Use of Transglutaminase for Improvement of Quality of Pastry Produced by Frozen-Dough Technology

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Abstract

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The improving effect on the quality of final products has been studied of the enzyme transglutaminase added in two concentrations, i.e. 4.5 mg or 7.5 mg/300 g of flour destined for the preparation of pastry dough. Changes of the sensory (sensory profile), nutritional (the contents of amino acids, especially that of lysine), and microbiological quality (total count, yeasts, moulds) were investigated as well as those of a_w and pH values of the pastry produced from the freezer-stored dough (–18 ± 2°C). It has been found that the sensory quality is favourably affected by the addition of transglutaminase (TGM) in the amount of 4.5 mg/300 g of flour and on the other hand that the protective effect on lysine increases if the applied TGM concentration is higher (7.5 mg per 300 g of flour). The microbiological quality, the pH and a_w values of dough, and the products have satisfied the criteria for the sanitary and hygienic requirements.

Keywords: pastry; transglutaminase (TGM); sensory evaluation; lysine; microbiology; a_w; pH

The important indicators of the quality (of the basic assortment) of cereal products are health safety and optimum sensory properties. The product quality is affected by many mutually associated external and internal conditions. The most important of them involve the raw material quality, the recipe, and the observance of the sanitary principles.

Food additives used more and more frequently for the protection of cereal products against ageing and deterioration include, apart from oxidative substances (L-ascorbic acid, azidocarbonamide), reducing substances (L-cystein), and emulsifiers (stearoyl natrium lactate, monoglyceride of diacetyltartaric acid), mainly enzymes (TENBERGEN 1999).

Enzymes are naturally present in foods such as wheat and soya as long as they are not removed or inactivated. For instance, amylases, proteases, hemicellulases, lipases, and oxidases have an influence on the whole process of the bakery products production.

Enzymes (and their mixtures) may find the following applications in baking:
– decolourizing (bleaching) of dough (oxidoreductases),
– improvement of the volume and texture of dough,
– substitution of bromides,
– maintenance of the shelf-life (CORSETTI *et al.* 2000; ROSSEL *et al.* 2001; SAHLSTRÖM & PRATHEN 1997; GROSSMAN & DE BARBER 1997; VEMULAPPALI & HOSENEY 1998; DELCROS *et al.* 1998; GÉLLINAS *et al.* 1998).

One of the significant and efficient enzymes applied and officially authorized in Japan for the improvement of the quality of cereal products is transglutaminase.

Transglutaminase (protein-glutamine-glutamyltransferase, EC 2.3.2.13) catalyses the acyl-transferase reaction between the carboxymidic group of peptides which contains glutamine residues (acyl donors), and various primary amines (acyl acceptors) including the amino groups of lysine bound in proteins. In the absence of substrates bearing amino groups transglutaminase catalyzes the deamination of glutamine residues in the course of which water molecules are used as acyl acceptors. Transglutaminase can modify proteins by the incorporation of amine, by cross-linking, and by deamination. These enzyme-catalyzed reactions may be used for the modification of food proteins which can lead, for example, to the

formation of texture as well as to the protective effect on lysine present in food proteins and thus to a higher retention of the nutritional value.

Transglutaminases are widely distributed in animal tissues and body fluids (AESCHLIMANN & PAULSSON 1994), plants, fishes (ARAKI & SEKI 1993), and microorganisms (ANDO *et al.* 1989; ZHU *et al.* 1995; SEGURO *et al.* 1995). GERRARD *et al.* (1998) applied transglutaminase in baking bread and croissants (GERRARD *et al.* 2001). The addition of transglutaminase (5000 ppm/weight of flour) revealed a positive effect on the texture and the distribution of pores

which resulted in higher loaves of bread and pastry. The best results in dough raising were achieved by the addition of transglutaminase to the dough which was subsequently frozen (the activation of the enzyme during freezing). The positive effect of the addition of transglutaminase persisted even during the storage of frozen croissants for 90 days with the bread crumb being better than that in the control. On the basis of this fact, our model experiment was used to study the changes in the sensory quality, the contents of amino acids (mainly lysine), the microbiological quality (total count of microorganisms,
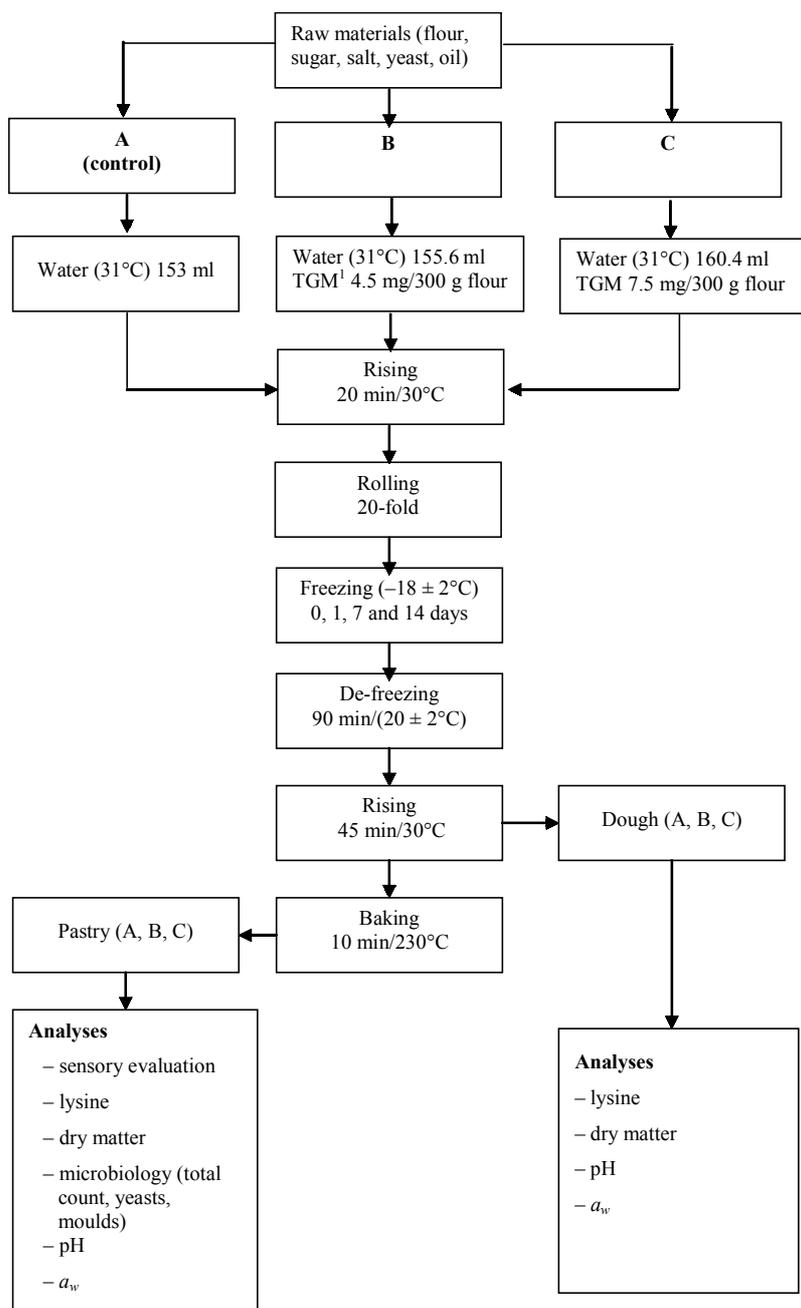
Fig. 1. Technological scheme of the samples preparation

[1] transglutaminase

number of yeasts and moulds), pH, and in the a_w value of pastry baked from frozen dough with the addition of two transglutaminase concentrations (4.5 mg or 7.5 mg/300 g of flour) which was stored for 14 days at –18 ± 2°C (sampling accomplished at intervals: 0, 1, 7, 14 days).

The addition of the selected enzyme and the examination of the sensory, nutritional and microbiological quality of the pastry produced from the freezer-stored dough have enabled us to assess and forecast the improvement of the quality of final baking products.

MATERIAL AND METHODS

Raw materials, formula of pastry

Wheat flour T 512 (300 g), salt (5.63 g), sugar (3.22 g), yeast (12.06 g), oil (7.5 g), transglutaminase (produced by ADIPO Ltd., Nitra, Slovak Republic) (4.5 and 7.5 mg/300 g of flour) (SMELÍK *et al.* 1987). The water amount was used in the dependence on the concentration of the enzyme added. The process of preparation and treatment of samples is shown in Fig. 1.

Notice

Dough before baking was stored in a freezing box Whirpool and the baking of 100 g loaves was carried out in an electric oven MORA 524 (10 min/230°C).

Methods

1. The sensory evaluation of the final products was accomplished by means of the 4-point hedonic scale determination and the profiling of tastiness was carried out by six panel assessors (POKORNÝ 1993); the height-width ratio was obtained by dividing the height by the width (mm) using the sliding measuring instrument and estimating the weight. After the division of those parameters, the optimum cambering of pastry was 0.65 (when it increased, the pastry became more cambered and when it decreased, it was upside down).

2. The contents of amino acids in the dough and the final products were determined according to the modified method of SPACKMAN *et al.* (1958) using the apparatus type AAA 339 M; the homogenized final products were analysed in two parallel determinations; dry matter of the samples for the evaluation of amino acids: preliminary drying at the temperature of 45°C and after homogenisation the completion of drying at 130°C for 60 min up to the constant weight (SMELÍK *et al.* 1987).

3. Aerobic mesophilic bacteria were determined by the plate count method, on the tryptone-glucose-yeast extract agar (Šarišské Michaľany, Slovak Republic) (STN ISO 4833, 1997).

4. Yeasts and moulds were determined by the plate count method on chloramphenicol-glucose-yeast extract agar (Šarišské Michaľany, Slovak Republic) (STN ISO 7954, 1997).

5. The measurement of the pH value was done with a pH meter OP – 211/l Radelkis (Hungary) with combined electrodes OP – 0808 P calibrated with buffers of pH = 6.865 and pH = 4.008 (AACC 1962).

6. The a_w value was determined according to the Slovak Standard STN 56 0030 – Determination of the water activity in foods (1996) (Rotronic AG, Switzerland).

7. The mathematical and statistical evaluation of the results ($\bar{x}$, *s*) (ECKSCHLAGER *et al.* 1980); in the case of the sensory evaluation $n = 6$; in the case of lysine and the microbiological evaluation, the average values of four replicates were graphically plotted.

RESULTS AND DISCUSSION

Sensory evaluation

Changes in the cambering (height-width ratio) and sensory properties were evaluated in three types of samples of pastry baked from dough stored at the freezing temperature of –18 ± 2°C for 0, 1, 7 and 14 days. The individual samples differed by the amount of transglutaminase added (4.5 or 7.5 mg/300 g of flour).

The assessors (6-member panel) evaluated the following sensory parameters: product – shape, odour, taste + crust – colour, thickness/hardness + crumb – elasticity, porosity, colour, hardness (resistance to bite), and the adhesive power (to the palate on a longer chewing). The evaluation was made using the 5-point hedonic scale where the maximum value of four points corresponded to the highest degree of the product quality, while the lowest degree of evaluation with zero points idicated essential qualitative deficiencies.

The values of cambering for three types of pastry (A, B and C) baked from the freezer-stored dough are listed in Table 1. The summarized results (*x*) of the sensory evaluation of the individual types of samples at the selected storage intervals are documented in Tables 2–4.

By evaluating the cambering of pastry, it was found that the 14-day freezer-storage brought about the following change of parameters in the baked pastry:

Table 1. Height-width ratio of pastry A, B, C depending on the time of storage of frozen dough

Storage (d)	A	B	C
0	0.72	0.68	0.62
1	0.69	0.64	0.62
7	0.67	0.62	0.59
14	0.70	0.62	0.60

A = pastry without transglutaminase (TGM)
B = pastry with TGM 4.5 mg/300 g flour
C = pastry with TGM 7.5 mg/300 g flour

Table 2. Sensory scoring evaluation ($\bar{x}$, $n = 6$) of the pastry A (without TGM[1])

Sensory parameter		Storage (d)			
		0	1	7	14
Product	shape	4.00 ± 0.00[2]	3.83 ± 0.41	3.67 ± 0.52	3.33 ± 1.21
Crust	colour	3.67 ± 0.52	3.33 ± 1.03	3.33 ± 0.82	2.67 ± 0.82
	thickness/hardness	3.67 ± 0.52	3.83 ± 0.41	3.17 ± 0.75	3.33 ± 0.52
Crust/Crumb	odour	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	3.67 ± 0.52
	taste	4.00 ± 0.00	3.83 ± 0.41	3.83 ± 0.41	3.50 ± 0.55
Crumb	elasticity	4.00 ± 0.00	4.00 ± 0.00	3.50 ± 0.55	3.67 ± 0.52
	porosity	3.67 ± 0.52	3.67 ± 0.52	3.50 ± 0.55	3.17 ± 0.41
	colour	4.00 ± 0.00	4.00 ± 0.00	3.67 ± 0.52	4.00 ± 0.00
	resistance to the bite	3.83 ± 0.41	4.00 ± 0.00	3.83 ± 0.41	3.17 ± 0.75
	adhesiveness to palate (on longer chewing)	4.00 ± 0.00	3.83 ± 0.41	4.00 ± 0.00	3.83 ± 0.41
Σ		38.84	38.32	36.50	34.34

[1]transglutaminase
[2] ± SD

Table 3. Sensory scoring evaluation ($\bar{x}$, $n = 6$) of the pastry B (TGM[1] 4.5 mg/300 g of flour)

Sensory parameter		Storage (d)			
		0	1	7	14
Product	shape	4.00 ± 0.00[2]	3.67 ± 0.82	4.00 ± 0.00	3.67 ± 0.82
Crust	colour	3.83 ± 0.41	3.33 ± 1.03	3.17 ± 0.75	3.00 ± 0.83
	thickness/hardness	3.67 ± 0.52	4.00 ± 0.00	3.67 ± 0.82	3.67 ± 0.82
Crust/Crumb	odour	4.00 ± 0.00	4.00 ± 0.00	3.83 ± 0.41	3.83 ± 0.41
	taste	3.83 ± 0.41	3.83 ± 0.41	4.00 ± 0.00	3.83 ± 0.41
Crumb	elasticity	4.00 ± 0.00	4.00 ± 0.00	3.83 ± 0.41	3.83 ± 0.41
	porosity	3.83 ± 0.41	4.00 ± 0.00	4.00 ± 0.00	3.67 ± 0.52
	colour	4.00 ± 0.00	3.83 ± 0.41	3.67 ± 0.52	4.00 ± 0.00
	resistance to the bite	4.00 ± 0.00	4.00 ± 0.00	3.67 ± 0.82	3.50 ± 0.55
	adhesiveness to palate (on longer chewing)	3.83 ± 0.41	3.83 ± 0.41	3.83 ± 0.41	4.00 ± 0.00
Σ		38.99	38.49	37.67	37.00

[1]transglutaminase
[2] ± SD

– in sample A (without enzyme) the width and height decreased with the prolongation of storage (height = 55.7 – 47.5 mm; width = 97.4 – 69.5 mm) and the camber ranged between 0.72 and 0.67 which means that the pastry became more cambered;

– in samples B (TGM – 4.5 mg/300 g of flour) and C (TGM – 7.5 mg/300 g of flour), the width and height increased after the first day of freezing and then a decrease of the parameters studied was recorded; however, the height-width ratio for sample B (0.62–0.68) showed that the cambering was optimum and for sample C it was weak;

– the weight of pastry was the highest after the first day of storage and later on it went up when the enzyme concentration was elevated (from 87.2 g to 93 g).

Table 4. Sensory scoring evaluation ($\bar{x}$, $n = 6$) of the pastry C (TGM[1] 7.5 mg/300 g of flour)

Sensory parameter		Storage (d)			
		0	1	7	14
Product	shape	4.00 ± 0.00[2]	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
Crust	colour	3.67 ± 0.52	3.33 ± 0.82	2.50 ± 0.55	3.00 ± 0.63
	thickness/hardness	4.00 ± 0.00	4.00 ± 0.00	3.67 ± 0.82	3.50 ± 0.84
Crust/Crumb	odour	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
	taste	4.00 ± 0.00	3.83 ± 0.41	3.33 ± 0.52	3.00 ± 0.89
Crumb	elasticity	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00
	porosity	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	3.83 ± 0.41
	colour	4.00 ± 0.00	4.00 ± 0.00	3.50 ± 0.84	3.83 ± 0.41
	resistance to the bite	4.00 ± 0.00	4.00 ± 0.00	3.83 ± 0.41	3.83 ± 0.41
	adhesiveness to palate (on longer chewing)	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	3.50 ± 0.00
Σ		39.67	39.16	36.83	36.49

[1]transglutaminase
[2] ± SD

The sensory point-evaluation permitted us to ascertain that the 14-day storage of dough under the freezer conditions caused the following changes in the pastry:
– for sample A (control – without enzyme), the most remarkable changes were observed in the product shape (0.67 points, further p), the crust colour (1.00 p) and in the crumb hardness (resistance to bite) (0.83 p) while the odour (0.33 p), elasticity (0.33 p), colour (0.33 p) and the adhesive power of crumb (to the palate on a longer chewing) (0.17 p) did not show any profound changes;
– the value of the total quality parameter ranged for sample A from 38.84 p (maximum – the zero day of storage) to 34.34 p (minimum – the 14th day of storage);
– for sample B (addition of 4.5 mg TGM/300 g of flour), the most crucial drop was registered in the assessment of the crust colour (0.83 p) whereas almost no drop was noticed in the product odour (0.17 p), colour (0.17 p) and the adhesive power (0.17 p) (of crumb to the palate on a longer chewing);
– for sample C (addition of 7.5 mg TGM/300 g of flour), the most significant decrease, equally as for sample B, was registered in the evaluation of the crust colour (1.17 p), and along with it also in the product taste (1.00 p); despite this fact, no changes were recorded in some other parameters (shape, odour and crumb elasticity);
– the value of the total quality parameter for the samples with the addition of transglutaminase ranged from 39.67 p (maximum – day zero of storage) to 36.49 p (minimum – the 14th day of storage) out of the total 40 p.

From the above-indicated findings it follows that:
– with respect to the product cambering, transglutaminase has the best effect after the first day of dough freezing, which can be given by that the dough needs some time for its activation (this observation is in agreement with the data reported by GERRARD *et al.* 2001);
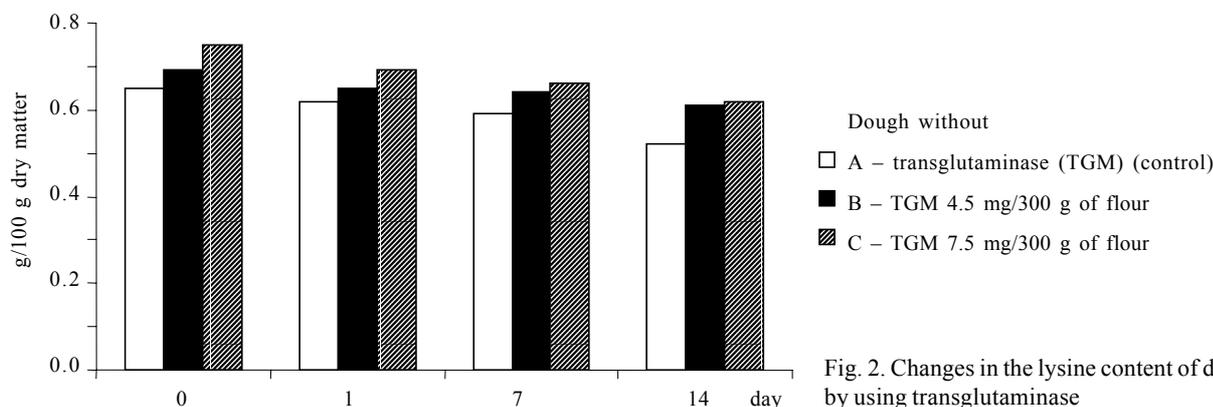

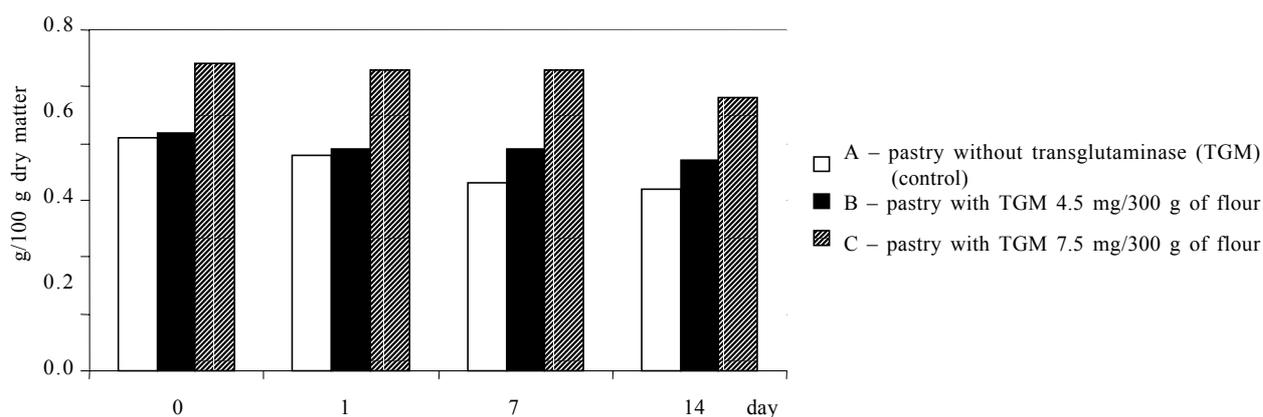Fig. 2. Changes in the lysine content of dough by using transglutaminase

Dough without
☐ A – transglutaminase (TGM) (control)
■ B – TGM 4.5 mg/300 g of flour
▨ C – TGM 7.5 mg/300 g of flour

Fig. 3. Changes in the lysine content of pastry by using transglutaminase

– freezing leads to a smaller pastry size which is probably caused by a decrease of the yeast activity caused by freezing;

– the sensory quality of all samples of pastry was falling with the prolongation of the storage; however, in the case of samples with the addition of transglutaminase, the evaluation was better than in the case of the control both before and after freezing;

– the best effect on the cambering of dough as well as on the sensory evaluation was achieved at the concentration of 4.5 mg TGM/300 g of flour;

– the dough at the concentration of 7.5 mg TGM/300 g of flour was very sticky and, therefore, its processing was difficult. The initial adhesive power was later partially reduced in the course of its further development. This is also in agreement with the literature data (GERRARD *et al.* 1998);

– freezing induced marbling of pastry which caused an unfavourable response of assessors.

Amino acid content

The amino acid content kept decreasing during the whole freezer-storage (from 10 to 34.4%) and also during baking which resulted in a decrease of the nutritional value of pastry. Both the dough and pastry contained all essential amino acids (leucine, isoleucine, lysine, valine, phenylalanine, threonine, methionine, etc.) as well as the majority of other amino acids. The dough and pastry samples contained amino acids such as glutamic acid together with glutamine, proline, and leucine in the highest amounts, and threonine, methionine and lysine in the lowest amounts (for an example, only lysine is indicated).

In the dough and pastry, the lower amount of the individual amino acids was caused by the addition of TGM. The lowest amount of amino acids was registered in samples at the proline concentration (55.2%). This fact cannot be confirmed in the case of amino acids such as serine, glutamic acid, alanine, valine and lysine the content of

which increased after the addition of transglutaminase (7.0–31.7%).

During the freezer storage, the content also decreased of lysine which belongs to the essential amino acids, and which is the most interesting for us from the viewpoint of the verified literature data. However, the lysine content in the samples with transglutaminase was higher than that in the control (A); in the case of sample B (TGM of 4.5 mg per 300 g of flour), the lysine content of dough was 5.8% and that of pastry – 1.2%; in the case of sample C (TGM of 7.5 mg/300 g of flour), the lysine content of dough was 14.2% and that of pastry – 33.1%, as can be clearly seen in Figs. 2 and 3. This was caused probably by the ability of transglutaminase to bind lysine present in food proteins and thereby to protect, the nutritional value (GERRARD *et al.* 2001).

Microbiological evaluation

Fig. 4 illustrates the results showing changes in the microbiological evaluation: total count, yeasts, moulds ($\bar{x}$, $n = 4$) (A, B, C) during the 14-day freezer-storage of dough.

The total count of microorganisms decreased in all samples in the course of the freezer-storage which was likely caused by a decrease of their activity as influenced by the low temperature.

According to the Food Codex (corn, soya and rice products, appendix No 8 to Chapter 4, 2nd part), bread and pastry are allowed to contain the total count of microorganisms equal to 2×10^2 CFU/g in 5 samples; in two samples tested, the admissible highest limit is 5×10^3 CFU/g.

Therefore, it is possible to state that all samples were during the whole storage period in the limits settled for the total count of microorganisms as defined by the Food Codex of the Slovak Republic (1998).

The values of the number of yeasts ranged from 40 to < 10 CFU/g. However, their number was decreasing with the length of their freezer-storage in the same way as in

moulds
yeasts
total count

A – pastry without transglutaminase (TGM) (control)
B – pastry with TGM 4.5 mg/300 g of flour
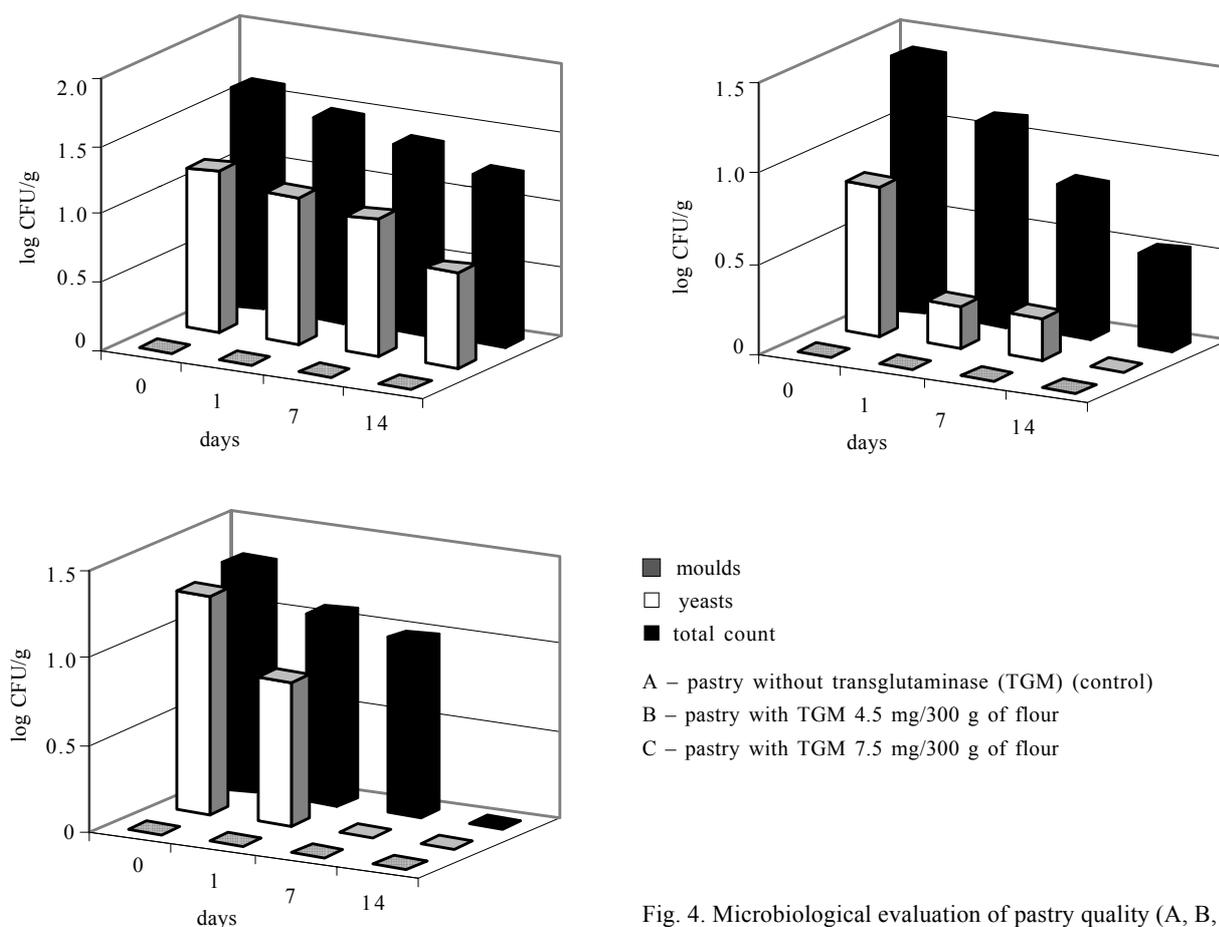C – pastry with TGM 7.5 mg/300 g of flour

Fig. 4. Microbiological evaluation of pastry quality (A, B, C)

the case of the total count of microorganisms. The highest admissible number of yeasts is not defined by the Food Codex.

As shown in Fig. 4, moulds did not appear at all, neither separately (< 10 CFU/g) nor in any analysed sample.

According to the Food Codex (corn, soya and rice products, appendix No 8 to Chapter 4, 2nd part of the Food Codex), bread and pastry are allowed to contain the highest admissible number of moulds corresponding to 0 CFU per g in 5 samples while one sample is allowed to have the highest number of 2×10^2 CFU/g.

On the basis of the above-indicated facts it is possible to declare that all samples satisfied the limits for the number of moulds as defined by the Food Codex of the Slovak Republic (1998) during the whole storage period.

a_w and pH values

a_w and pH values were measured as complementary characteristic parameters of the quality. The a_w values of dough ranged between 0.98 and 0.97 during the storage and those of pastry between 0.92 and 0.95. pH values recorded during the storage did not differ very much from one another and varied in the interval from 5.4 to 5.8 (dough and pastry).

Conclusion

Based on the results of this study, it may be concluded that the application of transglutaminase affected favourably the sensory and nutritional quality of pastry without negatively influencing the microbial quality. The application of a wider choice of enzymatic preparations in different concentration ranges would certainly provide the overall view and a better orientation in solving the particular range of problems in the near future.

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Súhrn

HOZOVÁ B., JANČOVIČOVÁ J., DODOK L., BUCHTOVÁ V., STARUCH L. (2002): **Použitie transglutaminázy na zlepšenie akosti pečiva vyrobeného technológiou mraziarensky skladovaného cesta**. Czech J. Food Sci., **20**: 215–222.

Cieľom modelového experimentu bolo zistiť zlepšujúci účinok prídavku enzýmu transglutaminázy (TGM) v dvoch koncentráciách: 4,5 mg a 7,5 mg/300 g múky do pečivového cesta – na akosť finálnych výrobkov. Sledovali sa zmeny senzorickej (senzorický profil), nutričnej (obsah aminokyselín, hlavne lyzínu), mikrobiologickej akosti (celkový počet, kvasinky, plesne), hodnôt a_w a pH pečiva vyrobeného z mraziarensky skladovaného cesta (–18 ± 2°C). Zistilo sa, že senzorickú akosť priaznivo ovplyvňoval prídavok TGM 4,5 mg/300 g múky; naopak s vyššou aplikovanou koncentráciou TGM (7,5 mg/300 g múky) stúpal protektívny účinok na lyzín. Mikrobiologická akosť, hodnoty pH a a_w cesta i výrobkov zapadali do intervalu kritérií zodpovedajúcich hygienickým požiadavkám pre tento druh výrobkov.

Kľúčové slová: pečivo; transglutamináza (TGM); senzorické hodnotenie; lyzín; mikrobiológia; a_w; pH

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