



Spaghetti from durum wheat: Effect of drying conditions on heat damage, ultrastructure and *in vitro* digestibility



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ABSTRACT

The effects of low (LT) or high (HT) temperature drying on ultrastructural, molecular and *in vitro* digestibility properties of cooked spaghetti were studied. Starch swelling and denaturation/aggregation of proteins occurring at diverse stages, LT or HT drying and cooking, resulted in different *in vitro* digestibility of spaghetti. For the first time, these differences were assessed in terms of the release of free AA and simple sugars. Indeed, at the end of *in vitro* digestion, the total amount of released maltotriose, maltose and glucose significantly differentiated digestates of LT and HT spaghetti (12.6 and 15.9 g 100 g⁻¹). In the same samples, diverse amounts (16.3 and 12.5 g 100 g⁻¹ protein) of free amino acids were found. Chemical artifacts occurring at protein level impaired release of lysine in cooked HT spaghetti after *in vitro* digestion. These results increase the knowledge on digestibility of LT and HT cooked spaghetti.

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1. Introduction

Dried pasta represents a basic food worldwide; it is prepared from dough obtained by mixing water with semolina from durum wheat (*Triticum turgidum* L. var. *durum*). The dough is continuously kneaded and extruded through a die that determines the shape of the final product. At industrial scale, pasta is finally dried to about 12% moisture according to different time/temperature/humidity cycles, which have been largely performed in Italy at low temperature (LT, 60–70 °C; 14 h) until the 1970s. More recently, adoption of high temperature (HT, >70 °C; 8 h) drying has reduced the processing time and generally improved cooking properties of pasta (Cubadda, Carcea, Marconi, & Trivisonno, 2007; Petitot, Abecassis, & Micard, 2009a; Petitot et al., 2009b). The phenomena involved in determining the behaviour of pasta components have been elucidated by investigating the ultrastructure of dried and cooked pasta by means of transmission electron microscopy and scanning electron microscopy (Cunin, Handschin, Walther, & Escher, 1995). As a result of these investigations, the structure of dried and cooked pasta has been generally described as a compact matrix with starch granules entrapped in a coagulated protein network, which especially forms and organises when HT drying is applied to pasta dried under low moisture (<15%) conditions (Bruneel, Pareyt, Brijs, & Delcour, 2010; Cunin et al., 1995). Under such

conditions, the cooked pasta achieves higher firmness, lower stickiness and minimally loses solids into cooking water (Petitot et al., 2009a, 2009b; Zweifel, Handschin, Escher, & Conde-Petit, 2003).

Due to the presence of reducing sugars and proteins, pasta is a food system easily prone to Maillard Reaction (MR), which can be enhanced by several biochemical and technological factors occurring in pasta production (De Noni & Pagani, 2010). Consequently, the heat damage of dried pasta involves protein glycosylation and formation of advanced glycation end products (AGEs) (De Noni & Pagani, 2010), which may affect protein digestibility (Seiquer et al., 2006). In this regard, the effect of drying treatments in modifying protein breakdown during digestion by formation of AGEs should be considered.

The molecular phenomena responsible for pasta (ultra)structure, especially during HT drying, could also affect the kinetics and degree of protein and starch breakdown in the gastrointestinal tract. Starch degradation during *in vitro* digestion of pasta was demonstrated to be affected by both its physical modification (mainly gelatinisation) and the compact protein matrix surrounding the starch granules, which may prevent amylolysis (Casiraghi, Brighenti, & Testolin, 1992; De Zorzi, Curioni, Simonato, Giannattasio, & Pasini, 2007). The size and shape of pasta as well as the spaghetti particle size have been reported to affect *in vitro* starch digestion by changing the surface-to-volume ratio and hence access by α -amylase (Aravind, Sissons, & Fellows, 2011). The influence of matrix modifications determined by processing conditions on the digestibility of pasta protein has been studied

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as well, and HT drying was shown to decrease the *in vitro* digestibility of proteins (De Zorzi et al., 2007; Petitot et al., 2009a, 2009b). Moreover, resistance of pasta proteins to digestion was shown to address sensitisation of humans to wheat proteins (De Zorzi et al., 2007; Petitot et al., 2009b; Simonato et al., 2001).

The overall effect of drying parameters on the digestibility of pasta was assessed by different *in vitro* digestion models and by evaluating different products arising from breakdown of pasta components (De Zorzi et al., 2007; Petitot et al., 2009b). Recently, a static protocol for *in vitro* digestion has been drafted and shared within the COST Action FA1005 (Dupont et al., 2011). Based on this protocol, the aim of this work was to assess the digestibility of cooked spaghetti dried under LT or HT conditions by measuring the release of amino acids and sugars in the digestates. The ultrastructure and the characteristics of starch and proteins of spaghetti before and after cooking were evaluated as well, with the aim to achieve a more detailed view of factors affecting their *in vitro* digestibility.

2. Materials and methods

2.1. Spaghetti production

Spaghetti were processed with a continuous pilot-scale plant (50 kg h⁻¹, Braibanti, Milan, Italy). Durum wheat semolina (protein content 12.0 g 100 g⁻¹ db; extraction rate 70%; Molino Grassi, Parma, Italy) was mixed with tap water at a semolina-to-water ratio of 10:2.67 to obtain a dough moisture of 32%, and mixed for 10 min. Spaghetti were extruded through a Teflon die (diameter 1.7 mm) at 10–11 MPa, 20 rpm and about 40 °C, and then dried in a pilot-scale drier (50 kg h⁻¹, Braibanti, Milan, Italy) until 12% moisture was reached. Drying at low (<62 °C) or high (<93 °C) temperature was applied to the pasta; the related time/temperature/moisture profiles are depicted in Fig. 1.

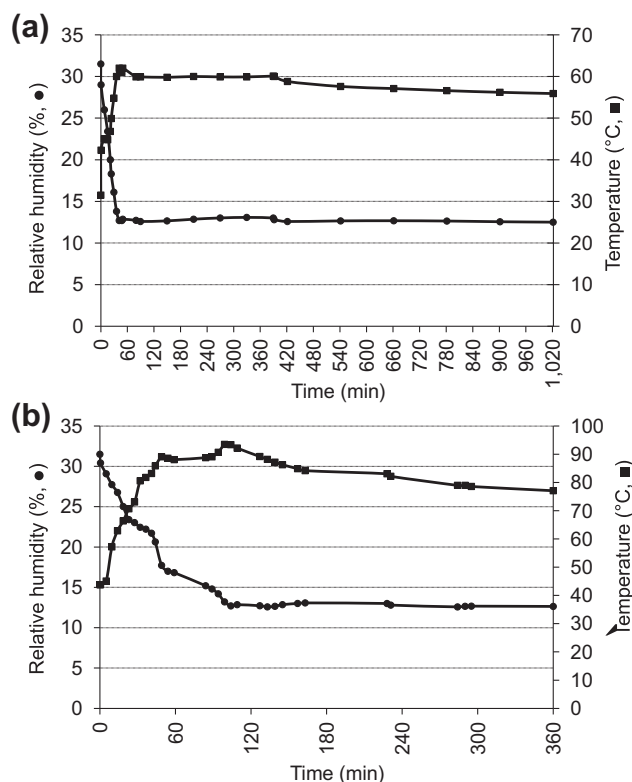


Fig. 1. Drying conditions of spaghetti processed in a semi-pilot plant according to low-temperature (A) and high-temperature (B) cycles.

2.2. Cooking of spaghetti

Dried spaghetti were cooked according to D'Egidio, Mariani, Nardi, Novaro, and Cubadda (1990) with minor modifications. In detail, dried spaghetti were cut to the same length (about 8 cm). Ten grams of spaghetti were then put into 100 mL boiling tap water without salt added. Optimal cooking times were 10.0 and 11.0 min for LT and HT dried spaghetti, respectively. The cooking time was determined by observing the disappearance of the white core of the pasta squeezed between two glass plates. Spaghetti were let to drain for 9 min and promptly submitted to analyses or freeze dried.

Water absorption was measured as the weight increase of spaghetti before and after cooking, and is expressed as percent weight gain with respect to the weight of uncooked spaghetti.

2.3. Pasting properties of spaghetti

The pasting properties of uncooked and cooked spaghetti were measured by a Brabender Micro-Visco-AmyloGraph (Brabender OHG, Duisburg, Germany) on 15 g of finely ground sample dispersed in 100 mL of distilled water, according to Bonomi et al. (2012). The following indices were considered: pasting temperature; peak viscosity (maximum paste viscosity achieved during the heating cycle); breakdown (decrease in viscosity during the first holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 95 °C); setback (increase in viscosity during cooling, corresponding to the difference between the final viscosity and the viscosity reached after the first holding period). Measurements were performed in triplicate.

2.4. Analysis of SDS-soluble, DTE-soluble and unextractable proteins

Proteins were extracted in triplicate from semolina, uncooked and cooked freeze dried spaghetti according to a modified method of Morel, Dehlon, Autran, Leygue, and Bar-L'Helgouac'h (2000) and described in Petitot et al. (2009b). The extraction procedure consisted of two successive extractions. The first step extracted SDS-soluble proteins and the second one extracted DTE-soluble proteins from the pellet of the first extraction. The remaining fraction that was extracted neither in SDS nor in DTE followed by sonication constituted the insoluble fraction or unextracted proteins. It represented proteins linked by covalent linkages other than disulfide bonds. The protein quantification in each extract (semolina, raw and freeze dried cooked pasta) was examined using total area from the SE-HPLC profile as described in Petitot et al. (2009b). The percentages of extracted proteins were calculated considering the semolina protein as 100% extractable. Assays were performed in triplicate.

2.5. Ultrastructural observations

Both uncooked and freeze-dried cooked spaghetti strands were mounted on aluminium stubs and sputter-coated with gold. On each stub, four cross-sectioned pieces (4–6 mm length; selected at random) of spaghetti were mounted. Pasta ultrastructure was imaged in the scanning electron microscope (SEM) LEO438 VP (LEO Electron Microscopy Ltd., Cambridge, UK), under high vacuum conditions (10⁻⁴ Pa) at an accelerating voltage of 15 kV.

2.6. *In vitro* static gastrointestinal digestion

To evaluate the digestibility of spaghetti, an *in vitro* static digestion protocol developed within the COST Action FA1005 was adopted (Dupont et al., 2011). In detail, simulated salivary (SSF), simulated gastric (SGF) and simulated duodenal (SDF) fluids were

prepared according to Kopf-Bolanž et al. (2012); Versantvoort, Oomen, van de Kamp, Rompelberg, and Sips (2005). Human salivary α -amylase was added to SSF (150 U mL^{-1}). Cooked pasta samples (5 g) were ground in a mincer in the presence of 5 mL of SSF at pH 7.0 for 2 min to reproduce mastication. Bolus derived from the oral digestion was mixed with 10 mL of SGF supplemented with porcine pepsin (1000 U mL^{-1} of SGF). The gastric phase digestion was performed at 37°C for 2 h at pH 3.0 (adjusted with 1 N HCl). Afterwards, 20 mL of SDF and bile salts extract (10 mM, Sigma–Aldrich, Milan, Italy) were added to the digestate. Enzymes for intestinal digestion were pancreatic α -amylase (200 U mL^{-1} of SDF), porcine trypsin (100 U mL^{-1} SDF), chymotrypsin (50 U mL^{-1} SDF), porcine intestinal lipase (2000 U mL^{-1} SDF) and co-lipase (molar ratio lipase/co-lipase: 1/2). The intestinal phase was performed at 37°C for 2 h at pH 7.0, and it was stopped by adding the protease inhibitor AESFB (Roche, Mannheim, Germany) to give 1 mM final concentration. The digestates were immediately frozen at -40°C and freeze-dried. All enzymes were purchased from Sigma–Aldrich (Milan, Italy) and digestions were performed in duplicate.

The contents of ϵ -pyrroline-lysine (ϵ -PL, ϵ -2-formyl-5-hydroxymethyl-pyrrolaldehyde), soluble sugars and free amino acids (AA) were determined in the digestates of spaghetti according to the methods described below.

2.7. Determination of starch hydrolysis during digestion

The degree of starch hydrolysis in spaghetti before and after different steps of *in vitro* digestion was assessed by determining the content of maltotriose, maltose and glucose. To this purpose, sugars were extracted and determined by HPLC as reported by Resmini, Pagani, Pellegrino, and De Noni (1993).

2.8. Determination of free amino acid released during digestion

The degree of protein hydrolysis during *in vitro* digestion was monitored by determining free AA content in digestates. This content was assessed in dried uncooked pasta samples as well, since moderate protease activity was reported for wheat kernel (Resmini et al., 1993).

Free AA were determined by ion-exchange chromatography, using a Biochrom 30 + amino acid analyser (Biochrom, Cambridge, UK), and applying the analytical conditions proposed by the manufacturer. Data were acquired and elaborated with EZChrom Elite™ software (Agilent Technologies, Santa Clara, CA). Six-point calibration curves were adopted for quantitation. For undigested samples, extraction of free AA was carried out on 1.5 g of ground sample, after dispersion in sodium citrate buffer (pH 2.2), followed by homogenisation and deproteinisation with 7.5% (w/v) 5-sulfosalicylic acid, according to the method described by Resmini et al. (1993). In the case of digested samples, 100 mg freeze dried digestate were diluted to 10 mL of citrate buffer (pH 2.2), and pH value was adjusted to 2.8 with 7.5% (w/v) 5-sulfosalicylic acid prior to injection.

2.9. Evaluation of heat damage of spaghetti

The formation of chemical artefacts potentially capable of affecting protein digestibility was assessed by determining the following molecules arising from MR. The level of furosine (ϵ -N-furouylmethyl-L-lysine, FUR) was determined in uncooked spaghetti by HPLC according to Resmini and Pellegrino (1994). To this purpose 500 mg of dried pasta were hydrolysed at 110°C for 23 h and then submitted to solid-phase extraction and HPLC analysis. The content of ϵ -PL in dried uncooked and in cooked digested spaghetti was assessed by adopting the method proposed by Resmini

and Pellegrino (1994). The level of glucosylisomaltol (GUI, 2-acetyl-3-D-glucopyranosyl-furan) was evaluated in dried uncooked and in cooked spaghetti by the HPLC method proposed by Pellegrino and Cattaneo (2001).

2.10. Statistical analysis

The dependent variables were processed by one-way analysis of variance (ANOVA) using Minitab software (release 14, 2004; State College, PA).

3. Results and discussion

3.1. Effect of drying on the ultrastructure of spaghetti

The ultrastructure of dried spaghetti before and after cooking was investigated (Fig. 2). Before cooking, the protein matrix of LT dried pasta looked discontinuous with protein aggregates unevenly distributed among starch granules (Fig. 2). During cooking, starch granules deformed and melted in some regions while proteins formed large aggregates unevenly distributed inside the product. This kind of structure was observed both in the core (Fig. 2) and with more evidence in the external regions of LT spaghetti (Fig. 2). Concerning the uncooked HT pasta, starch granules were surrounded by a more continuous protein network (Fig. 2). Although protein matrix was still recognisable after cooking, gradual structural changes were visible from the core to the external region, as observed by Cunin et al. (1995), and Petitot et al. (2009b). In the central regions, starch granules exhibited a limited degree of swelling (Fig. 2). Starch modification promoted by cooking was more evident in the external regions, although starchy material was mostly entrapped in the protein network (Fig. 2).

3.2. Effect of drying on starch and proteins of spaghetti

Pasting properties of uncooked and cooked spaghetti are shown in Table 1. The pasting temperature at which an initial increase in

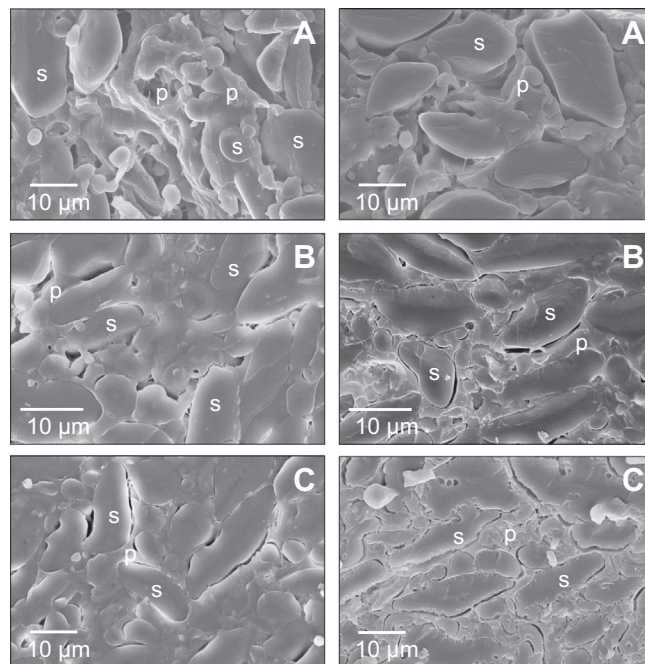


Fig. 2. SEM images of LT-dried (LEFT) and HT-dried (RIGHT) uncooked (A) and cooked spaghetti in the central (B) and external (C) regions of pasta strand. p, protein matrix; s, starch granule.

viscosity occurred was higher in HT uncooked spaghetti (80.1 °C) in comparison with LT sample (65.4 °C). Uncooked LT spaghetti had peak viscosity and final viscosity higher than the HT spaghetti. This means that starch in LT uncooked spaghetti presented a more relevant swelling capacity. On the contrary, the high pasting temperature of HT uncooked spaghetti could be related to both the presence of new bonds promoted by HT in the starch granules (Bonomi et al., 2012) and the more aggregated protein matrix formed upon drying, as suggested by Bruneel et al. (2010), and demonstrated by the data on protein extractability (Table 2). After cooking, the differences in pasting properties between LT and HT spaghetti greatly decreased and resulted from the diverse amount of adsorbed water, which was 18% higher in the LT sample. Durum wheat semolina was characterised by a high fraction of SDS-soluble proteins (81%), a low fraction of DTE-soluble proteins (19%) and a lack of unextractable proteins, in agreement with Petitot et al. (2009b). The dried spaghetti presented a higher protein aggregation, as shown by the decrease in SDS-soluble proteins in favour of DTE-soluble proteins. This may indicate formation of additional disulfide bonds during drying. This phenomenon was drastically enhanced with an increase of drying temperature. In HT spaghetti, SDS-soluble and DTE-soluble proteins reached indeed 23% and 65%, respectively, in agreement with the results obtained by Petitot et al. (2009b). In contrary to LT drying, HT drying led also to unextractable proteins (12%) that reached exactly the values obtained with drying at 90 °C by Petitot et al. (2009b). Cooking of LT pasta generated a decrease in SDS-soluble proteins (18%) in favour of DTE-soluble proteins (74%) and to a lesser extent unextractable proteins (9%), as described by Petitot et al. (2009b). This was also observed after cooking of HT spaghetti, in which SDS-soluble proteins decreased to 14% and DTE-soluble proteins increased to 79%. As reported by Petitot et al. (2009b), the cooking step reduced the variation in protein solubility previously observed after LT or HT drying, so that the same unextractable protein level (9%) characterised both LT and HT cooked samples. These data support the formation of the protein network in LT spaghetti to occur during the cooking process mainly.

3.3. Effect of drying on heat damage of dried spaghetti

In the present work, the occurrence of early and advanced MR products (MRPs) in dried spaghetti was studied. Furosine amount, as a measure of early MR extent, was 139 mg 100 g⁻¹ protein and 343 mg 100 g⁻¹ protein in LT and HT uncooked spaghetti, respectively. These values agreed with previous findings for pasta dried under LT and HT conditions (Cavazza et al., 2013; Giannetti, Mariani, & Mannino, 2013). The highest temperature (93 °C) during HT drying was applied to spaghetti having a moisture level <15% (Fig. 1). Such conditions favour the degradation of early MRPs to AGEs including ε-PL, which has been reported as a lysine-derived Maillard molecule resulting from the degradation of 3-deoxyosone (Resmini & Pellegrino, 1994). As expected, low level (0.72 mg kg⁻¹; 0.58 mg 100 g⁻¹ protein) of this AGE characterised LT spaghetti, whereas higher level (4.24 mg kg⁻¹; 3.39 mg 100 g⁻¹ protein) of ε-PL was recorded in the HT sample. The reaction of maltose with

free AA during pasta drying produces stable maltoglycosides (MGs) from 1-deoxyosone, as described for GUI by Resmini et al. (1993). This MG was absent or detected at 2 mg kg⁻¹ in LT and HT uncooked spaghetti, respectively. Glucosylisomaltol was no longer detectable in HT cooked spaghetti, since most of this molecule is lost in water during cooking (Resmini et al., 1993).

3.4. In vitro digestibility of spaghetti

3.4.1. Starch digestibility

The digestibility of starch can be affected by both drying and cooking of pasta (De Zorzi et al., 2007; Petitot et al., 2009b). Starch granules are gelatinised in cooked pasta and hence they are more prone to amylolysis during digestion (Petitot et al., 2009b). As previously discussed, after cooking the differences in pasting properties between LT and HT tended to level off. Ultrastructure images (Fig. 2) demonstrated starch granules to be gelatinised after pasta cooking in both LT and HT spaghetti. Higher deformation and melting swelling of starch granules was observed both in the core and in the external region of LT cooked spaghetti (Fig. 2).

On these bases, starch breakdown at molecular scale was followed by determining the levels of maltotriose, maltose and glucose at different steps of *in vitro* digestion of cooked spaghetti (Table 3). Levels of glucose and maltotriose, as well the total sugar content were not significantly ($p > 0.05$) different between LT and HT undigested cooked spaghetti. The revealed contents resulted from amylolytic activity occurring during mixing and kneading of dough (De Noni & Pagani, 2010). At the end of oral phase, amylolysis produced significantly ($p < 0.001$) different amounts of maltose and maltotriose in LT and HT spaghetti. In particular, starch breakdown in LT sample increased the levels of maltose and maltotriose to 1.39 and 1.54 g 100 g⁻¹, respectively, while in HT dried spaghetti the quantities of two sugars increased to 1.77 and 1.87 g 100 g⁻¹ (Table 3). After the same step of *in vitro* digestion, the content of glucose did not change in both LT and HT samples. These findings resemble those of Hoebler et al. (1998) who studied the release of sugars upon oral digestion of cooked spaghetti in human subjects. In particular, the authors observed spaghetti mastication to produce mainly maltose (3.6 g 100 g⁻¹ starch) and maltotriose (4.4 g 100 g⁻¹ starch), while the amount of glucose remained unchanged. According to Petitot et al., (2009b), the *in vitro* oral digestion of pasta led to about 22% of starch transformed in alcohol-soluble dextrins in 5 min, without significant differences between LT (<55 °C) and HT (<90 °C) dried pasta samples. As expected, the amount of released sugars did not increase after the gastric phase, since inactivation of salivary α-amylase occurs at gastric pH values (Groppe & Smith, 2013). Nevertheless, the gastric step of *in vitro* digestion weakens the pasta protein matrix surrounding gelatinised starch, which becomes more accessible to pancreatic amylase (Colonna et al., 1990; Petitot et al., 2009b). This explains the intense starch degradation observed at the intestinal step, with a level of maltotriose raised to 4.58 and 5.73 g 100 g⁻¹ and that of maltose to 7.40 and 9.34 g 100 g⁻¹ in LT and HT spaghetti, respectively (Table 3). Once again, amounts of maltose and maltotriose were significantly ($p < 0.05$) higher in the digestate of HT spaghetti.

Table 1
Pasting properties of uncooked and cooked spaghetti.

	LT-uncooked	HT-uncooked	LT-cooked	HT-cooked
Pasting temperature (°C)	65.4 ± 0.3 ^a	80.1 ± 1.2 ^b	67.8 ± 0.2 ^c	60.0 ± 0.8 ^d
Peak viscosity (UB)	343.0 ± 1.0 ^a	218.5 ± 2.5 ^b	182.0 ± 4.0 ^c	168.5 ± 0.5 ^d
Breakdown (UB)	123.5 ± 2.5 ^a	3.0 ± 0.5 ^b	Not determinable	4.0 ± 1.0 ^b
Setback (UB)	459.0 ± 1.0 ^a	543.0 ± 3.0 ^b	320.0 ± 1.0 ^c	311.0 ± 1.0 ^d

Means ($n = 3$) with the same subscript within a row are not significantly different ($p > 0.05$).

Table 2

Percentage of SDS-soluble, DTE-soluble and unextractable protein fractions in semolina and LT and HT uncooked and cooked spaghetti.

	Semolina	LT-uncooked	HT-uncooked	LT-cooked	HT-cooked
SDS-soluble	80.83 ± 0.02 ^a	72.31 ± 0.26 ^b	22.65 ± 0.11 ^c	18.21 ± 0.16 ^d	14.38 ± 0.33 ^e
DTE-soluble	19.17 ± 0.07 ^a	27.70 ± 0.27 ^b	65.17 ± 0.99 ^c	74.01 ± 0.05 ^d	79.37 ± 1.80 ^e
Unextractable	0.00 ± 0.00	0.00 ± 0.00	12.19 ± 0.87 ^a	9.18 ± 0.72 ^a	9.29 ± 0.58 ^a

Means ($n = 3$) with the same subscript within a row are not significantly different ($p > 0.05$).**Table 3**Comparison of glucose, maltose and maltotriose levels ($\text{g } 100 \text{ g}^{-1}$) between cooked LT and HT spaghetti: undigested and after different steps of *in vitro* digestion.

Sugar	LT-undigested	HT-undigested	Significance
Glucose	0.03 ± 0.01	0.03 ± 0.01	ns
Maltose	0.43 ± 0.01	0.49 ± 0.02	*
Maltotriose	0.15 ± 0.01	0.16 ± 0.01	ns
Total	0.61 ± 0.03	0.68 ± 0.04	ns
Sugar	LT-oral	HT-oral	Significance
Glucose	0.03 ± 0.01	0.03 ± 0.01	ns
Maltose	1.39 ± 0.04	1.77 ± 0.03	***
Maltotriose	1.54 ± 0.04	1.87 ± 0.06	***
Total	2.96 ± 0.09	3.67 ± 0.10	***
Sugar	LT-gastric	HT-gastric	Significance
Glucose	0.03 ± 0.01	0.03 ± 0.01	ns
Maltose	1.40 ± 0.06	1.75 ± 0.05	***
Maltotriose	1.53 ± 0.05	1.92 ± 0.04	***
Total	2.96 ± 0.12	3.70 ± 0.10	***
Sugar	LT-intestinal	HT-intestinal	Significance
Glucose	0.66 ± 0.02	0.82 ± 0.04	**
Maltose	7.40 ± 0.06	9.34 ± 0.10	***
Maltotriose	4.58 ± 0.04	5.73 ± 0.04	***
Total	12.64 ± 0.12	15.89 ± 0.18	***

Values are presented as means ± SD ($n = 6$). Level of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant.

The quantity of glucose was also significantly ($p < 0.05$) different, reaching 0.66 and 0.82 $\text{g } 100 \text{ g}^{-1}$ in LT and HT samples, respectively (Table 3). Overall, 12.6 and 15.9 $\text{g } 100 \text{ g}^{-1}$ of sugars were released in the digestates of LT and HT cooked spaghetti, respectively. These values differentiated ($p < 0.001$) the two samples and accounted for about half of the starch content of cooked pasta broken down to simple sugars. In this regard, the slightly firmer protein network formed in HT spaghetti (as a result of higher protein denaturation and formation of disulfide bonds, Table 2), and the lower starch granule swelling (Table 1) did not seem to prevent starch degradation to the studied sugars in cooked HT spaghetti.

Data from the literature are impossible to compare with our results since different amylolysis markers and *in vitro* digestion protocols were considered. Anyway, Petitot et al. (2009b) did not find HT and LT pasta samples to be significantly different in content of alcohol-soluble dextrans (degree of polymerisation 1–12) after *in vitro* pancreatin digestion. Our findings did not fit those of Colonna et al. (1990), who found HT drying to decrease the *in vitro* digestibility of starch in the derived cooked pasta, due to the intense starch encapsulation by protein. Nevertheless, these authors studied starch breakdown into oligosaccharides after removal of the protein network of cooked pasta by pronase. Casiraghi et al. (1992) confirmed lower starch degradation in HT pasta, but the ingestion of the same sample did not exert an effect on glycaemic response. According to Kim et al. (2008), the mechanical effects exerted on dough can also be responsible for starch digestibility in fresh cooked pasta. Indeed, the more the proteins dissociated from the starch granules with increasing sheeting passes, the less the protein matrix retarded starch breakdown during *in vitro* digestion

of fresh cooked pasta. As we previously demonstrated (De Noni & Pagani, 2010), only extrusion temperatures exceeding 50 °C cause abnormal swelling of starch granules and random protein coagulation. As our spaghetti were extruded at 40 °C, drying and cooking represented the paramount steps in modifying starch and protein characteristics, as well as their interactions, in the studied samples. In this regard, data concerning protein extractability of LT spaghetti indicated that the protein network mostly formed during cooking (Table 2). As a result, Bruneel et al. (2010) demonstrated that spaghetti dried under gentle conditions present a protein network more resilient and more suitable to restrict starch swelling during pasta cooking. In contrast, when proteins strongly polymerise during a severe drying cycle, they lack resilience to cope with starch swelling during cooking. This behaviour seems to be confirmed by the lower pasting temperature value of HT cooked pasta (60.0 °C) in comparison with LT cooked spaghetti (67.8 °C). This is the reason why starch in the HT cooked sample is more prompt to swell and likely to be more prone to amylolysis.

3.4.2. Protein digestibility

During drying, formation of disulfide bonds aggregates gluten proteins, lowering their solubility (Wagner, Morel, Bonicel, & Cuq, 2011). Most protein aggregation and insolubilisation occur during HT drying, whereas the solubility of LT pasta proteins does not change after drying and is almost abolished following cooking (Petitot et al., 2009b). The different protein aggregation in LT and HT spaghetti was revealed by SE-HPLC data, which showed a protein network with higher number of S–S bridges in the latter sample (Table 2). In addition, ultrastructure images evidenced a more organised protein network in HT (un)cooked spaghetti (Fig. 2).

The breakdown of protein aggregates upon *in vitro* digestion of cooked spaghetti was studied by monitoring the release of free AA. The levels of free AA in cooked undigested LT and HT spaghetti were 0.26 and 0.23 $\text{g } 100 \text{ g}^{-1}$ protein, respectively (Table 4). The total free AA content significantly ($p < 0.001$) differed between the two undigested samples, and it is likely related to proteolytic activities in sound durum wheat kernel, since moderate variation of free AA content occurs in dough and pasta during processing (De Noni & Pagani, 2010).

After the oral phase the free AA levels did not change (data not shown). During the successive gastric digestion pasta proteins were degraded by pepsin, and the quantity of free AA increased to 0.74 and 0.61 $\text{g } 100 \text{ g}^{-1}$ protein in digestates of LT and HT spaghetti, respectively (Table 4). With the exception of Trp, levels of the other free AA were significantly different in the two digestates. By measuring the number of NH_2 functions in digestates, Petitot et al. (2009b) observed about 8% of proteins to be degraded by pepsin (pH 2.0) after 180 min of *in vitro* digestion, with no statistically significant influence of the applied drying cycle. Only drastic drying (90 °C at low moisture content) significantly decreased protein digestibility of cooked pasta by 10%, probably due to the formation of highly aggregated proteins linked by strong covalent bonds (Petitot et al., 2009b).

At the end of the intestinal phase, the amount of free AA accounted for 16.3 and 12.5 $\text{g } 100 \text{ g}^{-1}$ protein in LT and HT spaghetti (Table 4), respectively, thus indicating ($p < 0.001$) the HT sample to

Table 4Comparison of free amino acid levels (g 100 g⁻¹ protein) between cooked LT and HT spaghetti: undigested, and after gastric and intestinal steps of *in vitro* digestion.

AA	LT-undigested	HT-undigested	Significance	LT-gastric	HT-gastric	Significance	LT-intestinal	HT-intestinal	Significance
Ala	0.029 ± 0.002	0.025 ± 0.002	***	0.052 ± 0.001	0.031 ± 0.002	***	0.387 ± 0.005	0.288 ± 0.004	***
Arg	0.020 ± 0.001	0.016 ± 0.001	***	0.033 ± 0.001	0.030 ± 0.001	***	2.109 ± 0.048	1.632 ± 0.048	***
Asn	0.051 ± 0.003	0.046 ± 0.002	*	0.047 ± 0.001	0.052 ± 0.002	***	0.259 ± 0.004	0.182 ± 0.003	***
Asp	nd	nd		0.040 ± 0.001	0.054 ± 0.002	***	0.128 ± 0.001	0.110 ± 0.003	***
Cys	nd	nd		nd	nd		nd	nd	
Cystine	nd	nd		nd	nd		nd	nd	
Gln	0.017 ± 0.001	0.009 ± 0.001	***	0.085 ± 0.006	0.046 ± 0.003	***	4.128 ± 0.179	3.168 ± 0.083	***
Glu	0.023 ± 0.001	0.031 ± 0.002	***	0.046 ± 0.001	0.073 ± 0.002	***	0.223 ± 0.004	0.147 ± 0.005	***
Gly	0.014 ± 0.001	0.011 ± 0.001	***	0.023 ± 0.001	0.014 ± 0.002	***	0.112 ± 0.002	0.088 ± 0.001	***
His	0.007 ± 0.001	0.006 ± 0.001	ns	0.028 ± 0.001	0.025 ± 0.000	***	0.344 ± 0.006	0.264 ± 0.004	***
Ile	nd	nd		nd	nd		0.675 ± 0.018	0.518 ± 0.011	***
Leu	0.010 ± 0.001	0.007 ± 0.001	**	0.036 ± 0.001	0.029 ± 0.001	***	2.164 ± 0.044	1.641 ± 0.033	***
Lys	0.009 ± 0.001	0.008 ± 0.001	ns	0.023 ± 0.001	0.034 ± 0.001	***	0.898 ± 0.017	0.624 ± 0.007	***
Met	nd	nd		0.014 ± 0.001	0.010 ± 0.001	***	0.391 ± 0.010	0.290 ± 0.011	***
Phe	0.010 ± 0.001	0.007 ± 0.001	***	0.058 ± 0.001	0.018 ± 0.001	***	1.412 ± 0.029	1.130 ± 0.015	***
Pro	0.029 ± 0.002	0.027 ± 0.002	ns	0.016 ± 0.001	0.007 ± 0.001	***	0.074 ± 0.003	0.036 ± 0.002	***
Ser	0.012 ± 0.001	0.014 ± 0.001	***	0.032 ± 0.001	0.027 ± 0.001	***	0.279 ± 0.005	0.202 ± 0.003	***
Thr	0.012 ± 0.001	0.004 ± 0.001	***	0.024 ± 0.001	0.022 ± 0.001	*	0.332 ± 0.005	0.258 ± 0.004	***
Trp	nd	nd		0.081 ± 0.002	0.083 ± 0.002	ns	0.334 ± 0.006	0.268 ± 0.007	***
Tyr	0.009 ± 0.001	0.011 ± 0.001	**	0.078 ± 0.002	0.033 ± 0.002	***	1.372 ± 0.046	1.080 ± 0.034	***
Val	0.009 ± 0.001	0.003 ± 0.001	***	0.025 ± 0.001	0.023 ± 0.001	**	0.719 ± 0.016	0.548 ± 0.019	***
Total	0.260 ± 0.020	0.225 ± 0.018	***	0.736 ± 0.017	0.611 ± 0.028	***	16.337 ± 0.208	12.473 ± 0.298	***

Values are presented as means ± SD (n = 6). nd, not detectable. Level of significance: *p < 0.05; **p < 0.01; ***p < 0.001; ns, not significant.

be slower to digest. Amounts of all free AA differed significantly ($p < 0.001$) between the two digestates. At this step of *in vitro* digestion, the decrease of protein digestibility of HT pasta was also reported by De Zorzi et al. (2007) and by Petitot et al. (2009b). Overall, our data confirm these findings and support the major role played by HT drying in lowering pasta protein digestibility.

Despite the limited human studies, Seiquer et al. (2006) reported MRPs to negatively affect digestibility and utilisation of proteins. In this regard, the formation of ϵ -PL and GUI in pasta can affect release and availability of AA (mainly Lys) by determining the formation of protein-bound AGEs and stable MGs. Indeed, HT drying impaired the release of Lys, the level of which (0.62 g 100 g⁻¹ protein) in the digestate of HT spaghetti was significantly ($p < 0.001$) lower than that of LT sample digestate (0.90 g 100 g⁻¹ protein). This difference partially derives from involvement of a larger number of Lys residues in the formation of Amadori compound and AGEs (De Noni & Pagani, 2010). Rombouts et al. (2011) found that heat treatment of gluten mainly involved Lys. Indeed, the level of free Lys decreased to 72% of the initial value in native gluten, mostly as the result of isopeptides and MRPs formation. Loss of free Lys in HT pasta can be also associated with the formation of GUI. According to Resmini et al. (1993), 0.03 g AA 100 g⁻¹ proteins were lost when about 2 mg kg⁻¹ GUI formed in dried pasta. We found the same level (2 mg kg⁻¹) of GUI in HT spaghetti. Therefore, this value would account only for a difference of 0.03 g Lys 100 g⁻¹ proteins in Lys content of LT and HT spaghetti digestates (Table 4). This value is negligible in comparison to our obtained difference in Lys content, 0.274 g Lys 100 g⁻¹ proteins (0.898 vs. 0.624). This difference could be attributed to the formation of other chemical artefacts involving Lys at a protein chain level.

Drying and cooking promoted formation of S–S bridges in spaghetti proteins and this phenomenon was enhanced with increasing drying temperature in HT spaghetti (Table 2). The consequent protein aggregation near these covalent linkages probably obstructed proteolysis accounting in this way for the absence of Cys and cystine in digestates of both LT and HT spaghetti (Table 4). Levels of these AA under the analytical detection limit cannot be excluded as well.

The release of the heat damage marker ϵ -PL was also monitored. This molecule is linked to the peptide chain via the ϵ -amino group

of lysine, and thus it may impede the degradation of proteins by modifying their structure. In the present study, 0.26 mg kg⁻¹ of ϵ -PL were released at the end of *in vitro* digestion of HT spaghetti, that accounted for about 6% of ϵ -PL content (4.24 mg kg⁻¹) of the related uncooked sample. Equally, Henle (2013) reported no more than 5% of protein-bound ϵ -PL to be released as free AA during simulated *in vitro* digestion of glycated casein. In this regard, the different level of protein-bound ϵ -PL (and protein glycation, as revealed by furosine level) found in LT and HT spaghetti could support the role of MRPs in lowering protein digestibility (Seiquer et al., 2006).

4. Conclusion

Our results confirm the deep changes which starch and proteins of spaghetti undergo during drying and cooking. Starch swelling as well as denaturation and aggregation of proteins occurring at diverse stages, LT or HT drying and cooking, determined different *in vitro* digestibility of spaghetti. For the first time, these differences were assessed in terms of the release of free AA and simple sugars after *in vitro* digestion. The role of some MRPs in lowering the release and availability of certain AA has been pointed out as well. The obtained data better address the knowledge of digestibility of LT and HT cooked spaghetti.

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