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SPECIAL ISSUE ARTICLE

Effect of Structural and Physicochemical Characteristics of the Protein Matrix in Pasta on In Vitro Starch Digestibility

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Abstract Pasta is a popular carbohydrate-based food with a low glycemic response. A continuous protein matrix which entraps starch granules and/or limits/retards starch hydrolysis by α-amylase is thought to be an important factor in explaining the slow digestion of starch in pasta. The characteristics of the protein matrix may also play an important role in determining the rate of starch digestion in pasta and therefore its glycemic response. In this study, the structural and physicochemical characteristics of the protein matrix of pasta were modified by varying the number of passes through sheeting rollers to investigate their effect on in vitro starch digestibility. The results show that the proteins dissociated from the starch granules with increasing sheeting passes thereby allowing an increased degree of digestion of starch.

Keywords Glycemic response · Pasta · Protein matrix · Starch digestibility · Structure

Introduction

Acute increases in postprandial plasma glucose and insulin levels after eating foods high in carbohydrate (high glycemic response) may increase the risk of metabolic diseases such as type 2 diabetes, cardiovascular disease, and obesity.[1] Therefore, low glycemic responses are considered favorable to health, and ways are being sought to reduce the glycemic impact of carbohydrate foods.

Pasta is a popular carbohydrate-based food which produces a low glycemic response.[2] Studies show that the glycemic index for different pasta remains in the low (0–55) to medium (56–69) range depending on whether glucose or bread is used as a reference for testing. A number of studies have endeavored to understand the reasons for the low glycemic response in pasta. Many factors have been suggested to explain the slow digestion of starch in pasta including the compact texture of the product produced by the pasta-making process and the larger food particle size arriving in the stomach. Pasta is ingested as a solid food with a compact texture and requires a low degree of mastication before swallowing, after which the pasta arrives in the stomach in the form of large solid particles. The compact texture and the larger particle size limit the surface area over which digestive enzymes are able to gain access to available starch, thereby limiting rates of digestion.[3] Granfeldt and Bjorck[4] showed that the 'lente' (i.e., slow release) properties of pasta were destroyed when the same ingredients were incorporated in soft-textured bread. The larger particle size has also been suggested to lower the rate of gastric emptying,[5] but Bornet et al.[6] showed that different model carbohydrate foods have the same rate of gastric emptying despite different glycemic responses. Another important factor suggested in explaining the slow digestion of starch in pasta is the presence of a continuous protein matrix, which entraps starch granules. This protein matrix has been shown to limit/retard the accessibility of starch to α-

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amylase.[7] However, the exact mechanisms by which this protein matrix slows starch digestion in pasta are still not fully understood.

The characteristics of the protein matrix may play an important role in determining the rate of starch digestion in pasta and therefore its glycemic response. Choi et al.[8] investigated the effect of changes in the structural characteristics of protein matrix in waxy sorghum flour on in vitro starch digestibility. They showed that the in vitro starch digestibility of sorghum flour significantly increased with the addition of the reducing agent sodium bisulfite during cooking, which prevented the formation of enzyme-resistant disulfide-linked protein polymers, thereby allowing digestive enzymes easy access to starch. It is widely known that wheat dough protein matrix undergoes structural and physicochemical changes during processing. However, little information is available on how these changes affect starch digestibility in food products. Such information would be very useful for understanding the mechanisms by which the protein matrix in foods slows starch digestion and therefore would assist food scientists to design new products featuring controlled starch digestion.

The aim of this study was to investigate how the structural and physicochemical characteristics of the protein matrix affect the degree of starch digestion in pasta. Processing a dough by repeated passing through sheeting rolls has been shown to change the protein structure.[9,10] Here, we attempted to modify the structural and physicochemical characteristics of the protein matrix of pasta by increasing the number of passes through sheeting rollers.

Materials and Methods

Pasta Production

Fresh pasta was prepared by mixing 300 g durum wheat semolina (85.4% dry matter, 68.6% starch, 11.9% protein; Weston Milling, Australia), 120 g water (20 °C) and 1.5 g salt in a Kenwood Chef KM002 mixer for 4 min on setting 1 with a hook attachment. The mix, which had a granular structure, was rested for 30 min and then passed through a Sinmag Mini Roll sheeter (85 mm roll diameter, 135 rpm roll speed) twice at a gap setting of 22 mm and once at each gap setting of 15 and 10 mm. The slab formed was further passed through the sheeter at a gap setting of 5 mm, folding it to double the thickness, turning the slab by 90° and repeating this procedure until the required number of passes (3 or 45 passes) was obtained. Immediately after sheeting, the sheet was processed through a sheeter gap setting of 3 mm and settings 1–3 on a pasta machine (Imperia, Torino, Italy), and samples were cut into fettuccini (6.5 mm×2 mm, 50 mm long) and frozen (−18 °C) until analysis. Three replicate runs were performed for each number of sheeting passes, giving three batch replicates per treatment.

In Vitro Starch Digestibility

An in vitro starch digestion method that mimics human digestion was performed according to a variation of the methods of Englyst et al.[11] and McCleary and Monaghan.[12] About 5 g of fettuccini was accurately weighed into 50 ml Falcon tubes, 45 ml of water was added, and the containers were placed in a boiling water bath for 10 min, which is a defined optimum cooking time for such samples (the time when the white central core of uncooked pasta disappears for the first time: AACC method 66–50). The cooked pasta was rinsed with cold water to stop cooking and drained for 2 min. A volume of 30 ml of water and 0.8 ml of 1 M HCl was added to the sample to attain pH 2.5 (±0.2), 1 ml of 10% pepsin dissolved in 0.05 M HCl was added, and the mixture was stirred slowly (130 rpm) at 15 s intervals. After digesting for 30 min at 37 °C, 2 ml of 1 M $NaHCO_3$ solution was added, and the digestions were adjusted to pH 6.5. Pancreatic digestion commenced with addition of 5 ml 5% pancreatin solution. The contents were immediately adjusted to a volume of 55 ml with distilled water and maintained at 37 °C with gentle stirring. Aliquots of digesta (1 ml) were removed at 20 and 120 min, each added to 4 ml absolute ethanol in 10 ml tubes, and immediately mixed thoroughly. A volume of 0.25 ml of 1% amyloglucosidase was then added, and after incubating for 10 min, 3 ml glucose oxidase-peroxidase reagent was added for measurement of free glucose. Starch measured in the 20 min supernatant was rapidly digested starch (RDS) and starch measured at 120 min was RDS+slowly digested starch (SDS). Resistant starch, starch remaining undigested after 120 min, was also measured. Mean values were calculated from measurements of three batch replicates.

Confocal Laser Scanning Microscopy

The pasta doughs were pre-stained by adding two fluorescent dyes, Fluorescein (sodium salt, 0.05% of flour weight, Sigma Chemicals, St. Louis, USA) and Rhodamine B (0.05% of flour weight, Sigma Chemicals) into the water before mixing. This allowed simultaneous observation of starch and protein under confocal laser scanning microscopy (CLSM). The Fluorescein stained the starch components, and the Rhodamine B stained the protein structures. Samples, which were frozen at −18 °C, were sectioned into 1-mm-thick slices with a razor blade, placed on glass cavity slides, and observed under a Leica TCS SP5 (Heidelberg, Germany) with dual excitation. A fluorescein isothiocyanate and a tetramethylrhodamine isothiocyanate filter block in the scanner were used for excitation of the

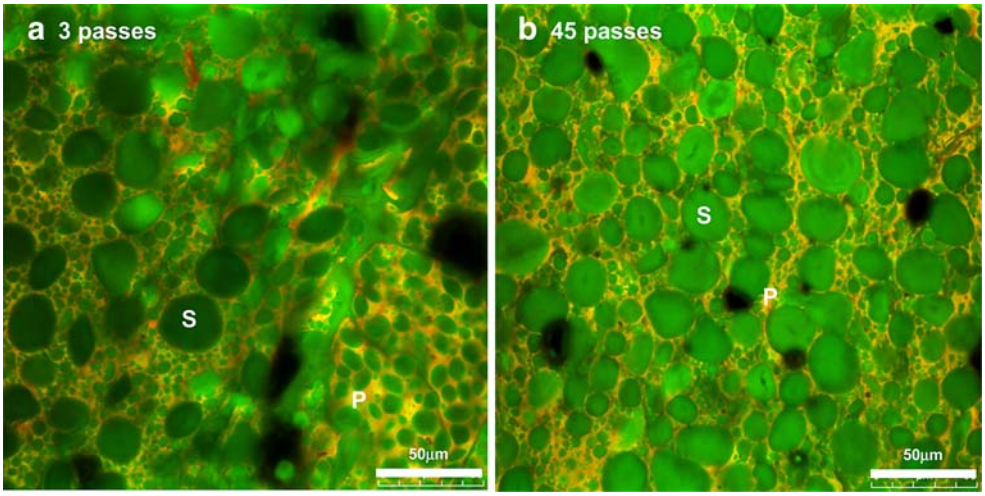

Fig. 1 Microstructure of fresh fettuccini after 3 and 45 sheeting passes. *S* Starch granules; *P* protein matrix. Micrographs are representative of at least nine sample sections from three batch replicates for each number of sheeting passes

dyes at wavelengths of 488 and 561 nm, respectively. For visualization of the structure of the cooked pasta, the cooked pasta slices were immersed in an aqueous solution of Fluorescein (sodium salt, 0.05% *w*/*v*) and Rhodamine B (0.05% *w*/*v*) for about 2 h and then rinsed in distilled water for 4 h. Several bulk sections were mounted onto the cavity glass slide. Post-staining is the most commonly used CLSM sample-preparation technique for cooked pasta and other food samples.[7,8,13] At least nine sample sections from three batch replicates were analyzed for each number of sheeting passes.

Size Exclusion-High Performance Liquid Chromatography

Samples were prepared for size exclusion-high performance liquid chromatography (SE-HPLC) analysis according to Sutton et al.[10] A sample was freeze-dried and suspended in 1 ml buffered SDS solution (0.5% *w*/*v*, 50 mM phosphate, pH 6.9) for 30 min at 20 °C. After centrifugation (10,000×*g*, 10 min), the supernatant (comprising the soluble proteins) was removed for the SE-HPLC analysis. The residual pellet from the first extraction was resuspended by 15 s of sonification (Branston sonifier, model 250, 3 mm micro tapered tip, 80 W) in a further 1 ml of the buffered SDS solution, and the supernatant (comprising the insoluble proteins) was taken for analysis after centrifugation (10,000×*g*, 10 min). Chromatography at 25 °C used a Waters 2690 solvent delivery/control/injection system equipped with a Waters 490 ultraviolet/visible detector and a 300×7.8 mm Bio-Sep 4000S column (Phenomenex, Torrance, CA, USA). Proteins (injection size 20 μl) were eluted isocratically with 50% (*v*/*v*) acetonitrile containing 0.1% (*v*/*v*) trifluoroacetic acid at a flow rate of 0.5 ml/min. Eluted components were detected by ultraviolet absorption at 210 nm.

Rheological Testing

A sample of dough removed from the main sheeted dough mass was subjected to a rupture stress test using an Instron

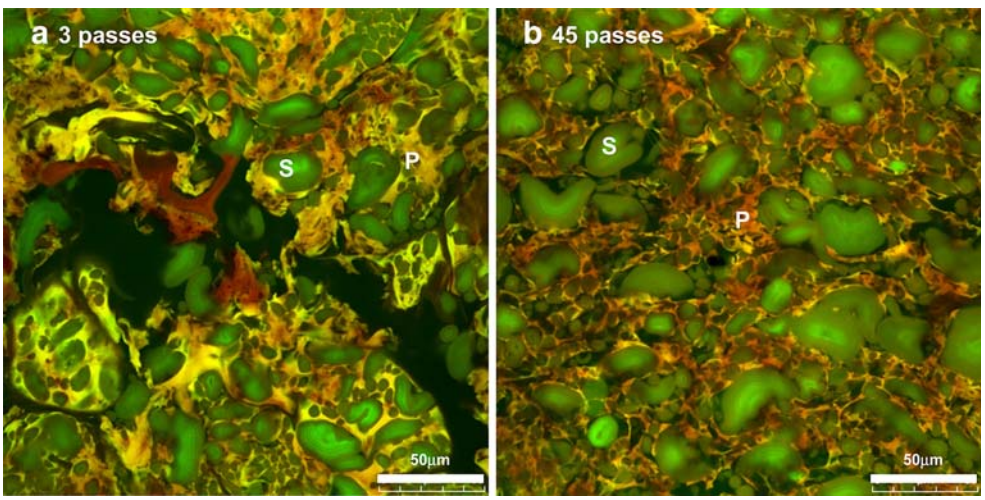

Fig. 2 Microstructure of cooked fettuccini after 3 and 45 sheeting passes. *S* Starch granules; *P* protein matrix. Micrographs are representative of at least nine sample sections from three batch replicates for each number of sheeting passes. The proteins in pasta after three passes remained closely associated with the starch granules after cooking (**a**), whereas the proteins in pasta after 45 passes dissociated from the starch granules, and the structure looked more open and less compact after cooking (**b**)

model 4444 universal testing machine.[9] A 110-mm-diameter disc was cut from the sheet and clamped between two Perspex plates. A cylindrical probe (diameter 35 mm) moved through circular holes (diameter 55 mm) in the plates to stretch the sheet at constant speed (50 mm/min) until rupture. The force on the probe was recorded as a function of time. From the data, the stress and strain at rupture were determined. The measurement was made after 30 min resting after the last sheeting pass. Ten dough discs were analyzed for each number of sheeting passes.

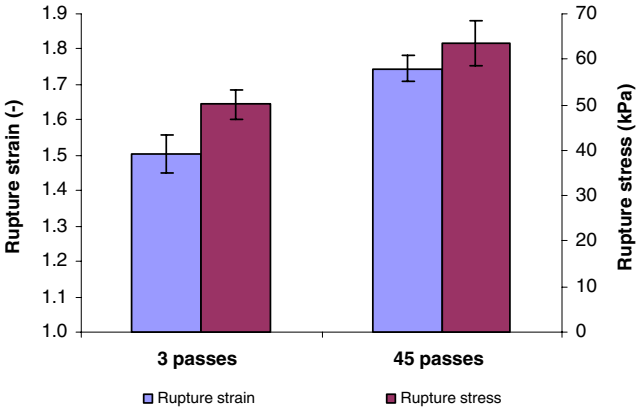

Fig. 4 Effect of increasing sheeting passes on elongation properties of pasta dough sheet. *Bars* indicate standard deviation of means

Results and Discussion

Changes in Structural Characteristics of the Protein Matrix During Sheeting

The structural characteristics of the protein matrix of pasta were examined using CLSM. Figures 1 and 2 show the microstructure of fresh and cooked pasta, respectively. The pasta dough protein was initially closely associated with the starch granules as is seen in wheat endosperm (Fig. 1a), and the proteins remained closely associated with the starch granules even after cooking (Fig. 2a). With increasing sheeting passes, the proteins and starch granules became distributed more uniformly throughout the dough (Fig. 1b). The proteins dissociated from the starch granules and became visibly aggregated during cooking, showing a clustering together of the starch granules (Fig. 2b).

Changes in Physicochemical Characteristics of the Protein Matrix During Sheeting

Changes in biochemical and rheological characteristics of the protein matrix of pasta during sheeting were assessed by SE-HPLC and rheological testing, respectively. Several authors have reported that dough develops a protein network during mixing or sheeting, which is associated with changes in the biochemical and rheological properties of gluten proteins (elasticity, extensibility, solubility, etc.). During mixing or sheeting, the dough became more elastic,[9] and the solubility of glutenin proteins (the elastic protein component in gluten) increased due to protein disaggregation and depolymerization of the glutenin polymer.[10,14,15] Figure 3 shows changes in the distribution of glutenin proteins in the pasta during sheeting from 3 passes and 45 passes. We observed that with increasing sheeting passes the quantity of the SDS-soluble polymeric glutenin increased, with a concurrent decrease in the SDS-insoluble polymeric glutenin. We also observed changes in the rheological properties of the dough sheet during sheeting (Fig. 4). The rupture stress and strain of the fresh pasta sheet increased with increasing passes through the sheeting rollers. These observations indicate development of a

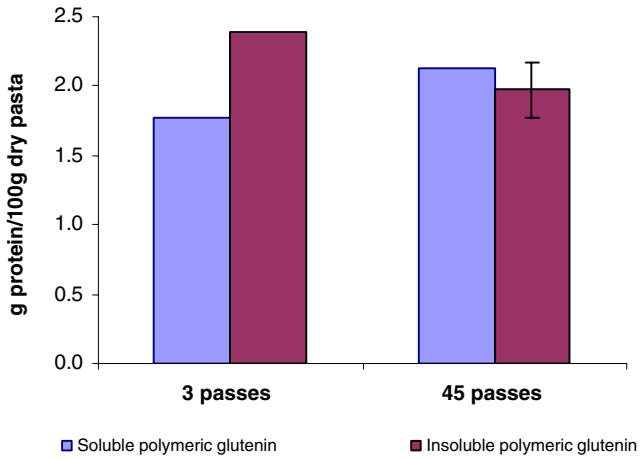

Fig. 3 Effect of increasing sheeting passes on protein composition. *Bar* indicates least significant difference at 95% confidence level

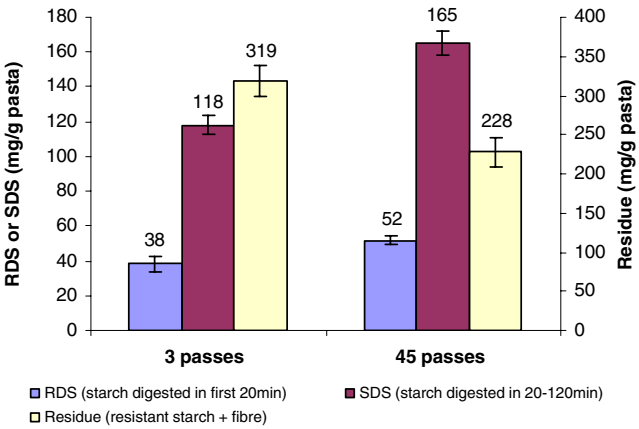

Fig. 5 Effect of increasing sheeting passes on in vitro starch digestion in pasta. *Bars* indicate standard deviation of means

protein network, which may result in increased exposure of the starch granules. The effect of the development of a protein network on the degree of starch digestion is discussed in "In Vitro starch Digestibility."

In vitro Starch Digestibility

Figure 5 shows the effect of increasing sheeting passes on in vitro starch digestion in pasta. The degree of digestion of starch increased with increasing sheeting passes. Both RDS and SDS increased in quantity, whereas resistant starch decreased. The microscopy evidence, shown in Fig. 2, strongly suggested that the protein in pasta dough was pulled away from the starch granules as sheeting proceeded. The reduction in cohesiveness between starch and protein may be responsible for the increase in starch accessibility to α-amylase, as shown by the digestibility results. These observations are consistent with a previous study by Jenkins et al.[16] who showed that the presence of a natural starch–protein interaction in white flour accounted for a decreased glycemic response, and the glycemic responses increased when the natural starch–protein interaction had been disrupted. In their study, bread made from gluten-free flour plus gluten has been shown to elicit a higher glucose response than an ordinary wheat bread. The natural starch–protein interactions in pasta dough here appears to be disrupted as sheeting proceeded, thereby increasing starch accessibility to α-amylase.

The microscopy result here matches an earlier study[17] where we observed that the protein in bread dough (62% water addition based on flour) was initially closely associated with starch granules but was pulled away from the starch granules and separated into strands as mechanical dough development proceeded. One may argue that mechanical dough development (MDD) is not the same as dough development by sheeting. However, in our previous study,[10] dough development using sheeting and MDD mixing were compared with respect to the effect the mixing method had on the biochemical and rheological properties of glutenin proteins, and we found that dough development can also be achieved using a sheeting process. Therefore, we assume that the protein in the pasta dough was also "developed" as sheeting proceeded. These microscopy observations are consistent with the biochemical and rheological results discussed in "Changes in Physicochemical Characteristics of the Protein Matrix During Sheeting." However, it should be noted that there was much less protein orientation observed in pasta dough than in bread dough with a fully developed gluten network. That is, the protein network in the pasta dough was much less "developed" than the bread doughs studied earlier, possibly as a result of the low moisture content of pasta dough (40% water addition based on flour).

Conclusions

Pasta is a popular carbohydrate-based food which produces a low glycemic response. Many factors have been suggested to explain the slow digestion of starch in pasta. This study suggests that the structural and physicochemical characteristics of the protein matrix play a role in determining the degree of starch digestion in pasta. In particular, we have shown that the presence of starch–protein interactions in pasta dough may be important for reducing the digestibility of starch in pasta. The proteins dissociated from the starch granules, and the natural starch–protein interaction may be destroyed as processing proceeds. Hence the starch present becomes more accessible to amylases. The present work is a preliminary observation to study the mechanisms by which the protein matrix of pasta controls starch degradation. Further research is under way to fully understand these mechanisms.

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References

1. E. Bonora, M. Muggeo, Postprandial blood glucose as a risk factor for cardiovascular disease in Type II: diabetes the epidemiological evidence. Diabetologia. **44**, 2107–2114 (2001)
2. T. M. S. Wolever, D. J. A. Jenkins, J. Kalmusky, C. Giordano, S. Giudici, A. L. Jenkins, L. U. Thompson, G. S. Wong, R. G. Josse, Glycemic response to pasta: effect of surface area, degree of cooking and protein enrichment. Diabetes Care. **9**, 401–404 (1986)
3. D. J. A. Jenkins, T. M. S. Wolever, A. L. Jenkins, R. Lee, G. S. Wong, R. G. Josse, Glycemic response to wheat products: reduced response to pasta but no effect of fibre. Diabetes Care. **6**, 155–159 (1983)
4. Y. Granfeldt, I. Bjorck, Glycemic response to starch in pasta: a study of mechanisms of the limited enzyme availability. J. Cereal Sci. **14**, 47–61 (1991)
5. C. Thomsen, O.W. Rasmussen, C. Christiansen, F. Andreasen, P. L. Poulsen, K. Hermansen, The glycaemic index of spaghetti and gastric emptying in non insulin-dependent diabetic patients. Eur. J. Clin. Nutr. **48**, 776–780 (1994)
6. F.R.J. Bornet, Y. Bizais, S.B.D. Varannes, B. Pouliquen, J. Delort-Laval, J.-P. Galmiche, Alpha-amylase (EC 3.2.1.1) susceptibility rather than viscosity or gastric emptying rate controls responses to starch in healthy humans. Br. J. Nutr. **63**, 207–220 (1990)
7. A. Fardet, C. Hoebler, P.M. Baldwin, B. Bouchet, D.J. Gallant, J. L. Barry, Involvement of the protein network in the in vitro degradation of starch from spaghetti and lasagne: a microscopic and enzymic study. J. Cereal Sci. **27**, 133–145 (1998)
8. S.J. Choi, H.D. Woo, S.H. Ko, T.W. Moon, Confocal laser scanning microscopy to investigate the effect of cooking and sodium bisulfite on in vitro digestibility of waxy sorghum flour. Cereal Chem. **85**, 65–69 (2008)
9. M.P. Morgenstern, H. Zheng, M. Ross, O.H. Campanella, Rheological properties of sheeted wheat flour dough measured with large deformations. Int. J. Food Prop. **2**, 265–275 (1999)

10. K.H. Sutton, N.G. Larsen, M.P. Morgenstern, M. Ross, L.D. Simmons, A.J. Wilson, Differing effects of mechanical dough development and sheeting development methods on aggregated glutenin proteins. Cereal Chem. **80**, 707–711 (2003)
11. H.N. Englyst, J. Veenstra, G.J. Hudson, Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycaemic response. Br. J. Nutr. **75**, 327–337 (1996)
12. B.V. McCleary, D.A. Monaghan, Measurement of resistant starch. J. AOAC Int. **85**, 665–675 (2002)
13. A. Fardet, P.M. Baldwin, D. Bertrand, B. Bouchet, D.J. Gallant, J. L. Barry, Textural images analysis of pasta protein networks to determine influence of technological processes. Cereal Chem. **75**, 699–704 (1998)
14. M. Hayta, M. Alpaslan, Effects of processing on biochemical and rheolofical properties of wheat gluten proteins. Nahrung/Food. **45**, 304–308 (2001)
15. J.H. Skerritt, L. Hac, F. Bekes, Depolymerization of the glutenin macropolymer during dough mixing. I. Changes in levels, molecular weight distribution, and overall composition. Cereal Chem. **76**, 395–401 (1999)
16. D.J.A. Jenkins, M.J. Thorne, T.M.S. Wolever, A.L. Jenkins, A.V. Rao, L.U. Thompson, The effect of starch–protein interaction in wheat on the glycemic response and rate of in-vitro digestion. Am. J. Clin. Nutr. **45**, 946–951 (1987)
17. M.P. Newberry, L.D. Simmons, M.P. Morgenstern, Confocal visualization of MDD dough development, in *The gluten proteins*, ed. by D. Lafiandra, S. Masci, R. D'Ovidio, (Royal Society of Chemistry, 2004), pp. 263–266