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## Use of selected sourdough lactic acid bacteria to hydrolyze wheat and rye proteins responsible for cereal allergy

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**Abstract** This work was aimed at showing the capacity of selected sourdough lactic acid bacteria to hydrolyze wheat and rye allergens. Hydrolysis was investigated after wheat sourdough fermentation and after treatment of wheat and rye sourdough breads with pepsin, trypsin and pancreatin, which mimicked the digestive process. As shown by immunoblotting with sera from allergic patients, wheat sourdough fermentation caused the disappearance of some IgE-binding proteins (albumins/globulins and gliadins mainly) with respect to the chemically acidified dough used as the control. The IgE-binding protein profile of wheat and rye sourdough breads differed from those of baker's yeast breads. The signals of the IgE-binding proteins contained in the sourdough breads disappeared after *in vitro* digestion with pepsin, trypsin and pancreatin. The same effect by digestive enzymes was not found for baker's yeast breads which showed persistent IgE-binding proteins. As shown by ELISA inhibition assays, the presence of IgE-binding low molecular weight proteins/peptides in sourdough breads significantly decreased with respect to baker's yeast breads. Proteolytic activity by selected sourdough lactic acid bacteria may have an importance during food processing to produce pre-digested wheat and rye dough which contains IgE-binding proteins degradable by digestive enzymes.

**Keywords** Bread · Lactic acid bacteria · Proteolysis · Sourdough · Wheat and rye allergens

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### Introduction

Food allergies are of concern to both medical care providers and the general population because of a rapidly increasing prevalence during the past few decades [1–3]. It is estimated that true food allergies affect up to 6–8% of children younger than 10 years of age and 1–4% of the adult population [4, 5]. The clinical classification of food allergy diseases is based mainly on the presence or absence of food specific immunoglobulin E (IgE) antibodies [6]. IgE-mediated food reactions occur in the presence of a rapid onset of symptoms after ingestion of a single, well-defined food, whereas non IgE-mediated disorders develop hours to days after food ingestion. IgE-mediated food allergy has been definitively linked to atopy; e.g., the predisposition to develop an allergic-type response to common environmental antigens (allergens).

Wheat flour (*Triticum aestivum* and *Triticum durum*) is largely consumed in the daily diet (e.g., leavened baked goods and pasta) and constitutes one of the most frequent causes of food allergy. Wheat proteins are divided into four solubility classes: water soluble albumins (about 15% of the total proteins), salt, e.g., 0.5 M NaCl-soluble globulins (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 (S–S) bonds. Immunoblotting with sera of patients suffering from allergic symptoms after wheat ingestion indicated that different protein components may contain IgE-reactive epitopes; LMW glutenin subunits and, especially, albumins, globulins, and gliadins [7–11].

Rye (*Secale cereale*) is an important cereal crop after wheat, rice and maize. A substantial part of the world rye harvested is used for bread making, especially in Central, Northern and Eastern European countries where more than 3 million tons of rye are used for bread making per year [12]. Typical rye proteins are classified according to their solubility into albumins, globulins, secalins and

glutelins. The secalins are divided into four groups, the high molecular mass secalins, 75-kDa  $\gamma$ -secalins (S-rich),  $\omega$ -secalins (S-poor), and 40-kDa  $\gamma$ -secalins (S-rich) determined by SDS-PAGE [13]. Rye flour has been considered a cause of food allergy also [7, 14, 15]. A list of identified allergens is reported by International Union of Immunological Societies Allergen Nomenclature Subcommittee [16]. Depending on the route of exposure, wheat and rye allergy may induce several clinical manifestations: classical food allergy affecting skin, gut or respiratory tract; exercise-induced anaphylaxis; occupational rhinitis or asthma and contact dermatitis. IgE antibodies play a fundamental role in all these clinical manifestations [17, 18]. Rye secalins were shown to cross-react with  $\omega$ -5 gliadin, one of the major allergen in wheat dependent exercise-induced anaphylaxis, thus suggesting that they may also elicit symptoms in patients affected by cereal allergy [7].

It has been shown that gastrointestinal enzymes (pepsin, pancreatin and trypsin) are not able to degrade cereal allergens which reach unaltered the intestine where they elicit the immune response [19].

As stated above, most of the wheat and rye flours are used for the manufacture of baked goods. Consequently, before coming in contact with their target organ, cereal allergens may undergo extensive modifications depending on the type of food processing. For instance, thermal processing to which baked goods are subjected during manufacture may influence differently the allergenicity of food proteins [20]. Recently, cereal food technology has changed by influencing dietary habits of entire populations. Cereal baked goods are currently manufactured by very accelerated processes where long-time fermentations by sourdough are almost totally replaced by the indiscriminate use of chemical and/or baker's yeast leavening agents. Under these technological circumstances, cereal components (e.g., proteins) are subjected to very mild or absent degradation during baked good manufacture [21]. Since one of the major requisites of cereal allergens is to show an inherent resistance to the gastrointestinal enzymes and to reach unaltered the intestine where they elicit the immune response [19], food technology options (e.g., use of selected lactic acid bacteria and related enzymes) to modify or eliminate epitopes in processed cereals should be at least investigated. Previously [21, 22], we showed that a pool of selected lactic acid bacteria had the capacity to extensively degrade wheat gliadins during sourdough fermentation and to decrease the human intolerance to gluten (coeliac disease).

This paper was aimed at showing the capacity of selected lactic acid bacteria used as starters for the manufacture of sourdough breads to hydrolyze wheat and rye flour allergens. Hydrolysis was investigated on baker's yeast bread also and, after baking, aliquots of breads were treated with digestive enzymes to mimic the gastrointestinal process and the persistence of IgE-reactive epitopes.

## Materials and methods

### Sera of patients with food allergy to cereals

Sera were kindly supplied by the Department of Onco-Immuno-Dermatology, IDI-IRCCS, Rome, Italy. Sera were from 10 patients with food allergy to cereals (e.g., wheat, barley and rye), suffering from various gastrointestinal symptoms (diarrhea, abdominal pain and/or bloating) after ingesting foods containing cereals. Food allergy to wheat was established by evidence of IgE-dependent sensitization by using the CAP system fluorescent enzyme immunoassay (CAP-FEIA) (Pharmacia, Uppsala, Sweden), by skin prick tests and by standardized double blind placebo control food challenges [23]. Specific IgE to barley, rice, rye, gluten, wheat and  $\alpha$ -amylase were tested (UniCAP specific IgE, Pharmacia & Upjohn, Uppsala, Sweden).

Human sera were pooled and used in immunoblotting experiments. The use of pooled sera allowed having a unique immunological map, targeting a large number of immunogenic epitopes [24, 25]. All IgE-binding bands recognized by single sera were present when pooled sera were examined in immunoblotting experiments. Pooled sera from healthy subjects were used as negative control.

### Micro-organisms and culture conditions

*Lactobacillus alimentarius* 15M, *Lactobacillus brevis* 14G, *Lactobacillus sanfranciscensis* 7A and *Lactobacillus hilgardii* 51B were selected previously on the basis of their capacity to hydrolyze gliadin fractions of wheat sourdoughs [22], and used in this study. The strains were routinely propagated for 24 h at 30 °C (*L. alimentarius* 15M, *L. brevis* 14G, *L. sanfranciscensis* 7A) or 37 °C (*L. hilgardii* 51B) in MRS broth (Oxoid, Basingstoke, Hampshire, England) with the addition of fresh yeast extract (5%, v/v) and 28 mM maltose at a final pH of 5.6. When used for wheat and rye sourdough fermentations, *Lactobacillus* cells were cultivated until the late exponential phase of growth (optical density at 620 nm, ca. 2.5) was reached (ca. 12 h).

### Bread making

The characteristics of the wheat and rye flours used were as follows: moisture, 12.8 and 10%; protein (N  $\times$  5.70), 10.7 and 10%, of dry matter (DM); fat, 1.8 and 2.5% of DM; ash, 0.6 and 1.5% of DM; and total soluble carbohydrates, 1.5 and 1.3% of DM, respectively

Two types of bread were manufactured from either wheat or rye flours. Baker's yeast bread was made by a mixture of 125 g of wheat or rye flour and 64 ml of tap water (containing 1.5% of baker's yeast) which was fermented for 2 h at 37 °C and baked at 250 °C for 15 min. Sourdough bread was made by a mixture of 125 g of wheat or rye flours and 297 ml of tap water (containing a cell concentration of

each sourdough lactic acid bacteria of ca.  $10^9$  CFU per g of dough). The semi-liquid dough was incubated for 24 h at 37 °C under stirring conditions (ca. 200 rpm). After fermentation, water was removed by freeze-drying and ca. 125 g of pre-treated wheat flour were mixed with 64 ml of tap water (containing 1.5% of baker's yeast) and used to produce bread as described earlier.

A semi-liquid dough, without bacterial inoculum, was chemically acidified to pH 4.0 with a mixture of lactic and acetic acids (molar ratio 4:1) and used as the control.

#### Extraction of proteins from sourdoughs and breads

After sourdough fermentation by selected lactic acid bacteria or chemical acidification, wheat flour proteins were separately extracted following the method originally described by Osborne and further modified by Weiss et al. [11, 26].

Extracted albumins, globulins and gliadins were stored at -20 °C and used for electrophoresis.

Total proteins were extracted from wheat and rye baker's yeast and sourdough breads also. Samples (100 mg) were suspended in 4 ml of 0.2 N HCl, homogenized with a Sterilmixer Lab (PBI International, Milan, Italy) and added of 2 ml of 0.6 M Tris-HCl, pH 7.4, containing 30% (w/v) glycerol, 6% (w/v) sodium dodecyl sulphate (SDS) and 6% (v/v) 2-mercaptoethanol. Samples were heated for 5 min at 100 °C. After centrifugation (12,000×g, 20 min), the supernatant (total protein extract) was stored at -20 °C and used for electrophoresis. Protein concentration of the extracts was determined by the Bradford method [27], using bovine serum albumin as the standard.

#### Bread digestion

Baker's yeast and sourdough breads were subjected to sequential protein hydrolysis by digestive enzymes according to the method described by Pasini et al. [28]. Briefly, 100 mg of bread were suspended in 4 ml of 0.2 N HCl (pH 2), containing 0.05 mg/ml of pepsin (EC 3.4.23.1) (Sigma Aldrich CO., St. Louis, MO), and homogenized in a Sterilmixer Lab (PBI International). After 30 min of incubation at 37 °C under stirring conditions (150 rpm), 1.15 ml of a solution of 1 M boric acid, 0.5 N NaOH, adjusted to pH 6.8 with 5 N HCl, 0.25 mg/ml of pancreatin (Sigma) and 0.0087 mg/ml of trypsin (EC 3.4.21.4) (Sigma) were added [29]. The resulting pH was 7.6. Pancreatic digestion was lasting 150 min. Digested samples were recovered after total digestion (30+150 min) and 2 ml of 0.6 M Tris-HCl, pH 7.4, containing 30% (w/v) glycerol, 6% (w/v) sodium dodecyl sulphate (SDS) and 6% (v/v) 2-mercaptoethanol were added. Samples were heated for 5 min at 100 °C. After centrifugation (12,000×g, 20 min), the supernatant (total protein extract) was stored at -20 °C and used for electrophoresis.

Electrophoresis and immunoblotting by sera of patients with food allergy to cereals

Albumin/globulin, gliadin and glutenin fractions extracted from wheat sourdough and chemically acidified dough, and total proteins extracted from wheat and rye breads (10–20 µl, containing ca. 15 µg of protein) were diluted with sample buffer (1:1), treated at 100 °C for 5 min and analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the Laemmli procedure [26].

The same extracts were analysed by immunoblotting to detect the IgE binding capacity of pooled human sera of patients with food allergy to cereals [30]. A semidry blotting technique was used. Protein bands separated by SDS-PAGE were transferred onto nitrocellulose sheets with a Trans-blot Cell (Bio-Rad Laboratories, Milan, Italy) by using a transfer buffer containing 48 mM Tris, pH 9.2, 39 mM glycine, 20% methanol (v/v) and 0.1% (w/v) SDS. The voltage was 50 V for 5 h. Blotting bands were visualized by soaking the membranes for a few minutes in Ponceau S (0.1% in 3% trichloroacetic acid) and marked with a pencil, before de-staining with water. Membranes were blocked with TBS (Tris Buffered Saline) containing 0.05% (v/v) Tween 20 (TBS-T) and 5% (w/v) skim milk powder (M-TBS-T) for 2 h, and incubated overnight with pooled human sera at room temperature, diluted 1:20 in TBS-T. After washing five times with M-TBS-T, blots were incubated at room temperature for 1 h with monoclonal anti-human IgE peroxidase-conjugate antibody (Sigma Chemical Co), diluted 1:5000 in M-TBS-T. After four washes in M-TBS-T and one in TBS, bound IgE were visualized by chemiluminescence using a luminol substrate of Supersignal Detection kit (Pierce Biotechnology Inc., Rockford, IL), according to the instructions provided by the manufacturer. The procedure was carried out at room temperature.

#### ELISA inhibition assays

The studies were performed by the method of Diaz-Perales et al. [31]. Polystyrene 96-well microtiter plates were coated with 50 µl of the total proteins extracted from wheat and rye breads at 15 µg/µl for 1 h at 37 °C. After blocking with 1% BSA in PBS, wells were incubated (14 h at 25 °C) with 10 µl of the serum pool previously pre-incubated with 40 µl of serial dilutions of baker's yeast and sourdough breads subjected to sequential protein hydrolysis by digestive enzymes (2180–8.5 µg/ml of low molecular weight proteins/peptides). Pooled human sera incubated without inhibitors was used as a control (0% of inhibition). Digested bread samples were dialyzed (membrane with a 10-kDa cut-off, Sigma) and the permeates were concentrated by freeze-drying and analyzed by ELISA-inhibition assays. The peptide concentration was determined by OPA method [32]. After washing with 0.1% Tween 20 in PBS, wells were incubated with a peroxidase-labeled anti-human IgE (Dako A/S; 1:500 dilution) for 1 h at 25 °C. Plates were washed again and then developed with 50 µl of peroxidase



substrate buffer (Dako, code S2045). After 30 min, the reaction was stopped with 50  $\mu$ l of 2 N HCl, and the Optical Density (OD) was measured at 492 nm. PBS with 1% BSA was used as a negative control. Assays were performed in triplicate. The OD obtained in ELISA assay without inhibitors was taken as 0% of inhibition and this value was used to determine the inhibition percentage of each sample.

ELISA inhibition measurement from three independent replicates were subjected to one-way ANOVA [33]; for multiple comparison the Tukey test was used and the alpha value for all experiments was set at 0.05, using the statistical software, Statistica for windows (Statistica 6.0 per Windows 1998).

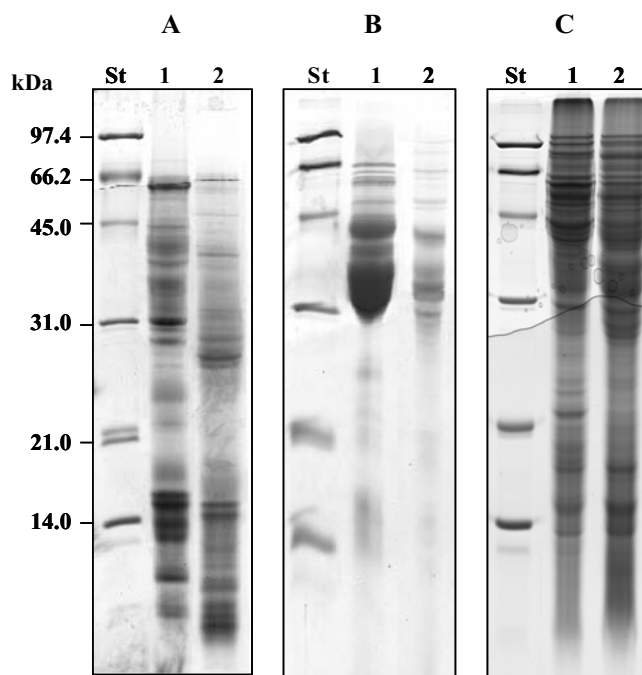
## Results

### Hydrolysis during wheat sourdough fermentation

After fermentation at 37 °C for 24 h, the cell concentration of lactic acid bacteria in wheat sourdough was ca.  $10^9$  CFU/g for each species used in the mixture. The pH of the sourdoughs was in the range 3.8–3.9. As shown previously [22], biological or chemical acidification caused a marked modification of the polypeptide pattern compared to the non-acidified dough; therefore, sourdoughs were compared to a chemically acidified and incubated dough to assess variations due to bacterial proteolysis mainly.

Preliminarily, wheat protein fractions were extracted from chemically acidified and fermented doughs, and analysed by SDS-PAGE (Fig. 1). Compared to the chemically acidified dough, sourdough fermentation with selected lactic acid bacteria caused a certain hydrolysis of albumins/globulins (Fig. 1A), and especially of gliadins (Fig. 1B). Hydrolysis was very low or absent towards glutenins (Fig. 1C). The same results were found by two-dimensional electrophoresis analyses (data not shown) which agreed with our previous findings [21, 22].

The related immunoblot analyses with sera of patients with food allergy to cereals (Fig. 2) showed that the albumin/globulin extract of the chemically acidified dough was characterized by several bands with molecular weights ranging from ca. 10 to 70 kDa. This profile can be well compared with the previous determination of an unidentified protein of ca. 67 kDa [34] and with the identification of peroxidase precursor (ca. 37 kDa) [9], xylanase inhibitor protein (ca. 33 kDa), putative transcription factor APFI (ca. 29 kDa), and wheat  $\alpha$ -amylase inhibitors protein family ( $\alpha$ -amylase/subtilisin inhibitor, WASI, ca. 19 kDa and  $\alpha$ -amylase inhibitor 0.19, ca. 13 kDa) (De Angelis et al., unpublished data). Sourdough fermentation caused the disappearance of some of the above IgE-binding proteins (e.g., ca. 13 kDa). The IgE-gliadin profile of the chemically acidified dough was characterized by intense signals in the range of 30–90 kDa. This was in agreement with some profiles of IgE-reactive epitopes from wheat gliadins [8, 35]. Compared to albumins/globulins, sourdough fermentation seemed to have a more extensive effect on the degradation of IgE-binding gliadin proteins. On the contrary, the



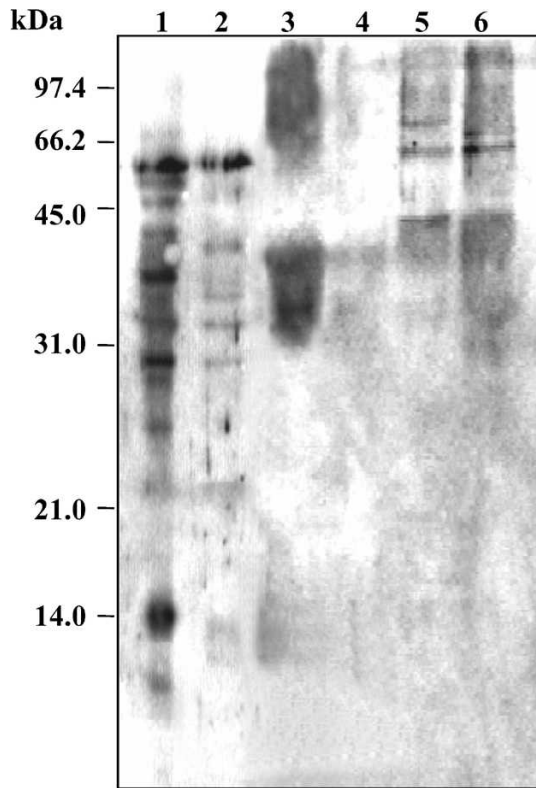
**Fig. 1** SDS-PAGE electrophoresis analysis of wheat protein fractions (ca. 15  $\mu$ g of protein) extracted from chemically acidified (lane 1) and fermented (lane 2) doughs. Albumins/globulins, gliadins and glutenins are shown in panels A, B and C, respectively. (St) molecular weight standard. Fermentation was carried out by selected lactic acid bacteria at 37 °C for 24 h

IgE-binding glutenin profile did not markedly differ between wheat chemically acidified and fermented doughs. Although differences were found between chemically acidified and fermented doughs, several IgE-binding proteins persisted after hydrolysis with selected sourdough lactic acid bacteria.

### Wheat and rye bread making and digestion

Wheat and rye flours were used for bread making by using baker's yeast or sourdough lactic acid bacteria. These two technological options were chosen to compare very fast and long-time, traditional processes, respectively. Baker's yeast and sourdough breads were then subjected to sequential protein hydrolysis by pepsin, trypsin and pancreatin to mimic the digestive process [28].

Figure 3A and B shows the SDS-PAGE and related immunoblot analysis of the total proteins extracted from wheat breads. Before *in vitro* digestion, pooled human sera showed an IgE-binding pattern which included proteins ranging from ca. 18–100 kDa. Other authors [8] showed that ca. 70% of wheat allergic patients have IgE antibodies directed against albumins/globulins mainly located in the migration zone between 30 and 70 kDa. Overall, IgE-binding profiles of baker's yeast wheat breads differed from those of the protein fractions extracted from sourdough (Fig. 2). It is well known that thermal processing during bread making has a marked influence on the type and characteristics of persistent epitopes [20, 24]. The intensity of

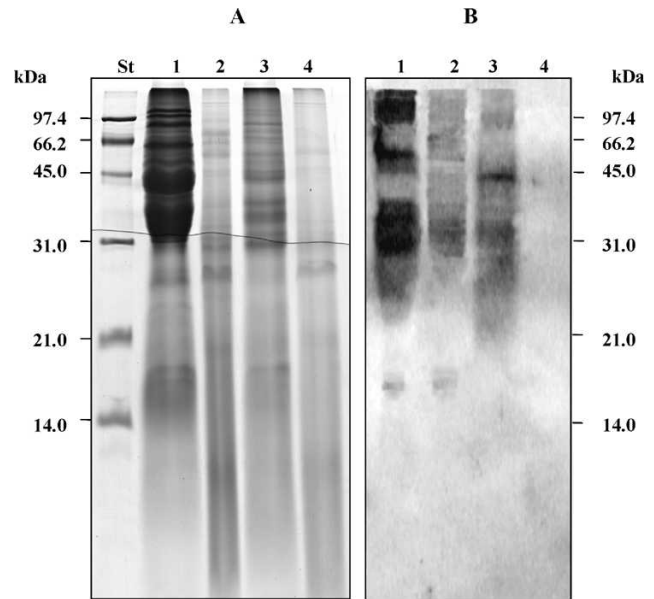


**Fig. 2** Immunoblot of wheat proteins (ca. 15 µg) extracted from chemically acidified (lanes 1, 3 and 5) and fermented (lanes 2, 4 and 6) doughs. Albumins/globulins, gliadins and glutenins are shown in lanes 1–2, 3–4 and 5–6, respectively. Fermentation was carried out by selected lactic acid bacteria at 37 °C for 24 h

the signals and the number of protein bands was markedly higher in the baker's yeast bread. Overall, baker's yeast does not cause appreciable proteolysis during dough fermentation [36], while the pool of selected lactic acid bacteria was shown to be capable of an intense proteolytic activity [21, 22]. The signals of the IgE-binding proteins contained in the sourdough bread disappeared after *in vitro* digestion with pepsin, trypsin and pancreatin. The same effect by digestive enzymes was not found for baker's yeast bread which showed persistent IgE-binding proteins.

Figure 4A and B shows the SDS-PAGE and related immunoblot analysis of the total proteins extracted from rye breads. Also in this case, it seemed that sourdough fermentation favoured a less complex pattern of IgE-binding proteins compared to baker's yeast bread. While IgE-binding proteins of ca. 70 and 35 kDa were detected in the baker's yeast bread, they almost totally disappeared in the rye sourdough bread after *in vitro* digestion by pepsin, trypsin and pancreatin. Secalins such as  $\gamma$ -70 and  $\gamma$ -35 were shown to be the major rye allergens probably involved in cereal-dependent, exercise-induced anaphylaxis [7].

In ELISA-inhibition studies, 2180 µg/ml of low molecular weight proteins/peptides from baker's yeast breads inhibited over 85% for wheat flour or 60% for rye flour of the IgE binding of pooled patient sera using total bread proteins as solid phase (Fig. 5A and B). When the sourdough bread extracts were used, the inhibition was less than 40%



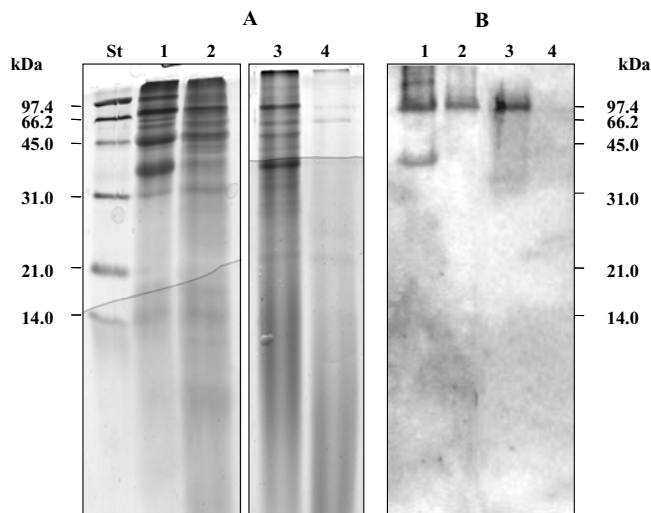
**Fig. 3.** SDS-PAGE electrophoresis analysis **A** and related immunoblot **B** of total proteins (ca. 15 µg) extracted from wheat breads before and after *in vitro* digestion. (St) molecular weight standard. Lanes 1: undigested baker's yeast bread; 2: baker's yeast bread digested with pepsin, trypsin and pancreatin; 3: undigested sourdough bread; and 4: sourdough bread digested with pepsin, trypsin and pancreatin. Sourdough bread was fermented by selected lactic acid bacteria at 37 °C for 24 h

for wheat flour and 15% for rye flour. No inhibition of the IgE binding of pooled patient sera was observed using 34 and 146 µg/ml of inhibitors for wheat and rye sourdough bread extracts, respectively.

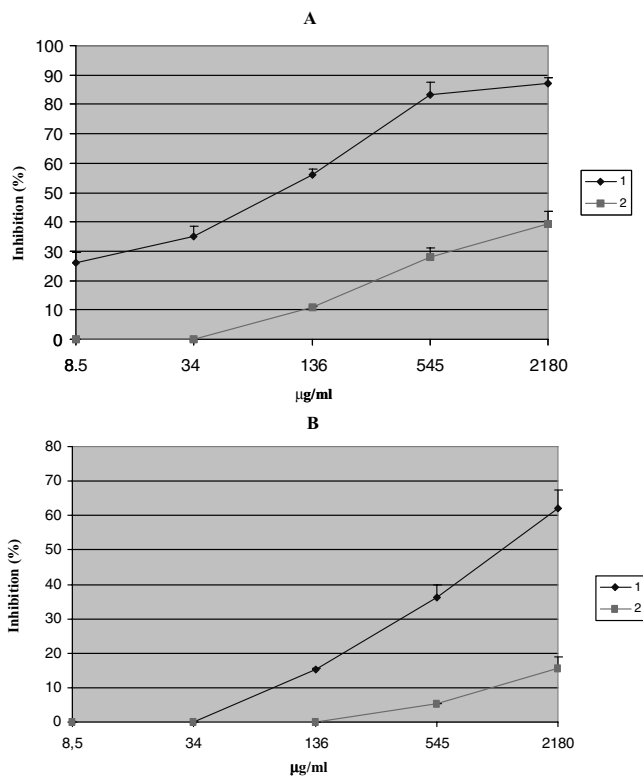
## Discussion

Currently, the only treatment for food allergy is the elimination of the offending food from the diet. Several proactive modalities such as peptide immunotherapy, DNA immunization with immunostimulatory sequences and anti-IgE therapy are under investigation to provide an effective treatment and prevention of food allergies in the near future [37]. Nevertheless, the study of the influence of cereal processing on the native structure of wheat and rye flour allergens and related IgE-reactive epitopes should be pursued.

First, this paper showed that selected sourdough lactic acid bacteria had the capacity to pre-digest wheat and rye flour during fermentation producing breads which did not contain IgE-binding proteins resistant to proteolysis by digestive enzymes. Proteolysis is also one of the mechanisms by which probiotic bacteria are involved in the management of food allergy [38]. Beyond stimulation of the immunological system, it is thought that strains of probiotic lactobacilli may aid in the host protection against allergenic sensitization by degradation of potentially allergenic epitopes in the intestinal lumen. Although a low incidence (0.006/100,000 children) has been reported for fatal and severe reactions to foods (e.g., exercise-induced anaphylaxis) [39, 40], the eventual role of probiotic bacteria is weakened under these



**Fig. 4** SDS-PAGE electrophoresis analysis **A** and related immunoblot **B** of total proteins (ca. 15  $\mu\text{g}$ ) extracted from rye breads before and after *in vitro* digestion. (St) molecular weight standard. Lanes 1: undigested baker's yeast bread; 2: undigested sourdough bread; 3: baker's yeast bread digested with pepsin, trypsin and pancreatin; and 4: sourdough bread digested with pepsin, trypsin and pancreatin. Sourdough bread was fermented by selected lactic acid bacteria at 37 °C for 24 h



**Fig. 5** ELISA-inhibition assays of the total proteins extracted from wheat **A** and rye **B** breads in the solid phase and related low molecular weight proteins/peptides (LMWP/Pep) as inhibitors. LMWP/Pep extracted from baker's yeast bread digested with pepsin, trypsin, and pancreatin, (1); and LMWP/Pep extracted from sourdough bread digested with pepsin, trypsin, and pancreatin, (2). Means ( $n=3$ ) and SEs (bars) are represented

circumstances. Overall, foods to be safe must be free of IgE-reactive epitopes after digestive processes and before reaching the intestinal lumen. The compliance to a wheat and rye-free diet is an extremely challenging task, given the problems related to cross-contamination, lack of clear food labelling policies and difficulties to establish a safe threshold for food allergy [41]. Compared to baker's yeast bread, selected sourdough lactic acid bacteria caused a modification/degradation of the proteins carrying IgE-reactive epitopes that favoured a subsequent hydrolysis by digestive enzymes. The individual strains which composed the selected pool or other sourdough lactic acid bacteria strains did not show the same capacity to hydrolyze or modify allergenic proteins carrying IgE-reactive epitopes during fermentation (data not shown). Previously [21, 22], it was shown that the selected pool of lactic acid bacteria used in this study possesses a complementary proteinase and peptidase activities against polypeptides (e.g., 33-mer) extremely resistant to proteolysis by digestive enzymes. As shown by immunoblotting with pooled sera from patients allergic to cereals, hydrolysis regarded some of the major wheat and rye albumin/globulin and gliadin allergens.

After dough leavening, baking is thought to likely increase allergenicity via the introduction of neo-antigens [20, 24]. The IgE-binding protein components of the unheated dough tended to disappear during *in vitro* digestion by pepsin, whereas permanence of IgE recognition of these proteins was evident for both the bread crumb and crust [24]. Compared to baker's yeast bread, the results of this study showed that long-time fermentation (24 h at 37 °C) with selected sourdough lactic acid bacteria favoured the degradation by digestive enzymes of the IgE-reactive epitopes which persisted after baking wheat and rye breads. ELISA inhibition assays showed the presence of IgE-binding low molecular weight proteins/peptides also in sourdough breads. Nevertheless, the inhibition was significantly ( $P<0.05$ ) decreased with respect to baker's yeast breads. It seems desirable to test the resulting products by means of other complementary methods, such as RAST assays, to ascertain the actual disruption of the main IgE-reactive epitopes.

Proteolytic activity by selected sourdough lactic acid bacteria may have an importance during food processing to produce pre-digested wheat and rye dough which contains IgE-binding proteins degradable by digestive enzymes.

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