

Ph.D. THESES

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M.Sc. in Bioengineering

**Effect of enzymatic hydrolysis, new, non-thermal storage process
and environmental factors on food proteins
and their immunoreactivity**

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Introduction and aims

The increasing population, changing environmental factors and the consumer's demand mean new challenges for scientists working in different specializations. We can contribute to cope with the tasks in food science by selection of raw or new materials for special purpose, development of new and more profitable processes and compliance with request of special consumer groups.

The raw materials of traditional and reform food contain cereal-, meat- and milk products, which are important protein and energy sources. The quality of these raw materials is affected by many factors from farm to fork. The evaluation of the effects on raw materials and products is essential in terms of food safety and healthy nutrition.

The significant role of cereals played in nutrition is due to their high energy level, good storage parameters and the broad range of bakery products. In this work the traceability of the effects of environmental factors in the protein patterns of different cereal (wheat and triticale) was investigated. The environmental stresses can modify plant metabolic pathways, thereby leading to decreased productivity. This can result in a serious loss of yield in case of cultivated plants. The selection and breeding of stress resistant cultivars are well recognised and widely used methods to solve this problem.

There are many kinds of metabolic pathway in responses to stresses. Some of them are the results of *up- and down-*regulation of gene transcription and in some cases the expression of the member of stress multigene family can be changed. The changes in gene expression and metabolic pathways and their interaction are responsible for adaptability of plant.

The aim of this work was to study the effects of enzymatic modification, high hydrostatic pressure and an agronomical factor (the fungicide treatment) on protein patterns and biological activity of raw materials. In the experimental I undertook the enzymatically modified epitopes in sodium-caseinate, the effects of high hydrostatic pressure on pork sausage paste and the fungicide treatment on wheat and triticale varieties.

Materials and methods

Sodium-caseinate hydrolisate

Commercial sodium caseinate isolate (SCI,) was hydrolysed with alcalase (2.4 FG, *Subtilisina carlsberg*, Novo Nordisk), pronase (from *Streptomyces griseus*) and papain (EC 3.4.24.4, both from Sigma) in a two-step process.

Meat samples

Raw batter of a fermented sausage paste was subjected to a hydrostatic pressure treatment of 600 MPa for 20 min in Stansted „Food Lab 900” equipment. The urea-soluble protein fractions of treated and untreated sausage batter were used for evaluations.

Cereal samples

The Cereal Research Non-Profit Company, Szeged, Hungary provided samples of wheat and triticale cultivars: Tisza wheat, Marko and Bogo triticale. In 2004, an increased dose of propiconazole (0,6 l ha⁻¹) was applied with an increased dose of tebuconazole (1,5 l ha⁻¹). The first application was performed at the growth stage 55, and the second at the growth stage 69. The control sample was triticale grains without fungicide treatment.

Sample preparation

Grain samples were ground by Hagberg – Perten mill, and the protein fraction of whole meal were prepared using an extraction method described by Osborne (1907).

SDS-PAGE

Proteins were separated by SDS-PAGE as described by Laemmli (1970).

Two-dimensional electrophoresis

In the 2D proteomics mapping, the first dimension (IEF) was run in the immobilized pH gradient (IPG) strip. Proteins were separated according to isoelectric point. In the second dimension the separation of proteins was carried out by SDS-PAGE. The gels were stained with Coomassie Blue R-250.

Immunoblotting

After electrophoresis, proteins were transferred onto a nitrocellulose membrane (Bio-Rad) by semidry blotting. Identification of immunoreactive proteins was performed using undiluted individual sera obtained from milk and cereal allergic patients.

Imaging of the gels and blots was carried out with Bio-Rad Gel Doc 2000 system.

Evaluation by software

The stained gels were scanned by Gel Doc 2000 (Bio-Rad) and analysed using Quantity One 4. 3. 0. (for SDS-PAGE gels) and PDQuest 7. 1. 0. (for two dimensional electrophoresis) software.

Mass spectrometry

Identification of proteins by Mass Spectrometry: Following the procedure described under 2D proteomics mapping, the proteins of interest were excised for in-gel trypsin digestion and were identified by a combination of MALDI TOF MS peptide mapping and MALDI-MS/MS mass spectrometric sequencing. Proteins were identified via processing MS/MS spectra in Agilent Spectrum Mill software using an up-to-date local copy of NCBI non-redundant database. Spectrum filtering and database search hit validation was carried out using the default values set in the Spectrum Mill software.

Measure of α -amylase enzyme activity

The α -amylase enzyme activity was determined using a commercial assay kit from Megazyme (Megazyme International Ireland Ltd., Bray, Ireland) according to the procedure described by manufacturer.

Statistical programme

Analyses of statistical estimate were conducted using MINITAB 13 (State College, PA) software.

New scientific results

1. Most of components obtained in the peptide mixtures were detected at less than 10 kDa molecular mass. As pointed out by immunreaction, the immunreactivity of alcalase-papain and pronase-alcalase hydrolysate decreased significantly.
2. The changes of 2D patterns of the urea-soluble proteins from pork paste, which are due to the effect of high hydrostatic pressure (600 MPa) were detected in the region of molecular mass of 34-50 kDa and the isoelectric point of 7.0-9.0. The conformation changes in pork paste proteins induced by high hydrostatic pressure were confirmed.
3. The immunreactivity of the urea-soluble proteins in pork paste was modified by high hydrostatic pressure (600 MPa) treatment. The high hydrostatic pressure altered epitope structures of some proteins in pork paste.
4. One chitinase, one enzyme inhibitor and one heat shock proteins showed IgE reactivity in wheat (*Triticum aestivum*) cultivar.
5. The examined Tisza wheat and Marko and Bogo triticales showed different response to fungicide treatments. In 2D patterns of water- and salt-soluble proteins in Tisza wheat there was alteration in two regions with components having isoelectric point of 5.0-9.0 and 5.0-8.0 molecular mass of 30-60 kDa and less than 22 kDa respectively. The protein patterns of Marko triticales were unchanged under fungicide treatment. The most of differences were established in the sensitive Bogo cultivars.

6. It was found that fungicide treatment led to changes in protein pattern of Bogó triticale cultivar. The increasing dose of applied fungicide (propiconazole and tebuconazole) induced the synthesis of dimer α -amylase inhibitors and endogen α -amylase/subtilisin inhibitors in Bogó triticale, while the activity of α -amylase was significantly decreased. The intensity of spots of two α -amylase/subtilisin inhibitor and two proteinase inhibitor isoforms increased.
7. The immunoreactivity of three proteins in water- and salt-soluble fraction of Bogó triticale was higher after fungicide treatment (MW: 15-16 kDa, pI: 6.6-6.7 and MW: 29-30 kDa, pI: 10.0) and two newly detected protein spots showed IgE reactivity (MW: 23-24 kDa, pI: 5.8-6.2).
8. The new proteins formed as a results of fungicide treatment were detected in the region of isoelectric point of 7.0-8.0 and molecular mass about 75 kDa in 2D patterns of alcohol-soluble fractions of Bogó triticale. The proteins identified as secalin precursors in gliadin fraction were detected only in the fungicide treated Bogó sample. The differences found in protein pattern of treated and untreated Bogó cultivars are attributable to the 10% infection of the untreated sample by *Fusarium sp.*. It seems that the response to the biotic stress is stronger than that to the abiotic one in Bogó triticale. As hypothesis the synthesis of secalin precursors may be blocked by biotic stress.

9. The IgA- and IgE-reactivity of some alcohol-soluble proteins of fungicide treated and untreated Bogo triticale showed differences against human seras. The differences of IgA reactivity were detected in the region of isoelectric point of 6.0-7.1 and molecular mass of 33-40 kDa, while the IgE reactivity of protein detected in the region of isoelectric point about 8.5 and molecular mass of 30-32 kDa in the untreated Bogo sample decreased significantly and the immun-reactivity, against human seras, of the proteins detected in the region of isoelectric point about 6.5 and molecular mass about 36 kDa increased after fungicide treatment.

Practical application of the results

The obtained results contribute to: detection and identification of stress proteins; to a better recognition of the effects of new, non-thermal storage processing method and changes in the environment on protein patterns; a preliminary index of plant allergens and their traceability. Furthermore, they provide data important for the research work in plant breeding and selection of varieties in the light of food safety and healthy nutrition.

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