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Improvement of gluten-free bread quality using transglutaminase, various extruded flours and protein isolates

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Summary
Different enzymes and other proteins are used to improve the quality of gluten-free breads, but their combinations and relative amounts need to be optimized to reduce the product cost and to improve the overall consumer acceptability. This paper aimed to investigate the feasibility of using extruded flours (rice, potato, corn, buckwheat) in combination with various proteins (egg-white powder, soya isolate, caseinate) and different amounts of transglutaminase (TG; 1 IU and 10 IU TG per gram of protein) to produce technologically and nutritionally improved gluten-free breads that may be useful for enhancing the diet of celiac sufferers. Recipe and protein addition interacted to significantly affect all physical properties of bread. TG addition reduced gluten content and increased crumb hardness and chewiness. The bread with the most desirable properties was prepared with extruded buckwheat extrudate, egg-white powder and 10 IU TG per gram of protein.

Keywords
gluten-free bread; transglutaminase; optimisation; protein addition; extruded flour; texture profile

Gluten-free alternatives for foods such as breads remain a manufacturing challenge. Wheat gluten is imperative for high bread quality because it provides a viscoelastic structure, a satisfactory feeling in the mouth and a spongy bread honeycomb. Gluten-free cereal products are protein-deficient and, in addition, cereals have a low biological value since they are deficient in lysine, threonine and tryptophan. This can substantially be improved by supplementing them with proteins isolated from non-wheat sources [1]. In particular, proteins isolated from legumes and dairy sources are most often added to gluten-free products [2]. Developing breads that can be labelled as sources of protein according to European legislation may be useful for improving the diet of celiac sufferers.

The quantity and type of added protein affect the quality of gluten-free bread. For instance, including 0.5% soya isolate in gluten-free bread improved texture, crumb grain and overall bread quality, and it also increased the specific volume [3]. Gluten-free bread volume increased by adding milk protein to a final level of 3% [4]. However, when the amount of added skim milk powder was raised from 1.2% to 4.8%, loaf height decreased [5]. Similarly, adding 13% soya protein isolate to gluten-free bread lowered the specific volume from 2 $l \cdot kg^{-1}$ to 1.59 $l \cdot kg^{-1}$ [6].

Addition of specific enzymes like transglutaminase may also improve the structure and overall quality of gluten-free breads by helping to form a protein network [2, 7]. Enzyme transglutaminase (TG, protein-glutamine γ-glutamyl-transferase, EC 2.3.2.13) catalyses mainly the covalent cross-linking of proteins via the ε-amino group of lysines on one protein to the γ-carboxyamide group of glutamines on the same or another protein [8]. When lysine residues act as acyl receptors in the reaction, intra- and intermolecular isopeptide bonds form. When the ε-amino group of lysine is

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the substrate, the two substrate proteins become covalently linked through a ε-(γ-glutamyl) lysine bond. Though this is the dominant reaction catalysed by TG, the enzyme also catalyses two other processes: in the presence of primary amines, it cross-links the amine to the γ-carboxyamide group of glutamine residues in proteins. If the substrate protein does not contain any primary amines, water serves as the acyl acceptor and TG catalyses deamidation of the γ-carboxyamide groups of glutamines, converting them into glutamate. Thus, TG can modify proteins by catalysing amine incorporation, cross-linking and deamination.

TG is used to improve the physical and texture properties of many protein-rich foods, but also to lower the gluten content in food [9], making it a useful ingredient in gluten-free products. Since the cross-linking kinetics of TG depend on the structures of the substrate proteins and the availability of lysine and glutamine residues [10], the content and source of proteins used in bread production greatly influence the formation of protein networks and therefore the final product characteristics [1]. The effects of 0.1, 1 and 10 IU TG on the characteristics of gluten-free breads have been tested [2, 11, 12], but an ideal content of TG has not yet been defined.

Gluten-free flours can be processed by extrusion cooking in order to improve their nutritional value and to simplify their production. Extruding flour destroys antinutritional factors, gelatinizes starch, increases soluble dietary fibre content and protein digestibility (PD) and reduces lipid oxidation [13]. Extrusion cooking is a physical approach for improving the properties of native starches without the need for chemical modification. It also ameliorates the negative effects of storing starchy food products, such as starch retrogradation. For example, gelatinizing rice by extrusion was shown to lead to three-dimensional networks that retain gases and expand during the fermentation and baking of gluten-free bread. CLERICI et al. showed that pregelatinizing rice flour by extrusion and simultaneously modifying it with an organic acid considerably improved the crust colour and texture characteristics of gluten-free bread [14].

The present study explored the way how the interaction of various proteins (egg-white powder, soya isolate and caseinate) with different contents of TG (1 IU and 10 IU per gram of protein) affected the quality of gluten-free breads made from different extruded flours. The physical and nutritional properties of the breads were investigated in order to define the optimal recipe that would give a product that closely resembled wheat bread and that was of high quality and high nutritional value. Bread of this kind may be useful for improving the diet of celiac sufferers.

MATERIALS AND METHODS

Ingredients

Gluten-free breads were made by mixing corn starch (Davert Muhle, Senden, Germany), rice extrudate (Davert Muhle) and the following extruded flours: extruded buckwheat flour (Dutch Organic, Barneveld, Netherlands), or extruded potato flakes (Naše Klasje, Zagreb, Croatia), or extruded corn flour (Naše Klasje). In order to increase the protein content, doughs were supplemented with soya isolate (Supro 620 IP, Ireks Aroma, Jastrebarsko, Croatia), egg-white powder isolate (Elcon PP, Zlatar Bistrica, Croatia) or sodium caseinate (Casein sodium salt from bovine milk, C8654, Fluka, Steinheim, Germany). Hydroxymethylpropylcellulose (Methocel K4M Food grade modified cellulose, Dow, Midland, Michigan, USA), guar gum (5% protein, 12% moisture, ≤0,5% ash, ≤0,5% fat, Ireks Aroma), glucose (D-(+)-glucose, Fluka), compressed yeast (*Saccharomyces cerevisiae*; Kvasac, Lesaffre International, Prigorje Brdovečko, Croatia) and salt (Solana Pag, Pag, Croatia) were incorporated in all recipes in constant amounts (Tab. 1). Microbial TG Activa WM was kindly donated by Ajinomoto company (Hamburg, Germany).

Experimental plan

According to the full factorial experimental plan of three independent variables (recipe, protein source and TG content), 27 types of bread were prepared (54 loaves in total, all types being prepared in duplicate). All the recipes contained rice extrudate and corn starch, but differed in the third ingredient (Tab. 1). Potato flakes and corn were added to a final content of 30%, while buckwheat extrudate was added to 15% with the remaining 15% made up with rice extrudate; a lower amount of buckwheat was used because of its strong taste and bitterness. Amounts of protein isolates added slightly differed from one recipe to another in an attempt to achieve similar protein contents in breads made by different recipes. Quantities of protein isolates added (Tab. 1) were calculated based on the protein content specified by the manufacturer (as shown in Tab. 2). The final protein contents of breads differed slightly from calculations, depending on the ingredients used and the moisture content. The quantity of TG added to the recipes (Tab. 1) was calculated according to the manufacturer's specification of TG

Tab. 1. Ingredients used in gluten-free bread production.

Ingredient	Recipe 1					Recipe 2					Recipe 3				
Extruded rice flour [g]	350					350					500				
Corn starch [g]	225					225					225				
Extruded potato flakes [g]	300					–					–				
Extruded corn flour [g]	–					300					–				
Extruded buckwheat flour [g]	–					–					150				
Hydroxymethylpropylcellulose [g]	5					5					5				
Guar gum [g]	15					15					15				
Glucose [g]	10					10					10				
Salt [g]	17					17					17				
Yeast [g]	15					15					15				
Water (ml)	1100					1100					1100				
Source of protein	Cas		Soy		Egg	Cas		Soy		Egg	Cas		Soy		Egg
Amount of protein added [g]	105.7		114.89		121.5	101.7		110.52		116.87	87.1		104.3		110.22
Transglutaminase [g]	0	1.5	15	0	1.5	15	0	1.5	15	0	1.5	15	0	1.5	15	0	1.5	15	0	1.5	15

Cas – sodium caseinate; Soy – soya isolate; Egg – egg-white powder.

activity (105 IU per gram). The specific activity of 1 IU TG per gram of protein of gluten-free bread was consistent with the addition of 1.5 g TG, while 10 IU TG per gram of protein of gluten-free bread was consistent with the addition of 15 g TG.

Bread making

TG was first dissolved in water and then added to the flour mixture. Yeast was dissolved in water with glucose and pre-fermented in a proofing cabinet at 35 °C and 85% relative humidity (RH) for 10 min before mixing with flour. Water at 30 °C was used to prepare dough. All ingredients were mixed in a spiral mixer (Diosna SP12, Diosna Dierks and Söhne, Osnabrück, Germany) for 7 min at 1,5 Hz and 6 min at 3 Hz. Bread dough was immediately divided into 500 g pieces, placed in baking tins (dimensions 20 × 9 × 7 cm) and fermented in a proofing cabinet at 35 °C, 85% RH for 40 min. Since the dough had a batter-like, almost liquid consistency, it was not formed before being placed into tins; instead, it was transferred directly with a spatula. The only exception was Recipe 1 using caseinate; in this case, the dough was firm enough that it could be formed before being placed into tins. Dough was baked for 80 min in a deck oven (Wiesheu, Affalterbach, Germany) with the lower heater at 210 °C, the upper heater at 200 °C, and 0.4 litre of steam released at the start of baking. The air valve was kept closed until the last 15 min. Breads were taken out of the pans and allowed to cool down to room temperature for 2 h. Breads of all recipes were prepared in duplicate and each batch was used in experiments.

Chemical analyses of ingredients and breads

Amino acid composition of ingredients was determined according to Csapo et al. [15] using an amino acid analyser (AAA400; Ingos, Prague, Czech Republic). Amino acid levels are expressed as grams per kilogram of protein (Tab. 2). Quantification of free amino groups (FAG) was done by spectrophotometric assay using the *o*-phthaldialdehyde (OPA) method according to [16], except that a spectrophotometer Helios Beta (Unicam, Cambridge, United Kingdom) was used instead of a microplate reader. Because a spectrophotometer requires more sample than a microplate reader, amounts of supernatant and reagent were three times larger those used in the original method.

Gluten quantity was determined by the ELISA-R5 method [17], based on the double antibody sandwich antigen-antibody reaction. Nitrogen content was determined by the Dumas method using a Leco instrument FP 328 (Leco instrumente, Mönchengladbach, Germany), calibrated using

EDTA. Crude protein content was calculated by multiplying the nitrogen content by the conversion factor of 6.25 (AACC-approved method 46-30 [18]).

PD was determined in vitro according to FAGEER et al. [19] by the protein digestibility-corrected amino acids score method. The method is based on the comparison between the content of the limiting amino acid in the tested protein and the content of the same amino acid in a reference sample. Lysine is the limiting amino acid for cereals. PD was expressed as the ratio of the digested protein to total protein in the sample. Moisture content of breads was determined by drying [20] and fat content by the Soxhlet extraction method [21]. All analyses were performed in duplicate.

Analyses of bread physical parameters

Bread volume was determined in duplicate by a rapeseed displacement method [22], and specific volume (volume-to-mass ratio) was calculated. Bread height and width were determined using a calliper in five replicates, and the average height-to-width ratio was calculated. Although breads were baked in tins, width of the loaves somewhat differed because some breads narrowed while other broadened during baking.

Texture profile analysis was performed in a double compression cycle using a TAHD. plus Texture analyser (Stable Micro Systems, Godalming, United Kingdom) with a cylindrical 25 mm probe, 25 kg load cell, speed of descent 1 mm·s^{-1}, 40% penetration depth and 30 s gap between compressions. Two loaves were prepared for each type of bread and three samples were taken from each loaf. A sample consisted of two slices of bread, 12.5 mm thick, taken from the middle of the loaf in order to acquire slices that were equal in size.

Statistical analysis and optimisation

The influence of independent factors (protein source, TG content, bread recipe) on bread properties was interpreted from analysis of variance (ANOVA) using a three-factor interaction model with backward elimination regression ($p < 0.05$). A multiple comparison analysis was

Tab. 2. Chemical composition of bread ingredients.

Ingredient	Rice extrudate	Corn starch	Buckwheat extrudate	Corn extrudate	Extruded potato flakes	Sodium caseinate	Soya isolate	Egg-white
Protein [g·kg^{-1}]	79.7 ± 0.5^{c}	1.5 ± 0.1^{e}	128.4 ± 0.1^{a}	84.4 ± 0.3^{b}	71.8 ± 0.2^{d}	920*	840*	800*
Gluten [mg·kg^{-1}]	6.63 ± 0.0^{b}	6.63 ± 0.03^{b}	7.19 ± 0.04^{a}	< 3	< 3	< 3	< 3	< 3
FAG [mmol·mg^{-1}]	0.67 ± 0.06^{c}	0.00 ± 0.00^{d}	1.49 ± 0.15^{b}	0.87 ± 0.07^{c}	2.79 ± 0.32^{a}	-	-	-
Amino acids [g·kg^{-1}]								
Asparagine	6.45	0.19	10.06	4.29	9.23	67.15	93.09	72.36
Threonine	3.09	0.06	4.75	2.69	2.85	38.81	31.00	36.49
Serine	3.71	0.13	5.69	3.7	2.62	51.12	42.45	46.77
Glutamine + Glu	11.47	0.40	18.27	14.13	7.07	204.49	144.91	86.54
Proline	3.31	0.03	4.15	7.63	2.64	125.36	49.64	33.79
Glycine	3.31	0.03	6.50	7.63	2.24	125.36	49.64	33.79
Alanine	3.81	0.14	4.43	5.51	2.25	27.42	32.05	39.02
Valine	3.07	0.04	4.59	2.84	3.04	42.17	26.03	31.69
Cysteine	0.62	0.00	1.23	0.70	0.29	1.49	3.27	7.40
Methionine	0.55	0.02	1.27	0.94	0.68	19.39	5.72	10.56
Isoleucine	2.06	0.07	3.32	2.07	2.11	33.32	26.98	25.00
Leucine	5.45	0.15	7.16	10.36	4.41	83.79	60.46	52.86
Tyrosine	2.98	0.03	2.91	3.32	2.11	45.57	26.12	22.60
Phenylalanine	3.48	0.09	4.88	3.74	2.74	43.82	39.07	37.02
Lysine	2.60	0.12	6.02	1.53	3.44	64.17	40.51	36.03
Histidine	1.61	0.10	2.43	1.85	1.05	24.09	16.93	11.82
Arginine	5.74	0.06	10.12	2057.00	2.53	27.67	50.23	28.76

* – values defined in manufacturer specification. Mean values followed by different letters in the same column are significantly different at 95% confidence level. Amino acids are expressed per kilogram of protein. FAG – free amino groups (expressed per milligram of lysine), Glu – glutamic acid.

carried out to assess significant differences among the samples. Fisher's least significant difference (LSD) test was used to describe means with 95% confidence. Experimental design, analysis and optimization were carried out using Design Expert 7.1.3. software (Stat-Ease, Minneapolis, Minnesota, USA).

RESULTS AND DISCUSSION

Amino acid composition

The amino acid composition of ingredients (Tab. 2) was similar to the literature [11, 23, 24]. In order to allow TG to catalyse reactions involving primary amines, the substrate protein must contain cysteine, histidine or asparagine residues [25]. Based on the reported amino acid contents of each type of flour, breads prepared according to Recipe 3 were expected to contain the highest levels of these three amino acids. Extruded flour containing the largest amount of lysine was buckwheat, followed by potato flakes, rice flour and corn extrudate (Tab. 2). Protein concentrate containing the largest amount of lysine was caseinate, followed by soya isolate and, finally, egg-white powder. Given that lysine is a limiting amino acid in cereals, the amino acid composition of the ingredients was used to estimate the lysine content of the final prepared breads. The lowest lysine content was predicted for breads from Recipe 1 containing added soya isolate, and the highest for breads prepared with buckwheat. Estimated lysine content ranged from 190 $g \cdot kg^{-1}$ of bread (Recipe 1, containing soya isolate and 10 IU TG) to 520 $g \cdot kg^{-1}$ of bread (Recipe 3 containing egg-white and no TG). These predictions for final lysine content should be used with caution, since lysine interacts with TG and is involved in Maillard reactions, so it is partially lost during breadmaking.

Quantity of free amino groups

Since TG catalyses covalent cross-linking reactions between amino groups of proteins, adding it to dough should reduce the number of free amino groups (FAG) [12]. Thus, the initial level of FAG should indicate the suitability of a given ingredient to act as a TG substrate. By this criterion, potato flakes, which were the flour type with the highest contents of glutamic acid and FAG (Tab. 2, 3), were expected to be the most desirable TG substrate. Surprisingly, breads with potato flakes were not of satisfactory quality. This is, to our knowledge, the first study to examine potato flakes in gluten-free bread, and although they did not show promising results in this study, their use should be further investigated with a careful recipe optimization. Corn and rice extrudate had similar low FAG contents, while corn starch had no detectable FAG.

ANOVA showed that the recipe, interaction of recipe and protein source, and interaction of TG content and protein source, had a significant influence on the amount of FAG. The interaction of TG content and recipe had a weaker influence (Tab. 4). For breads prepared using any of the three recipes and containing added caseinate, FAG was lower in the presence of TG than in its absence. In bread of Recipe 1, the level of FAG observed with egg whites was even lower after the addition of 1 IU TG, while in bread of Recipe 2 the same effect occurred after addition of 10 IU TG. Soya isolate unexpectedly led to higher FAG levels in the presence of TG in bread of Recipes 2 and 3. Similar results were observed in bread of Recipe 3 containing egg white and TG.

Quantity of gluten

A threshold of gluten sensitivity in celiac patients has not been determined conclusively. According to legislative guidelines of the European Union [26], up to 20 $mg \cdot kg^{-1}$ gluten is permitted in food for celiac disease (CD) patients, but information is lacking about the long-term risk posed to celiac patients by small doses of the gliadin fraction of gluten. Sensitivity to trace intake of gluten varies substantially from patient to patient [27, 28] and many individuals cannot tolerate even very small amounts of gliadin [29].

ANOVA showed that recipe, protein source and TG content significantly influenced the gluten content of bread (Tab. 4). The highest quantity of gluten was found in buckwheat extrudate, followed by corn starch and rice extrudate, which had similar gluten content. Potato flakes and corn extrudate had a gluten level below the limit of detection according to manufacturer's specifications, which was 3 $mg \cdot kg^{-1}$ of product (Tab. 2). Among the breads prepared without the addition of TG, the largest contents of gluten were found in Recipes 1 and 2 with the addition of sodium caseinate (Tab. 3). Since contents of gluten were considerably higher in these samples than in others, and with regard the fact that ingredients used for preparation of these breads were not the richest in gluten, it is possible that there was some cross contamination of the samples during the production of these breads. Smallest amounts of gluten were found in breads prepared with the addition of soya isolate in all three recipes.

The impact of microbial TG on gluten is still questionable. Several studies show contradicting

results. DEKKING et al. found that microbial TG had a broader substrate specificity than the tissue TG, deamidating both synthetic and natural gluten peptides that are recognized by gluten-specific T cells. Therefore, they concluded that microbial TG can enhance the immunogenicity of gluten and should not be used in food products intended for consumption by CD patients [30]. GIANFRANI et al. reported on the possibility of reducing gluten immunogenicity by transamidation using microbial TG. Deamidation of glutamine residues present in gluten proteins by tissue TG gives a negative charge to gluten peptides conferring high affinity to specific antigens. Using TG in a controlled system to bind free amino acids to glutamine amino groups in gluten proteins may prevent the immune recognition by specific antigen molecules in CD, due to steric hindrance by amino acid residues neighbouring the negatively charged glutamic acid residues [31]. Research results by ELLI et al. showed that modification induced by bacterial transglutaminase alone did not induce a reduction in immunostimulation when compared to unmodified gluten, whereas the addition of lysine to the enzymatic reaction abolished the release of specific antibodies, as well as the increase of TG activity [32]. We found that the gluten content was lower in the presence of TG than in its absence

Tab. 3. Chemical composition of gluten-free breads according to recipe, protein source and transglutaminase addition

Bread sample	Moisture [g·kg^{-1}]	Lipids [g·kg^{-1}]	Proteins [g·kg^{-1}]	PD [%]	FAG [mmol·mg^{-1}]	Gluten [mg·kg^{-1}]
R1-Cas-TG0	409 ± 2de	6.7 ± 0.0^{l}	91.5 ± 0.05^{m}	94.5 ± 0.2^{d}	2.39 ± 0.02^{a}	8.15 ± 0.07^{a}
R1-Soy-TG0	545 ± 2^{a}	6.2 ± 0.0^{m}	73.5 ± 0.02^{p}	84.3 ± 0.2^{j}	1.84 ± 0.01^{b}	5.3 ± 0.28^{f}
R1-Egg-TG0	392 ± 0^{i}	5.1 ± 0.0^{o}	99.9 ± 0.01^{i}	92.8 ± 0.1^{e}	1.77 ± 0.01^{b}	6.75 ± 0.07^{cd}
R1-Cas-TG1	404 ± 0^{e}	6.6 ± 0.1^{l}	93.8 ± 0.02^{k}	97.2 ± 0.1^{bc}	1.78 ± 0.05^{a}	7.25 ± 0.07^{c}
R1-Soy-TG1	530 ± 0^{c}	3.8 ± 0.1^{p}	74.7 ± 0.02^{o}	87.9 ± 0.3^{gh}	1.8 ± 0.03^{b}	4.2 ± 0.00^{h}
R1-Egg-TG1	397 ± 0^{f}	6.4 ± 0.0^{lm}	92.5 ± 0.03^{l}	94.1 ± 0.1^{d}	1.62 ± 0.07^{cb}	5.65 ± 0.07^{f}
R1-Cas-TG10	402 ± 0^{ef}	6.6 ± 0.1^{l}	86.9 ± 0.01^{n}	96.1 ± 0.2^{c}	0.76 ± 0.01^{d}	7.35 ± 0.35^{bc}
R1-Soy-TG10	540 ± 0^{b}	5.7 ± 0.0^{n}	72.9 ± 0.01^{r}	81.1 ± 0.2^{e}	1.7 ± 0.12^{b}	3.05 ± 0.07^{h}
R1-Egg-TG10	381 ± 1^{k}	7 ± 0.0^{k}	99.6 ± 0.01^{j}	95.8 ± 0.3^{c}	1.71 ± 0.04^{b}	4.05 ± 0.07^{ij}
R2-Cas-TG0	386 ± 0^{j}	8.6 ± 0.0^{g}	91.6 ± 0.01^{m}	84.2 ± 0.2^{j}	1.05 ± 0.06^{d}	8.15 ± 0.07^{a}
R2-Soy-TG0	392 ± 0^{i}	10.8 ± 0.0^{c}	73.5 ± 0.03^{p}	81.2 ± 1.1^{l}	1.01 ± 0.05^{de}	4.65 ± 0.21^{gh}
R2-Egg-TG0	395 ± 0^{g}	8.6 ± 0.0^{g}	99.9 ± 0.04^{i}	89.2 ± 0.2^{g}	0.12 ± 0.03^{g}	6.75 ± 0.00^{h}
R2-Cas-TG1	383 ± 0^{k}	8.2 ± 0.0^{h}	93.8 ± 0.02^{k}	87.2 ± 0.2^{h}	1.09 ± 0.12^{d}	3 ± 0.42^{k}
R2-Soy-TG1	378 ± 1^{l}	11.3 ± 0.0^{b}	74.7 ± 0.00^{o}	82.9 ± 0.2^{k}	1.16 ± 0.35^{fd}	3.9 ± 0.00^{h}
R2-Egg-TG1	385 ± 2^{j}	8 ± 0.0^{i}	92.5 ± 0.01^{l}	88.7 ± 0.2^{g}	0.12 ± 0.03^{g}	3 ± 0.00^{k}
R2-Cas-TG10	376 ± 0^{l}	7.5 ± 0.0^{j}	86.9 ± 0.01^{n}	91.3 ± 0.2^{f}	0.26 ± 0.10^{d}	3 ± 0.00^{k}
R2-Soy-TG10	399 ± 0^{f}	10.4 ± 0.0^{d}	72.9 ± 0.01^{r}	79.4 ± 0.1^{m}	1.68 ± 0.01^{b}	3.05 ± 0.00^{ij}
R2-Egg-TG10	395 ± 3^{g}	7.6 ± 0.0^{j}	99.6 ± 0.00^{j}	85.2 ± 0.1^{i}	0.03 ± 0.04^{h}	3 ± 0.00^{k}
R3-Cas-TG0	417 ± 1^{d}	8.7 ± 0.0^{g}	106.9 ± 0.00^{d}	96.3 ± 0.1^{c}	1.56 ± 0.25^{cb}	6.9 ± 0.14^{c}
R3-Soy-TG0	380 ± 0^{k}	10.5 ± 0.1^{d}	104.3 ± 0.14^{f}	92.1 ± 0.2^{ef}	0.76 ± 0.01^{d}	4.7 ± 0.00^{g}
R3-Egg-TG0	417 ± 1^{d}	8.3 ± 0.1^{h}	110.8 ± 0.01^{a}	96.2 ± 0.1^{c}	0.31 ± 0.04^{g}	7.6 ± 0.42^{b}
R3-Cas-TG1	395 ± 0^{g}	9.2 ± 0.0^{e}	101.3 ± 0.01^{h}	98.7 ± 0.1^{a}	1.49 ± 0.07^{cb}	7.15 ± 0.07^{c}
R3-Soy-TG1	400 ± 0^{f}	11.9 ± 0.1^{a}	107 ± 0.20^{d}	94.6 ± 0.2^{d}	0.77 ± 0.10^{d}	3.35 ± 0.21^{i}
R3-Egg-TG1	405 ± 0^{e}	9.4 ± 0.1^{e}	109 ± 0.01^{b}	98.4 ± 0.0^{ab}	0.96 ± 0.02^{de}	6.1 ± 0.14^{e}
R3-Cas-TG10	397 ± 1^{f}	9.1 ± 0.0^{ef}	102.7 ± 0.00^{g}	99.2 ± 0.1^{a}	1.22 ± 0.00^{d}	3.45 ± 0.07^{i}
R3-Soy-TG10	393 ± 0^{gh}	10.7 ± 0.1^{c}	106.3 ± 0.01^{e}	93.2 ± 0.1^{e}	1.28 ± 0.07^{d}	3.05 ± 0.07^{ij}
R3-Egg-TG10	395 ± 2^{g}	8.7 ± 0.2^{g}	107.2 ± 0.00^{c}	97.8 ± 0.1^{b}	2.52 ± 0.11^{a}	4.2 ± 0.14^{h}

Mean values followed by different letters in the same column are significantly different at 95% confidence level.
Designation of samples: recipe (R1 – recipe 1, R2 – recipe 2, R3 – recipe 3); protein source (Cas – caseinate, Soy – soya isolate, Egg – egg-white powder); transglutaminase (TG0 – 0 IU, TG1 – 1 IU, TG10 – 10 IU).
Moisture, lipids and proteins are expressed per kilogram of bread. FAG are expressed per milligram of lysine.

Tab. 4. Results of ANOVA analysis indicating significant influence of recipe, protein and transglutaminase and their interactions on the parameters of bread.

	Recipe	Protein	TG	Rec – Prot	Rec – TG	TG – Prot	R^2	$Adj\text{-}R^2$	$Pred\text{-}R^2$
	Statistical significance (p)								
Moisture	< 0.0001	< 0.0001	ns	< 0.0001	ns	ns	0.9768	0.9665	0.9477
Lipids	< 0.0001	0.0014	0.8333	0.0005	0.1217	ns	0.9535	0.8992	0.7645
Proteins	< 0.0001	< 0.0001	0.2130	< 0.0001	ns	0.0306	0.9859	0.9694	0.9284
PD	< 0.0001	< 0.0001	ns	0.0985	ns	ns	0.9141	0.8760	0.8068
FAG	0.0009	0.1585	0.9583	0.0332	0.0585	< 0.0222	0.9239	0.7527	0.1334
Gluten	0.315	0.0019	0.0001	ns	ns	ns	0.7317	0.6512	0.5110
Height to width	0.0027	0.0003	ns	< 0.0001	ns	ns	0.8311	0.7560	0.6200
Specific volume	< 0.0001	< 0.0001	ns	ns	0.0009	ns	0.9281	0.8962	0.8383
Hardness	< 0.0001	0.0002	0.0264	0.0005	ns	ns	0.9595	0.9342	0.8846
Resilience	< 0.005	< 0.0005	ns	< 0.001	ns	ns	0.9456	0.9214	0.8775
Springiness	0.0045	0.0005	ns	0.008	ns	ns	0.7634	0.6583	0.4678
Cohesiveness	< 0.0001	< 0.0001	ns	0.0178	ns	ns	0.9296	0.8984	0.8417
Chewiness	< 0.0001	0.0007	0.0276	0.0179	ns	ns	0.9144	0.8609	0.7562

$p < 0.05$.
TG – transglutaminase, ns – not significant, (Rec – Prot) – recipe and protein interaction; (Rec – TG) – recipe and TG interaction; (TG – Prot) – TG and protein interaction; R^2 – coefficient of determination; $Adj\text{-}R^2$ – adjusted coefficient of determination; t^2 – predicted coefficient of determination.

in all breads except for those prepared with corn extrudate, which was already low in gluten. On average, breads with 1 IU TG had by 27% lower gluten content than the same breads without TG, while breads with 10 IU TG had by 43% lower gluten content. Gluten content was even lower when TG was added in combination with egg white. In this case, 1 IU TG caused a 31% reduction, and 10 IU TG a 47% reduction.

Chemical composition of breads

Bread moisture content (Tab. 3) was significantly affected by recipe, protein source and their interaction (Tab. 4). Breads produced according to Recipe 1 with soya isolate had by 36% higher moisture content than the bread prepared with caseinate and egg-white powder. The moisture content was similar for the various breads (Tab. 3), except for breads containing soya isolate, which were wetter. This could be due to the water-binding ability of soya flour in baked goods [3, 33]. As a result, those breads were lower in quality and had a soggy crumb.

Fat content (Tab. 3) was highest in breads prepared according to Recipes 2 and 3 containing soya isolate, and lowest in bread of Recipe 2 containing caseinate and 10 IU TG. Most breads were of low fat contents (Tab. 3). Indeed, this was one of our goals in our efforts to define an optimal bread recipe for improving the diet of celiac sufferers. For this reason, we used egg-white powder (pure protein) instead of whole eggs.

Overall mean protein content was lowest after addition of soya isolate (84 $g \cdot kg^{-1}$ of bread), higher after addition of caseinate (95 $g \cdot kg^{-1}$ of bread), and highest after addition of egg-white powder (101 $g \cdot kg^{-1}$ of bread). Mean protein content of breads prepared according to Recipe 1 and Recipe 2 was 87 $g \cdot kg^{-1}$ of bread, while the average for Recipe 3 was 106 $g \cdot kg^{-1}$ of bread (Tab. 3). Protein provided on average 16% of the total energy content in all breads. Protein nutritional value depends on the quantity, digestibility and availability of essential amino acids. The quantity of protein in our gluten-free breads was similar to that in common wheat breads (Tab. 3). Bread prepared with buckwheat flour contained the highest amount of protein. In fact, buckwheat extruded flour is considered desirable for producing gluten-free bread because of its high nutritional value and high protein content [34]. All the breads produced in the present study can be considered sources of protein. This makes them useful for improving the diet of celiac sufferers, since gluten-free breads usually have low nutritional value [35].

Protein digestibility

PD is a measure of usability of the protein in the body. PD depended on the recipe and protein source. Overall, the best digestibility was deter-

mined for breads of Recipe 3 (average PD, 92%), followed by Recipe 1 (91.5%) and finally Recipe 2 (85.5%; Tab. 3). Usually, PD of buckwheat grain is relatively low (< 80%), but extrusion cooking can improve PD of cereals and pseudocereals. For example, after extrusion, PD of buckwheat increased by up to 10% [36] and PD of corn by up to 15% [24]. Extrusion may cause these effects by inactivating antinutritional factors that impair digestion and protein denaturation. For example, extrusion may expose enzyme-susceptible sites and break disulfide bonds that could otherwise re-form [13]. Baking might have also enhanced PD of our breads, since it is known that thermal treatment significantly improves PD. This most likely occurs because heat destroys heat-labile protease inhibitors and denatures globulins, which are highly resistant to proteases in their native state [37, 38].

Digestibility was the highest in breads prepared with added caseinate (average PD, 94%), followed by egg-white powder (93%) and finally soya isolate (86%). The fact that PD in breads varied with protein additives is in agreement with previous studies, which reported caseinate protein to have 94% digestibility [39], egg white to have 96% digestibility [40] and soya protein isolate to have 83% digestibility [41].

The influence of enzymatic cross-linking on protein digestibility can be considered a consequence of protein unfolding or denaturation (positive influence), as well as protein degradation or polymerization (negative influence). TANG et al. stated that covalent cross-linking by 20 IU of TG per gram of protein can significantly decrease in vivo digestibility of native soya protein isolate [42]. However, in another research by TANG et al. where a smaller dose of TG was tested (5 IU of TG per gram of protein), an improvement in trypsin digestibility was positively related to the extent of enzymatic cross-linking, or the longer periods of incubation with microbial TG [43]. Therefore, influence of enzymatic cross-linking on protein digestibility can be contradictory depending on the quantity of the enzyme applied. Our research shows no statistically significant effect of TG, but the addition of small amounts of TG did slightly improve PD. The ability of TG to improve digestibility only at low content may be caused by the fact that higher amounts of TG cross-link larger proteins, rendering them inaccessible to enzymes and therefore making them indigestible.

Specific volume and height-to-width ratio of bread loaves

Bread volume depends on the ability of the protein network to retain carbon dioxide produced during fermentation and a big loaf is considered desirable by consumers and bakers. Specific volume of loaves ranged from 1.5 $l \cdot kg^{-1}$ to 2.0 $l \cdot kg^{-1}$, which is a typical range for gluten-free bread. ANOVA showed that recipe, protein source and their interaction had a significant influence on the specific volume of gluten-free breads. Breads containing buckwheat extrudate (Recipe 3) generally had the largest volumes, followed by Recipe 2 and finally Recipe 1 (Tab. 5). Amongst protein isolates, use of egg white resulted in breads with the highest volume (average, 1.8 $l \cdot kg^{-1}$); caseinate was second best (average, 1.7 $l \cdot kg^{-1}$), while the use of soya isolate resulted in significantly lower volumes of loaves (average, 1.4 $l \cdot kg^{-1}$).

TG can lower the bread volume, in particular when used at higher content, presumably by catalysing formation of a protein network that does not occur in the absence of the enzyme [16]. TG did not significantly affect bread volume but, nevertheless, it reduced the volume of bread prepared according to Recipe 2 (Tab. 5). When soy isolate or egg white were added to the dough together with TG, the bread had a larger volume in the presence of 1 IU TG than in the presence of 10 IU TG. Similar results were observed in bread prepared according to Recipe 1 containing soya isolate. Soya proteins could show this effect due to their generally very poor foaming properties [2]. Also, it should always be kept in mind that the efficiency of TG in helping to form a protein network depends on the particular protein structure, and on the disposition of lysine and glutamine residues of the substrate protein. Some proteins such as caseinate are easily cross-linked by TG because lysine and glutamine are available while many other proteins have more stable structures that disable cross-linking reactions [1, 10]. As we expected, the same factors that influenced bread specific volume also affected the height-to-width ratio of the loaves (Tab. 5). The correlation coefficient between bread specific volume and height was 0.776 ($p < 0.001$).

Crumb texture of gluten-free breads

ANOVA showed that recipe, protein source and their interaction significantly influenced all the texture parameters of gluten-free bread, while TG exerted an influence only on hardness and chewiness.

Assuming that added proteins and TG interact in the dough, the simultaneous presence of both additives should lead to bread crumb hardening. GERRARD et al. detected a strong increase in crumb firmness (which is analogous to hardness) of wheat bread after adding 1 IU TG, indicat-

Tab. 5. Physical properties of gluten-free breads according to recipe, protein source and transglutaminase addition.

Bread sample	Height-to-width ratio	Specific volume [l·kg^{-1}]	Hardness [g]	Resilience	Springiness [%]	Cohesiveness	Chewiness
R1-Cas-TG0	0.53 ± 0.03^{b}	1.43 ± 0,01^{gf}	2886 ± 226^{g}	0.26 ± 0.01^{e}	90 ± 4^{d}	0.61 ± 0.02^{d}	1573 ± 121^{e}
R1-Soy-TG0	0.52 ± 0.1^{b}	1.37 ± 0,04^{g}	3469 ± 348^{e}	0.19 ± 0.01^{b}	87 ± 2^{e}	0.47 ± 0.02^{a}	1425 ± 145^{f}
R1-Egg-TG0	0.44 ± 0.02^{cb}	1.48 ± 0,01^{f}	5124 ± 392^{b}	0.22 ± 0.01^{j}	89 ± 2^{ed}	0.53 ± 0.01^{ed}	2404 ± 179^{b}
R1-Cas-TG1	0.48 ± 0.02^{b}	1.51 ± 0,04^{f}	3252 ± 153^{ef}	0.27 ± 0.01^{c}	89 ± 1^{ed}	0.53 ± 0.01^{k}	1765 ± 82^{d}
R1-Soy-TG1	0.52 ± 0.02^{b}	1.32 ± 0,05^{g}	3631 ± 222^{e}	0.18 ± 0.01^{h}	88 ± 3^{e}	0.44 ± 0.02^{i}	1424 ± 126^{f}
R1-Egg-TG1	0.48 ± 0.01^{b}	1.49 ± 0,01^{f}	4458 ± 287^{c}	0.22 ± 0.01^{a}	87 ± 2^{e}	0.52 ± 0.02^{j}	2045 ± 168^{c}
R1-Cas-TG10	0.53 ± 0.01^{b}	1.58 ± 0,01^{d}	3640 ± 197^{e}	0.27 ± 0.01^{k}	91 ± 1^{cd}	0.62 ± 0.01^{f}	2063 ± 121^{c}
R1-Soy-TG10	0.54 ± 0.02^{b}	1.35 ± 0,05^{g}	3903 ± 219^{d}	0.17 ± 0.01^{b}	89 ± 2^{ed}	0.44 ± 0.02^{c}	1530 ± 147^{ef}
R1-Egg-TG10	0.43 ± 0.02^{cb}	1.47 ± 0,01^{f}	5576 ± 356^{a}	0.22 ± 0.01^{d}	87 ± 2^{e}	0.53 ± 0.03^{b}	2574 ± 208^{a}
R2-Cas-TG0	0.52 ± 0.01^{b}	1.76 ± 0,01^{c}	1037 ± 50^{mn}	0.30 ± 0.01^{k}	89 ± 2^{ed}	0.67 ± 0.01^{k}	617 ± 27^{k}
R2-Soy-TG0	0.43 ± 0.02^{cb}	1.48 ± 0,03^{f}	2402 ± 153^{hj}	0.23 ± 0.01^{j}	85 ± 2f	0.53 ± 0.02^{g}	1085 ± 55^{h}
R2-Egg-TG0	0.73 ± 0.01^{a}	1.96 ± 0,06^{b}	1366 ± 133^{l}	0.41 ± 0.01^{o}	96 ± 1^{b}	0.76 ± 0.03^{gh}	993 ± 95^{ih}
R2-Cas-TG1	0.50 ± 0.00^{b}	1.61 ± 0,01^{d}	1241 ± 78^{lm}	0.32 ± 0.01^{g}	91 ± 2^{cd}	0.68 ± 0.01^{f}	767 ± 48^{j}
R2-Soy-TG1	0.45 ± 0.01^{b}	1.39 ± 0,01^{g}	2366 ± 63^{i}	0.23 ± 0.01^{h}	85 ± 3^{f}	0.53 ± 0.01^{j}	1067 ± 43^{h}
R2-Egg-TG1	0.64 ± 0.01^{ab}	1.79 ± 0,03^{c}	1834 ± 93^{j}	0.39 ± 0.00^{i}	94 ± 1^{bc}	0.73 ± 0.00^{k}	1262 ± 56^{fg}
R2-Cas-TG10	0.51 ± 0.01^{b}	1.56 ± 0,05^{df}	2544 ± 269^{h}	0.34 ± 0.01^{l}	91 ± 2^{cd}	0.71 ± 0.01^{k}	1636 ± 154^{e}
R2-Soy-TG10	0.45 ± 0.01^{b}	1.42 ± 0,02^{gf}	2687 ± 167^{h}	0.21 ± 0.01^{a}	85 ± 2^{f}	0.50 ± 0.01^{b}	113 ± 61^{h}
R2-Egg-TG10	0.67 ± 0.02^{a}	1.91 ± 0,04^{b}	1947 ± 139^{j}	0.39 ± 0.00^{l}	95 ± 1^{b}	0.73 ± 0.01^{g}	1 350 ± 84^{f}
R3-Cas-TG0	0.47 ± 0.02^{b}	2 ± 0,08^{ab}	1179 ± 138^{m}	0.29 ± 0.01^{b}	84 ± 2^{f}	0.63 ± 0.01^{cb}	622 ± 80^{k}
R3-Soy-TG0	0.51 ± 0.01^{b}	1.39 ± 0,05^{g}	1831 ± 129^{j}	0.25 ± 0.01^{c}	92 ± 4^{c}	0.57 ± 0.01^{i}	954 ± 74^{i}
R3-Egg-TG0	0.68 ± 0.01^{a}	2.08 ± 0,04^{a}	1690 ± 199^{kl}	0.42 ± 0.01^{n}	97 ± 2^{a}	0.76 ± 0.01^{k}	1247 ± 135^{f}
R3-Cas-TG1	0.54 ± 0.01^{b}	1.81 ± 0,04^{c}	714 ± 39^{o}	0.35 ± 0.01^{o}	93 ± 1^{c}	0.72 ± 0.01^{n}	476 ± 27^{l}
R3-Soy-TG1	0.50 ± 0.01^{b}	1.51 ± 0,05^{f}	1700 ± 14^{k}	0.25 ± 0.01^{m}	88 ± 1^{e}	0.56 ± 0.01^{d}	834 ± 52^{ji}
R3-Egg-TG1	0.66 ± 0.01^{a}	2.04 ± 0,05^{a}	1205 ± 10^{m}	0.43 ± 0.01^{i}	98 ± 2^{a}	0.77 ± 0.01^{m}	914 ± 80^{i}
R3-Cas-TG10	0.69 ± 0.03^{a}	2.15 ± 0,04^{a}	806 ± 125^{o}	0.41 ± 0.00^{i}	96 ± 1^{b}	0.80 ± 0.01^{h}	616 ± 92^{k}
R3-Soy-TG10	0.52 ± 0.01^{b}	1.51 ± 0,09^{f}	1752 ± 76^{jk}	0.28 ± 0.00^{f}	91 ± 1^{cd}	0.58 ± 0.01^{e}	935 ± 41^{i}
R3-Egg-TG10	0.65 ± 0.01^{a}	1.99 ± 0,01^{b}	1556 ± 108^{l}	0.41 ±0.01^{f}	96 ± 1^{b}	0.75 ± 0.01^{n}	1117 ± 68^{h}

Mean values followed by different letters in the same column are significantly different at 95% confidence level.
Designation of samples: recipe (R1 – recipe 1, R2 – recipe 2, R3 – recipe 3); protein source (Cas – caseinate, Soy – soya isolate, Egg – egg-white powder); transglutaminase (TG0 – 0 IU, TG1 – 1 IU, TG10 – 10 IU).

ing that a protein network was formed [9]. Renzetti et al. observed a similar effect on buckwheat flour, which they interpreted to mean that TG improved crumb structure and breadmaking potential by inducing a firm structure [44]. This made fresh bread easier to slice and butter, which is more desirable to consumers [9]. The hardest crumbs were measured among breads produced by Recipe 1 (Tab. 5). In all three recipes, protein addition hardened the crumb in the following order: caseinate < soya isolate < egg-white powder. Crumb was generally harder when TG was added, with the exception of Recipe 3, in which TG addition significantly decreased hardness ($p < 0.01$).

Resilience is the speed at which bread crumb returns to its original position, while springiness is the extent to which the bread crumb recovers after an applied force is removed [45]. The greatest resilience and springiness were determined for breads of Recipe 3 (Tab. 5). Generally, the greatest springiness was determined for bread of Recipe 3 containing egg-white powder and 1 IU TG. We found that TG did not influence the springiness or resilience of gluten-free bread, which is consistent with the results of Onyango et al. on the effects of TG on sorghum gluten-free bread [45]. Our results are in contradiction to those of Renzetti et al., who reported that TG improved the elastic

character of batter prepared from buckwheat and brown rice, while it decreased the elastic character of corn flour [36].

Crumb cohesiveness, which is measured by comparing how well the bread withstands a second deformation relative to how it behaved during the first deformation, reflects the internal cohesion of the bread material [45]. Adding caseinate and egg white did not affect cohesiveness, whereas adding soya isolate reduced it (Tab. 5). These results are consistent with those of MARCO and ROSELL [12], who observed a drop in cohesiveness when soya proteins and TG were added together. The main protein in soya flour is globular and most globular proteins cannot act as substrates for TG reactions [46]. Our experiments showed no significant effect of TG on crumb cohesiveness, similar to the findings reported by ONYANGO et al. [45].

Chewiness is the energy required to masticate bread into a state ready for swallowing; it is a product of hardness, cohesiveness and springiness. Chewiness parameters should be low to ensure consumer desirability. For most recipes in our study, both additions of TG increased chewiness, 10 IU more than 1 IU TG. The higher crumb chewiness may reflect an increase in the number of covalent bonds [11]. However, adding 1 IU TG to the dough of Recipe 3 containing caseinate or egg white did lead to a chewiness lower than that in the absence of TG (Tab. 5). Mean chewiness was the lowest in breads made according to Recipe 3, and highest in breads of Recipe 1 (Tab. 5).

Recipe optimization

Optimization was carried out taking into account the recipe, protein source and enzyme content of the breads. In order to determine the final desirability of breads, optimization of the recipes was carried out by setting the following conditions: to maximize specific volume, resilience, springiness, cohesiveness, height-to-width ratio and PD; and to minimize gluten content, crumb hardness and chewiness. Results showed the following ranking of breads by overall quality:

1. Recipe 3 with egg whites and 10 IU TG,
2. Recipe 3 with egg whites and 1 IU TG,
3. Recipe 3 with egg whites and 0 IU TG,
4. Recipe 2 with egg whites and 1 IU TG,
5. Recipe 2 with egg whites and 10 IU TG.

Results of the top three breads, i.e. those of the highest desirability, are shown in Fig. 1.

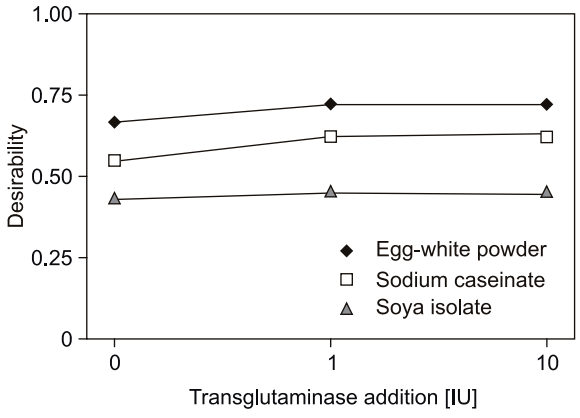

Fig. 1. Desirability of breads prepared according to Recipe 3 dependent of the protein isolate added and transglutaminase addition.

CONCLUSIONS

Egg-white powder and caseinate are desirable protein sources for enriching gluten-free bread, whereas soya isolate cannot be recommended. TG can be an effective additive at either 1 IU or 10 IU per gram of protein, depending on the physical property that the manufacturer wishes to modify, but the high cost of this enzyme means that 1 IU should be more than adequate. TG at 1 IU positively affected protein digestibility and lowered the gluten content at 10 IU as well. Best ingredients for the production of gluten-free bread and the best substrate for TG is the combination of extruded buckwheat and egg-white powder. Breads prepared with these components or corn extrudate, had improved properties and were very similar to common wheat bread. Potato flakes could be desirable for producing gluten-free bread because of their lysine and free amino group content, as well as technological feasibility, and its use needs to be further examined. The results of our study demonstrate that TG, in addition to affecting the quality of gluten-free bread, also shows a potential for making it safer for consumption by people with celiac disease.

REFERENCES

1. Marco, C. – Rosell, C. M.: Functional and rheological properties of protein enriched gluten-free composite flours. Journal of Food Engineering, *88*, 2008, pp. 94–103.
2. Moore, M. – Heinbockel, M. – Dockery, P. – Ulmer, H. M. – Ardent, E. K.: Network formation in gluten-free bread with application of transglutaminase. Cereal Chemistry, *83*, 2006, pp. 28–36.
3. Sanchez, H. D. – Osella, C. A. – De La Totre, M. A.:

Optimization of gluten-free bread prepared form cornstarch, rice flour and cassava starch. Journal of Food Science, *67*, 2002, pp. 416–419.
4. Gallagher, E. – Kunkel, A. – Gormley, T. T. – Arendt, E. K.: The effect of dairy and rice powder addition on loaf and crumb characteristics, and on shelf life (intermediate and long-term) of gluten-free breads stored in a modified atmosphere. European Food Research and Technology, *218*, 2003, pp. 44–48.
5. Schober, T. J. – Messerschmidt, M. – Bean, S. R. – Park, S. – Arendt, E. K.: Gluten-free breads from sorghum: quality differences among hybrids. Cereal Chemistry, *82*, 2005, pp. 394–404.
6. Marco, C. – Rosell, C. M.: Breadmaking performance of protein enriched, gluten-free breads. European Food Research and Technology, *227*, 2008, pp. 1205–1213.
7. Crocket, R. – Le, P. – Yael, V.: Effects of soy protein isolate and egg white solids on the physiochemical properties of gluten-free breads. Food Chemistry, *129*, 2010, pp. 84–91.
8. Yokoyama, K. – Nio, N. – Kikuchi, Y.: Properties and applications of microbial transglutaminase. Applied Microbiology and Biotechnology, *64*, 2004, pp. 447–454.
9. Gerrard, J. A. – Fayle, S. E. – Wilson, A. J. – Newberry, M. P. – Ross, M. – Kavale, S.: Dough properties and crumb strength of white pan bread as affected by microbial transglutaminase. Journal of Food Science, *63*, 1998, pp. 472–475.
10. Dickinson, E.: Enzymic cross-linking as a tool for food colloid rheology control and interfacial solubilisation. Trends in Food Science and Technology, *8*, 1997, pp. 334–339.
11. Renzetti, S. – Dal Bello, F. – Arendt, E. K.: Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten–free flours treated with a microbal transglutaminse. Journal of Cereal Science, *48*, 2008, pp. 33–45.
12. Marco, C. – Rosell, C. M.: Effect of different protein isolates and transglutaminase on rice flour properties. Journal of Food Engineering, *84*, 2008, pp. 132–139.
13. Singh, S. – Gamlath, S. – Walkeling, L.: Nutritional aspects of food extrusion: a review. International Journal of Food Science and Technology, *42*, 2007, pp. 916–929.
14. Clerici, M. T. P. S. – Airoldi, C. – El-Dash, A. A.: Production of acidic extruded rice flour and its influence on the qualities of gluten-free bread. Food Science and Technology, *42*, 2009, pp. 618–623.
15. Csapo, J. – Loki, K. – Csapo-Kiss, Z. – Albert, C.: Separation and determination of the amino acids by ion exchange column chromatography applying post-column chromatography applying post-column derivatization. Acta Agraria Kaposvariensis, *1*, 2008, pp. 5–29.
16. Gujral, H. S. – Rosell, C. M.: Functionality of rice flour modified with a microbial transglutaminase. Journal of Cereal Science, *39*, 2004, pp. 225–230.
17. Mendez, E.: Report of collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. European Journal of Gastrology and Hepatology, *14*, 2005, pp. 1053–1063.
18. AACCI Method 46-30.01. Crude protein – combustion method. In: Approved methods of analysis, 11th ed. St. Paul : AACC International, 2000, 2 pp.
19. Fageer, A. S. M. – Babiker, E. E. – El Tinay, A. H.: Effect of malt pre-treatment and/or cooking on phytate and essential amino acids contents and *in vitro* protein digestibility of corn flour. Food Chemistry, *88*, 2004, pp. 261.
20. AACCI Method 44-15.02. Moisture – air-oven methods. In: Approved methods of analysis, 11th ed. St. Paul : AACC International, 2000, 4 pp.
21. AACCI Method 30-10.01. Crude fat in flour, bread, and baked cereal products not containing fruit. In: Approved methods of analysis, 11th ed. St. Paul : AACC International, 2000, 2 pp.
22. AACCI Method 10-05.01. Guidelines for measurement of volume by rapeseed displacement. In: Approved methods of analysis, 11th ed. St. Paul : AACC International, 2000, 4 pp.
23. Belitz, H. – Grosch, W. – Schieberle, P.: Food Chemistry. 4th revised and extended edition. Heidelberg, Berlin: Springer, 2009. 984 pp. ISBN 978-3-540-69933-0.
24. Novotni, D. – Curic, D. – Gabric, D. – Cukelj, N. – Curko, N.: Production of high protein bread using extruded corn and soybean flour blend. Italian Journal of Food Science, *2*, 2009, pp. 123–133.
25. Blasko, B. – Madi, A. – Fesus, L.: Structural elements responsible for TG activity of protein disulphide isomerases and thioredoxins. Journal of Biological Regulators and Homeostatic Agents, *18*, 2004, pp. 1–8.
26. Commission Regulation (EC) No 41/2009 of 20 January 2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten. Official Journal of the European Union, L 16, 21.1.2009, pp. 3–5.
27. Stern, M. – Ciclitira, P. J. – Van Eckert, R. – Feighery, C. – Janssen, F. W. – Mendez, E. – Mothes, T. – Tang, C. – Li, L. – Yang, X.: Influence of transglutaminase-induced cross-linking on *in vitro* digestibility of soy protein isolate. Journal of Food Biochemistry, *30*, 2006, pp. 718–731.
28. Catassi, C. – Fabiani, E. – Iacono, G. – D'Agate, C. – Francavilla, R. – Biagi, F. – Volta, V. – Acconmando, S. – Picarelli, A. – De Vitis, I. – Pianelli, G. – Gesvita, R. – Carle, F. – Mandolesi, A. – Bearzi, I. – Fasano, A.: A prospective double-blind, placebo-controlled trial to establich a safe gluten threshold for patients with celiac disease. American Journal of Clinical Nutrition, *85*, 2007, pp. 160–166.
29. Hamilton, J. R. – McNeill, L. K.: Childhood celiac disease: response of treated patients to a small uniform daily dose of wheat gluten. Journal of Pediatrics, *81*, 1972, pp. 855.
30. Dekking, E. H. A. – Veelen, P. A. – Ru, A. – Kooy-Winkelaar, A. M. C. – Gröneveld, T. – Nieuwenhuizen, W. F. – Koning, F.: Microbial trans-

glutaminases generate T cell stimulatory epitopes involved in celiac disease. Journal of Cereal Science, *47*, 2008, pp. 339–346.
31. Gianfrani, C. – Siciliano, R. A. – Facchiano, A. M. – Camarca, A. – Mazzeo, M. F. – Constantini, S. – Salvati, V. M. – Maurano, F. – Mazzarella, G. – Iaquinto, G. – Bergama, P. – Rossi, M.: Transamidation of wheat flour inhibits the response to gliadin of intestinal T cells in celiac disease. Gastroenterology, *133*, 2007, pp. 780–789.
32. Elli, L. – Roncoroni, L. – Hils, M. – Pasternack, R. – Barisani, D. – Terrani, C. – Vaira, V. – Ferrero, S. – Bardella, M. T.: Imunological effects of transglutaminase-treated gluten in celiac disease. Human Immunology, *73*, 2012, pp. 992–997.
33. Gallagher, E. – Gormley, T. R. – Arendt, E. K.: Crust and crumb characteristics of gluten-free breads. Journal of Food Engineering, *56*, 2003, pp. 153–161.
34. Gambuś, H. – Gambuś, F. – Pastuszka, D. – Wrona, P. – Ziobro, R. – Sabat, R. – Mickowska, B. – Nowotna, A. – Sikora, M.: Quality of gluten-free supplemented cakes and biscuits. International Journal of Food Sciences and Nutrition, *60*, 2009, pp. 31–50.
35. Alvarez-Jubete, L. – Arendt, E. K. – Gallagher, E.: Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. International Journal of Food Sciences and Nutrition, *60*, 2009, pp. 240–257.
36. Rayas-Duarte, P. – Majewska, K. – Doetkott, C.: Effect of extrusion process parameters on the quality of buckwheat flour mixes. Cereal Chemistry, *75*, 1998, pp. 338–345.
37. Liener, I. E.: Legume toxins in relation to protein digestibility –a review. Journal of Food Science, *41*, 1976, pp. 1076–1081.
38. Walker, A. F. – Kochar, N.: Effect of processing including domestic cooking on nutritional quality of legumes. Proceedings of the Nutrition Society, *41*, 1982, pp. 41–51.
39. Guo, M. R. – Flynn, A. – Fox, P. F.: Heat induced changes in the nutritional properties of sodium caseinate. International Dairy Journal, *9*, 1999, pp. 243–247.
40. Calloway, D. H. – Margen, S.: Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. Journal of Nutrition, *101*, 1970, pp. 205–216.
41. Wu, W. – Hettiarachchy, N. S. – Kalopathy, U.: Functional properties and nutritional quality of alkali- and heat-treated soy protein isolate. Journal of Food Quality, *22*, 1988, pp. 119–133.
42. Tang, C. H. – Li, L. – Yang, X. Q.: Influence of transglutaminase treatment on the thermal properties of soy protein isolates. Food Research International, *39*, 2006, pp. 704–711.
43. Tang, C. H. – Sun, X. – Yin, S. W. – Ma, C. Y.: Transglutaminase induced cross-linking of vicilin-rich kidney protein isolate: Influence on the functional properties in vitro digestibility. Food Research International, *41*, 2008, 941–947.
44. Renzetti, S. – Dal Bello, F. – Arendt, E. K.: TG polymerisation of buckwheat (*Fagopyrum esculentum* Moench) proteins. Journal of Cereal Science, *48*, 2008, pp. 747–754.
45. Onyango, C. – Mutungi, C. – Unbehned, G. – Lindhauer, M. G.: Rheological and baking characteristics of batter and bread prepared from pregelatinised cassava starch and sorghum and modified using microbial TG. Journal of Food Engineering, *97*, 2010, pp. 465–470.
46. Kamiya, N. – Takazawa, T. – Tanaka, T. – Veda, H. – Nagamune, T.: Site specific cross-linking of functional proteins by transglutaminase. Enzyme and Microbial Technology, *33*, 2003, pp. 492–496.

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