

Review

Structuring of pasta components during processing: impact on starch and protein digestibility and allergenicity

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Pasta is a staple food known to have a low glycaemic index. This interesting nutritional property can be attributed to its specific structure, obtained after successive structural changes of its two main components, i.e. starch and proteins. This paper describes the state of art on protein and starch structuring during pasta processing and the inherent consequences on starch digestibility but also on protein digestibility and allergenicity. This review highlights the need for a multidisciplinary approach for the rational design of pasta, in order to control digestion and nutrient absorption through the food structure.

Introduction

Dry pasta is a traditional cereal-based food product that is becoming increasingly popular worldwide because of its convenience, palatability, and nutritional quality. Durum wheat (*Triticum durum*) semolina is recognised as the

most suitable raw material for pasta production due to its unique colour, flavour and cooking quality (Feillet & Dexter, 1996). In France and in Italy, dry pasta intended for the national market can only be made from durum wheat semolina (French law, 3 July 1934 modified by the law n° 99–574, 9 July 1999) (Italian law n°580, 4 July 1967 modified by the presidential decree n°187, 9 February 2001).

Starch (74–76% db) and proteins (12–15% db) are the major components of durum wheat semolina. Wheat starch is composed of small spherical B-type granules (average diameter 2–3 µm) and larger lenticular A-type granules (average diameter 30 µm) (Buleon, Colonna, Planchot, & Ball, 1998). Proteins can be fractioned into albumin (water-soluble), globulin (soluble in salt solution), gliadin (extractable in aqueous ethanol solution) and glutenin (soluble in dilute acid), according to the protein classification of Osborne (1907). Albumins and globulins are minor fractions (20%) as compared to monomeric gliadins and polymeric glutenins (80%). Glutenins are composed of high molecular weight (HMW) and low molecular weight (LMW) subunits linked together by disulphide bonds (Schofield, 1986).

Starch and proteins undergo successive structural changes during the pasta making process. The continuous production process is today performed *via* automation, giving rise to high productivity (2–5 tonnes/h). It involves four fundamental steps: hydration of semolina, mixing, forming (by extrusion or sheeting), and drying. During mixing, the water is distributed as evenly as possible throughout the semolina, thus promoting homogeneous particle hydration. The crumbly dough obtained is fed to an extrusion screw or a sheeter and formed into the desired shape. Fresh pasta is then dried in order to reduce its moisture content to about 12% (Dalbon, Grivon, & Pagani, 1996). Finally cooking in water gives pasta its ultimate structure.

The structure of cooked pasta is generally described as a compact matrix with starch granules entrapped in a protein network (Cunin, Handschin, Walther, & Escher, 1995; Pagani, Gallant, Bouchet, & Resmini, 1986). Its structure, as well as its composition, are responsible for the specific nutritional properties of pasta among cereal products (Colonna *et al.*, 1990; Granfeldt & Bjorck, 1991). Indeed, the unique feature of pasta is that it contains slowly digestible starch (Jenkins *et al.*, 1981; Monge *et al.*, 1990). Most authors to date have focused on the development or optimisation of the pasta manufacturing process so as to improve the sensorial quality of pasta (Abecassis *et al.*, 1994;

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Baiano & Del Nobile, 2006; Cubadda, Carcea, Marconi, & Trivisonno, 2007). However, modifying the process parameters could affect the pasta structure and therefore potentially alter the digestibility of the starch and protein fractions and the allergenicity of pasta (Simonato *et al.*, 2004).

It seems therefore essential to gain further insight into how the manufacturing process affects pasta structure and controls its nutritional quality. Knowledge must therefore be gathered about pasta processing, structuring and nutritional characteristics so as to be able to develop food products with both high sensorial and nutritional qualities. The main objectives of our review were therefore: (1) to draw up an overview on the development of pasta structure during each processing step, (2) to understand the role of process parameters on the final pasta structure, and (3) to present the inherent consequences on protein and starch digestibility and the potential allergenicity of pasta.

Structuring of pasta components during processing

Different methods can be used to monitor structural transformations in pasta components (mainly starch and proteins) that occur at different scales during processing. Microscopy is one of the most extensively used techniques to characterise the microstructure of pasta. Bright field light microscopy (BFLM) is generally used to locate proteins and starch. Polarized light microscopy (PLM) provides more detailed information about the molecular organisation of starch granules in pasta: ungelatinised native starch granules exhibit birefringence (or a “Maltese cross” pattern) under polarized light. Confocal laser scanning microscopy (CLSM) is often used because it is able to produce optical sections through a three-dimensional (3-D) specimen such as pasta. Scanning electron microscopy (SEM) observations are useful for assessing the topography and surface of pasta samples. Transmission electron microscopy (TEM) provides higher magnifications than SEM but requires extensive sample preparation to produce sections thin enough to be electron transparent.

At a lower scale, different techniques can be used to supplement microscopic observations. Starch gelatinisation can be evaluated by differential scanning calorimetry (DSC), X-ray diffractometry, colorimetric and enzymatic methods. DSC and X-ray diffractometry are used to quantify the endothermic melting and loss of crystallinity of starch granules, respectively. The colorimetric method quantifies amylose chains that are released during the gelatinisation process and which form a blue complex with iodine. Enzymatic methods measure the susceptibility of starch to hydrolytic enzymes (Baks *et al.*, 2007). Regarding the protein fraction, the denaturation and aggregation of proteins can be monitored by the loss of protein solubility. Size-exclusion (SE) HPLC and electrophoresis are conventionally used to better characterise proteins.

Structural transformations in semolina components can be monitored during each processing step (mixing,

forming, drying) and cooking using these techniques, as presented hereafter.

Mixing

When viewed under SEM, semolina presents irregularly shaped particles of variable sizes. The structure is compact, with few visible starch granules entrapped in a protein matrix (Aalami, Rao, & Leelavathi, 2007; Matsuo, Dexter, & Dronzek, 1978) (Fig. 1A). The right amount of water must be used to transform semolina into a homogeneous dough. The amount of water added to semolina generally ranges from 25 to 34 kg per 100 kg of semolina. It depends on the initial moisture content of semolina and on the final pasta shape (Dalbon *et al.*, 1996). For extruded spaghetti, 26.5 kg (per 100 kg of semolina) of water is generally added to semolina (14% moisture content) in order to obtain a dough with 32% water content, on a wet basis (or 47% water content on a dry basis)."

During the mixing stage, heterogeneous hydrated pea-sized lumps (of 2–3 cm dia.) are formed in the mixing chamber, but nonhydrated particles remain present in some areas. No major changes appear to occur at a microscopic level, i.e. under SEM, starch granules are still distinctly visible and firmly held within the protein matrix (Matsuo *et al.*, 1978). As water absorption takes place below 50 °C, starch granules show no important structural changes (Vansteelandt & Delcour, 1998), and no indication of gluten network formation is apparent (Matsuo *et al.*, 1978) due to the limited hydration level and low energy input (Icard-Vernière & Feillet, 1999). At a smaller scale, a slight increase (~3%) in the protein solubility in sodium dodecyl sulphate (SDS disrupts electrostatic, hydrophobic and hydrophilic interactions) is observed during mixing (Table 1), mainly affecting gluten proteins (Icard-Vernière, 1999). Although protein reactivity and denaturation is well documented, little information is available about protein structural transformations during the mixing step of pasta processing.

Forming

Pasta can be formed by either the sheeting or extrusion process (the most commonly used). The extrusion unit is composed of a cylinder fitted with an extrusion screw. The screw rotation pushes the dough towards the head press on which a die is set, where it takes its final shape. As the dough is transferred, pressure builds up and the temperature of the dough rises. The extrusion pressure (in the 5–10 MPa range in a single-screw extruder) seems to be essential to give the product the necessary compactness to stand up well to cooking (Kruger, Matsuo, & Dick, 1996). In the sheeting process, the dough is kneaded and rolled into a sheet by compression between two rotating cylinders. Three to five pairs of rolls, with decreasing roll gaps are used until the sheet reaches the desired thickness. The sheet is then cut into strands of the desired width and length. Extrusion and sheeting mainly differ with

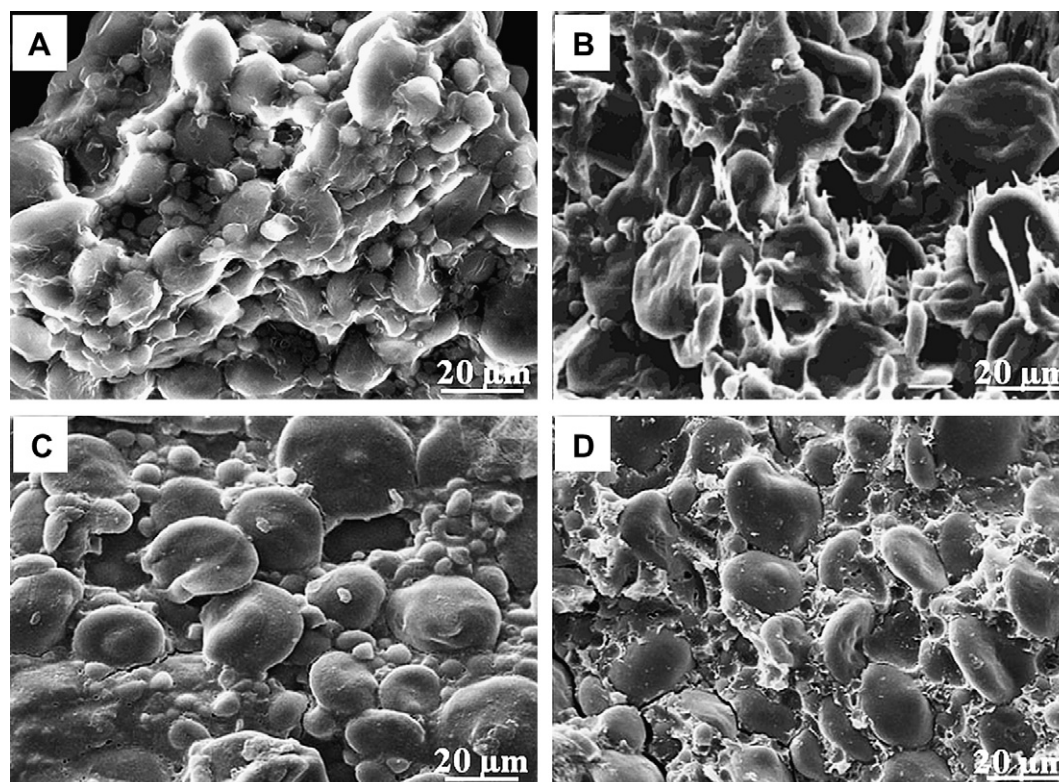


Fig. 1. Scanning electron microscopy of A) Semolina (Petitot *et al.*, unpublished data) B) freshly extruded pasta (Tudorica *et al.*, 2002), C) and D) Surface and cross section of dried spaghetti (85 °C) (Sadeghi & Bhagya, 2008).

respect to the mechanical energy used to form pasta. The total amount of energy transferred to the dough is higher with extrusion process and some part of the mechanical energy is dissipated as heat. Moreover, during extrusion, dough is submitted to shearing stress whereas sheeting exerts elongational stress. Such differences in stress, heat and pressure can result in the formation of different pasta structures.

Extrusion. In comparison to starchy foods produced by extrusion cooking, less attention has been devoted to the analysis of freshly extruded pasta.

Observed under SEM, freshly extruded spaghetti presents a compact internal structure with starch granules deeply embedded in a protein matrix and aligned along the direction of the flow (Matsuo *et al.*, 1978). The starch granules within the pasta are irregular in size and shape and appeared to be slightly swollen (Tudorica, Kuri, & Brennan, 2002) (Fig. 1B).

At the molecular level, the extent of protein denaturation can be detected by monitoring the loss of protein solubility in dilute acetic acid (Dexter & Matsuo, 1977) or in SDS (Icard-Vernière, 1999). Depending on the authors, different protein fractions are affected by extrusion. According to Dexter and Matsuo (1977), extrusion led to a loss of solubility of the globulin fraction without affecting the albumin, gliadin and glutenin fractions. This loss of solubility could

not be explained by polymerisation because no change in SS bonds and no change in the molecular weight distribution of proteins were detected. They hypothesised that globulins bind to the insoluble components of semolina. On the contrary, Icard-Vernière (1999) observed a decrease in protein solubility in SDS, mainly due to a decrease in glutenin aggregates solubility, that was concomitant with an increase in the insoluble fraction. This means that bigger insoluble glutenin aggregates were formed during extrusion. Generally speaking, extrusion is conducted at lower temperatures (≤ 50 °C) than those required to cause the insolubilisation of proteins (>60 °C) (Weegels & Hamer, 1998). However, as the dough is transferred in the extrusion worm, pressure builds up and the dough temperature rises locally. Structural transformations are therefore a consequence of both mechanical (shearing stress) and thermal forces involved during extrusion (Kruger *et al.*, 1996).

Concerning the starch fraction, mechanical forces can lead to moderate damage to starch granules (Icard-Vernière, 1999; Lintas & d' Appolonia, 1973). A local increase in temperature (>60 °C) due to mechanical forces can also lead to starch gelatinisation. DSC measurements revealed a lower gelatinisation enthalpy of extruded pasta compared to semolina (Vansteelandt & Delcour, 1998; Zweifel, Conde-Petit, & Escher, 2000) (Table 1). A reduced gelatinisation enthalpy could be explained by the presence of some gelatinised starch granules and/or some damaged starch

Table 1. Protein solubility and DSC analysis of starch during pasta processing

	Semolina	End of mixing	Extruded pasta	Dried pasta					Cooked pasta ^a
				LT	HT		VHT		
				< 55 °C	60–70 °C	80–85 °C	90–110 °C	180 °C	
Solubility in SDS (% of total protein content)	92.9(f)	96.3(f)	88.9(f)	70.0(g)	63.0(g)		22–28)(g)		~ 15.0(g) (OCT)
Solubility in acetic acid (% of total protein content)	71.3(a)			66.9(a)	64.8(a)	63.4(a)	46.7(a)		
	75.2(b)		66.2(b) 65.0(c)	66.0(b)					10.0(c)(OCT)
Gelatinisation Enthalpy (J/g db)	4.8(e)				2.7(e)	3.3(e)			
	6.2(g)			4.9(g)	4.9(g)		4.4–4.9(g)		
	12.4(h)		12.4(h)			12.2(h)			
	10.2(i)			9.6(i)		9.9(i)	9.9(i)		
	5.8(j)		5.4(j)	4.9(j)			5.2–6.8(j)		
Adapted from (a) Aktan and Khan (1992), (b) Dexter and Matsuo (1977), (c) Dexter and Matsuo (1979), (d) de Zorzi <i>et al.</i> (2007), (e) Güler <i>et al.</i> (2002), (f) Icard-Vernière (1999), (g) Petitot <i>et al.</i> (2009), (h) Vansteelandt and Delcour (1998), (i) Yue <i>et al.</i> (1999), (j) Zweifel <i>et al.</i> (2000).When several varieties of durum wheat were tested, average data were calculated.LT: Low temperature; HT: High temperature; VHT: Very high temperature; OCT: optimal cooking time. Optimal cooking time is indicated when the white core of the pasta disappeared when squeezed between two glass plates (Approved method 66–50 AACC 2000).									
^a Data are given for cooked pasta, whatever the drying profile.									

granules which require less energy to melt (Biliaderis, 1990).

Sheeting. CLSM observations of freshly sheeted durum wheat pasta revealed that the proteins are closely associated with starch granules. With increasing sheeting passes (from 3 to 45 passes), the proteins and starch granules became distributed more uniformly throughout the dough. At the molecular scale, it induced a higher glutenin solubility in SDS due to protein disaggregation and depolymerisation (Kim *et al.*, 2008). A moderate damage of starch was also reported (Zardetto & dalla Rosa, 2005).

Extrusion vs. sheeting

The comparison between the sheeting and the extrusion process dough remains difficult because of the lack of comparative studies. Pagani, Resmini, and Dalbon (1989) studied the microstructure of fresh roll-sheeted and extruded pasta prepared from the same blend of common wheat flour (*Triticum aestivum*). In freshly extruded spaghetti, the protein matrix looked discontinuous with protein aggregates unevenly distributed among the starch granules, whereas in fresh roll-sheeted pasta, a compact and continuous protein network was observed. More recently, Fardet *et al.* (1998) characterised the protein network of cooked sheeted lasagne and cooked extruded spaghetti, both made from durum wheat semolina. Lasagne exhibited a more porous and open protein/starch network and completely gelatinised starch granules in the centre of the strand. In contrast, spaghetti was characterised by a more compact structure with some ungelatinised starch granules in the central region (Fardet *et al.*, 1998). The contrasted results of Pagani *et al.* (1989) and Fardet *et al.* (1998) could be mainly explained

by the different raw materials used in both studies (common wheat flour vs. durum wheat semolina). At a lower scale, starch was found to be damaged by sheeting to a lesser extent than during extrusion (Zardetto & dalla Rosa, 2005), due to the lower “intrinsic stress” (lower temperatures and pressures and a shorter processing time) (Carini, Vittadini, Curti, & Antoniazzi, 2009).

Drying

Since the 1970s, technological improvements in the pasta making process have induced an increase in drying temperatures: from low temperature (LT) (40–60 °C/70–80% relative humidity (RH)/18–28 h), to high temperature (HT) (60–84 °C/74–82% RH/8–11 h), and very high temperature (VHT) (>84 °C/74–90% RH/2–5 h), thus leading to shorter drying times and improved hygienic standards (Pollini, 1996). Moreover, the use of higher temperatures was also beneficial for the overall cooking quality of the final product, with higher firmness, lower stickiness and lower cooking loss (Aktan & Khan, 1992; Zweifel, Handschin, Escher, & Conde-Petit, 2003). These improved properties are the results of structural modifications within pasta: depending on the temperature-moisture conditions applied during drying, physicochemical modifications (protein denaturation and starch swelling) occur in a different extent.

When viewed under SEM, the surface of dried pasta presents numerous starch granules of different sizes (Fig. 1C) (Cunin *et al.*, 1995; Sadeghi & Bhagya, 2008). In addition, they appear to be associated with a protein film (Cunin *et al.*, 1995; Sadeghi & Bhagya, 2008). Some cracks and small holes are apparent in the protein matrix at the surface (Fig. 1C). This is probably partly due to

surface tension in spaghetti dough during drying and partly due to shrinkage during sample preparation for microscopic observations (Sadeghi & Bhagya, 2008). The internal structure of dry pasta is characterised by starch granules deeply embedded in a protein matrix (Fig. 1D) (Cunin *et al.*, 1995; Sadeghi & Bhagya, 2008).

At the molecular level, up to a drying temperature of 70 °C, minor changes in the extent of protein denaturation in pasta is observed: about 65% of proteins are soluble in acetic acid (Aktan & Khan, 1992; Dexter & Matsuo, 1977), and 63–70% are soluble in SDS (Petitot *et al.*, 2009) (Table 1). At higher temperatures, the protein solubility decreases drastically: at 90 °C, only about 50% of proteins are soluble in acetic acid (Aktan & Khan, 1992; de Zorzi *et al.*, 2007) and about 25% of proteins are soluble in SDS (Petitot *et al.*, 2009), which represents a 20–60% decrease in protein solubility compared to LT drying. Lamacchia *et al.* (2007) characterised proteins of dried pasta by SE-HPLC and observed a progressive decrease in the small and large monomeric proteins and an increase in the molecular size of large polymeric proteins as the drying temperature increased from 60 to 90 °C. This was accompanied by an increasing amount of total unextractable polymeric protein (UPP) (20–100%), i.e. proteins that were not soluble in SDS or after sonication. Singh, Donovan, Batey, and MacRitchie (1990) postulated that sonication causes shear degradation of disulphide bonds that connect glutenin subunits together. Sonication would not affect other types of covalent linkages (peptide bonds). This means that protein aggregates stabilised by irreversible protein interactions probably occurred through interpeptide cross-linking or Maillard-type reactions, as observed by de Zorzi *et al.* (2007) and Petitot *et al.* (2009) in pasta dried at VHT or by Micard, Morel, Bonicel, and Guilbert (2001) in pure gluten treated under severe conditions (130 °C, 20% moisture content).

However, disulphide bonds remain the main links formed during pasta drying. According to Favier *et al.* (1996), glutenins are highly heat sensitive: at 80 °C, they form intermolecular disulphide bonds and become insoluble in thermally treated fresh pasta (29% moisture content). At higher temperatures (>80 °C), gliadins are also involved and form disulphide bonds with the glutenin complex. This is in accordance with studies carried out on pure gluten. Weegels, Verhoek, Degroot, and Hamer (1994) studied the reactivity of gluten and found that major changes occurred when gluten was heated at 80 °C and for a moisture content of 25–30% (w/w), which can be compared to the conditions applied during pasta drying at high temperature. A decrease in protein solubility was observed, affecting mainly the glutenin fraction. Gliadins appear to react at temperatures higher than 100 °C (Singh & MacRitchie, 2004). Glutenins are able to form both intermolecular and intramolecular disulphide bonds whereas gliadins form only intra-chain disulphide bonds that can participate in disulphide-sulphydryl interchange (Singh & MacRitchie,

2004). However these observations concern the effect of temperature on gluten proteins in model system, and not in a complex matrix such as pasta where the interaction between pasta constituents has also to be considered.

Changes in pasta structure during drying cannot solely be explained according to the extent of protein denaturation. Changes in the starch fraction can also occur and are commonly analysed by polarized light microscopy (birefringence study), differential scanning calorimetry (DSC) and X-ray diffractometry, which reflect different levels of ordered structures (Zweifel *et al.*, 2000). When viewed under polarized light, starch granules exhibited different birefringence levels in dry pasta. Most starch granules of LT dried pasta retained their birefringence (Altan & Maskan, 2005), whereas approximately 20% of starch granules of HT and VHT dried pasta either partially or completely lost their birefringence (Güler, Koksel, & Ng, 2002). Moisture content and temperature conditions may have been reached locally to induce the gelatinisation of some starch granules. DSC analysis of the thermal properties of starch led to the detection of two endothermic transitions: the first one could be attributed to the gelatinisation of starch and the second corresponds to the reversible dissociation of pre-existing amylose-lipid complexes. Drying may promote partial melting of starch (Altan & Maskan, 2005; Güler *et al.*, 2002; Yue, Rayas-Duarte, & Elias, 1999) (Table 1) and the formation of amylose-lipid complexes (Yue *et al.*, 1999). However, the impact of the drying cycle on gelatinisation enthalpy has yet to be clarified. Yue *et al.* (1999) and Petitot *et al.* (2009) did not find any significant difference among LT, HT and VHT dried pasta. In contrast, a higher gelatinisation temperature and enthalpy was found by Güler *et al.* (2002) and Zweifel *et al.* (2000) in pasta when increasing the drying temperature. Zweifel *et al.* (2000) hypothesised that VHT drying may favour the molecular rearrangement of starch polymers. When analysed by X-ray diffractometry, no clear effect of the drying temperature in crystallinity was detected (Baiano & Del Nobile, 2006; Güler *et al.*, 2002; Zweifel *et al.*, 2000).

Cooking

In durum wheat pasta, starch gelatinisation and protein coagulation are the main changes in pasta structure that occur during cooking. In the interspaces between granules, protein coagulation and interaction lead to a continuous and strengthened network, which traps the starch while the latter, by swelling and gelatinisation, occludes these interspaces. Structural transformations in starch and proteins are in the same range of temperature and moisture conditions; proteins appear to react at a slightly lower moisture level (Fig. 2) (Cuq, Abecassis, & Guilbert, 2003). Both transformations are competitive (both component compete for water) and antagonistic (the swelling of starch granules is opposed to the formation of the protein network) (Pagani *et al.*, 1986). The faster the starch swells, the slower the rate of protein interaction and the weaker the protein network

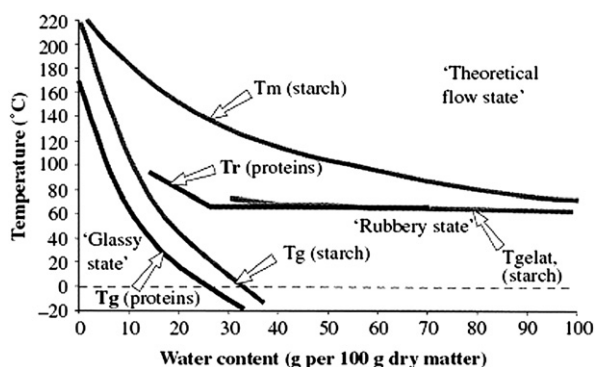


Fig. 2. Schematic representation of the temperature-water content state diagram for the main components in wheat flour (starch and gluten proteins). Tg is the glass transition temperature, Tm is the melting point, Tgelat is the starch gelatinisation temperature, and Tr is the minimum temperature for protein thermosetting (Cuq et al., 2003).

inside the spaghetti. Both transformations are controlled by water penetration inside the pasta strand during cooking. Until the optimal cooking time, the water uptake rate depends on the ability of water to diffuse through the matrix; and on the melting kinetics of the crystalline domains (del Nobile & Massera, 2000). Water, which acts as a plasticizer and increases polymer mobility, penetrates concentrically towards the centre of spaghetti with cooking time. The presence of a moisture gradient inside the strand can be revealed by magnetic resonance imaging (MRI) (Horigane et al., 2006; del Nobile & Massera, 2000). The lower moisture content in the core of pasta strand may lead to a stronger competition for water between protein and starch granules. The water may be not evenly distributed among components. A higher hydration of proteins locally may induce the formation of the protein network before the swelling of the starch granules. As a consequence of this moisture gradient, a continuous change in the structure from the outer surface towards the core characterizes the internal framework of cooked pasta (Heneen & Brismar, 2003).

Microscopic observations revealed that, on the smooth surface of cooked pasta, protein and starch are no longer distinguishable from one another (Fig. 3A), forming a thin film of about 1 μm thickness with some small cracks and open areas (Fardet et al., 1998; Heneen & Brismar, 2003).

After cooking pasta to its optimal cooking time, the internal structure of pasta can be divided into three concentric regions after cooking—an external region, an intermediate region, and a central region (Figs. 3 and 4) (Cunin et al., 1995; Heneen & Brismar, 2003; Petitot et al., 2009). In the external region, starch granules are largely deformed, swollen and it is still difficult to differentiate them from proteins when viewed under SEM or BFLM (Fig. 3D and 3G) (Fardet et al., 1998; Heneen & Brismar, 2003). The protein network is more clearly viewed under CLSM (Fig. 4). Whatever the drying profile, the external region

of cooked pasta is clearly distinguished from the intermediate and central regions (Petitot et al., 2009). It is characterised by a higher amount of thin protein films surrounding larger starch granules (Petitot et al., 2009). The intermediate region includes partly swollen granules embedded in a coagulated but dense protein network (Fig. 3C and 3F) (Fardet et al., 1998; Heneen & Brismar, 2003). The centre of the strand presents starch granules with a limited degree of gelatinisation (Fig. 3B and 3E), due to limited water absorption (Cunin et al., 1995).

At a smaller scale, the moisture content and temperature conditions during cooking are favourable for protein denaturation and aggregation. Indeed, a decrease in protein solubility in dilute acetic acid (Dexter & Matsuo, 1979) or in SDS (Petitot et al., 2009) was observed during cooking (Table 1). Depending on the previous drying profile applied, the loss of protein solubility due to cooking can be more or less accentuated. For example, when comparing the solubility of proteins in pasta dried at different temperatures, it appeared that pasta dried at VHT underwent less marked changes during cooking compared to LT and HT dried pasta because most of the proteins were already aggregated during the drying step (Petitot et al., 2009). Concerning the starch fraction, complete loss of birefringence is obtained when pasta is cooked to the optimal cooking time (Grzybowski & Donnelly, 1977; Marshall & Wasik, 1974). No gelatinisation endotherm is observed by DSC, suggesting the complete gelatinisation of starch in cooked pasta (Fardet et al., 1999; Petitot et al., 2009).

Cooking is an essential step in which major structural transformations occur. However, these transformations are also dependent on those occurring during the previous processing steps. Indeed, the effects of some process parameters such as forming and drying are still detected after cooking. For example, forming seems to mainly affect the pasta porosity. Extruded spaghetti exhibits a slightly less porous and less open protein/starch network than laminated lasagne made from durum wheat semolina (Fardet et al., 1998). Drying appears to be essential for maintaining the pasta structure, especially during overcooking: the protein network of LT (55 °C) dried and then cooked pasta, viewed on CSLM images, is partly disrupted and has lost its continuity. The application of VHT (100 °C) at a low moisture level preserves the microstructure of pasta, even after prolonged cooking (Zweifel et al., 2003). Moreover, the extent of starch swelling is lower in VHT (100 °C) pasta, with the effect being more marked for pasta when the VHT phase is applied at a low moisture level (Zweifel et al., 2003).

The final structure of pasta is therefore the result of successive changes occurring throughout the pasta making process and mainly affecting the starch and protein fractions. Modification of process parameters could therefore modify the pasta structure and impact its nutritional properties. Indeed, as discussed in the following section, the nutritional properties of pasta are closely linked to its structure.

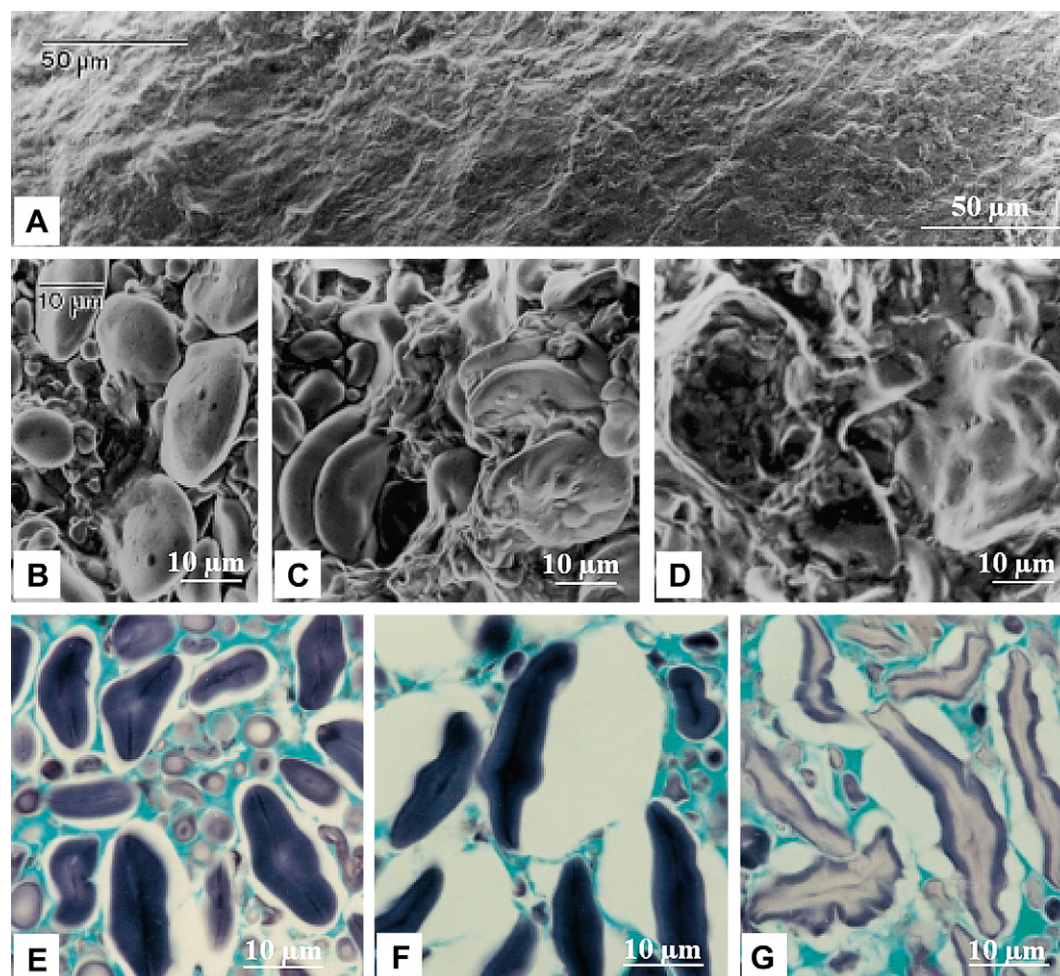


Fig. 3. Low voltage scanning electron microscopy (A, B, C, D) and light microscopy (E, F, G) of cooked HT-dried spaghetti. A: Surface of spaghetti, B–G: internal structure of spaghetti. From the left to the right: from the centre to the periphery of spaghetti, E–G: fast green-iodine staining: proteins stain green while starch granules stain blue-brown. Adapted from Heneen and Brismar (2003).

Microstructure of cooked pasta: impact on starch and protein digestibility and potential allergenicity

Pasta is a carbohydrate-based food (~70% starch) that is considered to be a source of slow-release carbohydrates, therefore possessing a low glycaemic index (GI) (Granfeldt & Bjorck, 1991; Jenkins *et al.*, 1983). The glycaemic index (GI) has been introduced in order to estimate the blood glucose response of foods after ingestion by humans. It is measured by the postprandial glycemic area of a test meal, expressed as the percentage of the corresponding area of the reference food (glucose of white bread). As human subjects would have to be recruited to measure GI, which is also time consuming, *in vitro* testing is commonly used as a faster method to predict *in vivo* GI (Casiraghi, Brighenti, & Testolin, 1992; Fardet *et al.*, 1999). This is based on *in vitro* measurement of the susceptibility of starch to digestive enzymes. Indeed, the glycaemic response and consequently the insulin demand appear to be closely related to the enzymic susceptibility of starch (Jenkins, Taylor, & Wolever, 1982). The enzymic susceptibility of starch

depends on the special organisation and structural state of pasta components. Hence, a modification in the pasta structure through a pasta processing change could have an impact on the rate of starch degradation, i.e. the pasta GI.

Numerous structural factors at the macroscopic, microscopic and molecular scales have been suggested to explain the slow rate of starch degradation in pasta.

At the macroscopic level (>100 µm)

The pasta size (i.e. vermicelli *vs.* spaghetti) and shape (i.e. macaroni *vs.* spaghetti), which depend on the forming step, seem to be of great importance. In spaghetti (large pasta size) starch was less susceptible to α -amylase (slower degradation rate) than in vermicelli (smaller pasta size). Higher surface to weight ratio of vermicelli *vs.* spaghetti may explain the higher accessibility of amylase to starch (Granfeldt, Bjorck, & Hagander, 1991). The effect of the pasta shape was investigated by Wolever *et al.* (1986). These authors found that macaroni produced a significantly higher *in vivo* glucose response than spaghetti (68 ± 8 *vs.*

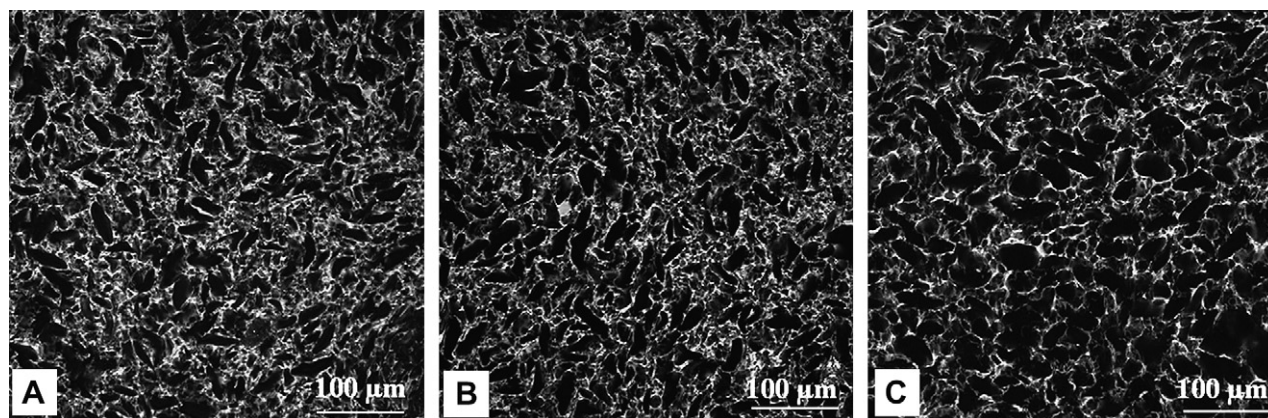


Fig. 4. Confocal laser scanning microscopy of HT-dried cooked pasta in the central (A), intermediate (B) and external (C) regions of pasta strand. Proteins were stained with fuchsin acid. White areas correspond to proteins and dark areas can be assimilated to starch granules (Petitot *et al.*, unpublished data).

45 ± 8) but did not find any correlation between the pasta GI and surface area of pasta.

Grinding spaghetti led to a higher enzyme susceptibility of starch granules compared to intact spaghetti (Colonna *et al.*, 1990). The destruction of pasta structure probably facilitated the diffusion of α -amylase. To assess the importance of the food structure with respect to the starch degradation rate, Granfeldt and Björck (1991) compared the carbohydrate digestion of spaghetti, spaghetti porridge and bread made from the same raw material. Spaghetti produced significantly lower GI (GI = 61) than bread (GI = 100), as a result of different structures obtained after different manufacturing and cooking processes. Bread is characterised by an open structure with large holes and highly swollen starch granules whereas pasta presents a denser structure (Brennan, Symons, & Tudorica, 2005). More interestingly, disintegration of spaghetti into a coarse 'porridge' significantly increased the glucose response (GI = 73) compared to intact spaghetti (GI = 61), thus highlighting the impact of the food structure, especially the particle size.

At the microscopic level (0.3–100 µm)

In order to demonstrate the protective role played by the protein network and fibres, several authors have studied the *in vitro* enzymic susceptibility of starch after removal of the protein network or inclusion of fibres. The encapsulation of starch granules by fibres (Tudorica *et al.*, 2002), and proteins (Colonna *et al.*, 1990; Fardet *et al.*, 1998) was found to limit the accessibility of α -amylase to starch.

Inclusion of high quantities of insoluble fibres (e.g. pea fibres) may disrupt the protein matrix, giving rise to a highly porous structure. The starch granules become more accessible, hence more susceptible to enzyme degradation (Tudorica *et al.*, 2002). In contrast, inclusion of soluble fibres (e.g. guar) may induce the entrapment of starch granules within a viscous protein-fibre-starch network, which acts as

a protective coat, leading to reduced glucose release (Tudorica *et al.*, 2002).

In order to highlight the protective role played by proteins, Colonna *et al.* (1990) removed the protein network of cooked pasta by Pronase® and found that starch degradation was increased. They also suggested that the application of high drying temperatures could lead to a high level of protein cross-linking, in turn leading to a more intense encapsulation of starch, thus decreasing its susceptibility to enzymes. Moreover, starch encapsulation by proteins could limit water absorption by starch granules, therefore limiting enzyme diffusion and decreasing α -amylolysis kinetics (Colonna *et al.*, 1990). However, according to Fardet *et al.* (1998), starch granules are not fully encapsulated by the protein network, whose porosity is high enough (0.5–40 µm) to allow α -amylase to diffuse freely. These authors suggested other hypotheses to explain the low GI of pasta: the tortuosity of the protein network which lengthens the pathway of α -amylases; and the presence of some high molecular weight starch polymers naturally resistant to enzymic attack.

At the molecular level (0.8–50 nm)

The physical structure of starch, such as its degree of gelatinisation (Holm, Bjoerck, & Eliasson, 1988), retrogradation (Akerberg, Liljeberg, & Björck, 1998), and amylose-to-amylopectin ratio (Akerberg *et al.*, 1998; Holm *et al.*, 1988) also influence the rate of starch degradation. The more gelatinised the starch is, the more available it is to amylases (Holm *et al.*, 1988). However, microscopic observations (Pagani *et al.*, 1986), DSC measurements (Petitot *et al.*, 2009) and X-ray diffractometry analyses (Colonna *et al.*, 1990) have shown that starch granules are completely gelatinised in pasta cooked to its optimal cooking time. The consumption of a high amylose pasta meal (amylose content $\geq 39.6\%$ of carbohydrate) (obtained by the replacement of 20% durum wheat flour by high amylose corn starch) induced a lower glucose and insulin response in

humans compared to those who had consumed a moderate amylose pasta meal (amylose content 25.9% of carbohydrates) (obtained by the replacement of 20% durum wheat flour by normal amylose corn starch) (Hospers, van Amelsvoort, & Weststrate, 1994). These authors suggested that a larger amount of slowly digestible amylose aggregates and resistant retrograded amylose starch may have been formed. Indeed, upon cooling, gelatinised starch retrogrades (especially amylose) and becomes resistant to hydrolysis (Svihus, Uhlen, & Harstad, 2005).

The gelatinisation of starch is not the factor limiting its degradation in cooked pasta. The use of high amylose starch and/or the presence a retrograded starch could induce lower starch hydrolysis in cooked pasta. Moreover, amylose is also prone to react with other food components such as lipids. The formation of these complexes was shown to reduce the rate of amylolysis in rats (Holm et al., 1983), probably due to the reduction of surface contact between the enzyme and its substrate and the decrease in starch swelling due to increasing hydrophobicity (Svihus et al., 2005).

As shown in this review, the slow rate of starch degradation could be explained by the specific structure of pasta at the macroscopic, microscopic and molecular scales. This specific structure is the result of successive structural changes occurring throughout the pasta making process. However, it is hard to establish a definite link between pasta processing and carbohydrate digestion because of the lack of studies. It has been demonstrated, for example, that an increase in screw speed (150 to 250 rpm) or temperature (40 °C to 70 °C) during extrusion does not seem to affect *in vitro* starch hydrolysis in extruded cooked pasta (Fardet et al., 1999). In sheeted pasta, Kim et al. (2008) suggested that several sheeting passes (3 to 45) contributed to the disruption of protein and starch interactions, leading to an increase in starch accessibility to α -amylase. The impact of pasta drying has also been studied. VHT dryings were shown to decrease the *in vitro* digestibility of starch in cooked pasta (Casiraghi et al., 1992; Colonna et al., 1990; Petitot et al., 2009), but no effect on *in vivo* GI was observed (Casiraghi et al., 1992). However, pasta products were tested by only six volunteers in this study. Heat is known to induce the formation of protein aggregates. Cysteine residues and disulphide bonds that are not accessible in the native conformation can become available and may react to form intermolecular cross-links (Visschers, 2005). Colonna et al. (1990) thus hypothesised that the formation of a high level of protein intermolecular cross-linking induced by VHT drying may lead to more intense starch encapsulation, thus decreasing its susceptibility to enzymes.

The structure of pasta has been well studied in order to identify the factors which control starch degradation. In comparison, the impact of processing on the protein digestibility has received less attention. Yet, the problem of protein degradability in the gastrointestinal tract is of particular relevance for proteins that act as food allergens after ingestion. Allergenicity of a food can be modified by changing the

IgE-binding epitopes of the potential allergens. An epitope is the distinctive molecular shape on the allergenic proteins which interacts with antibodies. Linear epitopes are made from a few amino-acids of the polypeptide chain in their linear order while conformational epitopes are made from a few amino-acids from different parts of the sequence, brought together by the folding of the polypeptide chain (Davis, 1998). No single figure can be traced to link structure and allergenicity: allergenicity results from a complex balance between formation and destruction of IgE-binding epitopes. Changing 3D-structure of allergens, for example by processes like thermal treatments, can lead to abolition of conformational epitopes and/or masking/demasking of epitopes. Obviously, allergenicity also relates to susceptibility of allergens to enzymes as digestion of proteins may lead to destruction of some epitopes. Then, mechanisms that increase resistance to enzymes (such as aggregation) are likely to increase allergenicity. In particular, thermal treatments can induce modifications of the physicochemical and immunological characteristics of the potential allergens by affecting the protein digestibility (Davis, 1998).

Only a few studies have been devoted to the characterisation of durum wheat (*T. durum*) allergens compared to bread wheat (*T. aestivum*) but it has been demonstrated that durum wheat semolina allergens have an allergenic potential similar to that of bread wheat flour (Simonato et al., 2004). Gluten proteins were suggested to be responsible for IgE-mediated reactions (exercise induced anaphylaxis and atopic dermatitis) after ingestion of wheat-based products (Simonato et al., 2004); in particular, gliadins were identified as important allergens (Rasanen et al., 1994; Varjonen, Vainio, & Kalimo, 1997, 2000). Since pasta processing modifies protein structure, it may also affect the potential allergenicity of wheat proteins. For example, Simonato et al. (2001) demonstrated that the baking process decreased the digestibility of the potential allergens in bread. These authors compared the protein digestibility and allergenicity of unheated bread dough, bread crumb and bread crust which can be considered as two heat treatments of different intensities (<100 °C and >180 °C respectively). High temperatures induced protein aggregation that involved probably mainly disulphide bonds and hydrophobic interactions in the bread crumb and strong irreversible interactions (different from hydrophobic interactions or disulphide bonds) in the bread crust (Pasini et al., 2001; Simonato et al., 2001). This protein aggregation prevented the complete proteolytic degradation of the allergenic proteins which could elicit the allergic response in the gastrointestinal tract. In contrast to bread, processing semolina into cooked pasta did not change the total protein digestibility, although cooked pasta was more resistant to pepsin digestion compared to semolina. Moreover, all the potential allergens (>1 kDa) were degraded after peptic/pancreatic digestion in both semolina and cooked pasta, although the presence of potentially allergenic protein fragments with very low Mr (<1 kDa) in digested pasta cannot be excluded

(Simonato *et al.*, 2004). However, as seen for bread, it was demonstrated that the application of a very high drying temperature (90–110 °C) decreased the *in vitro* digestibility of proteins in cooked pasta by about 10% compared to a low drying temperature (Petitot *et al.*, 2009; de Zorzi *et al.*, 2007). The higher resistance of proteins to digestion could be attributed to the presence of highly aggregated proteins stabilised by covalent protein interactions, such as interpeptide cross-linking and Maillard-type aggregates (Petitot *et al.*, 2009; de Zorzi *et al.*, 2007). Indeed, non-enzymatic browning related to the Maillard reaction readily occurs during pasta drying, especially at high and very high temperatures (Anese, Nicoli, Massini, & Lerici, 1999; Resmini & Pellegrino, 1994). In contrast to what was observed in bread, a modification of the degradability of proteins did not affect their allergenic properties after ingestion (Petitot *et al.*, 2009; de Zorzi *et al.*, 2007). More studies should be carried out to confirm these results. Moreover, the application of high drying temperatures could induce a decrease in the lysine bioavailability—about 30% of lysine could become unavailable in pasta processed under high temperature drying cycles (Resmini & Pellegrino, 1994). This decrease in lysine bioavailability could be due to the protein-carbohydrate Maillard reactions which involve reducing sugars and the terminal amino group of a free amino acid, in particular lysine (Acquistucci, 2000).

Conclusion

Among cereal products, pasta is an interesting source of slow-release carbohydrates. Attempts have been made to correlate the low glycaemic index of pasta with its structural properties at different scales. The compact structure of pasta, the encapsulation of starch by proteins and the physical structure of starch (retrogradation degree, amylose-to-amylopectin ratio) are the main hypotheses that have been put forward to explain the reduced enzymic susceptibility of starch in cooked pasta.

This specific structure is the result of successive changes occurring throughout the pasta making process. Forming and drying appear to be essential industrial steps which should be monitored in order to control the carbohydrate digestion of pasta. As the extrusion step gives pasta its compact structure, it would be interesting to determine whether a modification in the extrusion parameters could modify the density of extruded pasta and further decrease the digestibility of starch in cooked pasta. At the molecular level, starch and protein components undergo minor changes during extrusion. The drying step mainly affects the protein fraction. The use of high drying temperatures would allow preservation of the protein network at the microscopic scale, even after prolonged cooking. At the molecular level, the higher the drying temperature, the higher the protein insolubilisation and aggregation. Moreover, depending on the moisture and drying conditions applied, very high temperature may promote the formation of protein aggregates stabilised by irreversible protein interactions, probably through interpeptide cross-

linking or Maillard-type reactions. Structural modifications obtained especially at high drying temperatures could strengthen the protein network, and therefore better preserve starch from enzymic attack in cooked pasta. However, very high drying temperatures have been shown to decrease protein digestibility and lysine bioavailability (an essential amino acid). Moreover, the use of high drying temperatures would not affect the potential allergenicity of wheat proteins after ingestion.

Processes should thus be controlled so as to maintain or enhance the nutritional qualities of food products. This supposes the control of structural transformations of protein and starch fractions but questions remain unanswered. Should processes be optimised/developed in order to change the structural state of the protein or/and the starch fractions? Should starch granules be maintained in their native form (thus preventing starch damage and gelatinisation) or transformed into resistant starch? What is the best balance between the formation of a strengthened protein network (which protects starch from enzymic attack) and the decrease in lysine bioavailability, for example?

Regardless of the answer, control of the pasta nutritional properties would require control of the food structure, which depends on the process parameters applied. However, so far only a few authors have assessed, in a single study, the impact of processing on pasta structure as well as the inherent consequences on its nutritional properties. For pasta, as for any food, a multidisciplinary and integrated approach is now needed for rational food design, in order to be able to control digestion and nutrient absorption through the food structure. This implies that food should no longer be regarded as a simple source of essential nutrients but instead as an assembly of macromolecules forming a complex structure resulting from structural modifications occurring at different scales during the manufacturing process. Control of the nutritional properties of a food would require the control, adaptation of standard processes or even development of new processes.

Acknowledgements

This work was carried out under the « Programme National de Recherche en Alimentation et nutrition humaine », project « ANR-05-PNRA-019, PASTALEG » that received the financial support of the « ANR- Agence Nationale de la Recherche — The French National Research Agency »

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