[b]ND-Not determined.

that amylose-lipid complexes can also be formed during food processing (autoclaving and cooling). The influence of other lipids such as lecithin, palmitic acid, oleic acid and soya bean oil on RS formation has also been studied in smaller amounts (Holm *et. al.*, 1983). These lipids affected retrogradation to a lower extent than monoglycerides. Nevertheless, these authors found that pure potato amylose and oleic acid formed complexes highly resistant to amylolysis. On the other hand, the results obtained in this work have been compared with the Eerlingen data (Table 3a). Using the same amylose (25%) and lipid (5,10%) contents, differences in RS yields between added olive oil and sodium dodecyl sulfate (SDS) have been found. As can be observed, the decrease of RS yields is higher when SDS is added.

The influence of sugars on RS formation during autoclaving and subsequent retrogradation was studied using sucrose. As can be observed (Table 1), the RS yields decreased when sucrose was added. The effects of added soluble sugars (glucose, maltose, sucrose and ribose) on the gelatinization and/or retrogradation of different starches have been studied by different authors (Buck & Walker, 1988; I'Anson *et. al.*, 1990; Kohyama & Nishinari, 1991). The addition of these sugars has been found to reduce the level of crystallization and subsequently the yield of RS. The mechanism of retrogradation inhibition was considered as the interaction between sugar molecules and starch molecular chains which changes the matrix of gelatinized starch (the sugars act as antiplasticizers and increase the glass transition temperature, T_g).

However, a decrease in RS yield was observed for wheat starch while an increase was noticed for high amylose corn starch at higher sugar content by Eerlingen *et. al.*, (1994). These authors did not find any change of gelatinization, amylose or lipid content of either starch studied in response to impact of sugars on RS formation.

Table 3a. Comparison of RS yields reduced by lipids

Component added (%)	RS[a](%)	RS[b](%)
0	22	18.16
5	12	10.31
10	8	14.07

[a]Eerlingen values. 25% amylose (wheat starch). SDS added.
[b]Escarpa values. 25% amylose (potato starch). Olive oil added.

Table 3b. Comparison of RS yields reduced by sucrose

Starch:Water: Sucrose (w/w)	RS[a] (%)	RS[b] (%)
1: 10: 5[c]	3.5	2.8
1.8: 40: 0.2[d]	18.16	12.90

[a]RS yields with no added sucrose.
[b]RS yields with added sucrose.
[c]Eeerlingen values.
[d]Escarpa values. At higher sucrose content.

RS yields obtained in this work were reduced less than the Eerlingen values (Table 3b). In our case, amylose content in potato starch is the same as that in wheat starch (25%) and is free of lipids.

Recently, however, scanning electronic microscopy (SEM) studies reveal complete gelatinization in retrograded potato starch (Escarpa *et. al.*, 1996). Therefore, our data are in agreement with the Eerlingen values and show that the effect of sugars may be positive or negative depending on starch type. So, the values obtained for potato starch gels with added sucrose could be interpreted in terms of the impact of these sugars on the glass transition temperature of the polymer system and their subsequent role in the crystallisation process.

Other dietary constituent influences on RS formation.

The influence of certain micronutrients, such as calcium and potasium ions, on RS formation was investigated. The results show that the yields of RS decreased in starch gels in the presence of calcium and potassium ions. The probable adsorption of these ions might prevent the formation of hydrogen bonds between amylose and amylopectin chains. Studies carried out with cellulose show this (Torre *et. al.*, 1991).

The influence on RS formation of insoluble (lignin and cellulose) and soluble (pectins) fibre constituents has also been studied. The presence of these constituents decreases the yields of RS less than other constituents.

The amount of guar gum used in these mixtures was varied in order to obtain homogeneous gels. The presence of this additive led to a decrease in yields of RS.

The addition of catechin significantly reduced the yields of RS whereas the addition of phytic acid reduced the contents of RS to a minor exent. It is well know that these compounds can affect starch digestibility. The literature reveals that the addition of phytic acid reduces the wheat starch digestibility. However, catechin has no significant effect on starch digestibility (Thompson & Yoon, 1984).

Therefore, the results obtained in this work reveal that both phytic acid and catechin did not influence starch digestibility because the RS yields obtained with these antinutrients are lower than RS yields of retrograded potato starch .

Statistical analysis

In order to evaluate the influence of diet components on the yields of RS, a one-way analysis of variance (one-way ANOVA, $P \leq 0.05$) was performed.

Mutiple range analysis for RS values by percent component (Table 4) shows that the results did not differ significantly from the value proposed by the null hypothesis. One homogeneous group only was obtained. This fact reveals that the RS yields are independent of percent component employed and the water influence on RS yields is also independent of percents studied.

Table 4. Multiple range analysis for RS values by percent constituent

Percent	Count	Average	Groups
10	10	10.74	X
20	10	10.42	X
15	10	10.83	X
5	11	11.98	X

Contrast	Difference ± Limits
5–10	1.24 ± 2.70
5–15	1.14 ± 2.88
5–20	1.56 ± 2.99
10–15	−0.10 ± 2.94
10–20	0.31 ± 3.05
15–20	0.41 ± 3.20

$P \leq 0.05$

On the other hand, mutiple range analysis for RS values by diet component (Table 5) yields three homogeneous groups: Group A (nutrients, gum and pectins as dietary fibre components and phytic acid as antinutrient); group B (potassium ions, calcium ions and catechin), and group C (lignin, cellulose and starch with no added components). These results reveal that insoluble dietary components (lignin and cellulose) did not differ significantly whereas the values obtained for the groups A and B differed significantly from the value proposed by the null hypothesis. Significant differences between A and B groups were also found.

Table 5. Multiple range analysis for RS values by constituent

Constituent	Count	Average	Groups
Calcium	2	4.20	X
Catechin	4	7.26	XX
Potassium	4	8.12	X
Phytic Acid	4	10.85	X
Albumin	4	11.10	XX
Olive Oil	4	12.37	XX
Pectins	4	12.75	XX
Gum	2	12.80	XX
Sucrose	4	12.90	XX
Cellulose	4	13.57	XX
Lignin	1	14.10	XXX
Starch[a]	4	16.12	X

	Contrast	Difference ± Limits
Starch[a]	Albumin	−5.02 ± 2.57*
	Olive Oil	−3.75 ± 2.57*
	Sucrose	−3.22 ± 2.57*
	Potassium	−8.00 ± 2.57*
	Calcium	−11.92 ± 3.15*
	Cellulose	−2.55 ± 2.57
	Pectins	−3.37 ± 2.57*
	Gum Guar	−3.32 ± 3.15*
	Lingin	−2.02 ± 4.07
	Catechin	−8.87 ± 2.57*
	Phytic Acid	−5.27 ± 2.57*

*Denotes a statistically significant difference ($P \leq 0.05$).
[a]Control: Starch with no added constituent.

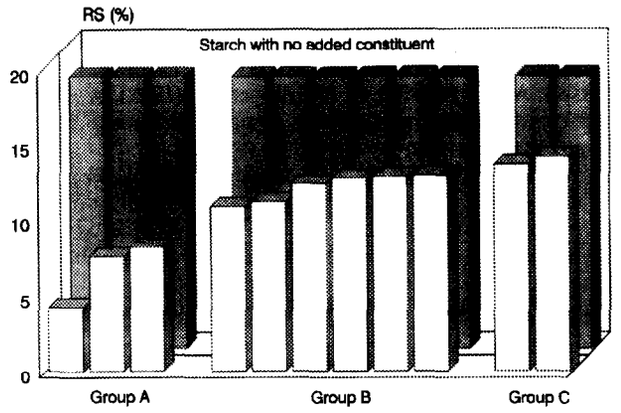

Fig. 1. Influence of diet constituents on RS formation.

Therefore a classification of the constituents studied could be proposed with regard to the results found: 1. components such as lignin and cellulose (no influence components) which had no influence on RS yields and 2. components which showed influence on RS formation, the high influence components (potassium ions, calcium ions and catechin) and the low influence components (nutrients, pectins, gum and phytic acid).

Finally Fig. 1 summarises the influence of the diet constituents studied on the formation of RS.

The systematic procedure used could be applied in foods. In the near future other techniques may be employed to elucidate the mechanisms involved.

ACKNOWLEDGEMENT

We acknowledge financial support for the research from Comision Interministerial de Ciencia y Tecnología (project ALI-0278).

REFERENCES

Berry, C. S. (1986). Resistant Starch: Formation and measurement of starch that survives exhaustive digestion with amylolitic enzymes during the determination of dietary fibre. *J. Cereal Sci.*, **4**, 301–314.

Biliaderis, C. G. (1991). The structure and interactions of resistant starch with foods constituents. *J. Physiol. Pharmacol.*, **69**, 60–78.

Buck, J. S. & Walker, C. E. (1988). Sugar and sucrose ester effects on maize and wheat starch gelatinization patterns by differential scanning calorimeter. *Starch/Starke.* **40**, 353–356.

Chandrashekar, A. & Kirleis, A. W. (1988). Influence of protein on starch gelatinization in sorghum. *Cereal Chem.*, **65**(6), 457–462.

Eerlingen, R. C., (1994). Formation, structure and properties of enzyme resistant starch. *Thesis.* Leuven University.

Eerlingen, R. C., Cillen, G. & Delcour, J. A. (1994*a*). Enzyme resistant starch. IV. Effect of endogenous lipids and added sodium dodecyl sulfate on formation of resistant starch. *Cereal Chem.*, **71**, 170–177.

Eerlingen, R. C., Van den Broeck, I., Delcour, J. A. & Levine, H. (1994). Enzyme resistant starch. VI. Influence of sugars on resistant starch formation. *Cereal Chem.*, **70**, 345.

Eliasson, A.-C. (1985). Starch-lipid interactions by differential scanning calorimetry. *Thermochimica Acta.*, **95**, 369–374.

Eliasson, A.-C., Finstad, H. & Ljunger, G. (1988). A study of starch- lipid interactions for some native and modified maize starches. *Starch/ Stärke.* **40**, 95–100.

Englyst, H., Kingman, S. M. & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European J. Clin. Nutr.*, **46 S**(2), S33–S50.

Escarpa, A., González, M. C., Mañas, E., García-Diz, L. & Saura-Calixto, F. (1996). Resistant starch formation: An standardrization of a high-pressure autoclave process. *J. Agric. and Food Chem.*, **44**, 924–928.

EURESTA (European Flair Action Concerted on Resistant Starch). *Newsletter III* (1992). **7**. Department of Human Nutrition. Wageningen Agricultural University.

Goñi, I., García-Diz, L., Mañas, E. & Saura-Calixto, F. (1996). Analysis of resistant starch: A method for foods and food products. *Food Chem.*, **56**, 445–449.

Holm, J., Björck, I., Asp, N.-G., Sjöberg, L.-B. & Lundquist, I. (1985). Starch availability *in vitro* and *in vivo* after flaking, steam cooking and popping of wheat. *J. Cereal Sci.*, **3**, 193–206.

Holm, J., Börck, I., Ostrowska, S., Eliasson, A-C., Asp, N.-G., Larsson, K. & Lundquist, I. (1983). Digestibility of amylose/ lipid complexes *in vitro* and *in vivo*. *Starch/Stärke.* **355**, 294–297.

I'Anson, K. J., Miles, M. J., Morris, V. J., Besford, L. S., Jarvis, D. A. & Marsh, R. A. (1990). The effects of added sugars on the retrogradation of wheat starch gels. *J. Cereal Sci.*, **11**, 243–248.

Mercier, C.(1980). Food process engineering Vol 1 (Linko, P., Malkki, Y., Olkku, J. & Larinkari, J. (eds.), Applied Science Publishers Ltd. London, pp 795–807.

Kohyama, K. & Nishinari, K. (1991). Effect of soluble sugars on gelatinization and retrogradation of sweet potato starch. *J. Agric. Food Chem.*, **39**, 1406–1410.

Sievert, D. & Pomeranz, Y. (1989). Enzyme resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical and microscopic methods. *Cereal Chem.*, **66**, 342–347.

Saura-Calixto, F. & Abia, R. (1991). Resistant starch: An indigestible fraction of foods. *Grasas y Aceites*, **42**, 239–242.

Slack, P. T., Baxter, E. D. & Wainwright, T. (1979). Inhibition by hordein of starch degradation. *J. Inst. Brew.*, **85**, 112–114.

Thompson, L. U. & Yoon, J. H. (1984). Starch digestibility as affected by polyphenols and phytic acid. *J. Food Sci.*, **49**, 1228–1229.

Torre, M., Rodriguez, A. R. & Saura-Calixto, F. (1992). Study of the interactions of calcium ions with lignin, cellulose and pectin. *J. Agric. Food Chem.*, **40**, 1762–1766.

Van Twisk, P. (1970). Neue erfahrungen bei der herstellung von stärke- verzuckrerungsprodukten aus maisgrits. *Starch/ Stärke.* **22**, 228–230.