Effects of Microbial Transglutaminase and Fermentation Type on Improvement of lysine Availability in Wheat Bread: A Response Surface Methodology

Neda Mollakhalili Meybodi[1], Amir Mohammad Mortazavian[2]*, Leila Mirmoghtadaie[2]*, Seyede Marzieh Hosseini[2], Seyyed Ali Yasini[3], Mohammad Hossein Azizi[4], Somayeh Mousavi Nodoushan[5]

1-Department of Food Science and Technology, Student Research Committee, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Science and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

2- Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Science and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

3- Department of Food Science and Technology, Islamic Azad University, Yazd, Iran.

4- Department of Food Science and Technology, Agriculture Collage, Tarbiat Modares University, Tehran, Iran.

5- Laboratory of Food, Cosmetic and Hygiene control, Shahid Sadoughi University of Medical science, Yazd, Iran.

Abstract

Background and objective: Lysine-glutamine crosslink formation catalyzed by microbial transglutaminase is supposed to affect improvement of lysine availability in wheat bread. Present study is done to investigate the effect of microbial transglutaminase and fermentation type in improvement of the lysine availability of wheat bread.

Material and methods: Lysine-fortified wheat breads were formulated using response surface methodology with composite-face central design. Statistical models were used to predict the impact of defatted soy flour level (0-50% w w^{-1}), microbial transglutaminase level (0-1.6% w w^{-1}) and fermentation type (yeast or mixed fermentation based on sourdough). Further information was provided on the individual role of independent variables in nutritional and structural characteristics of optimized formulation and blank and control samples. Experiments were carried out in triplicate and the mean values were analyzed using one-way analysis of variance and Tukey's test.

Results and conclusion: The suggested formula contained 26.64% w w^{-1} of defatted soy flour and 0.55% w w^{-1} of microbial transglutaminase, which was fermented using sourdough-based mixed fermentation and provided 0.16 mg 100 g^{-1} of available lysine and 2.09 cm^3 g^{-1} of specific volume. The highest lysine chemical score (22.79±0.16), essential amino acid index (35.31±0.37) and biological value (26.79±0.02) and the lowest lysine loss during the baking process seen in optimized formulation verified the effectiveness of microbial transglutaminase in lysine fortification of defatted soy flour/wheat breads (P ≤0.05). Considering rheology parameters and textural analysis, microbial transglutaminase treatment increased elastic modulus and β-sheet structure. These structural changes decreased final products digestibility, which can increase using mixed fermentation based on sourdough.

Conflict of interest: The authors declare no conflict of interest.

1. Introduction

Bread is the main foodstuff consumed worldwide. It is usually prepared using flour (mainly wheat flour), water, yeast and salt as its major ingredients. The distinctive characteristic of wheat protein to form a three dimensional, viscoelastic network (gluten) is recognized considerably important in proliferating various types of breads [1]. Indeed, the quality of wheat flour in baking breads is affected by its gluten quality and quantity. In general, the nutritional quality of wheat flour is poor (53 out of 100) regarding its low essential amino acid (EAA) contents, especially lysine contents [2]. Furthermore, the Maillard reaction during the baking process aggravates wheat lysine deficiency by decreasing its availability in food matrices. Nowadays, people are greatly concerned about their diets and know that appropriate diets are sources of (amino acids, minerals and vitamins as well as delivered energy sources. Despite frequency of protein deficiency in world especially in developing countries (due to the extensive use

of low-protein plant-based diets), the major concern seems not to be the overall protein requirements but the specific quantitative needs of EAAs and their balanced ratios [2]. Considering ubiquitous consumption of wheat breads with a poor nutritional quality, fortification of breads with lysine-rich sources have been reported in many studies [3,4]. Legumes are known as appropriate complementary to cereals due to their high lysine contents [5]. Therefore, special attentions have been devoted to soy flour, considering its high lysine proportions and improved protein efficiency ratios (PER) [6]. Notwithstanding suggested positive effects of lysine-rich source incorporation to wheat breads, it is not however so practical. Since lysine is highly susceptible to lose its availability via Maillard reaction, this is definitely developed, resulting a higher reactant availability [7]. Sensory characteristics of fortified products are also adversely affected, regarding wheat gluten dilution effects [8]. Transglutaminase enzymes seem appropriate to solve these problems.

Transglutaminase enzymes (e.g. protein-glutamine γ-glutamyltransferase EC 2.3.2.13) are naturally originated from mammalian tissue/blood, invertebrate, plant and microbial cells. However, microbial originated transglutaminase enzymes are preferred in food sciences due to their high mass production rates, activation independency to cofactors and no adverse effects on final products [9]. Transglutaminase enzymes isolated from microbial cells (microbial transglutaminase, MTG), as extracellular enzymes from various *Streptomyces mobaraensis*, are well-known for catalyzing two types of reactions of transamidation and deamidation. In fact, MTG catalyzes transamidation reactions to introduce new crosslinks; in which, the ɤ-carboxyamide groups of peptide-bound glutamine residues act as donors and primary amines as acceptors. Formation of new crosslinks is however efficient to improve rheological and textural characteristics of the fortified wheat breads [10]. Therefore, this may help improve availability of lysine in human body by keeping lysine as an endogenous amino acids and preventing its loss via Maillard reaction. Fermentation can affect MTG activity by either acidity change or substrate availability [11]. Hence, adding a mixture of defatted soy flour (DSF) (as a lysine-rich source) and MTG enzyme to wheat flour fermented by various processes seems an interesting approach to improve nutritional quality of wheat breads. Considering importance of wheat breads in human diets especially in developing countries, the poor nutritional quality of these breads due to low contents of the EAA of lysine and the necessity of preserving availability of lysine and sensory characteristics of the fortified wheat breads, the aim of this research was to develop lysine fortified wheat breads optimized by response surface methodology, using a combination of MTG enzyme, defatted soy flour

and fermentation type based on evaluating the effectiveness of MTG in improvement of lysine availability, specific volume and lysine bioavailability in final product.

2. Materials and methods

2.1. Bread ingredients

Defatted soy flour (8.56% w w^{-1} moisture and 22.54% w w^{-1} protein) was purchased from Top Soy (Tabriz, Iran), Wheat flour (12.2% w w^{-1} moisture and 11.07% w w^{-1} protein) from Roshan (Yazd, Iran), Microbial transglutaminase enzyme from Dor-Shimi-Marjan (Tehran, Iran), salt and sugar from Pars Namak Kaveh (Tehran, Iran); sunflower oil from Behshahr Industrial (Tehran, Iran), and dried instant yeast was obtained from Khuzestan Yeast (Dezful, Iran).

2.2. Bread preparation

A multi factorial based experimental design approach was set up to study and optimize the fermentation type and the level of MTG and defatted soy flour addition for the quality of bread-baking and improvement of lysine availability. Various levels of MTG and defatted soy flour were tested in a standard bread recipe used in industries. These breads were fermented either via yeast starter fermentation or mixed fermentation based on sourdough (MF-SD). The bread formulations were coded as summarized in Table 1. The amount of water is determined by similar consistency measured by farinograph (Brabender, Germany) [12].

2.2.1. Sponge-dough bread

Sponge-dough bread with a proportion of 60:40 was provided according to AACC Method 10.11.01. Two types of fermentation were used to prepare dough as follow [13].

2.2.1.1. Yeast starter fermentation

Sponge was obtained by 540 g wheat-soy flour (various ratio according to the formula), 60% of water (based on farinograph data) and 2.2 g 100 g^{-1} of flour active dry yeast. This was mixed in a bowl and kept for 4 h inside the fermentation cabinet set at 29±0.5°C. Dough formulation is also determined as 360 g of wheat-soy flour (various ratio according to the formula), MTG (based on the formula), 4.5 g of sugar (0.5% w w^{-1} flour basis), 9 g of salt (1% w w^{-1} flour basis), 27 g of canola oil (3% w w^{-1} flour basis) and the other portion of water. After mixing the dough ingredients for 2 min, the sponge was added in three separate fractions with continuous mixing for at least 5 min. The mixture is then placed at fermentation cabinet set at 29±0.5°C for 30 min. Afterward, the dough was divided into 900 g portions, sheeted, shaped and putted in baking pans with a temperature of 29±0.5°C for 30 min. Then the baking process is done in a convection oven (Model PFB-2, Duke manufacturing Company, St Louis, MO, USA) for 30 min at 220°C. The appropriate baking time was

reported based on the bread stickiness and ability to easily detach from the pan edges [13].

2.2.1.2. Mixed fermentation based on sourdough (MF-SD)

Sour-dough breads with a 60:40 proportion of sour to dough were provided by overnight fermentation. The sour was prepared by 540 g wheat-soy flour and 60% w w^{-1} water based on farinograph data. After mixing the ingredients for 2 min, it is putted in a bowl and held for 20 h in the fermentation cabinet with a temperature of 29±0.5°C. Then dry yeast were added at 2.2 g 100 g^{-1} flour and incubated for 4 h at 29±0.5°C. The dough preparation and baking process were similar to processes previously described [13].

2.3. Bread evaluation and optimization

2.3.1. Specific volume

Determination of breads specific volumes (which is calculated based on volume to weight ratio) were done in triplicate by seed displacement methodology (AACC Method 10-05.01), nearly 1h after leaving the oven [14].

2.3.2. Available lysine

Dried samples (powdered form) were mixed with 6% of aqueous sodium dodecyl sulphate (25 mg 3 ml^{-1}). The solution is then incubated for 30 min (with stirring for 30 s at every 10 min) and filtrated through filter (Wathman No.40, USA). Available lysine content was measured in filtrates using O-Phthalaldehyde assay. This assay estimates the level of lysine blockage via transglutaminase activity or baking process (Maillard reaction). The f assay was carried out as previously described by Michalska et al. [15]. The mixture containing 0.5 ml sample, 1 ml O-Phthalaldehyde reagent and 1 ml water was incubated for 3 min and its fluorescence readings were determined at λ_{Ex} = 340 and λ_{Em}= 455 nm, respectively. The quantification was carried out using the method of external standard and a calibration curve of N^a-acetyl-L-lysine at the range of 10 - 250 mM. Data (expressed as mg kg^{-1} protein) were the mean values of three replications.

The loss rate of the available lysine during dough preparation and bread baking (respectively indicating lysine cross-linked by MTG and lysine loss via Maillard reaction respectively) was calculated using the following formula [16].

Available lysine loss rate (%) =
$$\frac{(\text{Available lysine in dough/bread-available lysine in flour/dough}) \times 100}{\text{Available lysine in flour}}$$

2.4. Nutritional characterization

2.4.1. In vitro protein digestibility (IVPD)

The IVPD determination of samples was done based on a modified pepsin-pancreatin digestion method. Bread samples (1 g each) were incubated with pepsin solution (1

mg pepsin is dissolved in 10 ml of 0.1 M HCl) at 37°C for 3h and neutralized using 2M NaOH. Then, the pancreatin solution obtained by dissolving 4 mg pancreatin in phosphate buffer solution with a constant pH of 8 (7.5 ml) was added to the samples and left for 24 h at 37°C adjusted incubator. To prevent microbial growth, 1 ml of toluene was added to the samples. After 24 h, enzyme was inactivated using trichloroacetic acid (10 ml, 20% w v^{-1}), and precipitation of undigested protein using centrifugation at 5000 ×g for 20 min. The protein content of precipitate was extracted and determined. The IVPD was reported as the total protein fraction solubilized after enzyme hydrolysis. The supernatant, was used for amino acids profiling using a modified method of AOAC 982.30a [17].

2.4.2. Amino acid content

The amino acids contents were assessed using Amino Acid Analyzer (Biochrom 30 Series, Cambridge, UK). The supernatant provided by 1g of the sample was exposed to 5.7 M HCl (with a proportion of 1 ml 10 mg^{-1} of proteins) under nitrogen flow (to prevent amino acids degradation). This was and incubated for 24 h at 110°C. The solvent was vaporized using vacuum rotary and re-suspended in sodium citrate buffer with adjusted pH of 2.2 and filtered via 0.22 mm pore size filter (Millipore, USA). All amino acids were assessed individually. However, tryptophan quantification was not possible by this method. The amino acids content (mg 16 g^{-1} N) was calculated by considering amino acids and protein content of bread.

Chemical score described samples protein quality regarding its essential amino acid content compared to reference protein (hen's egg). The EAAs with the lowest chemical score are considered as limited essential amino acids. The chemical score of the most limiting EAA is considered as the protein score [17]. The essential amino acids index (EAAI) estimate the assessed proteins quality by considering the EAA ratio of the test and reference protein as follow:

$$EAAI = \sqrt[n]{\frac{(EAA_1 \times 100)(EAA_2 \times 100) \dots (EAA_n \times 100)_{[Sample]}}{(EAA_1 \times 100)(EAA_2 \times 100) \dots (EAA_n \times 100)_{[Reference]}}}$$

The biological value (BV) shows consumable fraction of the test proteins. This was calculated using the following equation:

BV = [1.09 × EAAI] -11.70

The PER estimates the nutritional quality of the proteins based on the amino acids profile of the samples after hydrolysis. The PER was calculated using a model developed by Ihekoronye [18] as follows:

PER= -0.468 + (0.454 × leucine) - (0.105 × tyrosine)

Nutritional index (NI) which shows qualitative and quantitative characteristics of the test proteins was calculated based on the following formula:

$$NI = EAAI \times protein\ (\%)/100$$

2.5. Fundamental rheological measurement

Oscillatory shear characterizations of samples were carried out using controlled shear/stress rheometer (Anton Paar MCR301, GmbH, Germany) and parallel plate geometry at 30°C. A sample was collected from the dough and was permitted to rest for 45 min on the plate. Strain sweep test was carried out to determine the linear viscoelastic region. Since, results indicated the linear behavior of dough at strain lower than 0.1%, frequency sweep test was carried out at a range of (0.1-100 Hz at constant strain (0.01%). Parameter damping factor (tan δ) and complex modulus (G*) were calculated respectively using the following formula [19]:

$$\tan \delta = \frac{G''}{G'} ; G* = \sqrt{G'^2 + G''^2}$$

2.6. Textural characteristics of breads

Instrumental texture parameters were calculated using texture profile analyzer (Stable Micro Systems Ltd., Surrey, UK) according to a modified AACC Approved Method 74-09 (2000). A piece of the crumb ($20 \times 20 \times 25$ mm) was pressed to 50% of its original height at speed of 1 mm s^{-1} with a 36 mm cylinder probe using 5 kg load cell. The analysis was carried out in six replicates at 25 ± 3°C on the bread slices. Hardness, cohesiveness, springiness and chewiness of the crumb were calculated by means of resulting Texture profile analysis curves [20].

2.7. Fourier transform infrared (FTIR)

The FTIR spectra was measured by Fourier transform spectrophotometer (PerkinElmer, West Midlands, UK) using potassium bromide (KBr, 200 mg) pellet method with KBr as control. Determination of each sample is done at 25°C using 16 scans in the range of 4000-400 cm^{-1} (with a resolution of 4 cm^{-1}). The curve-fitting analysis of amide I region was done by PeakFit software version 4.12 (SPSS Inc., Chicago, IL, USA), and its corresponding peak area was calculated [21].

2.8. Statistical analysis

All the experiments were carried out in three replications. One-way analysis of variance and Tukey's test was used to identify significant differences in treatment means. Furthermore, determining the linear correlations were reported. The adequacies of models were also investigated by variance analysis (F test), R^2 values, lack-of-fit tests and normal and residual plots. P≤0.05 is considered statistically significant. Data were processed using SPSS statistical software (SPSS Statistics 23.0, Chicago, IL, USA).

3. Results and discussion

Twenty-six samples were produced based on the experimental design and parameters of specific volume and available lysine of the samples were calculated (Table 1). Data were analyzed using specific software to validate suggested formulations of 'sequential model sum of squares' and 'lack of fit'. The lack-of-fit P-value, factor P-value, R^2 (R-squared) and adjusted R^2 of the suggested model were calculated for each function (Table 2). The P≤0.05 was considered statistically significant. Linear function for specific volume and quadratic function for available lysine were approved using specific software (P ≤0.05). Final equations with coded independent variables (A, defatted soy flour; B, microbial transglutaminase; C, fermentation type) were as following:

$$SV = 2.29 - 0.68 \times A - 0.18 \times B - 0.23 \times C$$

$$AL = 0.12 + 0.0241 \times A - 0.0062 \times B + 0.0425 \times C - 0.0001 \times AB + 0.0018 \times AC\ 0.0037 \times BC - 0.0180 \times A^2 - 0.0181 \times B^2$$

Numerical optimization was used to show precise values for independent variables providing the desired response. The available lysine (AL) and specific volume (SV) were calculated together. In general, 100 solutions were suggested by the software and the first suggestion (desirability value 1) was chosen. The suggested formula included 26.64% w w^{-1} DSF, 0.55% w w^{-1} MTG and MF-SD to provide 0.16 mg 100 g^{-1} of AL and 2.09 cm^3 g^{-1} of SV. Optimized levels of DSF, MTG and fermentation type were used to prepare breads in three replicates. The SV and AL values of optimized breads were calculated and the average and predicted values from the model were compared using one sample T-test. The verification results are presented in Table 3. No statistically significant differences (P>0.05) were observed between the values from validations. This indicates prediction effectiveness of the model achieved from optimization. The optimized bread, which is called F$_1$, was compared with blank and control samples to provide additional information on the individual roles of independent variables (DSF, MTG and fermentation type) on nutritional, textural and structural characteristics of the samples. These formulations were described as follows: Blank 1: MTG=0.55% w w^{-1}, DSF=0, yeast fermentation; Blank 2: MTG=0, DSF=26.64% w w^{-1} and sourdough fermentation; Control 1: DSF=0, MTG=0 and sourdough fermentation; and Control 2: DSF=0, MTG=0 and yeast fermentation); also called F$_2$, F$_3$, F$_4$ and F$_5$, respectively.

Table 1. Properties of different formulation of soy-wheat breads as suggested by mixture experimental design

Trial	Component proportion in lysine fortified wheat bread			Properties				
	X_1	X_2	X_3	Formula	Weight (g)	Volume (cm^3)	Specific volume (cm^3 g^{-1})	Available lysine (mg 100 g^{-1})
1	42.68	0.23	S	S_4T_2 (S)	749.06	1033.70	1.38	0.15
2	25	0.8	S	S_3T_3 (S)	673.98	1253.60	1.86	0.15
3	25	0.8	Y	S_3T_3 (Y)	698.23	1864.27	2.67	0.08
4	0	0.8	S	S_1T_3 (S)	694.67	1778.36	2.97	0.06
5	42.68	0.23	Y	S_4T_2 (Y)	721.08	1348.42	1.87	0.07
6	42.68	1.37	Y	S_4T_4 (Y)	719.76	1504.30	2.09	0.03
7	25	0.8	Y	S_3T_3 (Y)	683.17	1796.74	2.63	0.10
8	7.32	1.37	S	S_2T_4 (S)	649.63	1663.05	2.56	0.11
9	25	0.8	Y	S_3T_3 (Y)	703.16	1800.08	2.56	0.08
10	25	0.8	S	S_3T_3 (S)	689.38	1482.17	2.15	0.13
11	25	0.8	S	S_3T_3 (S)	706.62	1441.51	2.04	0.15
12	50	0.8	S	S_5T_3 (S)	765.78	666.23	0.87	0.16
13	7.32	0.23	Y	S_2T_2(Y)	694.63	2396.47	3.45	0.03
14	0	0.8	Y	S_1T_3 (Y)	685.14	2315.77	3.28	0.02
15	25	0	Y	S_3T_1 (Y)	694.63	2396.47	3.45	0.03
16	25	0.8	Y	S_3T_3 (Y)	707.56	1967.02	2.78	0.09
17	25	0.8	S	S_3T_3 (S)	671.75	1323.35	1.97	0.14
18	25	1.6	S	S_3T_5 (S)	694.62	1201.69	1.73	0.14
19	7.32	1.37	Y	S_2T_4 (Y)	701.87	2056.48	2.93	0.005
20	25	1.6	Y	S_3T_5 (Y)	715.78	1488.82	2.08	0.02
21	50	0.8	Y	S_5T_3 (Y)	765.23	795.84	1.04	0.10
22	25	0.8	S	S_3T_3 (S)	717.12	1635.03	2.28	0.14
23	25	0	S	S_3T_1 (S)	701.69	1564.77	2.23	0.15
24	42.68	1.37	S	S_4T_4 (S)	764.75	1404.71	1.79	0.15
25	42.68	0.23	S	S_4T_2 (S)	749.06	1033.70	1.38	0.15
26	7.32	0.23	S	S_2T_2 (S)	675.39	2154.49	3.01	0.12

X_1= defatted soy flour, X_2= microbial transglutaminase enzyme, X_3= fermentation type (Y=yeast starter, S= mixed fermentation based on sourdough)

Table 2. Statistical parameters of RSM optimization; considering available lysine and specific volume as dependent variables and A, B, C as independent ones

Dependent parameter	AL	SV
Model selection	Quadratic	Linear
Model p value	<0.0001	<0.0001
Lack of fit p-value	0.0567	0.072
Factors p-value		
A	0.0004	<0.0001
B	0.2403	0.03
C	<0.0001	0.0028
AB	0.99	-
AC	0.73	-
BC	0.47	-
A2	0.02	-
B2	0.01	
R^2	0.91	0.84
Adjusted R^2	0.86	0.81

P≤0.05 was considered statistically significant.
AL: available lysine; SV: specific volume
A: defatted soy flour, B: microbial transglutaminase and C: fermentation type

Table 3. Comparison of optimum point verification test results with the estimated values from the model

Response	Predicted value	Experimental results	Prediction error (%)	p-value
AL (mg 100 g^{-1})	0.16[b]	0.15±0.02[b]	6.25	0.22
SV (cm^3 g^{-1})	2.09[a]	2.07±0.06[a]	0.96	0.36

P≤0.05 was considered statistically significant.
AL: available lysine; SV: specific volume

3.2. Nutritional analysis

Proteins (qualitative and quantitative) are normally described as the key components in nutritional value characteristics of the breads. Therefore, nutritional characteristics of soy-wheat breads were assessed in the current study using bread protein fractions to investigate effects of DSF and MTG additions and various fermentation types on bread quality. Generally, quality of proteins is predictable using their amino acids compositions and limiting ones. Lysine is considered as the first limiting amino acids in breads not only due to its small quantity, but also as a result of nutritional availability loss induced by the Maillard reaction [7]. Lysine, as an EAA, is a primary amine that is available when its ε-amino group is free [15].

3.2.1. Loss of available lysine

Blockage of the lysine ε-amino group by Maillard reaction or MTG-mediated crosslink results in available lysine loss [22]. In this study, available lysine contents in flour, dough and breads were calculated (Figure 1). It has been suggested that differences in available lysine contents of flour and dough were mainly affected by the fermentation process and MTG enzyme activity. However, breads significantly differed from doughs in available lysine contents because of the Maillard reaction during the baking process [16]. Results indicated that DSF inclusion significantly increased the available lysine content of flour (P≤0.05). The value of AL in formulations substituted with 26.64% w w^{-1} DSF increased from 0.412 to 0.992 mg 100

g^{-1}; possibly due to its higher protein and lysine contents [23]. The loss rate in available lysine was assessed during dough preparation to investigate effects of the MTG enzyme as it introduced new crosslinks between glutamine and lysine residues [9,15]. Results demonstrated that MTG inclusion remarkably decreased AL content of the formulations. Nearly 65.42% lysine loss (between flour and dough) were observed in samples containing 0.55% w w^{-1} MTG while no lysine losses were seen in controls (F_4 and F_5). The AL content of dough is also affected by the fermentation type as AL contents of dough significantly increased in F_4 sample which is fermented by MF-SD, compared to that of yeast fermented samples (F_5). Estimation of available lysine contents of doughs and breads and their differences were considered as available lysine loss via Maillard reaction [16]. The highest lysine loss ratio was seen in DSF fortified wheat breads in absence of MTG (F_3); estimated nearly 69.97% compared to 50% in controls (absence of DSF). The higher lysine loss ratio in DSF substituted wheat breads was linked to its higher substrate availability. In fact, the lysine loss ratio increased significantly by DSF substitution and decreased significantly by MTG incorporation (P≤0.05). These possibly occurred because of their effects on substrate availability of Maillard reaction [7,22]. However, MTG addition with DSF substitution seems a good strategy to preserve chemical availability of lysine; a factor that is necessary to be monitored respecting is its subsequent biological availability.
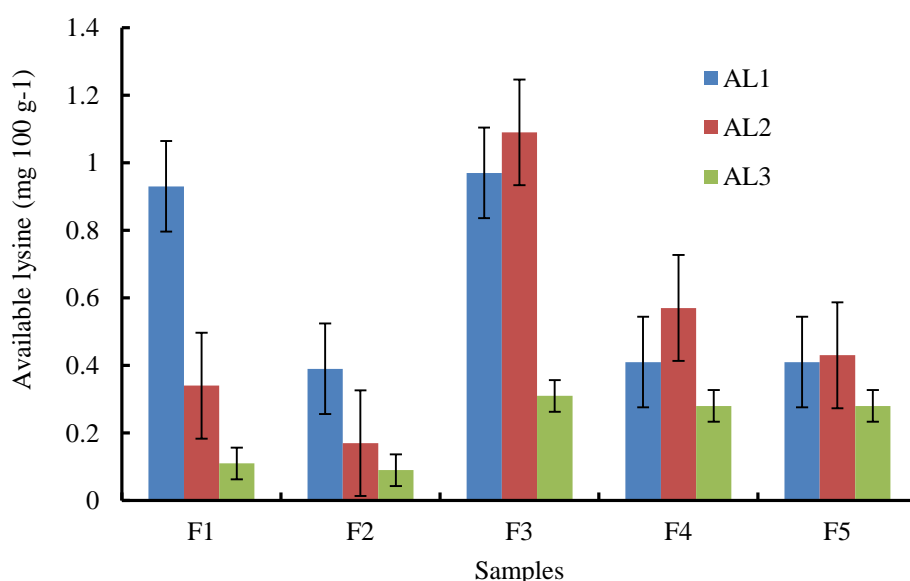
Figure 1. Comparison of available lysine content in flour (AL1), dough (AL2) and bread (AL3) of treatments

3.2.2. Amino acid profile

The protein content and amino acids profile of treatments were monitored to investigate their nutritional quality. Addition of DSF at 26.64% w w^{-1} increased wheat bread protein content from 10.86 to 14.65% of dry matter. The amino acids compositions of the optimized breads with blank and control samples are shown in Table 4. Considering effects of fermentation type, it was illustrated that formulations with MF-SD included higher amino acids contents, compared to that yeast leavened formulations did. Indeed, amino acids profiles of the formulations prepared by MF-SD process improved significantly, which was associated to higher proteolytic activity catalyzed by lactic acid bacteria and their further effective amino acids production rates [24,25]. In the present study, contents of EAAs, especially lysine, increased significantly in MF-SD fermented formulations. However, protein quantity and amino acids profile are described as indices to investigate nutritional statuses of the food products. These indices in combination with protein digestibility provide further precise information on nutritive values.

In vitro protein digestibility (IVPD) provides information on how protein hydrolysates endure digestive processes [17]. In the current study, the highest IVPD was reported for F_3 (treatment substituted with DSF and fermented by MF-SD in absence of MTG). Comparing treatments of F_3 and F_4 which differed only by their DSF contents, a small enhancement was observed in IVPD values by DSF addition which could be linked to effects of DSF on gluten weakening effects [26]. Considering the effects of fermentation type and DSF addition and MTG treatment on IVPD of the wheat breads, it was revealed that although MTG decreased IVPD of soy-wheat breads, MF-SD increased the value more successfully than that the yeast fermentation did. Moreover, the lowest IVPD was seen in F_2 (containing 0.55% MTG in absence of DSF fermented based on yeasts). The remarkably improved digestibility by MF-SD might be associated to proteolysis by sourdough lactic acid bacteria and activation of proteolysis by wheat/soy enzymes under acidic conditions of the sourdough fermentation [24]. The decreased IVPD seen in MTG treated samples verified the reverse correlation of food compactness and digestibility [27]. This result was similar to results by Giosafatto et al. from a study on digestibility of transglutaminase treated ovalbumin [28]. It is noteworthy that introduction of new bonds involved in β-sheet secondary structures resulted in further resistant structures to tryptic hydrolysis [29].

In contrast to lysine that entered the Maillard reaction, those involved in MTG mediated crosslinks are biologically available. The major difference between these two reaction products is that Maillard induced crosslinks are acid stable while MTG mediated crosslinks are not [30]. Therefore, lysine chemical scores can be helpful in effectiveness assessment of MTG enzyme performance. In this study, EAA profiles and their associated chemical scores were calculated based on EAA reference patterns (Table 4).

Table 4. Nutritional indices of breads made with optimized, block and control formulation

Properties	Trial				
	F_1	F_2	F_3	F_4	F_5
Protein content (%)	14.65±0.03^a	10.86±0.07^b	14.16±0.02^a	11.05±0.03^b	10.97±0.05^b
In vitro protein digestibility	65.27±0.17^b	51.11±0.16^f	73.27±0.30^a	70.26±0.16 ^{a}	58.24±0.17^d
Chemical score (%)					
Thr	56.10±0.16^e	25.05±0.05^d	31.05±0.14^c	40.95±0.01^e	10.09±0.02^b
Val	37.68±0.10^d	18.28±0.18^c	21.42±0.13^b	33.83±0.21^d	48.82±0.12^d
Met	12.19±0.41^a	7.92±0.31^a	6.47±0.08^a	12.34±0.13^b	5.41±0.18^a
Ile	38.35±0.17^d	43.83±0.17^e	34.81±0.19^c	37.49±0.01^{de}	41.34±0.04^c
Leu	40.85±0.14^d	31.57±0.09^c	41.25±0.20^b	36.56±0.02^d	39.52±0.16^b
Tyr+Phe	60.99±0.01^f	61.85±0.34^f	63.80±0.12^e	52.40±0.15^f	47.30±0.07^d
His	35.79±0.13^d	43.07±0.12^e	42.63±0.06^d	41.79±0.09^e	39.92±0.02^c
Lys	22.79±0.16^b	3.90±0.07^a	10.21±0.18^a	6.79±0.16 ^{a}	1.63±0.13^a
Protein score (%)	12.19±0.41	7.92±0.31	6.47±0.08	6.79±0.16	1.63±0.13
Essential amino acid index	35.31±0.37^a	21.84±0.12^{bc}	25.36±0.02^b	27.72±0.01^b	18.59+0.08 ^{c}
Biological value	26.79±0.02 ^{a}	12.11±0.03^c	15.94±0.01^b	18.51±0.09^b	8.57±0.07^c
Protein efficiency ratio	4.64±0.12 ^{b}	3.07±0.08 ^{d}	5.50±0.09 ^{a}	4.16±0.02^c	4.66±0.01^b
Nutritional index	5.17±0.01^a	2.37±0.04^d	3.59±0.09^b	3.06±0.03^c	2.03±0.06^e

Data are reported as average ± standard deviation

Different letters in each row means significantly different (P≤0.05) except chemical score data, which are compared in column (different letter in each column are significantly different (P≤0.05))

(F_1 (DSF: 26.64%, MTG: 0.55% and MF-SD), F_2 (MTG=0.55%, DSF= 0, YSF); F_3 (MTG=0, DSF=26.64%, MF-SD); F_4 (DSF: 0, MTG: 0, MF-SD); F_5 (DSF: 0, MTG: 0 and YSF) Thr: Threonine, Val: Valine, Met: Methionine, Ile: Isoleucine, Leu: Leucine, Tyr: Tyrosine, Phe: Phenylalanine, His: Histidine, Lys: Lysine

To assess effects of treatments on digestibility, supernatants (digestible protein fractions) were used after enzyme hydrolysis. While lysine was the first limiting amino acids in control and DSF substituted samples, this amino acid was second in optimized formulations (DSF substituted samples fermented by MF-SD and treated with MTG). The EAA and BV indices, as the major quality determining factors of the food proteins, were significantly the highest in breads prepared with optimized formulation of F_1 ($P\leq0.05$). The PER of the treatments ranged 3.07-5.50. Based on the data from Table 4, it is obvious that the PER quantity was changed significantly due to DSF addition, MTG treatment and fermentation type. Comparing treatments of F_3 and F_4, DSF increased PER significantly ($P\leq0.05$). Moreover, MTG decreased PER, compared to treatments of F_1 and F_3. The lowest PER was reported for F_2, which was treated with MTG and yeast fermentation in absence of DSF. The PER is highly dependent on sample digestibility; as reported by other studies [18]. However, MTG treatment decreased PER as the major indicator of protein quality. Use of MTG in fortified wheat breads is popular due to its positive effects on lysine chemical score as the first limiting amino acid. Lysine chemical score significantly increased in samples treated with MTG, which could be attributed to lysine blockage in ε-(γ-glutamyl) lysine moiety catalyzed by MTG. Lysine was categorized as the second limiting amino acid in DSF fortified formulations prepared via MTG treatment fermented by MF-SD; based on the sample amino acid scores of this study.

Nutritional index (NI), showing qualitative and quantitative factors, was markedly higher in fortified samples prepared with MTG (F_1) (Table 4). The NI effectively increased in fortified samples because of increased protein contents of the fortified samples. However, MTG treatment decreased digestibility of the proteins and consequently their bioavailability. Relatively, MF-SD can be considered as a modulating treatment to solve this problem. Regarding preventive effects of MTG on lysine EAA, the highest NI was seen in fortified samples prepared with MF-SD and treated with MTG. A negative correlation was seen between the lysine chemical score and lysine loss ratio via Maillard reaction ($R=-0.71$, $P\leq0.05$). In fact, MTG treatment increased the lysine chemical score by preventing the amino acid loss via Maillard reaction. A negative correlation was observed between the biological value and lysine loss via Maillard reaction ($R=-0.68$, $P\leq0.05$) since blocked lysine via Maillard reaction was no longer bioavailable [31]. Considering importance of digestibility in nutritional quality determination of the final products and its significant dependency to the structure as affected by the fermentation type, DSF addition and MTG treatment, the rheological, textural and structural characteristics of the

samples were provided to the best of the authors' knowledge.

3.3. Dough rheology

Effects of MTG and fermentation type on dynamic viscoelastic characteristics of wheat dough supplemented with DSF was investigated using oscillatory test with a frequency sweep of 0.01–10 Hz. Results showed that elastic and viscose moduli (G' and G") were frequency dependent and elastic modulus was higher than viscose modulus at all frequency ranges. Consequently, a solid, elastic-like structure was observed in dough (data not shown). Viscoelastic characteristics and deformation resistance of formulations were assessed using complex modulus (G^*) and damping factor (tan δ) (Figures 2a, b). Elastic and viscose parameters are known as quality determination factors in wheat dough since poor quality wheat doughs are less elastic and more viscous than high quality wheat doughs. The complex modulus and damping factor provided valuable information on the strength of samples, considering elasticity and the viscosity of the materials [19]. The optimized complex modulus must be considered in formulations since high complex moduli are too rigid to allow growth of gas bubbles needed in fermentation. Furthermore, low complex moduli produce poor quality doughs unable to restore gases [32]. The highest complex modulus were seen for dough samples prepared in absence of DSF and subjected to MTG treatment and yeast fermentation (F_2) and the lowest for samples fermented using sourdough in absence of DSF and MTG (F_4). The lowest and highest damping factors were seen for F_2 and F_3, respectively. Increased complex modulus and decreased damping factor were observed in F_1 treatments, compared to F_3. Increased complex modulus and dough stability by MTG treatment is believed to induce by increased elastic and viscous characteristics of the doughs. Decreased tan δ indicated that elastic modulus increased more relatively [19]. Indeed, the protein crosslinking reaction catalyzed by MTG treatment seems to be resulted in network structure and consequently changes in viscoelasticity characteristics of the composite doughs. Similar results were achieved from laccase treated oat doughs [32]. Comparing F_4 and F_5, it was revealed that MF-SD considerably decreased firmness and elasticity, regarding decreased complex modulus and increased damping factor. The elasticity decrease induced by sourdough is possibly induced by protein degradation effects of organic acids and higher protease activities as shown by other researchers [24]. In DSF addition, increased complex modulus and similar damping factors were observed in F_3 (containing DSF), compared to F_4. This behavior is assumed to be induced by the gluten weakening effects and its lower water incorporation [33]. Water molecules in high-moisture doughs usually behave as inert fillers, which decrease elastic and viscous moduli and consequently the complex modulus.

a

b
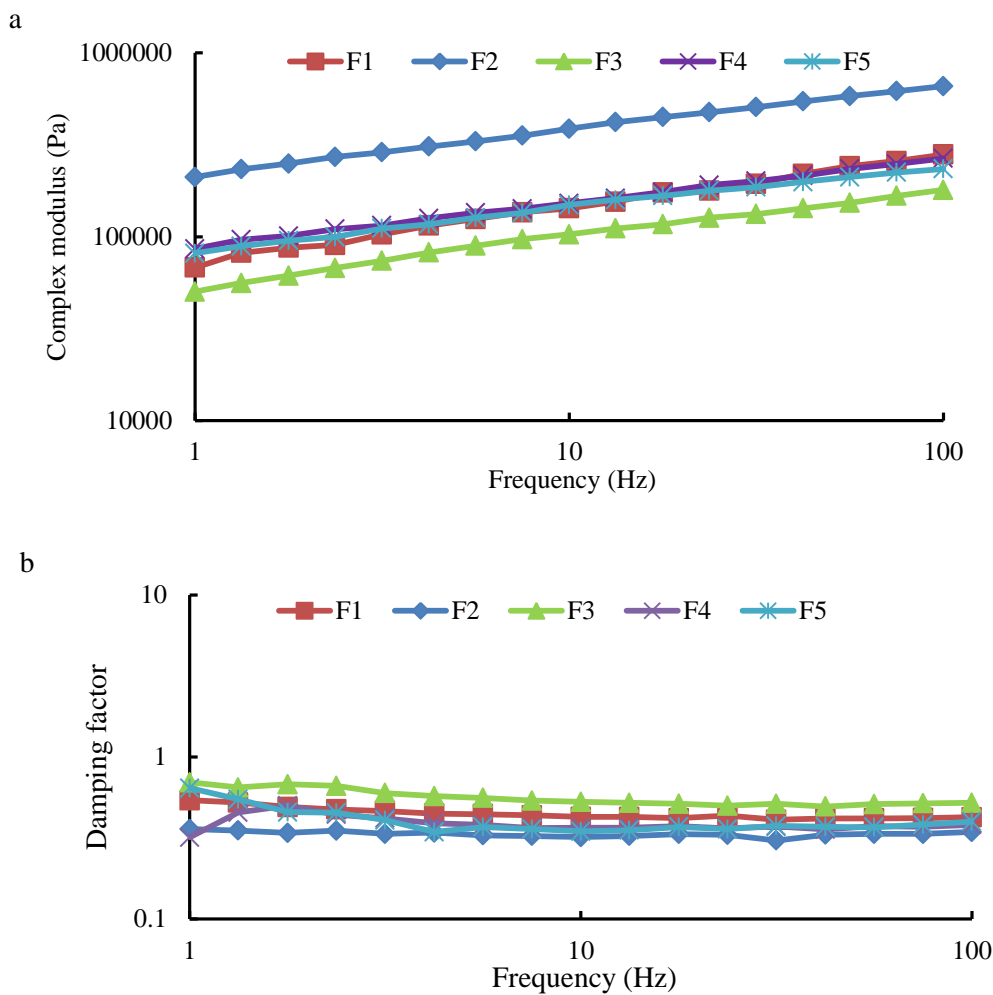
Figure 2a, b. Complex modulus (|G*|) and phase angle (tan δ) parameters of the investigated formula dough

3.4. Texture profile analysis

Texture profile analysis includes two reciprocating compressions of samples; similar to jaw action. Textural characteristics of the highlighted treatments verified significant effects of DSF addition, MTG treatment and fermentation type (Table 5). The lowest and highest hardness values were observed in F_4 and F_2, respectively. As previously stated, DSF increased sample hardness significantly ($P \leq 0.05$), which increased by MTG (F_1 hardness was lower than F_3 hardness). In fact, improving effects induced by MTG treatment depended on the type of flour, which resulted in further hardness in samples without DSF. High levels of cohesiveness and springiness are favored in wheat breads since these parameters characterize a lower disaggregation during the mastication. Gluten is well known to be responsible for cohesiveness and viscoelastic characteristics of the wheat breads. Hence, defatted soy flour can negatively affect the product cohesiveness [34]. However, MTG treatment improved the detrimental effects induced by DSF (comparison of F_1 with F_3) and negatively affected wheat bread cohesiveness in

absence of DSF (F_2). The MF-SD significantly decreased cohesiveness ($P \leq 0.05$) in accordance with protein network hydrolysis by the sourdough fermentation directly or pH mediated activation of proteolytic enzymes of the cereals [24]. No significant differences in cohesive contents of the optimized formulations (F_1) and control samples (F_4) verified synergic benefits of MF-SD in combination with MTG in DSF incorporated samples. Springiness which is also called elasticity indicated the recovery rate of a deformed material to its initial state after removal of deforming force. The highest springiness contents found in F_2 revealed the increased elasticity of gluten networks induced by MTG [33]. No statistically significant differences were reported in springiness contents of other formulations ($P > 0.05$). Chewiness, as an indicator of required energy to change a solid-state food to its swallowable form, shows food hardness, cohesiveness and springiness together. In the current study, the chewiness value was changed similar to hardness; as previously shown by Abdelghafor et al. [35].

Table 5. Texture profile analysis of breads made with optimized, block and control formulation

Trial	Properties			
	Hardness (g)	Springiness (-)	Cohesiveness (-)	Chewiness (g)
F_1	502.03±0.06 [c]	0.74±0.07 [b]	0.69±0.02 [b]	256.36±0.06 [c]
F_2	1272.85±0.08 [a]	0.88±0.01 [a]	0.48±0.01 [d]	537.65±0.01 [a]
F_3	1084.13±0.06 [b]	0.77±0.06 [b]	0.54±0.05 [c]	450.78±0.02 [b]
F_4	307.14±0.09 [d]	0.78±0.02 [b]	0.73±0.02 [b]	174.89±0.07 [d]
F_5	497.85±0.07 [c]	0.73±0.01 [b]	0.80±0.01[a]	290.74±0.03 [c]

Data are reported as average ± standard deviation

Different letters in each column means significantly different (P≤0.05)

(F_1 (DSF: 26.64%, MTG: 0.55% and MF-SD), F_2 (MTG=0.55%, DSF= 0, YSF); F_3 (MTG=0, DSF=26.64%, MF-SD); F_4 (DSF: 0, MTG: 0, MF-SD); F_5 (DSF: 0, MTG: 0 and YSF)

3.5. FT-IR analysis

Chemical composition and molecular structure changes in various bread formulations induced by MTG treatment, DSF addition and fermentation type can be verified using FT-IR spectroscopy (Figure 3a). In this study, freeze-dried samples were investigated to diminish the spectral interference of water molecules at amide I bands as the major protein secondary structure determinants (the O-H bending mode of water is at 1642 cm^{-1}) [35]. However, the major peaks were similar in various bread formulations, their amplitudes varied significantly. The spectra of MTG incorporated soy-wheat breads fermented by yeast starter fermentation or MF-SD showed the major bands at approximately 3800-3100 cm^{-1} (NH-stretching coupled with hydrogen bonding (amides A)), 3100-2800 cm^{-1} (asymmetric stretching vibration of CH and NH3$^+$ (amide B)), 1700-1500 cm^{-1} (amide-I, II) and 1200-800 cm^{-1} (C-C, C-O, C-H stretching and C-OH bending). The highest absorbance values at all bands were reported for MTG-treated samples (F_1 and F_2). Similarly, MTG enzyme treatments modified the chemical bonds such as N-H, C=O, C-H and C-N that were linked to these peaks. The absorbance of amide A region, which represents the asymmetric/asymmetric stretching of NH bonds and stretching vibration of free hydroxyls in amino groups, was significantly greater in samples treated with MTG (F_1 and F_2). Protein spectra are generally characterized by their strong amide I (1700-1600 cm^{-1}) and amide II (1600-1500 cm^{-1}) bands, indicating C=O stretching vibration and C-N stretching/N-H bending vibration, respectively. Increases in amide I and amide II peak amplitudes demonstrated formation of isopeptide bonds induced by MTG. Increases in amide I intensity were reported in glycerol incorporated gelatin films treated with MTG [36]. Effects of MTG treatments on the secondary structures and protein interactions were previously reported in literatures [37].

Shape of amide I region in 1700-1600 cm^{-1} wave number ranges was used to resolve the protein secondary structures. Investigation of secondary structures was carried out based on the facts that bands from 1640-1610 cm^{-1} were linked to β-sheets, bands from 1650-1640 cm^{-1} to random coils, bands from 1660-1650 cm^{-1} to α-helices and bands from 1700-1660 cm^{-1} to β-turns [38]. The deconvolution of secondary structures was carried out in F_1 and F_3 to show possible effects of MTG treatment on soy-wheat bread secondary structures (Figure 3b). The deconvolution of amide I bands in F_1 and F_3 was composed of four components, located at 1623, 1642, 1658 and 1684 cm^{-1}, respectively (Figure 3b). However, bands from 1623 and 16583 cm^{-1}, which are amide groups respectively involved in β-sheet and α-helix structures, were significantly higher in F_1, compared to F_3. Furthermore, bands from 1642 and 1684 cm^{-1}, respectively corresponding random coils and β-turns, were quite higher in F_3. This was similar to other studies which showed increased β-sheets and α-helices in whole wheat doughs using MTG and GOX treatments [38]. The β-sheet and α-helix structures are considered as the most stable and ordered conformation structures, respectively. Despite the flexible and open nature of α-helix, β-turn, and random coil structures, the β-sheet structures are moderately stable [39]. Consequently, it is suggested that decreased tryptic digestibility by MTG treatment was induced by decreased chain flexibility via formation of isopeptide bonds. This finding is similar to rheology finding (increased elastic modulus and decreased damping factor in MTG treated samples) as the elastic energy is stored better via development of β-sheet structures in doughs at the expense of β-turn conformation [38]. In addition, no obvious differences were observed in peak intensities from 1447, 1530 and 1658 cm^{-1}, representing C-N, C=N and C=O groups, respectively. These functional groups are associated to Maillard reaction products [24]. Despite efficiency of MTG in preserving availability of lysine EAA, no significant differences were seen in Maillard reaction rate of MTG treatments, since other amino acid side chains such as those in arginine and tryptophan are susceptible to Maillard reaction [40].
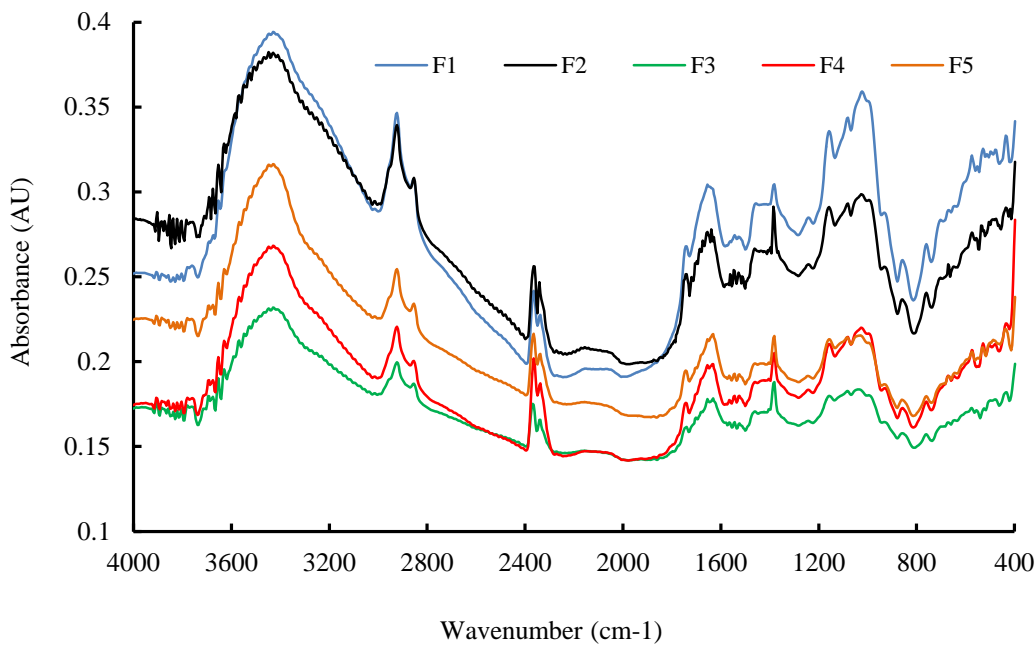
Figure 3a. FTIR spectra of optimized bread (F_1), block (F_2, F_3) and control (F_4, F_5) samples
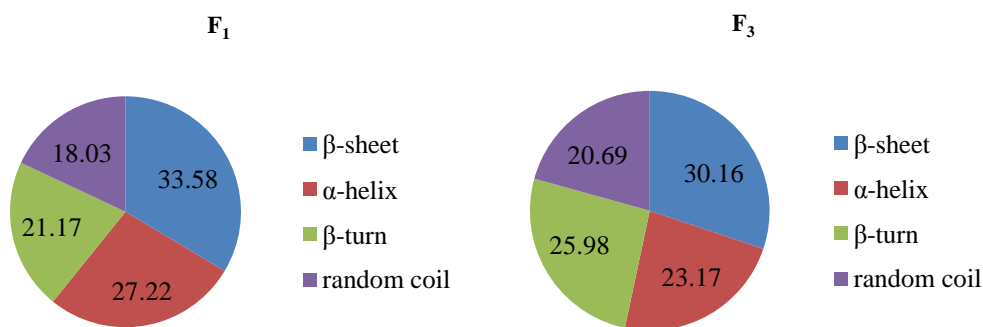
Figure 3b. Examples of FT-IR deconvolution of Amide I region of F_1 and F_3 (estimate as secondary structure area/total amide I band area)

4. Conclusion

In general, the aim of this study was to investigate the effectiveness of microbial transglutaminase enzymes in lysine fortification of the wheat breads substituted by defatted soy flour as a lysine-rich source fermented by yeast starter fermentation or sourdough-based mixed fermentation. Results demonstrated that EAA and nutritional indices increased significantly in optimized formulations, containing 26.64% w w^{-1} defatted soy flour and 0.55% w w^{-1} microbial transglutaminase and fermented by sourdough-based mixed fermentation (F_1), compared to blank and control samples. The highest chemical score of lysine was seen in F_1. However defatted soy flour is a lysine-rich source, its addition increased the lysine loss ratio during baking process; improved by microbial

transglutaminase incorporation. Biological values of the blocked lysine were however assessed by digestibility of the final products. Considering the rheological, textural and structural characteristics, it was revealed that the novel crosslinks introduced by the microbial transglutaminase increased β-sheet structures and consequently decreased their flexibility and tryptic digestibility. The microbial transglutaminase anticipated compactness was developed using sourdough-based mixed fermentation. In conclusion, the highest biological value, lysine chemical score and nutritional and amino acid indices and the lowest available lysine loss during baking process were observed in F_1, containing defatted soy flour and microbial transglutaminase and fermented using sourdough-based mixed fermentation.

5. Acknowledgements

6. Conflict of interest

The authors declare no conflict of interest.

References

1. CauvainSP. Breadmaking: Improving Quality.Elsevier, Australia, 2012: 35-48.

2. Albert C, Gombos S, Salamon R, Csiki Z, Prokisch J, Csapo J. Production of highly nutritious functional food with the supplementation of wheat flour with lysine. Acta Univ Sapientiae Aliment. 2017; 10(1): 5-20.
doi: 10.1515/ausal-2017-0001.

3. Alamu EO, Popoola I, Maziya Dixon B. Effect of soybean (*Glycine max* (*L.*) *Merr.*) flour inclusion on the nutritional properties and consumer preference of fritters for improved household nutrition. Food Sci Nutr. 2018; 6(7): 1811-1816.
doi: 10.1002/fsn3.751.

4. Kolapo AL, Sanni MO. A comparative evaluation of the macronutrient and micronutrient profiles of soybean-fortified Gari and tapioca. Food Nutr Bull. 2009; 30(1): 90-94.
doi: 10.1177/156482650903000110.

5. Yamsaengsung R, Schoenlechner R, Berghofer E. The effects of chickpea on the functional properties of white and whole wheat bread. Int J Food Sci Tech. 2010; 45(3): 610-620.
doi: 10.1111/j.1365-2621.2009.02174.x.

6. Angioloni A, Collar C. High legume-wheat matrices: An alternative to promote bread nutritional value meeting dough viscoelastic restrictions. Eur Food Res Technol. 2012; 234(2): 273-284.
doi: 10.1007/s00217-011-1637-z.

7. Corke H, De Leyn I, Nip WK, Cross N. Bakery Products: Science and Technology: John Wiley and Sons, USA, 2008: 301-381.

8. Eduardo M, Svanberg U, Ahrne L. Effect of hydrocolloids and emulsifiers on the shelf-life of composite cassava-maize-wheat bread after storage. Food Sci Nutr. 2016; 4(4): 636-644.
doi: 10.1002/fsn3.326.

9. Yokoyama K, Nio N, Kikuchi Y. Properties and applications of microbial transglutaminase. Appl Microbiol Biotechnol. 2004; 64(4): 447-454.
doi: 10.1007/s00253-003-1539-5.

10. Heil A, Ohsam J, van Genugten B, Diez O, Yokoyama K, Kumazawa Y, Pasternack R, Hils M. Microbial trans-glutaminase used in bread preparation at standard bakery concentrations does not increase immune-detectable amounts of deamidatedgliadin. J Agr Food Chem. 2017; 65(32): 6982-6990.
doi: 10.1021/acs.jafc.7b02414.

11. Nore S, Eva Van der M, Sami H, Joran V, Kevin JV, Christophe MC. Bread dough and baker's yeast: An uplifting synergy. Compr Food Sci Food Saf. 2017; 16: 850-867.
doi: 10.1111/1541-4337.12282

12. Linlaud NE, Puppo MC, Ferrero C. Effect of hydrocolloids on water absorption of wheat flour and farinograph and textural characteristics of dough. Cereal Chem. 2009; 86(4): 376-382.
doi: 10.1094/CCHEM-86-4-0376.

13. Gamel TH, Abdel Aal ESM, Tosh SM. Effect of yeast-fermented and sour-dough making processes on physicochemical characteristics of β-glucan in whole wheat/oat bread. LWT Food Sci Technol. 2015; 60(1): 78-85.
doi: 10.1016/j.lwt.2014.07.030.

14. Gerrard JA, Brown PK, Fayle SE. Maillard crosslinking of food proteins III: The effects of glutaraldehyde, formaldehyde and glyceraldehyde upon bread and croissants. Food Chem. 2003; 80(1): 45-50.
doi: 10.1016/S0308-8146(02)00233-9.

15. Michalska A, Amigo Benavent M, Zielinski H, Del Castillo MD. Effect of bread making on formation of maillard reaction products contributing to the overall antioxidant activity of rye bread. J Cereal Sci. 2008; 48(1): 123-132.
doi: 10.1016/j.jcs.2007.08.012.

16. Lopez EP. Influence of the addition of lupine protein isolate on the protein and technological characteristics of dough and fresh bread with added brea gum. Food Sci Technol. 2014; 34: 195-203.
doi: 10.1590/S0101-20612014005000016.

17. Rizzello CG, Lorusso A, Montemurro M, Gobbetti M. Use of sourdough made with quinoa (*Chenopodium quinoa*) flour and autochthonous selected lactic acid bacteria for enhancing the nutritional, textural and sensory features of white bread. Food Microbial. 2016; 56: 1-13.
doi: 10.1016/j.fm.2015.11.018

18. Ihekoronye A. A rapid enzymatic and chromatographic predictive model for the In-vivo rat-based protein efficiency ratio. Int J Food Sci Technol. 1981; 21(1): 81-87.
doi: 10.1111/j.1365-2621.1986.tb01933.x

19. Upadhyay R, Ghosal D, Mehra A. Characterization of bread dough: Rheological properties and microstructure. JFood Eng. 2012; 109(1): 104-113.
doi: 10.1016/j.jfoodeng.2011.09.028

20. Katina K, Heinio RL, Autio K, Poutanen K. Optimization of sourdough process for improved sensory profile and texture of wheat bread. LWT Food Sci Technol. 2006; 39(10): 1189-1202.
doi: 10.1016/j.lwt.2005.08.001

21. Luo Y, Li M, Zhu KX, Guo XN, Peng W, Zhou HM. Heat-induced interaction between egg white protein and wheat gluten. Food Chem. 2016; 197: 699-708.
doi: 10.1016/j.foodchem.2015.09.088

22. Fu L, Wang C, Wang J, Ni S, Wang Y. Maillard reaction with ribose, galacto-oligosaccharide or chitosan-oligosaccharide reduced the allergenicity of shrimp tropomyosin by inducing conformational changes. Food Chem. 2019; 274: 789-795.
doi: 10.1016/j.foodchem.2018.09.068.

23. Serrem CA, de Kock HL, Taylor JRN. Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. Int J Food Sci Technol. 2011; 46(1): 74-83.
doi: 10.1111/j.1365-2621.2010.02451.x.

24. Huma N, Shahid M. Impact of mixed lactic acid bacterial (lab) culture on flavoring profile and quality attributes of

spring wheat sourdough bread. Pak J Agr Sci. 2016; 53(1): 225-231.
doi: 10.21162/PAKJAS/16.4599.

25. Thiele C, Ganzle MG, Vogel RF. Contribution of sourdough lactobacilli, yeast, and cereal enzymes to the generation of amino acids in dough relevant for bread flavor. Cereal Chem. 2002; 79(1): 45-51.
doi: 10.1094/CCHEM.2002.79.1.45

26. Rajiv J, Lobo S, Jyothi Lakshmi A, Venkateswara Rao G. Influence of green gram flour (*Phaseolus aureus*) on the rheology, microstructure and quality of cookies. J Texture Stud. 2012; 43(5): 350-360.
doi: 10.1111/j.1745-4603.2012.00346.x.

27. Romano A, Giosafatto CVL, Di Pierro P, Romano R, Masi P, Mariniello L. Impact of transglutaminase treatment on properties and In vitro digestibility of white bean (*Phaseolus vulgaris L.*) flour. Food ResInt. 2016; 88: 239-246.
doi: 10.1016/j.foodres.2016.02.014.

28. Giosafatto CVL, Rigby NM, Wellner N, Ridout M, Husband F, Mackie AR. Microbial transglutaminase-mediated modification of ovalbumin. Food Hydrocoll. 2012; 26(1): 261-267.
doi: 10.1016/j.foodhyd.2011.06.003

29. Tang CH, Sun X, Yin SW, Ma CY. Transglutaminase-induced cross-linking of vicilin-rich kidney protein isolate: Influence on the functional properties and In vitro digestibility. Food Res Int. 2008; 41(10): 941-947.
doi: 10.1016/j.foodres.2008.07.015.

30. Gan CY, Cheng LH, Azahari B, Easa AM. In-vitro digestibility and amino acid composition of soy protein isolate cross-linked with microbial transglutaminase followed by heating with ribose. Int J Food Sci Nutr. 2009; 60(7): 99-108.
doi: 10.1080/09637480802635090.

31. Wada Y, Lonnerdal B. Effects of different industrial heating processes of milk on site-specific protein modifications and their relationship to In vitro and In vivo digestibility. J Agr Food Chem. 2014; 62(18): 4175-4185.
doi: 10.1021/jf501617s.

32. Flander L, Holopainen U, Kruus K, Buchert J. Effects of tyrosinase and laccase on oat proteins and quality parameters of gluten-free oat breads. J Agr Food Chem. 2011; 59(15): 8385-8390.

doi: 10.1021/jf200872r.

33. Ndayishimiye JB, Huang WN, Wang F, Chen YZ, Letsididi R, Rayas Duarte P, Tang XJ. Rheological and functional properties of composite sweet potato - wheat dough as affected by transglutaminase and ascorbic acid. J Food Sci Technol. 2016; 53(2): 1178-1188.
doi: 10.1007/s13197-015-2004-z.

34. Boukid F, Carini E, Curti E, Bardini G, Pizzigalli E, Vittadini E. Effectiveness of vital gluten and transglutaminase in the improvement of physico-chemical properties of fresh bread. LWT. 2018; 92: 465-470.
doi: 10.1016/j.lwt.2018.02.059.

35. Abdelghafor RF, Mustafa AI, Ibrahim AM, Krishnan PG. Quality of bread from composite flour of sorghum and hard white winter wheat. Adv J Food Sci Technol. 2011; 3(1): 9-15.
doi: 10.1.1.1028.2394.

36. Zhang X, Wenhang, Wang W, Wang Y, Wang Y, Wang X, Gao G, Chen G, Liu A. Effects of nanofiber cellulose on functional properties of heat-induced chicken salt-soluble meat protein gel enhanced with microbial transglutaminase. Food Hydrocoll. 2018; 84: 1-8.
doi: 10.1016/j.foodhyd.2018.05.046.

37. Rostamzad H, Paighambari SY, Shabanpour B, Ojagh SM, Mousavi SM. Improvement of fish protein film with nanoclay and transglutaminase for food packaging. Food Packaging Shelf. 2016; 7: 1-7.
doi: 10.1016/j.fpsl.2015.10.001.

38. Niu M, Xiong L, Zhang B, Jia C, Zhao S. Comparative study on protein polymerization in whole-wheat dough modified by transglutaminase and glucose oxidase. LWT. 2018; 90: 323-330.
doi: 10.1016/j.lwt.2017.12.046.

39. Song CL, Ren J, Chen JP, Sun XH, Kopparapu NK, Xue YG. Effect of glycosylation and limited hydrolysis on structural and functional properties of soybean protein isolate. J Food Meas Charact. 2018; 12(4): 2946-2954.
doi: 10.1007/s11694-018-9910-5.

40. Van Boekel M. Formation of flavour compounds in the Maillard reaction. Biotechnol Adv. 2006; 24(2): 230-233.
doi: 10.1016/j.biotechadv.2005.11.004.

اثرات ترانس‌گلوتامیناز میکروبی و نوع تخمیر بر بهبود دسترسی به لیزین در نان گندم:روش سطح پاسخ

ندا ملاخلیلی میبدی¹، امیرمحمد مرتضویان²*، لیلا میرمقتدایی²**،سیده مرضیه حسینی²، سید علی یاسینی³، محمد حسین عزیزی⁴، سمیه موسوی ندوشن⁵

۱- گروه علوم و صنایع غذایی، کمیته تحقیق دانشجویی، انستیتو تحقیقات تغذیه ای و صنایع غذایی کشور، ، دانشکده علوم تغذیه و صنایع غذایی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران.

۲- گروه علوم و صنایع غذایی، انستیتو تحقیقات تغذیه ای و صنایع غذایی کشور، دانشکده علوم تغذیه و صنایع غذایی، دانشگاه علوم پزشکی شهید بهشتی، تهران، ایران.

۳- گروه علوم و صنایع غذایی، دانشگاه آزاد اسلامی، واحد یزد، یزد، ایران.

۴- گروه علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران.

۵- آزمایشگاه کنترل مواد غذایی، آرایشی و بهداشتی،دانشگاه علوم پزشکی شهید صدوقی یزد، یزد، ایران.

چکیده

سابقه و هدف: به نظر می رسد ایجاد اتصال عرضی گلوتامین-لیزین کاتالیز شـده توسـط آنـزیم تـرانس گلوتامینـاز میکروبی جهت بهبود دسترسی به لیزین در نان گندم تاثیر داشته باشد. هدف از این مطالعه بررسی اثر آنزیم تـرانس گلوتامیناز میکروبی و نوع تخمیر در بهبود دسترسی لیزین نان گندم است.

مواد و روش ها: نان‌های گندم غنی شده با لیزین به روش سطح پاسخ با طراحی مرکب مرکزی فرمولـه شـدند. از مدل های آماری برای پیش بینی تأثیر سطح آرد سویای چربی گرفته (۰ تا ۵۰ درصد وزنی-وزنی)، سـطح آنـزیم ترانس گلوتامیناز میکروبی (۰ تا ۱/۶ درصد وزنی-وزنی) و نوع تخمیر (با مخمر یا تخمیر ترکیبی بر پایه خمیر ترش) استفاده شد. سایر اطلاعات براساس نقش متغیرهای مستقل به تنهایی بر ویژگی‌های سـاختاری و تغذیـه‌ای فرمـول بهینه و نمونه های کنترل تهیه شد. آزمون‌ها در سه بار تکرار انجام و میانگین‌ها با استفاده از آزمون‌های واریانس یک طرفه و توکی بررسی آماری شدند.

یافته‌ها و نتیجه‌گیــری: فرمـول پیشـنهادی دارای ۲۶/۶۴ درصد وزنی-وزنی آرد سـویای بـدون چربـی، ۰/۵۵ درصد وزنی-وزنی آنزیم ترانس گلوتامیناز میکروبی به منظور فراهم نمودن ⁻¹(۱۰۰g) mg ۰/۱۶ لیزین در دسترس و ⁻¹g cm³ ۲/۰۹ حجم ویژه بـود. بـالاترین نمـره شـیمیایی لیـزین (۲۲/۷۹ ±۰/۱۶)، انـدیس آمینـو اسـید ضـروری (۰/۳۷± ۳۵/۳۱) و ارزش زیستی (۰/۰۲ ±۲۶/۷۹) و کمترین میزان افت لیزین طـی فرآینـد پخـت در فرمولاسـیون بهینه، کارآیی آنزیم ترانس گلوتامیناز میکروبی در غنی‌سازی گندم حاوی آرد سویای چربی گرفته با لیـزین را تاییـد کرد (P≤۰/۰۵). با توجه به شاخص‌های رئولوژیکی و آزمون بافت، تیمـار بـا تـرانس گلوتامینـاز میکروبـی مـدول‌هـای الاستیک و ساختار صفحه ای بتا را افزایش داد. این تغییرات ساختاری قابلیت هضم محصول نهایی را کاهش داد، کـه می‌تواند با استفاده از روش تخمیر ترکیبی بر پایه خمیر ترش افزایش یابد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.