

POTENTIAL ALLERGENS IN DURUM WHEAT SEMOLINA AND PASTA: FATE DURING COOKING AND DIGESTION

I POTENZIALI ALLERGENI DELLA SEMOLA DI GRANO DURO E DELLA PASTA: EVOLUZIONE DURANTE LA COTTURA E LA DIGESTIONE

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ABSTRACT

The aim of this study was to identify the potential allergens of durum wheat semolina and pasta and to determine their fate during pasta cooking and proteolytic digestion. By using sera of patients with food allergy to wheat, the potential allergens of durum wheat semolina were detected by IgE-immunoblotting and compared to those detectable in a commercial pasta sample. IgE binding to some semolina bands disappeared in raw pasta, whereas binding to other semolina allergens re-

RIASSUNTO

Lo scopo di questo lavoro è l'identificazione dei potenziali allergeni presenti nella semola di grano duro e nella pasta e lo studio della loro evoluzione durante la cottura e la digestione proteolitica. Utilizzando sieri di pazienti con allergia alimentare al grano, sono stati individuati, tramite IgE immunoblotting, i potenziali allergeni presenti nella semola di grano duro e in un campione di pasta commerciale. Alcune bande riconosciute dalle IgE nella semola non erano più rilevabili nella pa-

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mained unaffected. Cooking pasta did not change the IgE binding pattern. However, in contrast to what was previously observed for bread, *in vitro* digestion of cooked pasta resulted in almost the complete disappearance of its potential allergens with Mr > 1 KDa, indicating that they may be completely degraded before reaching the intestine. Therefore, although durum wheat seems to contain potential allergens similar to those of bread wheat, patients with food allergy to wheat may tolerate pasta better than bread.

sta, mentre altri allergeni rimanevano inalterati. La cottura della pasta non aveva effetto sul legame con le IgE. Comunque, al contrario di quanto precedentemente osservato per il pane, la digestione *in vitro* della pasta cotta provocava la quasi totale scomparsa dei potenziali allergeni, indicando che essi potrebbero essere completamente degradati prima di raggiungere l'intestino. Quindi, anche se il grano duro sembra contenere allergeni simili a quelli individuati nel grano tenero, i pazienti con allergia alimentare al grano potrebbero tollerare meglio la pasta del pane.

INTRODUCTION

It is estimated that more than 2% of the adult population of the developed countries suffer from food allergy and the incidence of this pathology seems to be continuously increasing (ORTOLANI *et al.*, 2001). Wheat is often included in the list of the most allergenic foods. In fact, in addition to problems derived from contact with or inhalation of wheat flour particles, ingestion of wheat-based foods, such as bread or pasta, can cause different problems of an allergic nature. These include atopic dermatitis (SAMPSON, 1992; VARJONEN *et al.*, 2000), food-dependent, exercise-induced anaphylaxis (FDEIA) (GUINNEPAIN *et al.*, 1996; VARJONEN *et al.* 1997), or gastrointestinal (GI) symptoms; the latter suggest an involvement of wheat proteins in eliciting an intestinal IgE-mediated reaction (SAMPSON, 1989; SIMONATO *et al.*, 2001a).

The proteins of wheat endosperm are classically divided into four solubility classes (OSBORNE, 1907): water-soluble albumins (about 15% of the total proteins), salt (e.g. 0.5 M NaCl-soluble globulins (about 5% of the total proteins), water/salt-insoluble monomeric gliadins

(about 40% of the total proteins) and the polymeric glutenin, which is made up of high- and low-molecular-weight subunits (HMW-GS and LMW-GS, respectively) linked together by disulphide (SS) bonds. While most of the components of the soluble protein fraction have a physiological function (enzyme, enzyme inhibitors), gliadins and glutenin, also known as prolamins or gluten proteins, are the storage proteins of the seed. These latter proteins are considered the key factors that allow the wheat flour to be processed into a visco-elastic dough which can be transformed into leavened products or pasta (MACRITCHIE, 1992).

The main allergens of bread wheat flour (*Triticum aestivum*) have been identified in previous studies. They are proteins belonging to both the soluble (PFEIL *et al.*, 1990; WEISS *et al.*, 1997) and insoluble fractions (SANDIFORD *et al.*, 1997, SIMONATO *et al.*, 2001a). It has also been suggested that the soluble proteins, due to their solubility in "physiological" conditions, are mainly involved in causing the allergic symptoms that arise after flour inhalation, whereas the insoluble gluten proteins are those that elicit allergic reactions after ingestion of wheat-based products (BALDO and WRI-

GLEYS, 1978; SUTTON *et al.*, 1982, VARJONEN *et al.*, 1997, SIMONATO *et al.*, 2001a). These foods are always prepared by using some type of thermal processing, such as cooking, baking, extrusion, etc., and therefore it is important to know how these treatments can affect the allergenicity of the flour proteins. It is well-known that these proteins are modified after heat processing and the extent of the changes depends on the intensity of the heat treatment. These modifications involve not only protein breakdown, but also aggregation, cross-linking and Mailard-type reactions (HANSEN *et al.*, 1975).

While numerous studies have been conducted on the allergenic proteins of bread (hexaploid) wheat (*Triticum aestivum*) flour, only a few data are available on the potential allergens of durum (tetraploid) wheat (*Triticum turgidum* subsp. *durum*) semolina (KUSABA-NAKAYAMA *et al.*, 2000; SANCHEZ-MONGE *et al.*, 1997; SIMONATO *et al.*, 2002). These two species are closely related and the proteins of durum wheat are very similar to those of bread wheat (AUTRAN *et al.*, 1979). Therefore, a similar reactivity toward the serum IgE of allergic patients would be expected. However, the most important foods prepared with durum and bread wheat (i.e. pasta and bread, respectively) undergo different thermal treatments during processing. The temperatures used in pasta preparation (including both industrial drying and home cooking) are generally lower than those used in bread-making. In a previous paper, PASINI *et al.* (2001) showed that bread-baking strongly affects the immunological and physicochemical properties of bread wheat proteins. The degradability of the proteins by the GI proteases is reduced and the IgE binding pattern is modified; the extent of these effects depends on the degree of thermal treatment (SIMONATO *et al.*, 2001b). Variations in the potential allergenicity of durum wheat proteins during pasta-making and cooking are

not known. Using sera of allergic patients who suffer from GI symptoms after ingesting wheat-based food, the IgE-binding proteins found in both durum wheat semolina and a commercial pasta sample were analysed and the effects of pasta cooking and gastrointestinal digestion on the fate of the potential durum wheat allergens were studied.

MATERIALS AND METHODS

Patients

Eighteen adults free of any gut disease who reported gastrointestinal symptoms (diarrhoea, abdominal pain and/or bloating) after ingesting food containing wheat were selected (SIMONATO *et al.*, 2001a). Nine of these patients were positive in wheat-specific IgE detection using the CAP-FEIA system (Pharmacia, Uppsala, Sweden), while the others were negative or not determined by the same test (SIMONATO *et al.*, 2001a) (Table 1). Sera belonging to the two groups were pooled and used in immunoblotting experiments.

Chemicals

Pepsin (E.C. 3.4.23.1) from hog stomach (≈ 3000 U/mg) was from Fluka (Milan, Italy). Pancreatin from porcine pancreas and anti-human IgE peroxidase conjugate antibody were from Sigma (Milan, Italy). Nitrocellulose membranes were from Sartorius (Firenze, Italy). The GelCode Glycoprotein staining kit and the Super Signal detection kit were from Pierce (Rockford, IL, USA). All the other chemicals were of analytical grade.

Sample preparation

The water/salt-soluble and salt-insoluble (prolamins) proteins of durum wheat semolina (cv. Lira) were extracted as previously described (SIMONATO *et al.*, 2001a).

Table 1 - Allergological features of the patients.

Pool	Patient	Total IgE (kU/L)	SkinPrickTest wheat ^a	Specific IgE ^b	
				wheat	gluten
1	1	265	Pos.	2	0
	2	2.713	Pos.	2	1
	3	1.053	Pos.	3	0
	4	359	Pos.	3	0
	5	114	Pos.	1	0
	6	470	n.d.	2	0
	7	n.d.	Pos.	3	0
	8	370	Pos.	5	0
	9	37	Neg.	1	0
2	10	2	Neg.	0	0
	11	3	Neg.	0	0
	12	12	Neg.	0	0
	13	2.118	Neg.	0	0
	14	14	n.d.	0	0
	15	42	n.d.	0	0
	16	271	Neg.	0	0
	17	323	Neg.	0	0
	18	508	Pos.	0	0

(a) Pos.: positive reaction; Neg.: negative reaction; n.d.: not determined; (b) CAP-FEIA values.

Briefly, five grams of semolina were extracted with 50 mL of 0.5 M NaCl for 1 h at 4°C. After centrifugation (12,000 g, 20 min at 4°C), the supernatant containing the water/salt soluble proteins was collected and stored at -20°C. The residue was washed twice with 0.5 M NaCl (100 mL) and once with water. The washed residue was then extracted overnight with 50 mL of 80 mM Tris-HCl, pH 8.0, 50% (v/v) propan-2-ol, containing 20 mM dithiothreitol at room temperature with continuous stirring. After centrifugation (as above), the supernatant, containing the salt-insoluble proteins (prolamins), was collected and stored at -20°C.

A commercial pasta sample (Penne) was used either raw or after cooking in boiling salted water for 10 min. The cooked pasta was then freeze-dried. Raw pasta and freeze-dried cooked pasta were ground to a fine powder in a mortar and sieved.

Total proteins were extracted from 100 mg of semolina and from raw and cooked pasta powder with 4 mL of 100 mM Tris-HCl buffer, pH 8.3, containing 6 M urea, 2.5% (w/v) sodium dodecyl sulphate (SDS), 2.5% (v/v) 2-mercaptoethanol (2-ME) and 10% (w/v) glycerol and heated for 5 min at 100°C. After centrifugation (12,000 g, 20 min), the supernatant (total protein extract) was collected and stored at -20°C.

Protein digestion

In vitro protein digestion of durum wheat semolina and cooked pasta was performed as previously described (PASINI *et al.*, 2001). Briefly, 60 mg of sample were suspended in 4 mL of 0.2 N HCl (pH 2.0) containing 0.05 mg/mL of pepsin. After 30 min at 37°C, 1.15 mL of 1 M boric acid, 0.5 N NaOH, adjusted to pH 6.8 with 5 N HCl and containing 0.25

mg/mL of pancreatin were added. The resulting pH was 7.6. The reaction was performed at 37°C in a shaking water bath and stopped at different times (0, 15 and 30 min of pepsin attack and 15, 30, 90, 150 min of pancreatic digestion) by adding 1 volume of 20% (w/v) trichloroacetic acid (TCA). After standing for 1 h, the samples were centrifuged (8,000 g, 10 min) and the pellets were recovered. Pellets were mineralized according to the method of HACH *et al.* (1985) and nitrogen was quantified by the AOAC method 33.056 (AOAC, 1984).

When the digested samples were to be analysed by electrophoresis, the reaction was stopped after 30 min of pepsin attack and after 150 min of pancreatic digestion by adding 0.5 volumes of 0.6 M Tris-HCl pH 7.4, containing 30% (w/v) glycerol, 6% (w/v) SDS and 6% (v/v) 2-ME. The samples were then heated to 100°C for 5 min, centrifuged and then loaded on the gel.

Electrophoresis

Water/salt-soluble proteins, prolamins and total proteins were analysed by SDS-PAGE according to LAEMMLI (1970), with a total polyacrylamide concentration of 16%. Before electrophoresis the samples were treated as follows: an aliquot of the water/salt-soluble protein solution was diluted with 0.25 volume of a 0.5 M Tris-HCl buffer, pH 7.2, containing 10% (w/v) SDS, 10% (v/v) 2-ME, 40% (w/v) glycerol and heated for 5 min in a boiling water bath.

Prolamins were precipitated from 1 mL of solution by adding 9 mL of cold acetone. After standing at 4°C for 15 min, the suspension was centrifuged and the supernatant removed. The air-dried pellet was then solubilised with 2.5 mL of 0.1 M Tris-HCl, pH 7.2, containing 2% (w/v) SDS, 2% (v/v) 2-ME and 8% (w/v) glycerol and heated as above. Electrophoresis was run at a constant 50 mA.

Digested samples and undigested con-

trols were analysed by tricine-SDS-PAGE (SHÄGGER and VON JAGOW, 1987) in a 16% total polyacrylamide gel as previously described (PASINI *et al.*, 2001). Electrophoresis was run at a constant 30 mA.

Gels were stained with Coomassie Brilliant Blue according to KOENIG *et al.* (1970) or used for blotting. The molecular weight standard proteins (Bio Rad) were phosphorylase B (97.4 k), bovine serum albumin (66.2 k), ovalbumin (45 k), carbonic anhydrase (31 k), soy trypsin inhibitor (21.5 k) and lysozyme (14.4 k).

IgE Immunoblotting

Detection of IgE binding to durum wheat semolina and cooked pasta proteins before and after digestion was performed as previously described (SIMONATO *et al.*, 2001b). Briefly, after PAGE separation, proteins were transferred onto nitrocellulose membranes by semi-dry electro-blotting, blocked with tris buffer saline (TBS) containing 0.05% Tween 20 (TBS-T) and 5% skim milk powder (M-TBS-T) for 4 h and then incubated overnight with pooled sera from the allergic patients diluted with 0.5 volumes of M-TBS-T. After 5 washings with M-TBS-T, blots were incubated for 1 h with an anti-human IgE peroxidase-conjugate antibody diluted 1:6000 in M-TBS-T. After 4 washings with TBS, peroxidase activity was visualized by chemio-luminescence using the Super Signal detection kit, following the manufacturer's instructions.

Pooled sera from both healthy subjects and patients with wheat-unrelated allergy were used as negative controls.

Carbohydrate detection

Carbohydrates were stained on electrophoretic gels by the periodate method, using the GelCode Glycoprotein staining kit.

RESULTS

Electrophoretic analysis of semolina, raw and cooked pasta proteins

The electrophoretic analysis of semolina and pasta proteins showed the classical main protein components of durum wheat, including water/salt soluble proteins (ranging from 60 to about 14 kDa) and salt-insoluble components, namely HMW-GS (Mr 92-100 kDa), LMW-GS (Mr 45-60 kDa) and gliadins (Mr 31-45 kDa) (Fig. 1A).

No clear differences could be observed among the total protein extracts of semolina, raw and cooked pasta samples, although some loss in HMW-GS band staining along with the appearance of aggregated protein material at the top of the gel seemed to be present in both raw and cooked pasta samples (Fig. 1A, asterisk). Staining the gel for carbohydrate detection indicated the presence of sugars at the top of the resolving gel only in

the pasta sample (Fig. 1B, asterisk). This suggests that at least part of the protein aggregates detected by Coomassie staining contained some glycation products, which were probably derived from Maillard-type reactions that occurred during pasta processing (DEXTER *et al.*, 1984).

Moreover, the low-molecular albumin fraction (around 14-16 kDa), corresponding to the alpha-amylase inhibitor protein family (CARBONERO and GARCÍA-OLMEDO, 1999), were detected in both the semolina and pasta samples (Fig. 1A).

IgE immunoblotting analysis of semolina, raw and cooked pasta proteins

Pooled sera of the selected patients, all suffering from GI symptoms after wheat ingestion (SIMONATO *et al.*, 2001a), were used to evaluate the IgE binding to semolina and raw and cooked pasta proteins (Fig. 2). When semolina

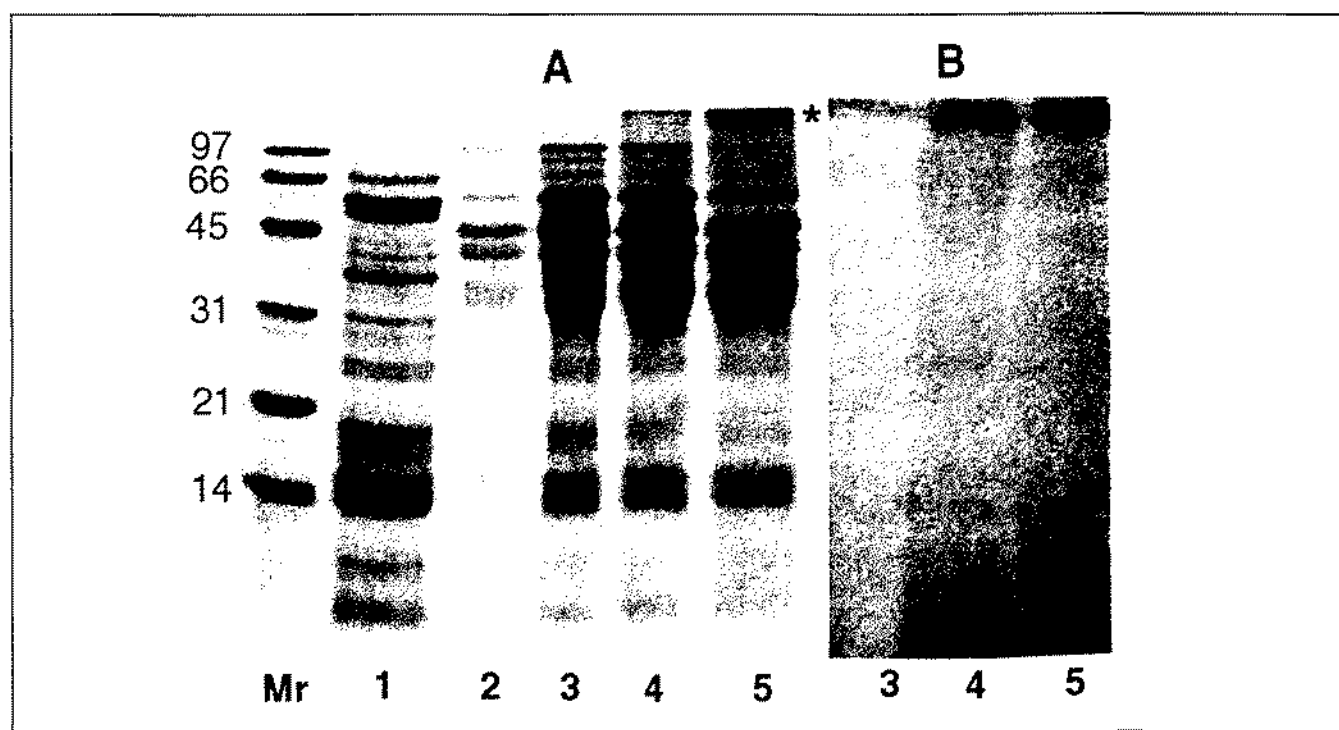


Fig. 1 - SDS-PAGE analysis of durum wheat and pasta proteins. Mr: molecular weight standard proteins. Lanes 1 and 2: water/salt-soluble and insoluble proteins from semolina, respectively. Lanes 3, 4 and 5: total proteins from semolina and raw and cooked pasta, respectively. Part A: staining for total proteins, Part B: staining for carbohydrates.

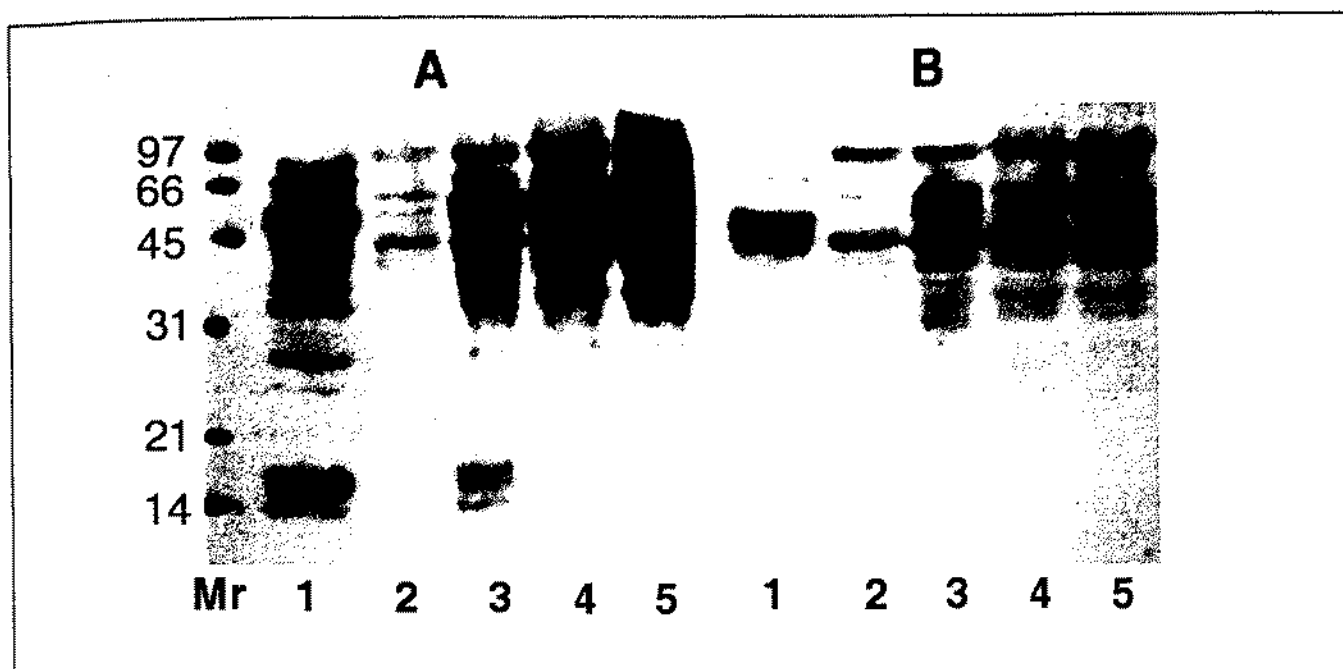


Fig. 2 - IgE immunoblotting with pooled sera of patients with food allergy to wheat. Part A: CAP-positive patients. Part B: CAP-negative patients. Samples are the same as in Fig. 1.

samples were examined, both sera pools showed IgE binding to both water/salt-soluble and salt-insoluble (prolamins) protein bands. In particular, a salt-insoluble component with an Mr around 42 kDa was strongly detected in all the samples. A LMW-GS with a similar Mr was previously shown to be a major allergen of wheat flour (SIMONATO *et al.*, 2001a; MARUYAMA *et al.*, 1998). In contrast, the low-Mr albumin components, which correspond to the major allergens involved in baker's asthma (GOMEZ *et al.*, 1990), were recognized by the pooled sera of the CAP-positive patients (Fig. 2A) but not by the pooled sera of the CAP-negative patients (Fig. 2B). These results confirmed the results obtained with bread wheat (SIMONATO *et al.*, 2001a). IgE binding to these bands was not detectable in either raw or cooked processed pasta. Although these protein components were detected by Coomassie staining in both raw and cooked processed pasta (Fig. 1, lanes 4 and 5), IgE binding to these bands was lost in these samples (Fig. 2, lanes 4 and 5). This indicates that the heat processing used in

pasta drying is sufficient to eliminate the potential allergenicity of the major allergens involved in baker's asthma (SIMONATO *et al.*, 2001b). Finally, the pattern of IgE binding to pasta proteins was not affected by 10 min of cooking in boiling water (Fig. 2, lanes 4 and 5). When control sera from two patients with wheat-unrelated allergy were used, no IgE reacting bands were detected.

In vitro protein digestion of durum wheat semolina and cooked pasta

Since the degradability of a protein during GI digestion can influence its allergenic potential (ASTWOOD *et al.*, 1996) and since the digestibility of wheat proteins can be affected by heat treatment (HANSEN and JOHNSTON, 1976; JIDEANI *et al.*, 1994; PASINI *et al.*, 2001), *in vitro* digestion experiments were performed on unheated durum wheat semolina and on cooked pasta. To simulate the physiological digestion process, samples were treated sequentially with pepsin (at pH 2) and pancreatin (at pH 7.6) for differ-

ent times, and the digestibility was calculated by measuring the 10% TCA-insoluble nitrogen (TIN). With this system, it was possible to measure the protein material with a molecular weight > 400 Da (GREENBERG and SHIPE, 1979) which, in this case, included the undigested proteins as well as the proteolytic fragments that were large enough to elicit an immunological response in the gut.

After 30 min of pepsin attack, the TIN of durum wheat semolina was reduced to 27% of the initial value, whereas the TIN of the cooked pasta sample was reduced to 50% of the initial value (Fig. 3). These data confirm that heat treatment affects the degree of hydrolysis of wheat proteins by pepsin (HANSEN and JOHNSTON, 1976; PASINI *et al.*, 2001). The pancreatin attack further decreased the TINs of semolina and the pasta samples and, after the entire digestion treatment, the TINs of both semolina and pasta tended to be around 20% of the initial value. This indicates that the pancreatic enzymes can eliminate the difference in digestibility between the two samples.

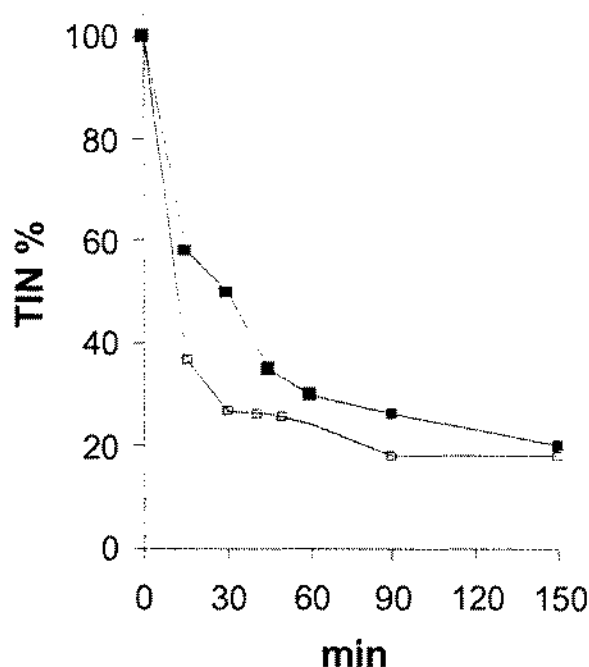


Fig. 3 - TCA-insoluble nitrogen (TIN) during *in vitro* digestion of durum wheat semolina (■) and pasta (□).

Undigested control samples and samples taken after 30 min of pepsin digestion and after 120 min of pancreatin treatment were analysed by tricine-SDS-PAGE, a technique which allows polypeptides with an Mr as low as 1 kDa to be detected (SHÄGGER and VON JAGOW, 1987). When digested samples of semolina were examined (Fig. 4A), the bands corresponding to the storage proteins (HMW-GS, LMW-GS and gliadins) disappeared after pepsin digestion. In contrast, protein bands with an Mr around 15 kDa seemed to be more resistant to proteolysis and some bands with Mr values lower than 14 kDa were detected up to the end of the digestion process (Fig. 4A). These results resembled those previously found for bread wheat flour (PASINI *et al.*, 2001). The electrophoretic patterns obtained with cooked pasta during digestion were different from those of semolina (Fig. 4A and B), which confirmed the results obtained by TIN quantification. The differences between semolina and pasta became more evident after pepsin attack (Fig. 4A and B, lanes 2). While the two samples looked more similar at the end of the digestion process, the bands that accumulated at Mr < 14 kDa were less detectable in the cooked pasta samples than in the semolina (Fig. 4A and B, lanes 3). Moreover, the aggregated protein material at the top of the gel in the undigested pasta sample disappeared at the end of proteolytic digestion. This was detected by staining the gel for both protein and sugars (Fig. 4B and C, respectively).

IgE binding to proteins of semolina and cooked pasta and to their digestion products

Pooled sera of the two groups of wheat-allergic patients were tested for IgE binding to the proteins of both semolina and cooked pasta before and after *in vitro* digestion (Fig. 5). During the *in vitro* digestion process, the IgE binding to sem-

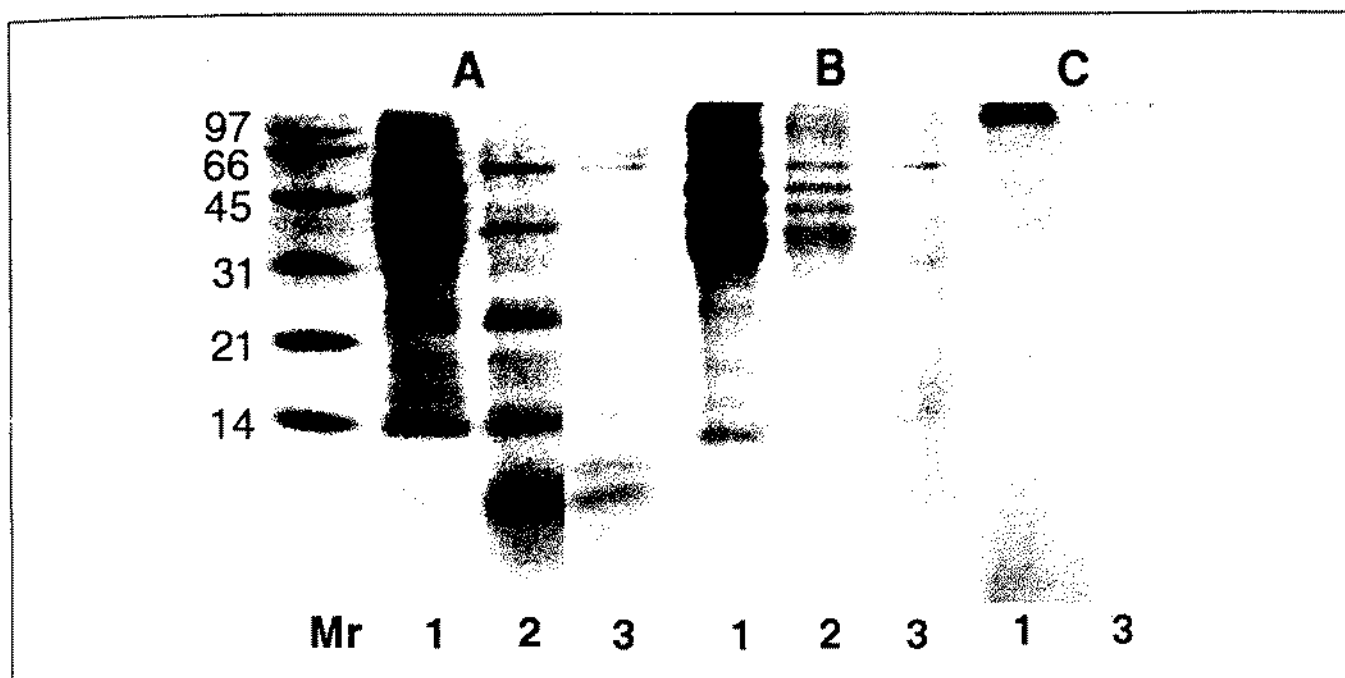


Fig. 4 - Tricine-SDS-PAGE analysis of durum wheat semolina and pasta proteins during *in vitro* digestion. Mr: molecular weight standard proteins. Lanes 1, 2 and 3: samples taken before digestion, after 30 min of pepsin attack and at the end of the digestion process, respectively. Part A: semolina, staining for total proteins. Part B: pasta, staining for total proteins. Part C: pasta, staining for carbohydrates.

olina and processed pasta proteins progressively decreased. This binding almost completely disappeared after 120 min of pancreatin attack, indicating that all the potential allergens with Mr > 1 kDa were degraded during digestion in both semolina (Fig. 5A and C) and cooked pasta (Fig. 6B and D). This result was obtained with both the wheat-CAP-positive (Fig. 5A and B) and CAP-negative (Fig. 5B and D) patients.

DISCUSSION

For some people who do not suffer from any gut disease ingestion of wheat-based food can cause gastrointestinal (GI) symptoms, such as abdominal pain, bloating, and diarrhea (SIMONATO *et al.*, 2001a). After examining 18 patients who suffer from these problems, half of them resulted negative in specific IgE detection to wheat by CAP-testing, but all showed serum IgE binding to soluble and insoluble bread (hexaploid) wheat pro-

teins in immunoblotting. Therefore a food allergy to wheat could be suspected as being the cause of the GI symptoms in all of these patients (SIMONATO *et al.*, 2001a).

By using the pooled sera from these CAP-positive and CAP-negative patients, we studied the potential allergenicity of durum wheat semolina and commercial durum wheat pasta ingested by humans (i. e. after home cooking) and processed in the GI tract.

Durum wheat semolina has an allergenic potential similar to that of bread wheat flour. (SIMONATO *et al.*, 2001a) at least in terms of the ability of its proteins to bind serum IgE in patients with food allergy to wheat. This is not surprising because the proteins of durum and bread wheat are very similar at the molecular level (AUTRAN *et al.*, 1979). As already shown for bread wheat (SIMONATO *et al.*, 2001a), in addition to proteins belonging to the albumin/globulin fraction, several components of the insoluble protein fraction (prolamins)

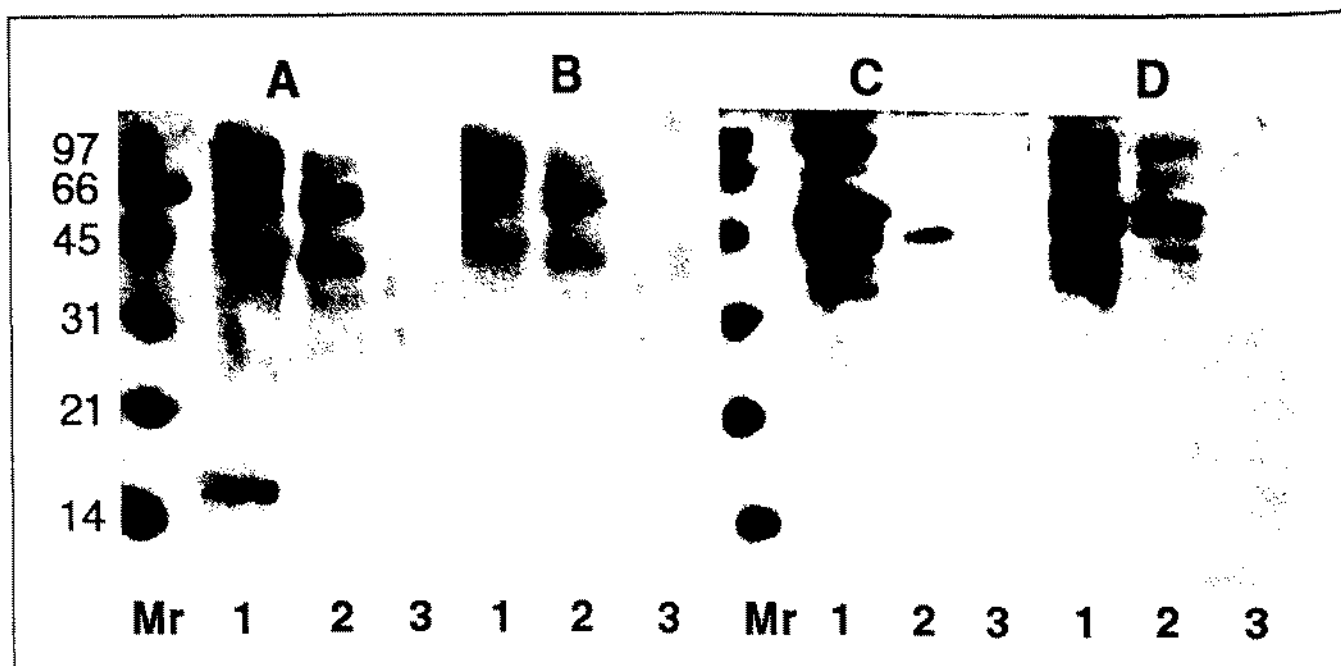


Fig. 5 - IgE-immunoblotting analysis of durum wheat semolina and pasta proteins, separated by Tricine-SDS-PAGE, during *in vitro* digestion. Pooled sera of patients with food allergy to wheat were used. Part A: semolina, CAP-positive patients. Part B: pasta, CAP-positive patients. Part C: semolina, CAP-negative patients. Part D: pasta, CAP-negative patients. Lanes 1, 2 and 3: samples taken before digestion, after 30 min of pepsin attack and at the end of the digestion process, respectively.

were recognized in immunoblotting by the IgE of the pooled sera. This confirms that members of the prolamin superfamily act as food allergens when passing through the GI tract (MILLS *et al.*, 2003). However, the results also suggest that the low Mr albumins involved in bakers' asthma, which are members of the alpha-amylase inhibitor protein family (GOMEZ *et al.*, 1990), are not potential allergens for patients with allergic symptoms after wheat ingestion. This has already been shown for bread wheat (SIMONATO *et al.*, 2001b). Although these proteins represent strong potential allergens in semolina, they were not bound by IgE after semolina processing into pasta (Fig. 2). Considering the immunological heat-lability of these proteins (PASINI *et al.*, 2001), this fact could be due to the heat treatment used in pasta drying. Although no information was available about the temperatures used during the drying process of the commercial pasta sample used in this study, it is known that modern pasta drying

technologies use temperatures ranging from 75° to 100°C and even higher. Our results suggest that this type of heat-processing is sufficient to eliminate IgE from binding the allergens belonging to the alpha-amylase inhibitor protein family. Therefore, despite the fact that some authors have identified these components as being involved in food allergy to wheat (JAMES *et al.*, 1997; ARMENTIA *et al.*, 2002), it seems that this form of allergy is mainly related to other allergens, particularly insoluble proteins. (SUTTON *et al.*, 1982).

The commercial pasta sample was boiled in water to study the effects of the cooking process on the potential allergens of durum wheat semolina. In comparison to those of raw pasta, the proteins extracted from the cooked pasta sample showed the same IgE binding pattern, indicating that boiling pasta in water does not have any allergological effect and that the changes in IgE binding of semolina proteins occur only during the heat treatment used in pasta drying.

In addition to changes derived from heat-processing (DAVIS and WILLIAMS, 1998; SIMONATO *et al.*, 2001b), the potential allergenicity of a food can also be modified during its passage through the GI tract. The degradability of the allergenic structure is so important that protease resistance is one of the parameters considered when assessing the potential allergenicity of the proteins of genetically-modified foods (METCALFE *et al.*, 1996, FAO/WHO, 2001). In fact, the capacity of a food allergen to elicit an immunological reaction at the level of the intestinal mucosa is related to the capacity of its allergenic epitopes to escape the degradation process during their transit in the GI tract (ASTWOOD *et al.*, 1996). In view of this fact, we studied the *in vitro* digestibility of the potential allergens found in durum wheat as they are ingested by humans, i.e. in the form of home-cooked pasta.

It is well-known that heat processing can affect the digestibility of the wheat flour proteins (HANSEN and JOHNSTON, 1976; JIDEANI *et al.*, 1994). In previous papers we showed that bread-baking affects the protein digestibility in relation to the level of the heat treatment. It also modifies the physical-chemical and immunological properties of the wheat proteins (PASINI *et al.*, 2001), causing the formation of undigestible high-molecular-weight aggregates that also contain sugar, which were recognized by the serum IgE of wheat allergic patients (SIMONATO *et al.*, 2001b).

The results of the *in vitro* digestion process used in the present study also indicate that the gastric digestibility of cooked pasta proteins was less than that of the proteins of durum wheat semolina. However, following the action of the pancreatic proteases, the digestibility of semolina and cooked pasta proteins tended to approach the same value by the end of the digestion process. The lower pepsin degradability of the cooked pasta proteins, compared to that of the

semolina, could be due to reduced accessibility of the protease to its sites of action, which could be a result of a chemically reversible heat-induced protein aggregation that occurs during pasta processing. However, when the pepsin-digested pasta sample was treated with pancreatin, the proteolytic degradation values were similar to those of the semolina. This could be due to the fact that, unlike pepsin (an endoproteolytic enzyme), pancreatin is a mixture of various proteases (including enzymes with exopeptidase activity such as carboxypeptidases A and B), which can improve the action on the protein aggregates present in processed pasta.

When the potential durum wheat allergens were analysed by IgE-immunoblotting, the results in our *in vitro* system indicated that the allergens in cooked pasta were almost completely destroyed after peptic/pancreatic digestion. This confirms that processing semolina into pasta does not reduce its digestibility, in contrast to what has been observed in bread (SIMONATO *et al.*, 2001b, PASINI *et al.*, 2001). Therefore, after passing through the GI tract, it is unlikely that the pasta proteins maintain the potential that would elicit an allergic response in the gut, although the presence of potentially allergenic protein fragments with very low Mr (< 1 KDa) in digested pasta cannot be excluded.

In conclusion, these results suggest that, although bread and durum wheat seem to contain similar potential allergens, wheat-allergic patients, under certain conditions, may tolerate pasta better than bread, because the thermal conditions of pasta preparation allow the GI degradability of these allergens.

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