Modification of food products properties by use of transglutaminase
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ABSTRACT
The splitting enzymes are used usually in food technologies. A new direction of food enzymology is treatment of raw materials by binding enzymes. We have investigated the action of transglutaminase (TG), [EC 2.3.2.13] as regards technological and functional properties of bread, milk and combined products. TG catalyzes acyl transfer reaction between glutamine γ-amine group of the first substrate and ε-NH₂-groupe belonging to lysine of second substrate. Here was used preparation of microbial TG (Activa®), derived by Ajinomoto Co. It was established TG in concentrations 0,025-0,3% improves physical and textural properties of dough and quality of bakery foods, explaining by generation of proteinase resistant isopeptide bonds in gluten proteins. The conditions of milk serum proteins binding by TG were studied and curd enriched with serum proteins was obtained. It is known defect of gliadin digestion leading to autoimmune intestines damage – celiac disease; to reduce immune affinity of gliadine it was connected to milk serum proteins by TG. The lowering of immune activity of gliadin combined with milk proteins opens a new possibility for manufacturing of gliadin contained products for nutrition of celiac disease patients.

Keywords: whey proteins; gluten; celiac disease

INTRODUCTION
The modern trend of food industry development is deriving of functional food products with regular properties. Enzymes are the tools useful for very thin, correct and purposeful manipulations of raw materials. The ancient processes of brewing and cheese-making rely on enzyme activity at various stages of manufacture. But the traditional products are performed by endogenous enzymes naturally occurring in the plant and animal tissues or in the microorganism’s cells. Majority of enzymes applied in food industry are hydrolases such as glycosidases, and in part proteases used for the meat tenderizing. The new direction of catalytic technology is the use of enzymes for covalent modification of protein structure without its cleavage. Transglutaminase (TG), [EC 2.3.2.13] is showed to be useful for this aim. TG is the family of enzymes that are widely distributed in living nature. They are found in all animal and vegetable tissues, and in microorganism cells, and take part of important physiological functions, including apoptosis, blood clotting, wound healing [1]. TG produces the both inter- and intra-molecular cross-linking bonds in the proteins. This enzyme can catalyze the protein to protein cross-linking by formation of covalent isopeptide bonds between the lysine and glutamine amino acid residues in proteins by scheme:

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\text{Gln} \quad \text{C} \quad \text{NH}_2 + \text{H}_2\text{N} \quad \text{Lys} \quad \text{TG} \quad \text{Glu} \quad \text{C} \quad \text{NH} \quad \text{Lys} + \text{NH}_3
\]

In the beginning of the past century TG was applied for food processing. Microbial TG is used in food industry at present time. It was at first derived Ando et al. [2] from bacterium Streptomyces moharaensis (previously termed Streptovercillium moharaense). TG has the revolutionary ability to improve the physical properties of various protein containing food products. This enzyme shows high activity in a wide pH-range, between pH-value 5 and 8. The optimum temperature is 50 – 55 °C. Shleikin et al. [3] have developed compositions of fish cutlets from minced fish (35 – 55 %) with pea flour (15 – 20 %) and soy-bean textured flour (3 – 6 %) addition. TG was added in amount 0.5 – 2.5 %. To be fermented with TG the semi-products were held round the clock at +2 °C. Measurement of samples elasticity was conducted by penetrometer in the same conditions. It has been established that control samples have crumbly, pasty texture, but experimental samples were compact and elastic. In cutlet samples prepared with addition soy-bean texturized flour were noted definite
consistency improvement respectively control samples. Samples elasticity increases proportionally to amount TG used. In sample with TG elasticity strength twofold exceeds the same of control sample. Sensory quality characteristics of semi-products are higher for TG-fermented samples than for control. TG samples treated by TG have also more high water binding power and water-holding capacity than control samples. Thus by using of TG is possible improvement of structure, enlarged output and nutritional value of product.

Aim of this work is to develop new protein products by combination of animal and vegetable raw materials. The designing of new combined products possessing with functional significance from different protein sources is important task from nutritional, medical, economic and ecological points of view.

MATERIALS & METHODS

Two microbial TG preparations (Activa® MP) EB and YG were purchased and partially were gifts from Ajinomoto Co., Inc. (Hamburg, Germany) with a declared activity of TG 100 U g⁻¹ powder; WPC was received from Friesland Foods Domo (Netherlands) and gluten was received from local manufacturer. The incubation of specimens was carried out with 38 °C and pH 7.4. Colorimetric Lowry method for determining of soluble protein concentration and enzyme multiplied immunoassay (ELISA) were used. For this purpose were derived monoclonal antigliadin rat antibodies. All experiments were carried out in triplicate. The results are analyzed statistically by Student’s test with comparison of differences between the means of the treatments and controls at the significance level of P < 0.05.

RESULT & DISCUSSION

Making combining products is important from economical and nutritional points of view. By combining animal and vegetable raw materials it is possible to regulate amino acid, lipid and mineral composition of foods, to enrich food with vitamins, microelements, dietary fibers. It is important for healthful and dietary meals. Milk is beautiful framework for making combining products with functional properties, which by daily usage for meal, are able to show control action on defined system of organism or on human body en bloc. Whey protein is a full-value, high quality protein with a rich amino acid profile. It contains the full spectrum of amino acids including essential amino acids and branched-chain amino acids, such as leucine or isoleucine, which are important in tissue growth and repair. In part leucine is a key branched-chain amino acid in protein synthesis and has recently been identified as playing a critical role in insulin and glucose metabolism. In particular this work deals with combined proteins making from whey protein concentrate and wheat gluten. Celiac disease is gluten-induced enteropathy caused by inflammatory response to ingestion of gluten in genetically susceptible individuals. According to Ciclitira [4] celiac disease was believed to affect 1:1500 individuals throughout Europe, with a higher prevalence (1:150) in Ireland. As stated by Reif and Lerner [5], TG of human tissues is the key player in celiac disease. It is important to modify gluten to prevent toxic action of immune active peptides. We suggest develop a combined product consisting of gluten proteins (gliadins, glutenins) and whey globulins, and having modified biological activity. It is well known that milk whey proteins (α-lactalbumin, β-lactoglobulin and immunoglobulins) have the high splitting rate and very bright biological activity. Shleikin et al. [6] indicated cross-linking effect of TG on gluten and whey proteins by electrophoresis. It has opened the perspective to develop a new product with enriched protein composition and with reduced immune activity of gluten. In these experiments WPC and gluten in equilibrium 1:1 were mixed with TG and sustained in above specified conditions during 60 minutes. After incubation the samples were centrifuged by 113 g. The supernatant was decanted and water soluble protein amounts were determined according Lowry method. These residual protein concentrations are represented in Fig. 1.

![Figure 1. Dependence of soluble proteins amount upon incubation time of WPC and gluten fermented with 1 % TG.](image-url)
It is observed significant decrease in presence of soluble proteins in whey depending on time of incubation. It is suggested WPC-gluten combined protein products are formed under TG action. The immune activity of these products to antigliadin antibodies was measured with ELISA. Results are given in Fig. 2, 3.

**Figure 2.** Dependence of immune activity of WPC and gluten products derived with 0.5% TG upon incubation time.

![Graph showing immune activity vs. incubation time](image)

**Figure 3.** Cooperative incubation of WPC and gluten for 60 min. Results of enzyme multiplied immunoassay (ELISA).

![Graph showing immune activity vs. TG concentration](image)

Samples of incubation WPC and gluten were analyzed by ELISA on XGY1, XGY6, XGY16 – antigliadin antibodies (Fig. 2, 3). There were noted decreasing of optical density of samples both with increasing reaction time (Fig. 2) and TG concentration (Fig. 3). Immune activity of modified proteins corresponds to optical density of conjugates. So, there were made protein conjugates with reduced toxicity of gluten. By this way may be manufactured new kinds of food products with reduced immune activity of gluten that may help to solve the nutrition problem of patients suffered of celiac disease.

**CONCLUSION**

We established that the combined animal and vegetable proteins containing products can be produced by use of enzymatic cross-linking of proteins with TG. The product developed by this method from milk serum proteins and gluten has reduced affinity to antigliadin antibodies, that is important for patients of celiac disease. Our further investigation will deal with combined protein products made with TG that has important nutritional, medical and economic significances.

**REFERENCES**


