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Modification of pasta structure induced by high drying temperatures. Effects on the *in vitro* digestibility of protein and starch fractions and the potential allergenicity of protein hydrolysates

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ABSTRACT

The effects of drying on pasta structure, starch and protein digestibility and potential allergenicity were investigated. Pasta was dried at low (55 °C, LT), high (70 °C, HT) and very high temperature (90 °C) applied either at the beginning (VHT) or at the end of the drying profile (VHT_LM). Changes in dried and in cooked pasta structure were determined regarding protein solubility, thermal properties of starch, microscopic and rheological measurements. Changes were moderate up to 70 °C and increased at higher temperatures and especially for VHT_LM drying.

VHT_LM drying tended to decrease starch digestibility and significantly decreased protein digestibility of cooked pasta by 10% probably due to the formation of highly-aggregated proteins linked by very strong covalent bonds. None of the drying profiles were found to remove the allergenic properties of pasta. IgE-reactive peptides from prolamins and albumins/globulins fractions were found in all digestion juices; VHT-LM drying increased the latter.

1. Introduction

Pasta is a traditional and highly popular cereal-based food product because of its convenience, nutritional quality and palatability (Cubadda, Carcea, Marconi, & Trivisonno, 2007). Pasta is obtained after kneading semolina and water, extruding and drying. The most noticeable recent innovation in pasta production is the application of high temperature in the drying process (Aktan & Khan, 1992; Cubadda et al., 2007; Degidio, Mariani, & Novaro, 1993; Güler, Koksel, & Ng, 2002; Novaro, d' Egidio, Mariani & Nardi, 1993; Zweifel, Conde-Petit, & Escher, 2000). This innovation was found to affect positively pasta qualities (higher firmness, lower cooking loss and lower stickiness) (Baiano & Del Nobile, 2006; De Stefanis & Sgrulletta, 1990; Zweifel, Handschin, Escher, & Conde-Petit, 2003), especially when high temperature is applied during the final stages of the drying process (Anese, Nicoli, Massini, & Lerici, 1999).

Up to now, of all the major quality factors, nutritional properties of pasta have received the least attention by researchers. Among cereal products pasta appears to possess unique nutritional features in that the starch is slowly digested and absorbed in the small intestine, hence promoting a low plasma glucose response (Bjorck, Liljeberg, & Ostman, 2000; Jenkins et al., 1983). The low glycaemic index (GI) of pasta is generally attributed to its compact structure (Barkeling, Granfeldt, Björck, & Rossner, 1995; Bjorck, Granfeldt, Liljeberg, Tovar, & Asp, 1994; Granfeldt & Bjorck, 1991; Granfeldt, Bjorck, & Hagander, 1991; Wolever et al., 1986), but other numerous factors have been suggested to explain the different rates of starch degradation. Indeed, the surface area accessible to digestive enzymes (Granfeldt et al., 1991), the encapsulation of starch granules by fibres (Brennan, Blake, Ellis, & Schofield, 1996) and proteins (Colonna et al., 1990; Fardet et al., 1998b), and the physical structure of starch, such as its degree of gelatinisation or retrogradation, and its amylase:amylopectin ratio (Akerberg, Liljeberg, & Björck, 1998; Holm & Bjoerck, 1988) were demonstrated to affect starch digestion. Some work has been initiated to study the effect of process parameters on the nutritional quality of pasta. For example, high drying temperatures were shown to decrease the *in vitro* digestibility of starch (Casiraghi, Brighenti, & Testolin, 1992) and proteins (De Zorzi, Curioni, Simonato, Giannattasio, & Pasini, 2007). Although some hypotheses have been suggested to explain the relationship between pasta structure and its nutritional properties, there is still limited data on the subject. The objective of this work was to better characterise, by a multi-scale approach, the structure of pasta dried with different drying profiles, and to study the repercussions on the *in vitro* digestibility of carbohydrate and protein fractions, and the potential allergenicity of digested pasta.

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2. Materials and methods

2.1. Pasta manufacturing

2.1.1. Pasta production

Spaghetti was processed with a continuous pilot-scale pasta extruder (Bassano, Lyon, France). Durum wheat semolina (Panzani, Marseille, France) (5 kg) was hydrated with tap water to obtain a moisture content of 47 g/100 g db and then mixed for 15 min at 60 rpm. The product was then extruded at 31 rpm and 40 °C. Extruded spaghetti was then dried in a pilot-scale drier (AFREM, Lyon, France), in order to reach 12% moisture. Four drying profiles were applied: low temperature, 55 °C (LT), high temperature, 70 °C (HT), very high temperature, 90 °C, applied either at the beginning of the cycle, when the moisture content of pasta is high (about 20%) (VHT), or at the end of the drying cycle, when the moisture content of pasta is low (about 12%) (VHT_LM). The relative humidity and temperature inside the dryer and the moisture content of pasta (evaluated according to AACC method (44-15)) were monitored during drying.

2.1.2. Pasta cooking

Dried spaghetti was cooked in Evian water containing 0.7% (w/v) of sodium chloride with a water:solid ratio of 20. Optimal cooking time (t) was indicated when the white core of the pasta disappeared when squeezed between two glass plates AACC method 66-50. All analyses on cooked pasta were made on pasta cooked at (t + 1) min.

2.2. DSC measurements on dried and cooked spaghetti

Differential scanning calorimetry (DSC) measurements were conducted on a DSC 2019 modulated (TA Instruments, New Castle, DE, USA), calibrated with indium and an empty pan as a reference. Durum wheat semolina, dried pasta and freeze-dried cooked pasta samples were ground and sieved to pass through a 250 μm mesh screen. Ground samples were accurately weighed in aluminium hermetic pans. Water was added with a micropipette with a water:solid ratio of 4. Then the pans were sealed, reweighed and allowed to stand for 1 h at room temperature. The analyses were performed from 10 to 120 °C at a heating rate of 10 °C/min using an empty pan as a reference. For each endotherm, the onset (T_o), peak (T_p) and conclusion (T_c) temperatures and the gelatinisation enthalpy (ΔH) were computed by using the TA Instruments analysis software program. Temperature ranges ($T_r = T_c - T_o$) were calculated. Each experiment was repeated four times for each sample. Data were subjected to analysis of variance (ANOVA) followed by the Fisher's least significant difference (LSD) test to compare means at the 5% significance level, using Microsoft Xlstat software 2008 (Addinsoft, Paris, France).

2.3. Protein extractability of dried and cooked spaghetti

2.3.1. Protein extraction procedure

Proteins were extracted in triplicate from semolina, dried pasta and freeze-dried cooked pasta, according to a modified method of Morel, Dehlon, Autran, Leygue, and Bar-L'Helgouac'h (2000). The first extraction was conducted at 60 °C for 80 min with a sodium phosphate buffer containing 1% sodium dodecyl sulphate (SDS, 0.1 M) and a solid:solvent ratio of 8 mg/ml, in order to extract SDS-soluble proteins. SDS disrupts the electrostatic, hydrophobic and hydrophilic interactions occurring between proteins. After centrifugation, the pellet was suspended at 60 °C for 60 min with 5 ml of the SDS-phosphate buffer, containing 20 mM dithioerythritol (DTE), and sonicated for 5 min, in order to extract SDS-insoluble proteins, referred to as DTE-soluble proteins. The combination of sonication and DTE causes the degradation of disulphide bonds that connect the glutenin subunits together (Singh, Donovan, Batey, & MacRitchie, 1990). The remaining pellet made of unextractable proteins represents proteins linked by covalent linkages that were not affected by sonication and DTE (e.g., isopeptides bonds).

Once corrected for their different solid:solvent ratios during extractions, areas (in arbitrary units) of SDS-soluble and DTE-soluble proteins were added, and the sum (i.e., total extractable proteins) was expressed as percents of the corresponding area calculated for semolina (on equivalent dry protein basis). Data were subjected to analysis of variance followed by the Fisher's least significant difference test, to compare means at the 5% significance level by using Microsoft Xlstat software 2008 (Addinsoft, Paris, France).

2.3.2. Size distribution measurement

The size distribution of proteins in cooked pasta was studied by size-exclusion high performance liquid chromatography (SE-HPLC). The SE-HPLC apparatus (Waters model LC Module1 plus) was equipped with an analytical column, TSK G4000-SW (7.5 × 300 mm) and a guard column, TSK G3000-SW (7.5 × 75 mm), (both Merck, Darmstadt, Germany) as previously described Morel et al. (2000). Each SE-HPLC profile of SDS-soluble and DTE-soluble proteins was arbitrary divided into five peaks (S1–S5 and P1–P5, respectively). Apparent molecular weights were estimated by calibrating the column with protein standards, according to Redl, Morel, Bonicel, Vergnes, and Guilbert (1999). Fraction S1 corresponded to polymeric proteins eluted at the void volume of the column (blue dextran, M_r = 2000 kDa). Fraction S2 corresponded to proteins ranging from $M_r \approx 780$ to 95 kDa. Fractions S3 and S4 corresponded to proteins ranging from $M_r \approx 95$ to 52 kDa and from 52 to 21 kDa, respectively. Fraction S5 corresponded to the smallest monomeric proteins (M_r < 21 kDa). The second extract, obtained after solubilisation by the combined action of DTE and sonication, allowed the characterisation of SDS-insoluble proteins whose molecular weight exceeded 2000 kDa before sonication and solubilisation in DTE. Fraction P1 corresponded to M_r > 2000 kDa. Fraction P2 corresponded to protein ranging from $M_r \approx 780$ to 116 kDa. Fractions P3 and P4 corresponded to proteins ranging from $M_r \approx 116$ to 62 kDa and from 62 to 21 kDa, respectively. P5 corresponded to proteins of M_r < 21 kDa.

2.4. Microscopic characterisation of the protein network in cooked spaghetti

2.4.1. Sample preparation

After cooking (t + 1), water was decanted and pasta was cooled for 15 min before sectioning in a covered container at 25 °C. Pasta was cut into small pieces and frozen quickly at −40 °C in water plus a cryoprotector (OCT, Cellpath, Newtown, UK). Samples were cut at −20 °C, using a microtome (Microm HM 560, Walldorf, Germany), to obtain 15 μm thick transverse sections. After drying they were stained for 10 min in a 0.01% (w/v) fuchsin acid solution diluted in 1% (v/v) acetic acid, as described by Fardet et al. (1998a). Sections were then rinsed 3 times for 2 min in distilled water.

2.4.2. Image acquisition

Images were acquired using an inverted confocal laser scanning microscope (CLSM) (Zeiss Axiovert 200M) with attached Zeiss LSM510 META imaging system (Carl Zeiss, Jena, Germany) in the Montpellier RIO Imaging (MRI) facility. The excitation wavelength was 543 nm and the light emitted over 560 nm was selected by a long pass filter. A ×20 lens coupled to a numeric zoom of 1.1 allowed the acquisition of 1024 × 1024 pixels images large of

410 μm. The pixel size was therefore equal to 0.4 μm. Grey levels were coded on 8 bits, giving an intensity scale from 0 (black) to 255 (white).

For each transverse section, images were acquired at 3 locations within the pasta: external, intermediate and central zones, without any overlapping between the images. For each sample of cooked pasta dried with one of the four drying profiles, 3 strands were cut and 3 sections per strand were observed. Therefore 108 images were collected and used for subsequent mathematical treatment.

2.4.3. Image processing

Images were pre-processed in order to normalise grey levels over the surface and between images. A FFT filtering was performed before a normalisation procedure considering a shading over the image as already made on CSLM images of tomato cells (Guillemin, Devaux, & Guillon, 2004). The protein network was characterised by analysing the grey-level granulometry based on mathematical morphology (Devaux, Robert, Melcion, & deMonredon, 1997; Rouille, Della Valle, Devaux, Marion, & Dubreil, 2005), with squared structuring elements. The sum of the grey-level values (the volume, V) in the images was calculated after each erosion/dilation step and the erosion/dilation curves were obtained according to the equation:

$$g(i) = \frac{|V(i) - V(i+1)|}{V_0 - V_f}$$

where V_0 represents the volume of the original image, V_f the volume after the last erosion/dilation step and i one of the steps of the image processing. The g curves measure the proportions of the grey-level volume that were modified between steps i and $(i+1)$, i.e., between two successive erosions or dilations. Erosion/dilation curves were analysed by principal component analysis (PCA). A similarity map could be drawn from principal component scores, whereas the loading analysis revealed the weight of each erosion or dilation step in their computation.

All the image analysis and data treatments were performed with Matlab (v7.0.4) software (The MathWorks, Paris, France) using dedicated toolboxes (image processing and PLS toolbox v3.5 (Eigenvector Research Inc., Manson, WA, USA).

2.5. Rheological properties of cooked spaghetti

2.5.1. Sample preparation

A TA-XTplus (Stable Micro Systems, Godalming, UK) texture profile analyser equipped with a Windows version of Texture Expert software package (Stable Micro Systems, Godalming, UK) was used to evaluate textural properties of cooked spaghetti. After cooking at $(t+1)$, pasta was allowed to equilibrate at ambient temperature for 20 min in a covered container at 25 °C before texture analysis. The variables were recorded through five measurements for each pasta, cooked on two different occasions, totalling 10 measurements per pasta each. Data were subjected to analysis of variance (ANOVA) followed by the Fisher's least significant difference (LSD) test, to compare means at the 5% significance level by using Microsoft Xlstat software 2008 (Addinsoft, Paris, France).

2.5.2. Texture profile analysis

The TA-XTplus was equipped with a 35 mm cylindrical probe (ref. P/35, Stable Micro Sytems). The probe compressed a single strand of spaghetti at a constant rate of deformation (1 mm/s) to 70% of the initial spaghetti thickness. The probe was retracted and held stationary 10 s before performing a second compression to 70% of the original spaghetti thickness. From a texture profile analysis (TPA) curve, textural parameters of hardness, cohesiveness, resilience and springiness were obtained (Epstein, Morris, & Huber, 2002). Spaghetti hardness was defined as the maximal peak force attained during the first compression. Cohesiveness was calculated as the ratio of the area under the second peak to the area under the first peak. Resilience was defined as the ratio of the area under the second half of the first peak to the area under the first half of the same peak. Springiness was calculated as the ratio between the distance of the first half of the second peak to the distance of the first half of the first peak.

2.5.3. Tensile test

The TA-XTplus was equipped with tensile grips (ref. A/SPR, Stable Micro Sytems). The initial distance between the two tensile grips was 15 mm. The test was performed at a constant rate of deformation (3 mm/s). Breaking stress ($N\,m^{-2}$) and breaking strain (%) were recorded from the stress–strain curve. The energy ($J\,m^{-3}$) stored in the sample until fracture, which corresponds to the area under the stress–strain curve, was also calculated.

2.6. In vitro digestion of cooked and minced spaghetti

2.6.1. Enzymes

Porcine pancreatic α-amylase (EC 3.2.1.1) from Fluka Biochemika (10080, 1217678, 43.6 U/mg), pepsin (EC 3.4.23.1) from Sigma (P7000, porcine stomach mucosa, 61K0197, 596 U/mg) and pancreatin (EC 232.468.9) from Sigma (P7545, 045K0673, 8 USP) were used. Enzymes were prepared in phosphate buffer (5 mM, pH 6.9, NaCl 1 M, $CaCl_2$ 4 mM). Pepsin solution and supernatants of α-amylase and pancreatin solutions (centrifuged 10 min, 1500*g*, 10 °C) were stored at 4 °C until use (i.e., at most 3 h).

2.6.2. In vitro digestion procedure

Cooked pasta $(t+1)$ was ground in a meat mincer to reproduce buccal mastication (Hoebler, Devaux, Karinthi, Belleville, & Barry, 2000). Minced pasta (15 g) was mixed 15 min with 25 ml of phosphate buffer (5 mM, pH 6.9, NaCl 1 M, $CaCl_2$ 4 mM) at 37 °C, before being digested for 5 min at 37 °C with porcine pancreatic α-amylase (200 U/g wheat starch). pH was adjusted to 2 with 1 N HCl and pepsin was added (73,400 U/g wheat protein). Gastric digestion lasted 0 (inactivated pepsin), 30 or 180 min and was stopped by addition of 2 N KOH to raise pH to 7. Intestinal digestion by pancreatin (1 g/g wheat starch) lasted 0 (inactivated pancreatin), 10, 30 or 180 min and was stopped by placing the reactor for 5 min in boiling water. Cooled down digests were homogenised (Kinematica AG, PT3000, Lucerne, Switzerland) and stored at −20 °C.

2.6.3. Determination of carbohydrate hydrolysis during digestion

Hydrolysis of starch during digestion was studied by the percentage of starch transformed into alcohol-soluble dextrins (PASD); dextrins with a degree of polymerisation between 1 and 10–12 are soluble in 80% ethanol. Equivalent glucose concentration in ethanolic extracts and samples were determined by the phenol–sulphuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.6.4. Study of protein hydrolysis during digestion

Digestion extracts were prepared from 1.5 g homogenised digests in 10 ml tetraborate buffer (0.1 M, pH 9.3, 1% (w/v) SDS, 5% (v/v) 2-mercaptoethanol) gently stirred for 16 h at ambient temperature and centrifuged (5000*g* for 10 min at 10 °C then 10,000*g* for 20 min at 10 °C). The number of free and NH_2 functions in amino acids, peptides and proteins was determined on digestion extracts as previously described (Frister, Meisel, & Schlimme, 1988) at the initial phase of digestion (t_0), during digestion (t_x) and after a total hydrolysis (HCl 6 N, 24 h at 105 °C) (t_{total}). The degree of hydrolysis (DH) was calculated according to the equation:

$$DH(\%) = \frac{[NH_2]_{(t_x)} - [NH_2]_{(t_0)}}{[NH_2]_{(t_{total})} - [NH_2]_{(t_0)}} \times 100$$

As low remaining protease activity was previously reported for pig pancreatic α-amylase contrary to human salivary α-amylase (Fardet et al., 1998b), t_0 corresponding to the end of the buccal phase was chosen as the reference for protein hydrolysis.

The soluble peptides released during the digestion were analysed by gel filtration; homogenised digests were centrifuged twice (first at 1500*g* for 10 min and then at 10,000*g* for 10 min at 20 °C), to prepare the so-called digestion juices. High-performance liquid chromatography was carried out with an Alliance 2795 HPLC System (Waters S.A.S., Saint-Quentin En Yveline, France) onto a Superdex™ Peptide 10/300 GL with a fractionation range of M_r 100 to 7000 and 13 μm average particle size. Digestion juice was mixed (v/v) with trifluoroacetic acid (TFA) 0.2%, filtered through Millipore 45 μm and samples of 100 μl were loaded onto the column, which was eluted with 30% acetonitrile–0.1% TFA at 0.5 ml/min for 60 min. Dual detection at 220 and 280 nm was performed.

2.7. IgE dot blotting and competitive ELISA

Sera were obtained with informed consent from four patients with food allergy to wheat and one patient allergic to Graminaceae without food allergy (control serum). All patients with allergy to wheat were children and suffered from atopic eczema/dermatitis syndrome associated for two of them with asthma, and one had digestive symptoms upon pasta consumption. The serum from the last patient was used for IgE dot-blotting experiment. A pool of the four sera was used for competitive ELISA.

For IgE dot blotting, 2 μl of digestion extracts or digestion juices from pasta and four controls (gliadin fraction 0.5 mg/ml, purified α-gliadin 0.5 mg/ml, albumin/globulin fraction 0.2 mg/ml and a mixture of α-amylase, pepsin and pancreatin, each at a concentration corresponding to the digestion assay) were applied to a nitrocellulose sheet (0.2 μm, Sartorius, Goettingen, Germany) and the sheets were air-dried at 37 °C for 2 h. Fixation of IgE (polyvinylpyrrolidone (PVP 40, Sigma, P0930) as blocking agent, human serum diluted 20 times) was revealed by luminescence (peroxidase-conjugated anti-human IgE (P 0295, Dako, Glostrup, Denmark, 100,000 times diluted), SuperSignal West Dura Extended Duration substrate (34075, Pierce, Rockford, IL, USA), according to instructions of the manufacturer). Images were acquired using a luminescent image analyser (LAS-3000, Fujifilm, Saint-Quentin en Yvelines, France) in high resolution binning mode and 5 min exposure time and were exported using Multi Gauge software (version 3.0: Fujifilm Corporation, Tokyo, Japan).

For competitive ELISA, digestion juices from pasta and pool of human sera (10 and 15 times diluted in PBS-T-G (0.5% gelatin (Sigma, G2500) in PBS buffer containing 0.1% Tween-20), respectively) were incubated for 3 h at 37 °C. Wheat proteins solution (purified α-gliadin or γ-gliadin or LMW glutenins; albumin/globulin fraction; 5 μg/ml) were coated on 96-well white plates Microfluor 2 (Thermo LabSystems, Franklin, MA, USA) for 2 h at ambient temperature and blocked with PBS-T-G. Mixtures of digestion juices and human sera were then added and incubated for 15 hours at 37 °C. Fixation of IgE was revealed by fluorescence using goat anti-human IgE alkaline phosphatase conjugate (ε-chain specific, Sigma A-3525) 500 times diluted and 4-methylumbelliferyl phosphate (4-MUP) substrate (M-3168, Sigma) diluted 5 times. After incubation in the dark for 90 min, fluorescence was measured at 440 nm (excitation 360 nm) with an FL_X800 microplate reader and KC4 software (BioTek Instruments, Colmar, France).

Data from *in vitro* digestion of cooked pasta and competitive ELISA were subjected to ANOVA and subsequent LSD test at the 5% significance using Statgraphics Plus 3.0 software (Manugistic Inc., Rochville, MD, USA). For ANOVA on *in vitro* digestion data, effects (pasta drying, pepsin time and pancreatin time) and all their interactions were first included then non-significant interactions ($p > 0.05$) were excluded. Principal components analysis of smoothed SE-HPLC profiles of digestion juices were performed in the Matlab environment using Matlab software (v7.0.4) (The MathWorks, Paris, France) and SAISIR (2008).

3. Results

3.1. Effect of the drying profile on the constituents of dried spaghetti

3.1.1. Starch

The gelatinisation of starch was determined by DSC measurements. Table 1 shows the changes in DSC gelatinisation endotherms from durum wheat semolina and dried spaghetti samples as a function of the drying profile.

Whatever the drying profile used, dried pasta presented a lower gelatinisation enthalpy (4.9 J/g), compared to semolina (6.2 J/g), which is in accordance with previous studies (Güler et al., 2002; Vansteelandt & Delcour, 1998; Yue, Rayas-Duarte, & Elias, 1999; Zweifel et al., 2000). The reduced gelatinisation enthalpy in dried pasta could be explained by the presence of partially gelatinised starch granules, which require less energy to melt (Biliaderis, 1990).

Moreover, in VHT pasta, the lower gelatinisation enthalpy was accompanied by a higher onset (T_o) and peak (T_p) temperatures (56.4 and 63.6 °C, respectively, *vs.* 54.2 and 61.8 °C for semolina) and reduced temperature range (14.0 °C *vs.* 16.1 °C for semolina) as already observed (Güler et al., 2002; Vansteelandt & Delcour, 1998; Yue et al., 1999). This may indicate that in VHT pasta, starch molecules had more mobility and underwent conformational reorganisation, resulting in an increase in crystalline stability and homogeneity, as previously suggested (Yue et al., 1999). In comparison, the other dried pasta underwent less marked changes.

3.1.2. Proteins

Protein molecular weight distributions of durum wheat semolina and dried pasta were examined using SE-HPLC. Areas of SE-HPLC elution profiles from different protein extracts were used to measure protein extractability, which reflects protein aggregation. Fig. 1A shows the percentage of proteins soluble in SDS, in DTE followed by sonication and of the unextractable protein fraction. Durum wheat semolina was characterised by a high fraction of SDS-soluble proteins (81%), a low fraction of DTE-soluble proteins (19%), and no traces of unextractable proteins. All dried pasta presented a higher protein aggregation, as shown by the decrease in SDS-soluble proteins in favour of DTE-soluble proteins. This may indicate the formation of additional disulphide bonds. This phenomenon was enhanced with increasing drying temperatures, and particularly when the VHT was applied at a high pasta moisture content. Although all dried pasta presented a significant

Table 1
DSC measurements obtained from the first endothermic peak of DSC on dried spaghetti dried with one of the four drying profiles (LT, HT, VHT_LM or VHT). Means ($n = 4$) with the same superscript within a column are not significantly different ($p > 0.05$). T_o, onset temperature; T_p, peak temperature; T_c; conclusion temperature, $T_r = T_c - T_o$, temperature range, ΔH gelatinization enthalpy.

	Drying profiles	T_o (°C)	T_p (°C)	T_c (°C)	T_r (°C)	ΔH (J/g, db)
Semolina		54.2^{b}	61.8^{bc}	70.3^{ab}	16.1^{b}	6.2^{a}
Dried pasta	LT	53.4^{b}	61.2^{c}	70.0^{ab}	16.5^{ab}	4.9^{b}
	HT	53.5^{b}	61.8^{bc}	69.4^{bc}	15.9^{b}	4.9^{b}
	VHT	56.4^{a}	63.6^{a}	70.4^{a}	14.0^{c}	4.9^{b}
	VHT_LM	54.3^{b}	61.9^{b}	68.9^{c}	14.6^{c}	4.4^{b}

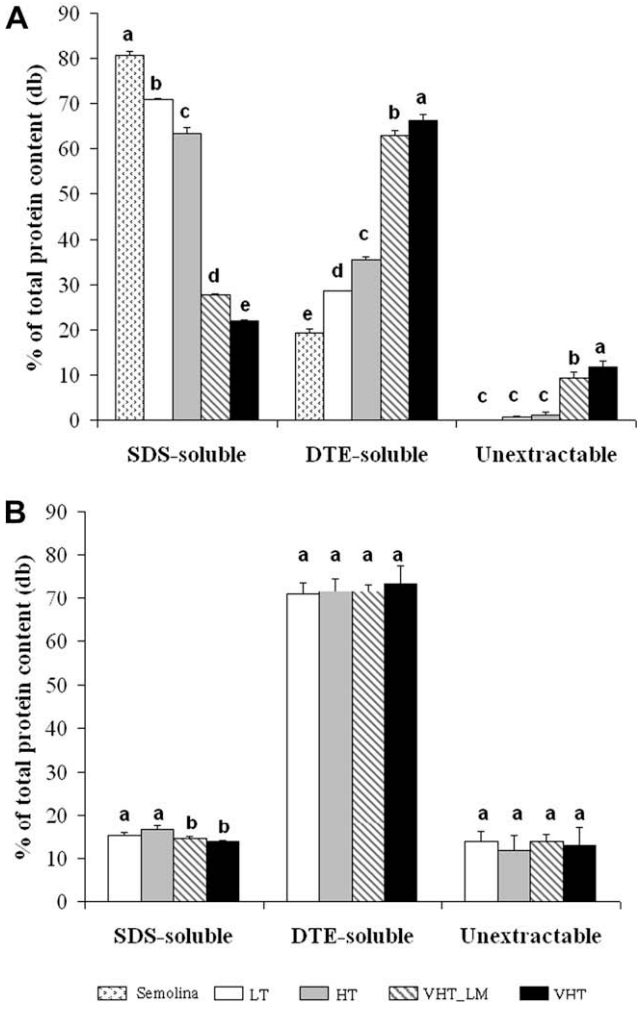

Fig. 1. Peak areas of SE-HPLC elution profiles of SDS-soluble, DTE-soluble and unextractable protein fractions in semolina and dried pasta (A) and in cooked pasta (B). Means ($n = 3$) with the same superscript within a graph (A or B) are not significantly different ($p > 0.05$).

difference in protein solubility, 2 main groups of pasta could be distinguished: a group formed by LT and HT dried pasta (70% and 64% of proteins soluble in SDS; 28% and 35% soluble in DTE, respectively) and another one formed by VHT_LM and VHT dried pasta (28% and 21% of proteins soluble in SDS; 64% and 68% soluble in DTE, respectively). Moreover, the application of VHT_LM and VHT dryings resulted in the formation of other covalent bonds (e.g., isopeptide bonds), as shown by the presence of unextractable proteins in high proportions (10% and 12%, respectively).

3.2. Effect of the drying profile on cooked spaghetti

3.2.1. Characterisation of the main constituents of cooked spaghetti

3.2.1.1. Starch. After cooking at ($t + 1$), no gelatinisation endotherm was observed on pasta, whatever the drying profile used. In all pasta, starch was completely gelatinised, as already observed (Colonna et al., 1990; Fardet, Hoebler, Armand, Lairon, & Barry, 1999).

3.2.1.2. Proteins. The analysis of SDS-soluble, DTE-soluble and unextractable proteins in cooked pasta as a function of the drying profile is presented in Fig. 1B. Pasta cooking generated a decrease in SDS-soluble proteins in favour of DTE-soluble proteins and, to a lesser extent, of unextractable proteins. This phenomenon was especially pronounced for LT and HT cooked pasta in which cooking generated a decrease in SDS-soluble fraction from 71% and 63% to 16% and 17%, respectively. In the same way, DTE-soluble fraction increased from 29% and 35% to 72% for both cooked pastas. As a result, the cooking step levelled down the variation in protein solubility which was observed after drying, except for a small but significant lower SDS protein solubility in VHT and VHT_LM cooked pasta (about 14%). This lower proportion of SDS-soluble fraction is counterbalanced by a higher proportion of DTE-soluble and unextractable fractions.

A more detailed analysis of SE-HPLC elution profiles from cooked pasta was then conducted. Elution profiles of SDS-soluble (Fig. 2A) and DTE-soluble fractions (Fig. 2B) were divided into five major fractions: S1–S5 and P1–P5, respectively. Elution profiles of SDS-soluble and DTE-soluble proteins in LT and HT cooked pasta were similar. Changes appeared to occur at higher drying temperatures. Compared to LT pasta, SDS-soluble proteins in VHT_LM pasta presented a significant higher proportion of fraction S1, and a lower proportion of fractions S3, S4 and S5. The same trend was observed in VHT pasta but to a lesser extent. Moreover, the DTE-soluble extract of VHT pasta presented a higher proportion of protein aggregates with a molecular weight between 62 and 780 kDa (fractions P2 and P3) whereas in VHT_LM, the DTE-soluble extract was characterised by a high proportion (14%) of very large protein aggregates (>116 kDa) (Fig. 2B).

3.2.2. Pasta microstructure

Typical CLSM images of cooked pasta samples are shown Fig. 3. Considering the staining procedure, the protein network appeared in white, whereas non-fluorescent (dark) area could be mainly related to starch granules (Fardet et al., 1998a; Zweifel et al., 2003). Whatever the drying profile applied to fresh pasta, a continuous protein phase was visible. A method for objective description of protein network at the microscopic scale was developed, based on digital image analysis, by evaluating the grey-level granulometry. Erosion/dilation curves were built by successive application of opening and closing morphological operators (Fig. 3d). The left side (dilation) gave information on dark area, whereas the right side (erosion) reflected the protein film thickness, considering that one erosion step makes the film lose 2 pixels, i.e., 0.8 μm (Devaux et al., 1997; Rouille et al., 2005).

In order to provide an overall comparison of the images, the 108 erosion/dilation curves were processed by principal component analysis (PCA) (Fig. 4A). The first two principal components (PC) accounted for 97% of the total variability. Whatever the drying profile, the external regions of pasta (coded "e") were clearly distinguished from the central (coded "c") and intermediate regions (coded "i") by a lower PC1 score (Fig. 4A). In order to identify which parts of the erosion/dilation curve were contributing to the location effect, the first PC loading was examined (Fig. 4B). External regions of pasta were characterised by a lower amount of small dark area (<6 μm) and a higher amount of larger dark area (>6 μm) that could be related to higher starch swelling. They were also characterised by a higher amount of the thinnest protein films (thickness < 4 μm). The protein distribution was therefore dependent on the location within pasta as already shown (Fardet et al., 1998b; Heneen & Brismar, 2003; Zweifel et al., 2003). On the contrary to Fardet et al. (1998b) and Zweifel et al. (2003), but similarly to Heneen and Brismar (2003), similar protein distributions were observed in the intermediate and central regions of pasta. This difference could be explained by sample preparation for microscopic analyses that was similar to Heneen and Brismar (2003) but slightly different from Fardet et al. (1998b) and Zweifel et al. (2003).

For each location (central, intermediate and external zones), an effect of the drying profile could be observed. Whatever the location, the volumes calculated from the CLSM images were lower in pasta dried at higher temperatures (HT, VHT and VHT_LM),

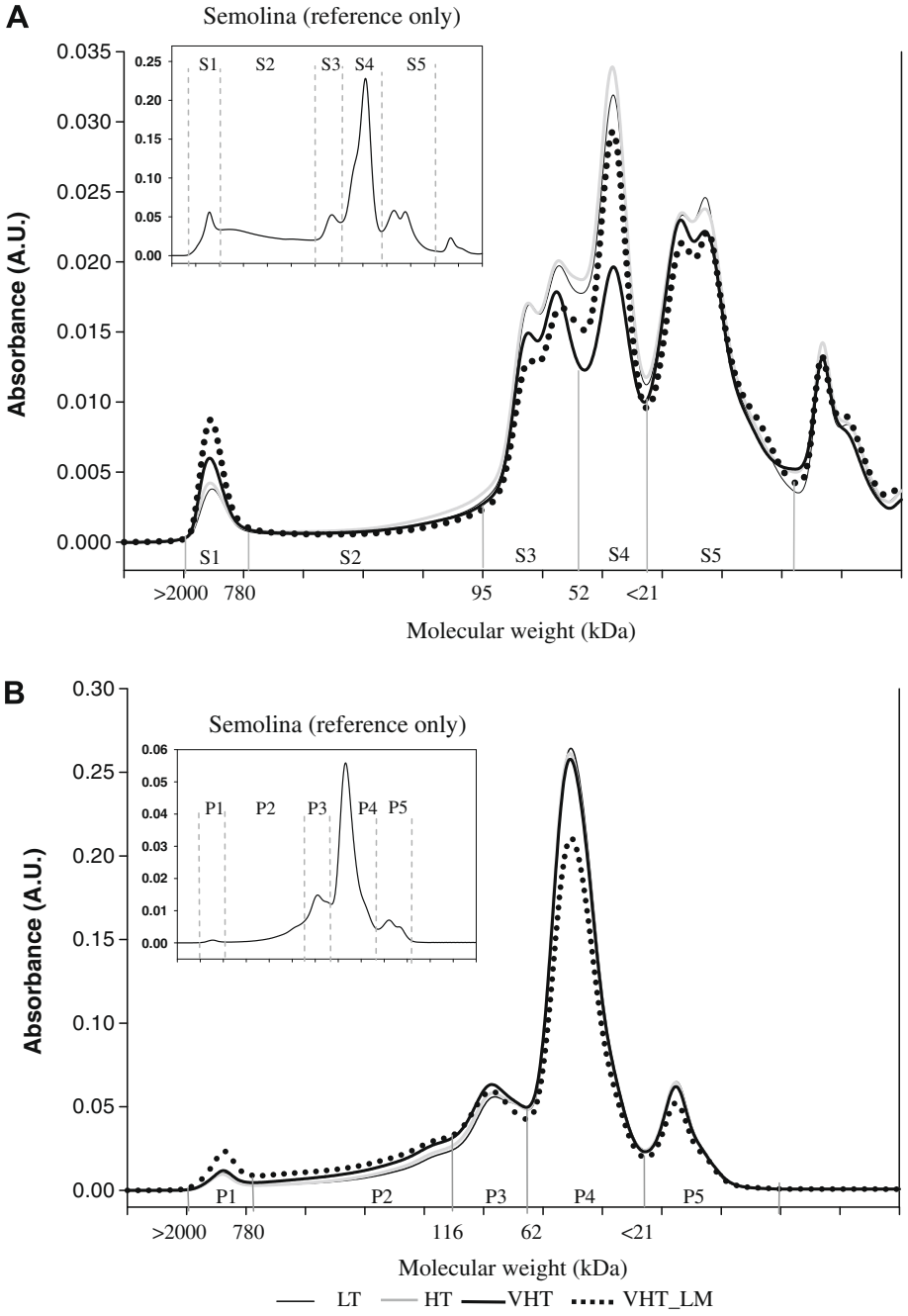

Fig. 2. SE-HPLC elution profiles ($n = 3$) of SDS-soluble (A) and DTE-soluble (B) protein fractions in cooked pasta dried with one of the four drying profiles.

implying a lower amount of proteins in these images (data not shown). In external regions, granulometric curves calculated from images of HT, VHT and VHT_LM drying profiles were significantly distinguished from LT drying profile (without any distinction between HT, VHT_LM and VHT dryings) (Fig. 4A). Similar trends were also observed for images acquired in the centre or intermediate zones of pasta.

Pasta dried at high and very high temperatures (HT, VHT and VHT_LM) were characterised by a higher amount of the thinnest films (<4 μm) and a lower amount of the smallest dark area. Images from high and very high temperature drying profiles exhibited significantly ($p < 5\%$) lower PC1 and higher PC2 scores. High temperature drying profiles brought about a loss of the smallest starch granules, as observed by Zweifel et al. (2003). The greatest thinning of the protein network in these pasta could be due to the highest swelling of starch granules whose structure could have been affected by higher temperature during drying.

3.2.3. Rheological properties of pasta

3.2.3.1. Texture profile analysis (TPA). TPA analysis of cooked pasta samples are presented in Table 2. Four parameters were extracted from TPA curves: hardness, cohesiveness, resilience and springiness. Pasta hardness represents the force required to compress a strand of pasta between molar teeth. Cohesiveness represents the ability of the material to stick to itself. Springiness and resilience assess the ability of pasta to regain its original shape after compression. LT cooked pasta presented the lowest values for hardness, cohesiveness and resilience. Conversely, VHT_LM cooked pasta presented the highest values but was not significantly different from HT for cohesiveness and VHT for springiness. It was not

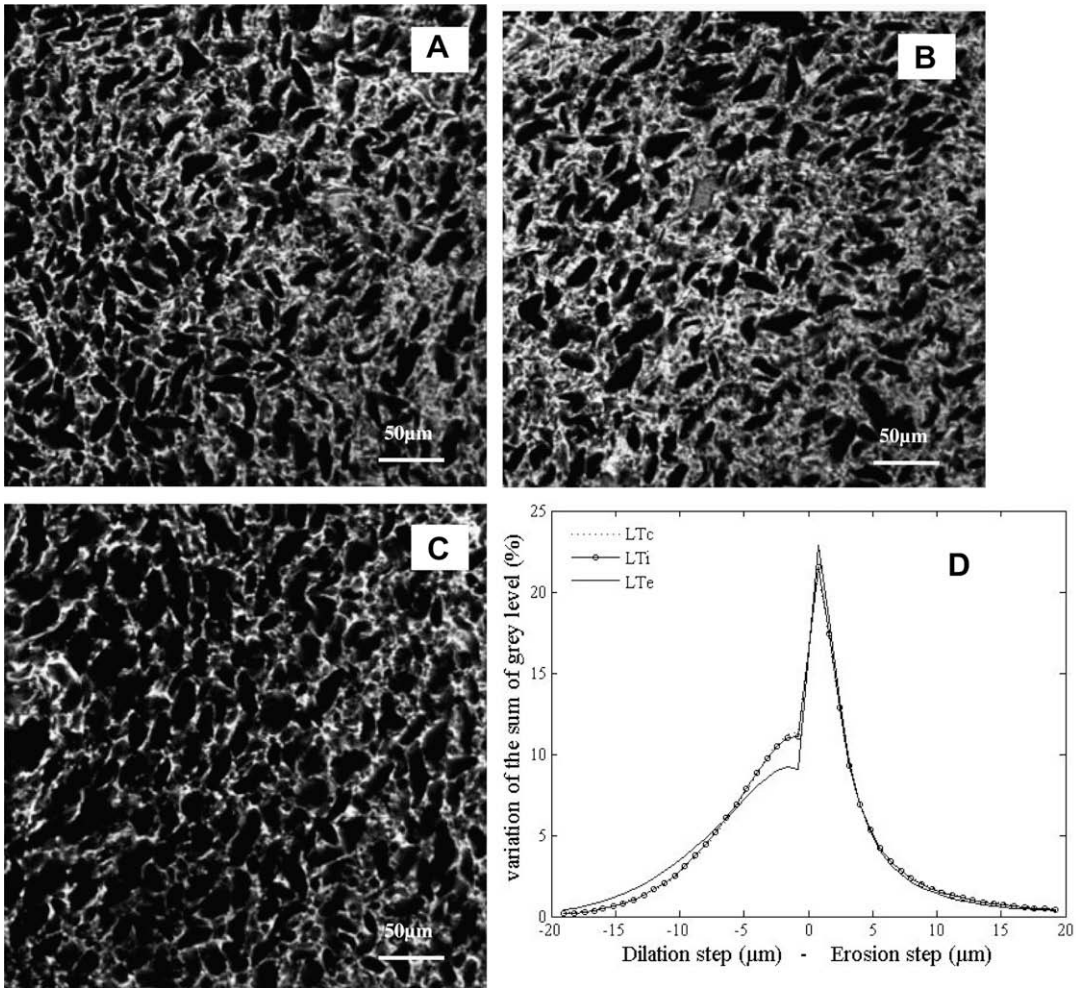

Fig. 3. Typical images (acquired by CLSM) of the LT cooked pasta in the central (A), intermediate (B), and external (C) zones and granulometric curves (D) corresponding to the three images (LTc, LTi and LTe: curves for LT cooked pasta in the central, intermediate and external zones of pasta).

possible to distinguish HT and VHT cooked pasta. TPA test can be related to the nature and arrangement of the structural elements that constitute the food (Olivera & Salvadori, 2006). We can conclude that the application of different drying profiles distinguished 3 groups of spaghetti (LT, HT/VHT, VHT_LM) with different structure arrangements, probably affecting the protein network.

3.2.3.2. Extension test. Cooked pasta samples were submitted to an extension test (Table 2). Breaking strain and breaking stress were recorded at the breaking point of the pasta strand and breaking energy corresponded to the total area under the strain/stress curve. The energy required to break cooked pasta was higher for VHT and VHT_LM cooked pasta (5.9 and 6.2 J/m^3) than for HT and particularly LT cooked spaghetti (5.0 and 3.7 J/m^3, respectively). This higher breaking energy was due to both a high breaking strain (304% and 317%) and a high breaking stress (3.8 and 4.1 $\times$ 10^5 N/m^2 for VHT and VHT_LM samples, respectively).

3.3. Effect of pasta drying on the in vitro digestion of cooked and minced pasta

Since the drying profile can affect wheat protein digestibility (De Zorzi et al., 2007; Hansen & Johnston, 1976), pasta dried at different temperatures (LT, HT, VHT, VHT_LM) were digested by an *in vitro* multi-enzymatic system simulating the digestion process in the bucco-gastrointestinal tract. Contrary to a previous study on pasta (De Zorzi et al., 2007), care was taken to apply the digestion process on structurally and morphologically relevant states of pasta by mimicking the particle size distribution obtained after human mastication of spaghetti (Hoebler et al., 2000). Cooked and minced pasta samples were successively treated by α-amylase at pH 6.9 (buccal digestion), pepsin at pH 2 (gastric digestion) and pancreatin at pH 7 (duodenal digestion); protein and polysaccharide degradation were analysed in the course of digestion.

3.3.1. Effect of pasta drying on hydrolysis of starch and proteins

Results of protein and starch hydrolysis are presented in Table 3. During the *in vitro* bucco-gastric digestion, α-amylolysis of starch led to about 22% of starch transformed in alcohol-soluble dextrins in 5 min; VHT drying profile tended to increase this percentage but this was not statistically significant. Inactivation of enzymes of the buccal digestion at pH 2 explains that the percentage of alcohol-soluble dextrins remained unchanged whatever the duration of the pepsin phase. Wheat proteins were slightly digested by pepsin during the gastric digestion, 3% in 30 min, 8% in 180 min, with no statistically significant influence of the drying profile of pasta.

During the *in vitro* duodenal digestion, proteins and starch were largely digested (at 180 min of pancreatin phase, mean values of the degree of hydrolysis (DH) for proteins and of the percentage of alcohol-soluble dextrins (PASD) for starch were 63% and 86%, respectively). ANOVA revealed an influence of the drying profile

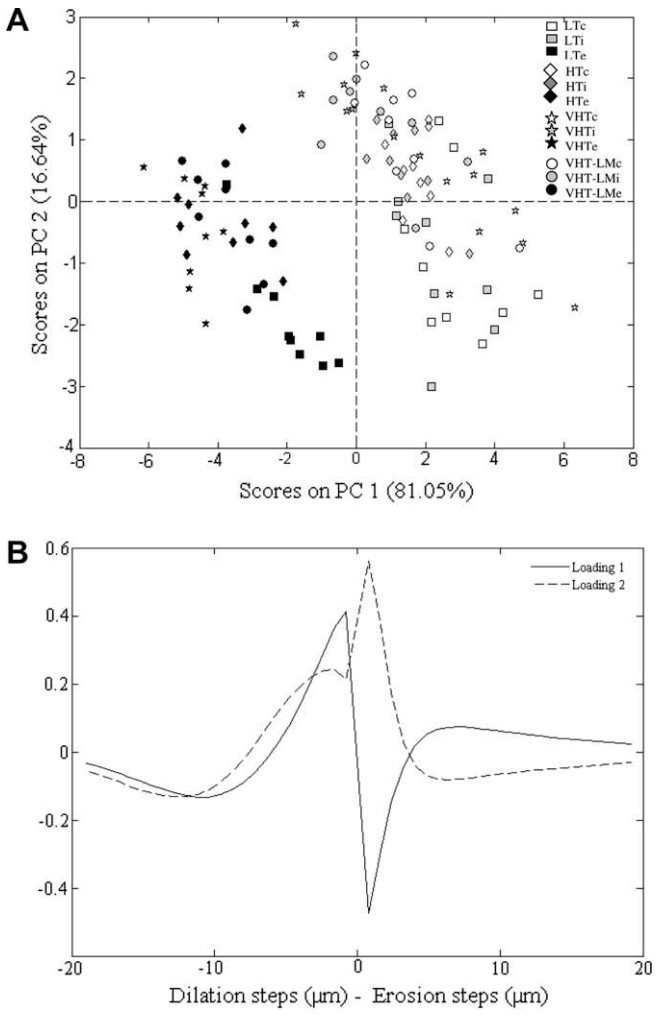

Fig. 4. Principal component analysis of the granulometric curves from LT, HT, VHT and VHT_LM cooked pasta in the central (c), intermediate (i) and external (e) zone of pasta strand. (A) and loadings 1 and 2 corresponding to principal components 1 and 2 (B). Components 1 and 2 accounted for 81.05% and 16.64% of the total variance.

with no significant interactions with other factors for DH of proteins. VHT_LM drying profile led to a significantly reduced protein digestion (mean DH value of 34%; 18%, 29% and 55% at 10, 30 and 180 min, respectively, for VHT_LM compared to a mean DH value of about 40% for the other pasta samples and 19–23%, 32–33% and 65–66% at 10, 30 and 180 min respectively). For starch digestion, results were not as significant as for protein digestion but the tendency was similar, VHT_LM (mean PASD value was 66%, 84% at 180 min) differed from VHT process (71% and 89%) but both of them were not significantly different from LT and HT profiles (mean values 70% and 69% respectively and 86% and 87% at 180 min). Whatever the drying profile, hydrolysis of the proteic network by pepsin during the gastric phase increased the proteic digestion during the pancreatic phase (mean values 34%, 38% and 43% corresponded to 14%, 22% and 26% at 10 min; 28%, 30% and 37% at 30 min; 61%, 62% and 66% at 180 min of pancreatic phase for no prehydrolysis by pepsin and 30 or 180 min of pepsinolysis, respectively). Whatever the drying profile, hydrolysis of the proteic network by pepsin during the gastric phase speeded up the starch digestion (significant interaction pepsin * pancreatin on PASD) during the pancreatic phase (47%, 67%, 85% for no prehydrolysis by pepsin and 62%, 73% and 85% for 180 min of pepsinolysis, respectively, at 10, 30 and 180 min of pancreatin phase). Such an increased rate of starch digestion by prehydrolysis of proteic network has already been shown in pasta (Colonna et al., 1990; Fardet et al., 1998b; Granfeldt et al., 1991) and other products, such as rice products (Mujoo & Ali, 1998).

3.3.2. Effect of pasta drying on composition of digestion juices

Digestion juices were analysed by SE-HPLC during *in vitro* digestion. SE-HPLC profiles of digestion juices at 220 nm are illustrated in Fig. 5A for LT pasta. As expected, digestion shifted profiles toward lower molecular weight and increased amount of material in digestion juices. SE-HPLC profiles of digestion juices at 220 nm were then analysed by PCA (Fig. 5B and C) and ANOVA were performed on the principal components. On the first PCA plot (Fig. 5C), SE-HPLC profiles of digestion juices gathered as a function of the digestion step. On the first dimension (80% of variance), large differences between profiles from each pancreatic time (30 and 180 min) and the group of profiles from the end of buccal and gastric phases were found. The second dimension (13% of variance) distinguished profiles at the end of the buccal phase (−0.224) from profiles at the end of the gastric phase (0.205) and from the two sets of profiles from the pancreatic phase (0.043 and −0.024 for 30 and 180 min of pancreatic digestion, respectively). Only profiles of pancreatic digests were significantly influenced by the drying profile, resulting in lower and higher first component, respectively, for VHT and VHT_LM profiles (Fig. 5C). Taking into account that higher first component value appeared linked to lower digestion, this result agrees with lower DH values previously found for VHT_LM pasta (29% and 55% at 30 and 180 min, respectively) compared to 33% and 65% at 30 and 180 min, respectively for VHT. No significant effect of the drying profile was found on the second component. Both quantitative and qualitative differences in the composition of polypeptides in digestion juices could result in such differences in SE-HPLC profiles. Pancreatic digestion juices of VHT and VHT_LM pasta were then further analysed by reversed-phase HPLC but no qualitative differences could be found (results not shown), only quantitative differences appeared to be responsible for differences found on SE-HPLC profiles of digestion juices.

3.3.3. Effect of pasta drying on residual allergenicity of digestion extracts and juices

Wheat proteins from pasta were first investigated for their IgE binding during digestion by dot blotting (data not shown). As

Table 2
Textural characteristics of cooked pasta from texture profile analysis (TPA) and tensile test. Means ($n = 10$) with the same superscript within a column are not significantly different ($p > 0.05$).

Drying cycle	TPA				Tensile test		
	Hardness (*N*)	Cohesiveness	Resilience	Springiness	Breaking stress ($N/m^2 \times 10^5$)	Breaking strain (%)	Breaking energy ($J/m^3 \times 0^5$)
LT	3.14^{c}	0.80^{c}	0.61^{c}	0.98^{b}	2.9^{c}	272^{b}	3.7^{c}
HT	3.40^{b}	0.87^{ab}	0.66^{b}	0.99^{b}	3.5^{b}	301^{a}	5.0^{b}
VHT	3.45^{b}	0.85^{b}	0.65^{b}	1.02^{ab}	3.8^{ab}	304^{a}	5.9^{a}
VHT_LM	3.71^{a}	0.89^{a}	0.70^{a}	1.05^{a}	4.1^{a}	317^{a}	6.2^{a}

Table 3
Statistical analysis of the variations of the degree of hydrolysis of proteins (DH) and of the percentage of alcohol-soluble dextrins (PASD) during the bucco-gastric and duodenal phases. Results of the ANOVA and subsequent LSD test.

Hydrolysis during the bucco-gastric phase				Comparison of means – LSD test[B]						
	Analysis of variance[A]			Effect of drying				Effect of hydrolysis by pepsin (min)		
	Effect	df	*p*-value	LT	HT	VHT	VHT_LM	0	30	180
Proteins	A: Drying	3	0.551	6.3[a]	6.0[a]	4.5[a]	5.8[a]			
	B: Pepsin time	1	0.0001					0	3.2[a]	8.1[b]
Polysaccharides	A: Drying	3	0.213	20.8[a]	20.8[a]	23.8[a]	21.0[a]			
	B: Pepsin time	2	0.945					21.4[a]	21.5[a]	21.8[a]

Hydrolysis during the duodenal phase				Comparison of means – LSD test[B]							
	Analysis of variance[A]			Effect of drying				Effect of hydrolysis (min)			
	Effect	df	*p*-Value	LT	HT	VHT	VHT-LM	0	10	30	180
Proteins	A: Drying	3	0.008	39.7[a]	39.1[a]	40.6[a]	34.2[b]				
	B: Pepsin time	2	0.000					34.3[a]		37.9[b]	43.0[c]
	C: Pancreatin time	3	0.000						20.6 [a]	31.7 [b]	62.9 [c]
Polysaccharides	A: Drying	3	0.152	70.2 [ab]	69.4 [ab]	71.2 [b]	66.5 [a]				
	B: Pepsin time	2	0.001					66.1 [a]		68.5 [a]	73.4 [b]
	C: Pancreatin time	3	0.000						53.3 [a]	68.3 [b]	86.4 [c]
	Interactions BC	6	0.002								

[A] Main effects (pasta drying, pepsin time and pancreatin time) and all their interactions were first included in the model then non-significant interactions ($p > 0.05$) were excluded.
[B] Within the same row, the values with the same superscript are not significantly different ($p > 0.05$). For each analysed effect, mean value for all tested conditions for the other effects is indicated.

expected, IgE binding was detected with the serum from a patient allergic to wheat-based food, whereas no signal was detected for the patient allergic to Graminaceae, except for a faint signal for the albumin/globulin fraction control. Whatever the drying profile, IgE binding to extracts decreased as digestion proceeded to be hardly detected at the end of the pancreatic phase in tested conditions. However, at all tested steps of digestion, IgE binding was still detected in digestion juices. Juices progressively concentrated solubilised proteic fragments, some of which still kept IgE binding capacities that could be a risk for allergic patients whenever they were not fully processed by peptidases from the brush border. These results show that IgE-reactive peptides that resist the digestion process are formed whatever the drying profile, in agreement with previously reported data (De Zorzi et al., 2007).

To compare IgE reactivity of proteic fragments in digestion juices from different pastas and to get an insight of the proteic origin of these fragments, competitive ELISA was then performed with digestion juices and a pool of sera of 4 patients suffering from food allergy to wheat against wheat protein fractions. Higher concentration of IgE-reactive peptides in digestion juice will lead to higher inhibition of wheat protein recognition, provided that IgE interact with preserved epitopes of tested protein. Linked to IgE reactivity of the pool against wheat protein, competitive ELISA was performed for the prolamin fraction against α and γ-gliadins and low molecular weight glutenins, and against the albumin/globulin fraction. As shown in Table 4, inhibition varied, depending on wheat fraction. Direct comparison of inhibition percentage must be restricted to proteins with similar abundance in semolina and IgE reactivity; α-gliadin and albumins/globulins fraction meet these conditions. Inhibition by digestion juice of LT pasta was thus found higher for α-gliadin than for soluble albumins/globulins at the end of gastric phase (66% and 28%) but effects were similar at the end of the intestinal phase (16% and 14%). Reduction of IgE reactivity with digestion also depended on the wheat fraction. For LT pasta, for example, reduction of inhibition by a factor 4 for α-gliadin, 2 for albumins/globuins fraction and low molecular weight glutenins occurred but no reduction was found for γ-gliadin. This last result was found whatever the drying profile. Compared to LT pasta, the drying process led to reduced allergenicity of digestion juices from the end of the gastric phase in the case of VHT profiles (lower inhibition of α-gliadin recognition for VHT and VHT_LM pasta and of low molecular weight glutenins recognition for VHT_LM pasta). For digestion juices from the end of the intestinal phase, recognition of gliadins was not modified by drying process but allergenicity was increased with higher inhibition of low molecular weight glutenins recognition for HT and VHT_LM pasta and of albumins/globulins fraction for VHT_LM pasta. VHT_LM pasta exhibited the lowest reduction of allergenicity upon digestion for α-gliadin (by 2) and albumins/globulins fraction (by 1). To conclude on these results, digestion juices from pasta were found to inhibit recognition of each tested wheat fraction by IgE from allergic patients, showing that these juices gather proteic fragment with allergenic potentialities originated from different wheat fractions. Impacts of drying profile on allergenic properties of digestion juices depended on digestion step and on which wheat protein is involved; that is to say that drying process can lead to various results (increase, decrease, no change) depending on sensitisation profile of allergic patients but no tested drying profile was found to remove allergenic properties of wheat proteins.

4. Discussion

The first objective of this study was to investigate the effects of pasta drying on the structure of dried and cooked pasta, characterised by a multi-scale approach. Pasta dried at low temperature (LT) showed small but significant differences in starch and protein structures, when compared to semolina. Starch presented a reduced gelatinisation enthalpy, which may indicate the presence of partially gelatinised starch. LT dried pasta was also characterised by a higher protein aggregation, probably through disulphide bonds (protein solubility in SDS was decreased by 10% in favour of DTE solubility). An increase in the drying temperature up to 70 °C had a moderate impact on starch and protein structures. However, the application of a very high temperature (VHT and VHT_LM), especially at high pasta moisture content (VHT), induced significant reorganisation of starch and protein fractions. The results suggested that starch from VHT pasta presented a more stable and homogeneous crystalline structure. VHT_LM and VHT drying induced a high protein aggregation, as already shown in pasta (Aktan & Khan, 1992;

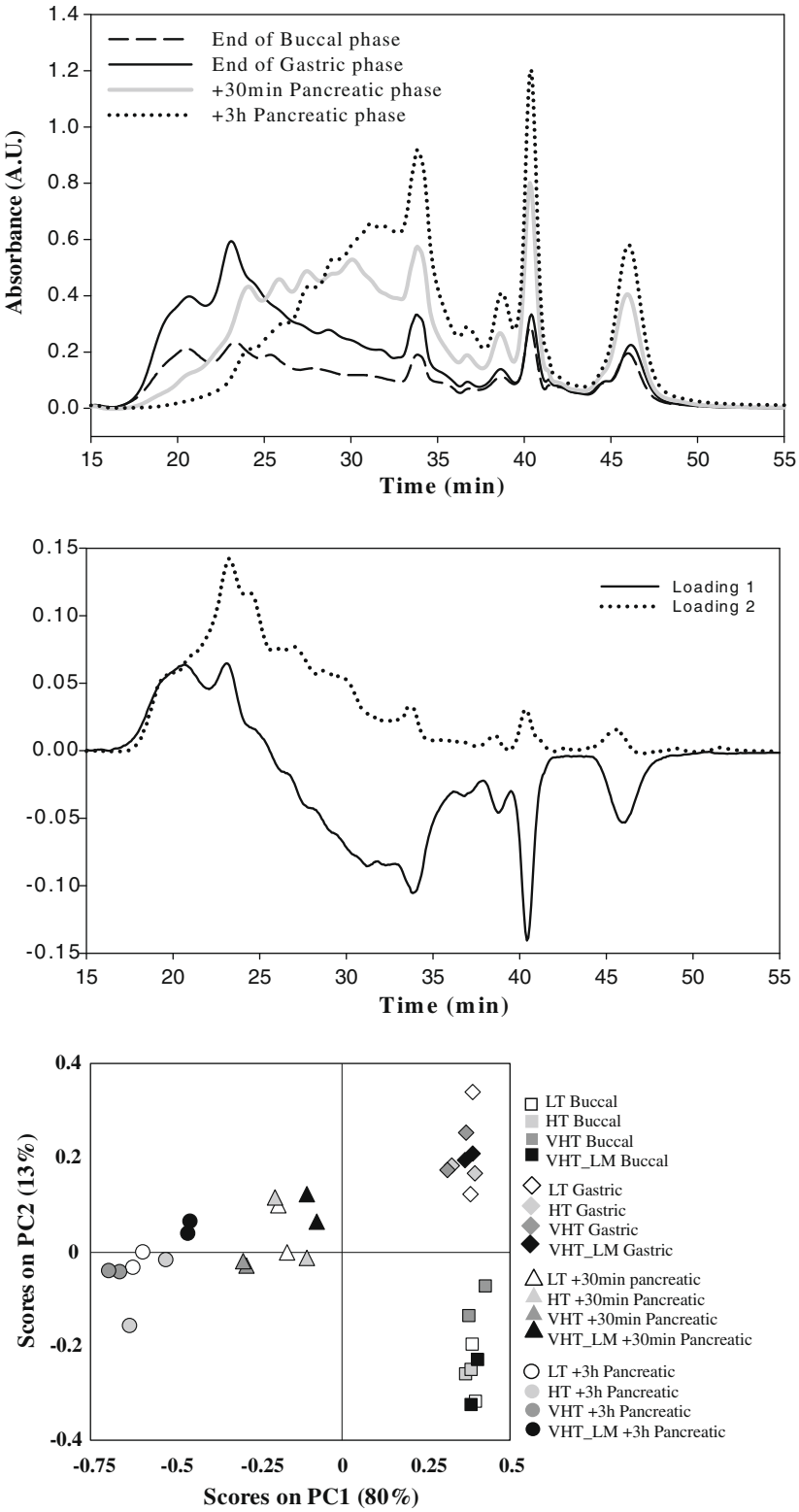

Fig. 5. SE-HPLC profiles at 220 nm of digestion juices of LT pasta in the course of *in vitro* digestion (A) and (B) loadings and (C) first plan of the principal components analysis (PCA) of SE-HPLC profiles of LT, HT, VHT and VHT_LM cooked pasta at the end of buccal phase, gastric phase, after bucco-gastric phase + 30 min of pancreatic phase and after bucco-gastric-phase + 3 h of pancreatic phase.

Lamacchia et al., 2007; Zweifel et al., 2003). This protein aggregation probably occurred through the formation of additional disulphide bonds and other covalent bonds (i.e., isopeptide bonds).

Further structural changes occurred during the cooking step. On the basis of DSC measurements, starch was totally gelatinised in all cooked pastas. This was not surprising since they were cooked until the disappearance of the white core, corresponding to ungelatinised starch. Cooking led to a marked protein aggregation in LT and HT pasta, probably through disulphide bonds but also through other covalent bonds. In contrast, proteins from VHT and VHT_LM

Table 4
Results of competitive ELISA with digestion juices from pasta and a pool of sera from allergic patients to wheat. Percentage of inhibition obtained with digestion juices at the end of the gastric phase (5 min of hydrolysis by α-amylase and 3 h by pepsin) and at the end of the intestinal phase (5 min of hydrolysis by α-amylase, 3 h by pepsin and 3 h by pancreatin) are presented. Means ($n = 2$) with the same superscript by protein are not significantly different ($p > 0.05$).

	Fluorescence intensity without inhibitor	Digestion juice from the end of	LT	HT	VHT	VHT_LM
α-Gliadin	31,753	Gastric phase	66^{a}	64^{a}	44^{b}	40^{b}
		Intestinal phase	16^{cd}	14^{cd}	10^{d}	19^{c}
γ-gliadin	9989	Gastric phase	88^{a}	79^{bc}	80^{abc}	81^{ab}
		Intestinal phase	78^{bc}	75^{bc}	72^{c}	77^{bc}
Low MW glutenins	12,118	Gastric phase	93^{a}	91^{ab}	90^{ab}	87^{b}
		Intestinal phase	51^{d}	66^{c}	46^{d}	63^{c}
Albumin/globulin fraction	31,732	Gastric phase	28^{a}	26^{ab}	24^{ab}	24^{ab}
		Intestinal phase	14^{c}	16^{c}	14^{c}	21^{b}

pasta underwent less marked changes during cooking probably because most of the proteins were already aggregated during the previous drying step. Cooking reduced differences in protein structure created by the different drying profiles and led to an apparent similarity between cooked pasta. However, the analysis of protein size distribution revealed that VHT_LM cooked pasta had a higher proportion of larger protein polymers. This small difference seen at a molecular level may induce important changes at a macroscopic one.

Indeed, an increase in drying temperature corresponded to an increase in pasta hardness, cohesiveness, resilience and springiness (TPA test) and in breaking energy (tensile test). In particular, VHT_LM drying induced an increase of 15% in pasta hardness and of 40% in pasta breaking energy compared to LT drying. It is suggested that more severe drying conditions may promote the formation of a strong protein network, responsible for the higher resistance of pasta to compression and tensile forces.

The microstructure of all cooked pasta was characterised by large structural differences between the external and the central regions. This result can be a consequence of the gradient in moisture distribution created during cooking (Baiano & Del Nobile, 2006; Horigane et al., 2006). The core and intermediate regions of cooked pasta presented a dense protein network surrounding voids assimilated to starch granules with varying sizes. The external zone was characterised by a looser protein network, surrounding larger voids assimilated to highly swollen starch granules. This is in accordance with previous studies on pasta (Cunin, Handschin, Walther, & Escher, 1995; Heneen & Brismar, 2003; Zweifel et al., 2003). The increase in drying temperature did not create much differences in pasta microstructure.

The second objective of our study was to determine whether structural changes, induced by pasta drying, could modify the digestibility of protein and starch fractions and to know whether structural changes in the protein fraction induced by pasta drying and/or the digestion process could abolish the presence of IgE-reactive peptides.

The bucco-gastric digestion of cooked pasta led to 22% starch hydrolysis and a limited protein hydrolysis (8%), whatever the drying profile. The effect of the drying profile on protein and starch digestibility was more pronounced at the duodenal phase. In particular, in VHT_LM cooked pasta protein and starch hydrolysis was reduced by 10% and 3%, respectively, after three hours of intestinal phase, in comparison with LT and HT pasta. VHT_LM cooked pasta was also characterised by a high proportions of protein aggregates in protein extracts and of larger peptides in digestion juices. Moreover, none of the drying profiles tested was found to abolish allergenic properties, digestion juices from VHT_LM pasta were even found to contain more IgE-reactive peptides from albumins/globulins fraction. These results are in agreement with two previous studies on pasta where drastic thermal treatment was shown to (1) decrease the in *vitro* digestibility of starch (Casiraghi et al., 1992) and (2) reduce the *in vitro* digestibility of wheat proteins without affecting their potential allergenicity (De Zorzi et al., 2007).

The application of a very high temperature, especially at low pasta moisture content (VHT_LM) has contributed to the formation of a different structure, certainly responsible for the lower protein and starch hydrolysis. This difference was not observed at a microscopic scale but at a molecular one. No major changes were observed on the spatial distribution of proteins. However, molecular rearrangements of proteins have led to the formation of large protein aggregates which have probably contributed to the moderate decrease in the *in vitro* protein and starch hydrolysis and increase in the residual allergenicity. However, it would be interesting to confirm these results *in vivo*.

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