

This article was downloaded by: [University of Oslo Library]

On: 18 June 2009

Access details: Access Details: [subscription number 907468096]

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Food and Agricultural Immunology

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713422299>

Decrease of wheat flour allergenicity via lactic acid fermentation

Joanna Leszczyska ^a; Anna Diowks ^b; Agata cka ^a; Magorzata Bryszewska ^a; Katarzyna Wolska ^a; Wojciech Ambroziak ^b

^a Institute of General Food Chemistry, Technical University of Lodz, Lodz, Poland ^b Institute of Fermentation Technology and Microbiology, Technical University of Lodz, Lodz, Poland

Online Publication Date: 01 June 2009

To cite this Article Leszczyska, Joanna, Diowks, Anna, cka, Agata, Bryszewska, Magorzata, Wolska, Katarzyna and Ambroziak, Wojciech(2009)'Decrease of wheat flour allergenicity via lactic acid fermentation',Food and Agricultural Immunology,20:2,139 — 145

To link to this Article: DOI: 10.1080/09540100902889944

URL: <http://dx.doi.org/10.1080/09540100902889944>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Decrease of wheat flour allergenicity via lactic acid fermentation

Joanna Leszczyńska^{a*}, Anna Diowks^b, Agata Łacka^a, Małgorzata Bryszewska^a, Katarzyna Wolska^a and Wojciech Ambroziak^b

^a*Institute of General Food Chemistry, Technical University of Lodz, 4/10 Stefanowskiego Str., 90-924 Lodz, Poland;* ^b*Institute of Fermentation Technology and Microbiology, Technical University of Lodz, 171/173 Wolczanska Str., 90-924 Lodz, Poland*

(Received 12 March 2009; final version received 12 March 2009)

Results of the research on the lowering of allergenicity of wheat flour fermented using homo- and heterofermentative lactic acid bacteria and mixed cultures with yeast were discussed. The gliadins' immunoreactivity was measured using the indirect non-competitive enzyme-linked immunosorbent assay method. The biggest decrease of the immunoreactivity of gliadin fraction was observed in the case of fermentation performed with the use of mixed cultures of lactic acid bacteria and yeast. This way of wheat flour modification seems to be promising, because fermentation is a natural process causing not only lowering of the allergenicity, but also improving organoleptic and nutritional values of obtained products.

Keywords: gliadins; lactic acid bacteria; fermentation; wheat flour; ELISA

Introduction

Necessity of keeping a rigoristic gluten free diet by people with intolerance of gluten peptides considerably limits an assortment of allowed food products. It also causes restriction in the content of proteins, food fibres, vitamins, microelements and many other ingredients necessary for human health as bread cereals are the basic source of them in everyday food (Kunachowicz, 1998). Thus, a lot of actions are undertaken to obtain flour with a reduced level of immunoreactivity.

Many investigators have intensively studied the phenomenon of wheat flour peptides' allergenicity. Simonato investigated the changes in immunoreactivity of flour, which was exposed to typical for bread technological treatment and digested in the digestive system (Simonato et al., 2001). Hypoallergenic wheat flour with a lowered immunoreactivity of glutenin fraction was obtained after treatment with different enzymes: pepsin, trypsin, collagenase, elastase, transglutaminase, bromelain, chymotrypsin, papain, subtilisin and actinase (Leszczyńska et al., 2002; Tanabe, Arai, & Watanabe, 1996; Tanabe et al., 1996, 2000; Watanabe, Ikezawa, & Arai, 1994; Watanabe, Watanabe, Sonoyama, & Tanabe, 2000). The influence of specific chemical reactions (Gerrard, Brown, & Fayle, 2003), gamma irradiation and microwave heating on the gluten immunoreactivity, especially in the case of omega

*Corresponding author. Email: slezanko@snack.p.lodz.pl

and alpha gliadins (Leszczyńska, Łacka, Szemraj, Lukamowicz, & Żegota, 2003a,b), as well as the lactic acid fermentation (Di Cagno et al., 2004) were also investigated.

Lactic acid fermentation, which is performed in sourdough environment, is a natural, though a little forgotten, step of the process in traditional technology of rye and mixed bread and in some regions of the world also of wheat bread production (Diowksz & Ambroziak, 2006). An intensive hydrolysis proteins is performed in this process at a degree depending on individual features of the microbiological organisms – lactic acid bacteria active in the fermentation process (Di Cagno et al., 2002). This was the reason for undertaking the study of reducing the wheat flour allergenicity as the result of fermentation performed by the chosen strains of lactic acid bacteria.

Materials and methods

Wheat flour (Type 500) from “Kruszynek” mill, containing 18.8% of gluten, 0.51% of ash and 14.9% of water was exposed to lactic acid fermentation. Fermentation tests of wheat flour were carried on with dough prepared with the yield of 200 and with several monocultures of lactic acid bacteria (*Lactobacillus*). In this study, six homofermentative strains (*Lb. plantarum*), five heterofermentative strains (three *Lb. brevis* and two *Lb. sanfranciscensis*) and bakery yeasts (*Saccharomyces cerevisiae*) were used. A 24 h inoculum containing 10^9 cfu/cm³ was added to the dough in amount of 2% (v/v). Fermentations were conducted for 24 hours at 30°C. The number of bacteria and yeast cells in sourdough was determined by the plate count method on MRS medium and was expressed as colony forming units (cfu)/g of sourdough. The total acidity of sourdough was also determined by the titration method and was expressed as ml of 0.1 mol l⁻¹ NaOH used for 10 g of sourdough.

Immunoreactivity analysis

To estimate the immunoreactivity of gliadin fraction isolated from modified flour the indirect enzyme-linked immunosorbent assay (ELISA) technique was used, in which human sera containing antigliadin antibodies and monoclonal antibodies against human immunoglobuline IgG conjugated with an alkaline phosphatase were used (Leszczyńska et al., 2003a,b). Gliadins were isolated from the sourdoughs according to Osborne's procedure (Osborne, 1907). Monoclonal antibodies anti-human IgG conjugate with alkaline phosphatase from mouse, clone GG-5 (Sigma, A 2064), were used for ELISA. All human sera containing antigliadin antibodies were taken from sensitive patients at the Polish Mother's Memorial Hospital.

Microtitre plates EB 92029330 (Labssystem, Helsinki, Finland) were coated overnight at 4°C with 100 µl of the antigen solution (100 times diluted extracts of gliadins obtained from samples) in 0.1 M carbonate buffer (pH 9.6), containing about 1.5 mg of protein. Plates were washed and free binding sites were blocked by incubation of the plates with a 3% solution of low fat milk in phosphate buffer (pH 7.2), containing 0.1% Tween-20 for 2 h. This was followed by removal of buffer solution, rinsing the plates five times and further incubation with human sera containing antigliadin antibodies diluted with phosphate buffer for 1 h at room temperature. The plates were washed again and 100 µl of 1000-fold diluted solution of anti-IgG antibodies conjugated with an alkaline phosphatase was added. After

incubation of the plates for 1 h and rinsing with phosphate buffer the bound phosphatase activity was determined by reaction with p-NPP with Multiscan MC reader at 405 nm. A 0.1% milk solution in phosphate buffer (pH 7.2), containing 0.05% Tween-20 was used for all washings and dilutions of antibodies.

The remaining immunoreactivity of fermented samples was estimated in ratio to unfermented wheat flour acidified with lactic acid to pH 4.0, accounted for peptide content determined by Pierce's method using the BCA Protein Assay Reagent Kit (Pierce) according to the instruction of the producer.

A-PAGE

Gliadin peptides extracted from fermented samples were exposed to A-PAGE electrophoretic separation. Electrophoresis was performed in polyacrylamide gel (aluminium lactate buffer, 2.5 g/l, lactic acid up to pH 3.1). A volume of 50 µl lactate buffer, containing 0.6 mg of methyl green and 0.5 ml of glycerol were mixed with 50 µl of gliadin extract, then transferred onto the gel. Electrophoresis was run in a minigel setup of Desaga apparatus (Desaga, Heidelberg, Germany) at 300 V for 30 minutes. The analysis of the obtained electrophoregrams was performed using the computer program GelScan ver.1.45 (Kucharczyk, Techniki elektroforetyczne, Sp. z o.o.).

Results

Data shown in Table 1 characterised the progress of lactic acid fermentation in terms of increased bacteria number and their acidifying activity. For the most of tested microorganisms a similar yield of lactic acid bacteria with value about 10^9 cfu/g was found. Only in three samples the number of lactic acid bacteria was by one order lower (*Lb. plantarum* LOCK 0861, *Lb. brevis* DSM 1269 and *Lb. plantarum* DSM 2648). In the first two cases, it was accompanied also with a low acidity of the sourdough after 24-hour fermentation, which was about one half lower than in the case of the other fermentations. In both cases, there was a noticeable difference in the final consistence of the sourdough, which was still dense, whereas well matured sourdough gained softer consistency.

The decrease of immunoreactivity of isolated gliadin fraction is shown in Table 2 with the biggest reduction of immunoreactivity seen in the case of *Lb. plantarum* AD-98, *Lb. plantarum* LOCK 0860, *Lb. plantarum* 0861, *Lb. sanfranciscensis* DSM 20663 and *Lb. plantarum* DSM 2646 (3, 6, 7, 8 and 11). Observed decreasing of immunoreactivity of gliadin fraction in fermentated samples *Lb. plantarum* AD-98, *Lb. plantarum* LOCK 0860 and *Lb. sanfranciscensis* DSM 20663 (3, 6 and 8) is positively correlated with a great increase of number of bacteria, noticeable acidity and it is also a consequence of hydrolysis of peptides (additional bands of peptides on electrophoregrams) (Figure 1). However, in spite of weak increase of number of bacteria and lack of proteolysis, there is a considerably reduction of immunoreactivity, as seen in the case of *Lb. plantarum* 0861 and *Lb. plantarum* 2048 (7 and 11). On the other hand, hydrolysis of polypeptides in the case of *Lb. sanfranciscensis* MW-94 and *Lb. brevis* DSM 1269 (1 and 10) did not cause a noticeable decrease of immunoreactivity.

Table 1. Characteristics of growth of lactic acid bacteria in sourdoughs.

No.	Strain	Number of bacteria cells (cfu/g)	Total acidity (ml 0.1 mol l ⁻¹ NaOH/10g sourdough)
1	<i>Lb. sanfranciscensis</i> MW-94	1.77×10^9	9.60
2	<i>Lb. brevis</i> MS-99	1.62×10^9	10.70
3	<i>Lb. plantarum</i> AD-98	2.05×10^9	10.15
4	<i>Lb. plantarum</i> LOCK 0858	2.35×10^9	10.10
5	<i>Lb. plantarum</i> LOCK 0859	2.10×10^9	9.48
6	<i>Lb. plantarum</i> LOCK 0860	2.76×10^9	10.10
7	<i>Lb. plantarum</i> LOCK 0861	3.65×10^8	4.45
8	<i>Lb. sanfranciscensis</i> DSM 20663	1.10×10^9	10.43
9	<i>Lb. brevis</i> DSM 1267	1.18×10^9	9.53
10	<i>Lb. brevis</i> DSM 1269	7.43×10^8	5.55
11	<i>Lb. plantarum</i> DSM 2648	4.88×10^8	9.13

Selected strains of lactic acid bacteria with the strongest immunoreactivity reduction were used to create mixed cultures for fermentation of wheat flour. The mixed cultures were also associated with bakery yeast *Saccharomyces cerevisiae*. The immunoreactivity of gliadin fraction in the resulted sourdoughs was examined (Table 3).

It seems, that not only lactic acid fermentation of flour with *Lb. plantarum*, *Lb. sanfranciscensis* or *Lb. brevis* reduces the immunoreactivity of gliadins, but also the same effect can be seen in the case of the presence of *Saccharomyces cerevisiae* yeast. However, co-fermentation led by mixed cultures of lactic acid bacteria and yeast considerably increases this effect. The reduction of immunoreactivity in this case was

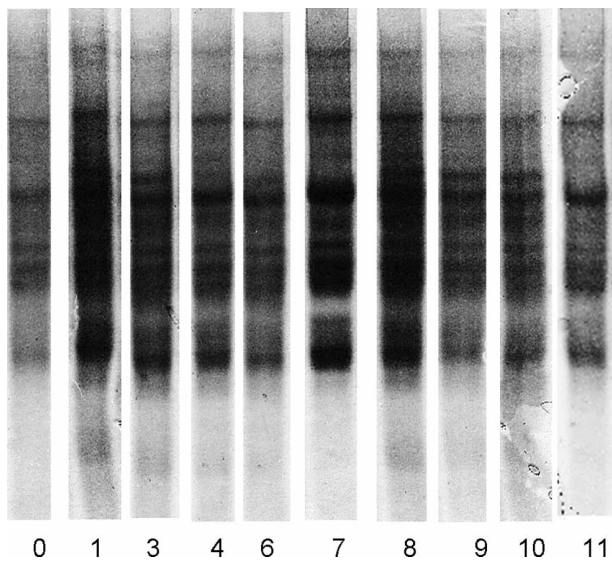


Figure 1. A-PAGE of the fermented wheat flour samples (0-reference, numbers of the bacteria used according to the tables).

Table 2. Residual immunoreactivity (%) in the samples of the fermented wheat flour.

No.	Strain	Number of the human sera	
		S 467	S 918
1	<i>Lb. sanfranciscensis</i> MW-94	88, 74	90, 36
2	<i>Lb. brevis</i> MS-99	83, 78	86, 12
3	<i>Lb. plantarum</i> AD-98	78, 30	78, 46
4	<i>Lb. plantarum</i> LOCK 0858	85, 37	87, 54
5	<i>Lb. plantarum</i> LOCK 0859	94, 19	85, 04
6	<i>Lb. plantarum</i> LOCK 0860	84, 14	64, 08
7	<i>Lb. plantarum</i> LOCK 0861	78, 40	77, 10
8	<i>Lb. sanfranciscensis</i> DSM 20663	76, 51	77, 82
9	<i>Lb. brevis</i> DSM 1267	94, 72	81, 98
10	<i>Lb. brevis</i> DSM 1269	93, 49	84, 88
11	<i>Lb. plantarum</i> DSM 2648	78, 16	74, 18

the highest. Stronger proteolysis performed by mixed cultures than by individual strains results from the action of the complex of proteolytic enzymes with wide specificity, produced by different strains of lactic acid bacteria (De Angelis et al., 2006).

The health effect of lactic acid fermentation of wheat flour was also investigated by other researchers (Hammes & Tichaczek, 1994; Tieking, Korakli, Ehrmann, Ganzle, & Vogel, 2003) and especially the influence of such modified peptides of gliadin fraction on patients with coeliac disease was examined (Di Cagno et al., 2002, 2004). It was found, that fermentation with *Lb. alimentarius*, *Lb. brevis*, *Lb. sanfranciscensis* and *Lb. hilgardii* caused degradation of albumin, globulin and gliadin, whereas glutenin did not undergo proteolysis. Gliadins were the fraction with the most effective degradation during sourdough fermentation, especially for IgE-binding gliadin peptides (Rizzello, De Angelis, Coda, & Gobbetti, 2006). It is also worth to notice that 31–34 fragment of A-gliadin toxic for patients ill with coeliakia was hydrolysed. It was also found that the combination of proteolysis and acidification with bacteria led to emerging of shorter and stronger gluten with more flexible structure.

Table 3. Decrease of the immunoreactivity of wheat flour fermented with bacteria and mixed cultures (%).

Strain	Number of human sera		
	S 902	S 467	S 721
<i>Lb. plantarum</i> AD-98	65, 4	64, 9	77, 1
<i>Lb. sanfranciscensis</i> MW-94	68, 9	64, 2	77, 8
<i>Lb. brevis</i> MS-99	83, 8	63, 1	90, 8
<i>Saccharomyces cerevisiae</i>	71, 8	69, 1	80, 2
Lp+Ls+Lb 1:1:1	74, 9	66, 7	83, 6
(Lp+Ls+Lb)+Sc (1:1)	45, 5	57, 7	73, 7

The results of our investigation and literature data suggest that fermentation as a method of reduction of wheat flour allergenicity seems to be promising because of its natural character and improving both: organoleptic and healthy features of the product. The obtained modified wheat flour can be used as an additive to mixtures prepared for allergic people, especially that not all patients show the same sensitivity to that allergen and not always the complete elimination of gluten from the diet is necessary.

References

- De Angelis, M., Rizello, C.G., Fasano, A., Clemente, M.G., De Simone, C., Silano, M., et al. (2006). VSL#3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for celiac sprue. *Biochimica Biophysica Acta*, 1765, 80–93.
- Di Cagno, R., De Angelis, M., Auricchio, S., Greco, L., Clarke, C., De Vincenzi, M., et al. (2004). Sourdough bread made from wheat and nontoxic flours and started with selected Lactobacilli is tolerated in celiac sprue patients. *Applied and Environmental Microbiology*, 70, 1088–1096.
- Di Cagno, R., De Angelis, M., Lavermicocca, P., De Vincenzi, M., Giovannini, C., Faccia, M., et al. (2002). Proteolysis by sourdough LAB: Effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. *Applied and Environmental Microbiology*, 68, 623–633.
- Diowski, A., & Ambroziak, W. (2006). Sourdough. In Y.H. Hui, H. Corke, I. De Leyn, W. Nip, & R.B. Swanson (Eds.), *Bakery products. Science and technology* (pp. 365–380). USA: Blackwell.
- Gerrard, J.A., Brown, P.K., & Fayle, S.E. (2003). Maillard crosslinking of food proteins II: The reactions of glutaraldehyde, formaldehyde and glyceraldehyde with wheat proteins *in vitro* and *in situ*. *Food Chemistry*, 80, 35–43.
- Hammes, W.P., & Tichaczek, P.S. (1994). The potential of lactic acid bacteria for the production of safe and wholesome food. *Zeitschrift für Lebensmittel – Untersuchung und – Forschung*, 198, 193–197.
- Kunachowicz, H. (1998). Wartość odżywcza dostępnych w Polsce produktów bezglutenowych oraz zasady żywienia w celiakii w aspekcie zapewnienia odpowiednio zbilansowanej diety. *Żywność Człowieka i Metabolizm*, 25, 21–26.
- Leszczyńska, J., Łącka, A., Pytasz, U., Lukamowicz, J., Szemraj, J., & Lewiński, A. (2002). Influence of proteolysis on gliadin immunoreactivity. *Polish Journal of Food and Nutrition Sciences*, 11, 145–148.
- Leszczyńska, J., Łącka, A., Szemraj, J., Lukamowicz, J., & Żegota, H. (2003a). The influence of gamma irradiation on the immunoreactivity of gliadin and wheat flour. *European Food Research and Technology*, 217, 143–147.
- Leszczyńska, J., Łącka, A., Szemraj, J., Lukamowicz, J., & Żegota, H. (2003b). The effect of microwave treatment on the immunoreactivity of gliadin and wheat flour. *European Food Research and Technology*, 217, 387–391.
- Osborne, T.B. (1907). *The proteins of the wheat kernel*. Washington, DC: Carnegie Institute, Publ. No. 84.
- Rizzello, C.G., De Angelis, M., Coda, R., & Gobbetti, M. (2006). Use of selected sourdough lactic acid bacteria to hydrolyze wheat and rye proteins responsible for cereal allergy. *European Food Research and Technology*, 223, 405–411.
- Simonato, B., Pasini, G., Giannattasio, M., Pruffo, A.D.B., De Lazzari, F., & Curioni, A. (2001). Food allergy to wheat products: The effect of bread baking and *in vitro* digestion on wheat allergenic proteins. A study with bread dough, crumb and crust. *Journal of Agricultural Food Chemistry*, 49, 5668–5673.
- Tanabe, S., Arai, S., & Watanabe, M. (1996). Modification of wheat flour with bromelain and baking hypoallergenic bread with added ingredients. *Bioscience, Biotechnology and Biochemistry*, 60, 1269–1272.
- Tanabe, S., Tesaki, S., Yanagihara, Y., Mita, H., Takahashi, K., Arai, S., et al. (1996). Inhibition of basophil histamine release by a haptenic peptide mixture prepared by

- chymotryptic hydrolysis of wheat flour. *Biochemical and Biophysical Research Communications*, 223, 492–495.
- Tanabe, S., Watanabe, J., Oyama, K., Fukushi, E., Kawabata, J., Arai, S., et al. (2000). Isolation and characterization of a novel polysaccharide as a possible allergen occurring in wheat flour. *Bioscience, Biotechnology and Biochemistry*, 64, 1675–1680.
- Tiekink, M., Korakli, M., Ehrmann, M.A., Ganzle, M.G., & Vogel, R.F. (2003). *In situ* production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. *Applied and Environmental Microbiology*, 69, 945–952.
- Watanabe, M., Ikezawa, Z., & Arai, S. (1994). Fabrication and quality of hypoallergenic wheat products. *Bioscience, Biotechnology and Biochemistry*, 58, 2061–2065.
- Watanabe, M., Watanabe, J., Sonoyama, K., & Tanabe, S. (2000). Novel method for producing hypoallergenic wheat flour by enzymatic fragmentation of the constituent allergens and its application to food processing. *Bioscience, Biotechnology and Biochemistry*, 64, 2663–2667.