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Effect of Trans Glutaminase and Glucose Oxidase Addition on Rheological and Baking Characteristics of Wheat and Oat composite flour

Sayran Sardar [1] & Mohammed Wajeeh Zainulabideen [2]

1 Crop Science Department. College of Agricultural Science. University of Sulaimani. Sulaimani-Iraq
2 Food Science Department. College of Agricultural Science. University of Sulaimani. Sulaimani-Iraq
Email: sayran,hasan@univsul.edu.iq

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Abstract

The effect of Transglutaminase (TGase) and Glucose oxidase (GOase) on white, wheat, oat and their composite flours were studied. The chemical content significantly differed among the treatments, especially protein percentages which was ranged between 9.58±0.52 for white flour and 12.67±0.10 for Aras flour. Substitution 30% of white and Aras flours by oat flour caused increasing of all Amylograph parameters while addition of TGase and GOase highly decreased the breakdown of viscosity during stability of paste heating. In the same manner addition, the both enzymes increased Farinograph water absorption and somewhat stability time. However, the effect of two enzymes on the other Farinograph parameters depended on the quality of the studied flour. Addition of TGase and GOase, in general, improved loaf specific volume and volume to protein, in addition to their improving to loaf curst color and crust uniformity. This study was carried out at University of Sulaimani / Faculty of Agricultural Sciences –Food Science department, to study the effect of transglutaminase (TGase) and Glucose oxidase (GOase) on dough rheology and baking test of wheat (*Triticum aestivum L.*, type; Aras cultivar) and Oat (*Avena sativa L.*) and then the results were compared with studied wheat white flours.

Introduction

There is a growing demand in the new generation to produce healthier foods that have good sensory qualities. The nutrients in cereals are distribute unevenly throughout the grain and are known to have potential for reducing the risk of some diseases. Thus, the diet with regular inclusion of cereals can contribute much to health promotion and disease prevention ***[1]***. In the new trend of present studies, composite flour bread was prepared using refined wheat flour with different cereal flour, included oat flour, with an aim to formulate an enriched flour, which is high in protein, fiber content. These flours not only increase the bread rheology, but also increase the nutritional value of the product ***[2]***. Oats (*Avena sativa L.*) have received considerable attention for their high content of dietary fibers, phytochemicals and nutritional value. It is believe that consumption of oats possesses various health benefits such as hypocholesterolaemic and anticancerous properties. Owing to their high nutritional value, oat-based food products like breads, biscuits, cookies, breakfast cereals, flakes and infant food are gaining increasing consideration. The nutritional benefits of oat have attracted attention from researchers worldwide and have resulted in the increased interest of food industry in using oats as food ingredient in various food products including infant foods ***[3]***. Enzyme – catalyzed protein crosslinking, such as glucose oxidase and trans glutaminase, are used recently on wheat (*Triticum aestivum L.*, type; Aras cultivar) and Oat (*Avena sativa L.*) to improve the texture of volume bread crumb, water absorption capability and the baking quality of normal and low quality characteristic of bread made of wheat - oat composite flour. Addition of enzymes improved the bread sensory quality and got higher overall acceptability, especially xylanase ***[4]***. The aim of this research was to study the role of enzymes catalyzed protein crosslinking, trans glutaminase (TGase) and glucose oxidase (GOase), in the improvement of pan loaf made of wheat and oat composite flour.

Materials and Methods

A. Raw material

Austrian type of oat grains was purchased from Debbane Freres, Lebanon market. Wheat grains, (Aras) local cultivar, bread wheat type was obtained from bakrajo agricultural research center, Sulaimani, Kurdistan Region-Iraq. As for the White flour (15) kg trademarked America's Gold medal, produced in (U.A.E.) by Emirates Grain Products Company L.L.C. was purchased from the local market in Sulaimani city. Trans-glutaminase :(TGase) derived from streptoverticillium sp. was obtained from Ajinomoto Co. (Shanghai Biocaxis Chemicals Co., LTD) which its activity 50 U/g protein. Glucose Oxidase: GBK- BG100 Glucose Oxidase (GOase) was purchased from Baishengyuan, Shush an, District, Hefei, Anhuri Co. China, which its activity of 5000U/g protein. Instant Yeast (saf + plus) from S.I.leaftre/Turkey: Iodized Salt (ZER) from Turkey; Sugar (Al-Osra) from the Kingdom of Saudi Arabia; the Oil (Al-afia) from Jeddah/KSA and Milk type (Nido) nestle from Dubai-U.A.E, were purchased from the local market to be used in preparing pan bread loaves.

B. Chemical analysis

All chemical analyses, moisture, protein, crude lipids, ash, and dietary fibers for white, Aras, and oat flour, were achieved according to AACC methods Nos. 44-0.1.01, 46-10.01, 30.25.01, 0.8-01.01, 32.10.01respectively (AACC, 2000) ***[5]***. Nitrogen free extract (carbohydrate) calculated by difference.

C. Preparation of composite flour

Wheat-Aras and oat grains was milled in local disc mill, and then they were sieved in 150-micron sieve. Composite flour samples were prepared by substitution of 30% of white or Aras flour by oat flour.

D. Rheological method

According to AACC methods Nos.22-12.0.1 and 54-21.0.2 (AACC, 2000) [5], Amylograph and Farinograph tests respectively were used to determine the paste and dough rheology of white, Aras and oat composite flour with or without TGase and GOase addition.

E. Baking test

Loaf pan bread for all flour samples was prepared according to the (AACC, basic straight-dough bread-baking method and long fermentation method No. 10-09.01) (AACC, 2000) [5]. Bread quality analysis included: weight, volume (determined by seed displacement in a loaf volume meter), and specific volume (volume/weight) and volume/ protein content (AACC, Method No. 10-05.01, 2000) [6]. The sensory evaluations for studied samples of pan bread (loaf) were conducted according to the (AACC, sensory evaluation method, No. 33-50.02) (AACC, 2000) [5]. Each samples was randomly coded (A-B-C-…..-S); panel member was permitted to form his judgment of bread samples individually based on 10 characteristics (crust color, crust uniformity, crust thickness, crumb color, crumb texture, crumb cell uniformity, taste, flavor, chewing and tenderness, overall acceptable). Panelists were asked to give the scores between (1 to 5) to the coded samples, with (5) related to the best samples and (1) to the worst one.

Statistical analysis

Experimental results were represented as the mean of three replication (± SD) (Standard Deviation). Statistical evaluation was carried out by the analysis of variance (ANOVA) using (CRD) design followed by comparing tests using LSD test at the probability (95%,) of confidence level. All the analyses were conducted using the statistical software, SAS [7], with the probability of ($p \leq 0.05$) & ($p \leq 0.01$) being considered significant.

Results and Discussion

A.Chemical composition:

chemical composition of studied flour (Table -1) showed that all studied flours contained proper amounts of moisture that allows to store these products safely without fungi spoilage (Samuel and Ugwuanyi, 2014) [8]. However, the significant difference in white flour moisture content does not has any significant effect on flour quality. There results also showed that there was a significant difference between the protein content of Aras flour which was 12.67% and oat and white flour which were 10.51 and 9.58% respectively Iraqi flour specifications it considers (10%) protein in white flour as adequate for Iraqi bread (Iraqi specification for bread No.677, 1979). The crude lipid percentages as shown in (Table-1) significantly differ ($P \leq 0.01$) among the studied flours, it was between (2.03±0.08)% for white flour and (5.49±0.98)% for Oat flour. Oat grains characterize by their high content of lipids specially in endosperm [9]. Ash percentages, showed also in Table 1, a significantly differ ($P \leq 0.01$) among the types of flour. In the same manner, crude fiber increased significantly ($P \leq 0.01$) in oat flour compared to white and Aras flours which this result agreement with (Liu, 2010) [10]. The nitrogen free extract percentage (carbohydrate without fiber) significantly differ ($P \leq 0.01$) between white flour and the other types of flours which ranged between (71.79±0.32)% for oat flour to (75.60±0.60)% for white flour(Tab-1).

Table -1 : Chemical Composition of Studied Cereal Flour

Flour samples	*Moisture%*	*Protein %*	*Crude Lipid %*	*Ash %*	*Crude Fiber%*	*Nitrogen free Extract%*
White	*12.26±0.30(a)*	*9.58±0.52(c)*	*2.03±0.08(c)*	*0.35±0.55(c)*	*0.16±0.005(c)*	*75.60±0.60 (a)*
Aras	*8.76±0.35(b)*	*12.67±0.10(a)*	*3.08±0.63(b)*	*1.28±1.28(b)*	*1.45±0.30(b)*	*72.73±1.52(b)*
Oat	*8.06±0.41(b)*	*10.51±0.30(b)*	*5.49±0.98(a)*	*1.50±0.03(a)*	*2.62±0.238(a)*	*71.79±0.32(b)*
LSD (P≤0.05)	*0.882*	*0.873*	*1.656*	*0.164*	*0.550*	*1.543*
LSD (P≤0.01)	*1.337*	*1.323*	*2.509*	*0.249*	*0.833*	*2.337*

* Each value represents the means of three replications ± standard deviation.
** Different letters within a column shows significant differences between the treatment means ($P \geq 0.05$) & ($P \geq 0.01$).

B.Rheological properties

Amylograph

Amylograph results (table-2) showed that there were clear differences among studied flours in most amylograph parameters. However, white and Aras flours had the same pasting (gelatinizing) temperature (61.5)° C while oat flour had a higher pasting temperature (64.5)° C. There was wide difference between white flour and wheat flour in the most amylograph parameters. Except pasting temperature, for the white flour had the higher amylographical values compared to Aras flour. Hot and cold peak viscosities were 145 and 180 AU respectively for Aras flour while it was 470 and 1040 AU respectively for white flour. This behavior may due to the activity of α- amylase, which decreases these two parameters value. The results also showed that all amylograph values were higher than white and Aras flour that not due to the low activity of α- amylase only but also due to its high content of β - glucan. ***[11]*** Found that β-glucan increases viscosity of liquid and paste during heating and cooling. These results are in agreement with Choi, et al. (2012) ***[12]*** who stated that Oat cultivars shows higher peak viscosity, but lower breakdown and setback, indicating that they easily swell. The effect of TGase and GOase on amylograph parameters of oat flour was studied. The results showed that there was an effect of each enzyme on amylograph parameters, and this may be due to their important role in increasing the stability of hot paste viscosity during heating for 20 minutes. Break down viscosity values was reduced, in the oat flour treated with TGase and GOase, from 430 AU to -10 and 100 AU respectively. The considerable effect of TGase on amylograph parameters, not only reduces the value of break down viscosity but also increased the hot paste viscosity, which was 700 AU to be 1050 AU. This finding may be important for many reasons, one of them related to the role of TGase and GOase in assistance oat starch pastes to resist the thermal effect on it. The second reason is that most of researchers studied the effect of these enzymes on protein structure and its function in food, without concerning their effects on starch paste rheology. However, it was though that this phenomenon might related to their role in increase of preserving the swollen starch granules from the early breakdown (table-2).

Table -2 : Amylograph Parameters for white, Aras, oat and their composite flour.

Treatments	Pasting temperature°C	PeaK viscosity AU	Hot paste Viscosity AU	Break down Viscosity AU	Cold Paste Viscosity AU	Set back viscosity AU
White flour	61.5	470	370	100	1040	670
Aras flour	61.5	145	90	55	180	90
Oat flour	64.5	1130	700	430	>1200*	Uncountable *
Oat f.+0.03%TGase	70.5	1040	1050	-10**	1250	210
Oat f.+0.03%GOase	69	1110	1010	100	1140	130

* Values exceeded the capability of equipment to determine.

** Increasing of hot paste viscosity took place without broke down in viscosity.

Farinograph test

Farinograph results (table -3) showed that there were significant differences between the tested samples in most parameters. The range of water absorption was between 69.20 % for white flour to 86.10% for oat flour. The results of development time showed that white flour had the lowest development time value 2.05 min. compared to Aras flour 4.55 min. Amount and the type of protein in addition to the flour content of dietary fibers specially β- glucan are the main reason for increasing of development time ***[13;14]***. The results also indicated that Aras and oat flours behave as a weak flour because they had low value of stability time and high value of MTI which were 2.55±0.07min., 1.75±0.21min., 89±1.41FU and142±11.3 FU respectively compared to white flour 11.7±1.41min and 31.5±3.53FU. ***[15]*** stated that oat flour has pronounced effects on farinograph dough properties yielding a higher water absorption and dough development time and lower dough stability and extensibility compared to the control white flour.

Table -3 :Farinograph Parameters for white, Aras, Oat and their composite flour.

Flour and composite flour	***Water absorption%***	***Development time min.***	***Stability min.***	***Mixing Tolerance index (MTI) FU***	***Time to breakdown min.***	***Farinograph quality number***
White	*69.2±0.28(de)*	*2.05±0.21(f)*	*11.7±1.41(a)*	*31.5±3.53(c)*	*4.85±0.63(de)*	*48.5±6.36 (de)*
Aras	*76.5±0.70(bc)*	*4.55±0.07(c)*	*2.55±0.07(d)*	*89±1.41 (b)*	*5.85±0.07(d)*	*58.5±0.70(d)*
Oat	*86.1±1.41(a)*	*3.6 ± 0.28(e)*	*1.75±0.21(d)*	*142±11.3 (a)*	*4.5±0.28(e)*	*45 ±2.82(e)*
White+30%Oat	*70.5±0.35(d)*	*5.5±0.28 (b)*	*2.85±0.07(d)*	*64.5±3.53(b)*	*8.35±0.07(b)*	*83.5±0.70(b)*
Aras+30%Oat	*79.4±0.07(b)*	*4.45±0.07(cd)*	*1.85±0.07(d)*	*123±2.12(a)*	*5.25±0.35(de)*	*52.5±3.53(de)*
LSD (P≤0.05)	*3.423*	*0.459*	*1.683*	*29.165*	*1.060*	*10.597*
LSD (P≤0.01)	*4.917*	*0.659*	*2.417*	*41.899*	*1.522*	*15.224*

* Each value represents the means of three replications ±standard deviation.

** Different letters within a column shows significant differences between the treatment means (P≥ 0.05).

C. Effect of Trans glutaminase (TGase) and Glucose oxidase (GOase) addition on farinograph parameters.

The effect of TGase and GOase on some treatments of white, Aras and their composite oat flours on farinograph parameters was studied. The results (table 4) showed addition of different concentrations of TGase (0.03, 0.07 and 0.15% on the flour bases) did not increase the differences among the three concentrations, thus the lower concentration (0.03%) was selected. The overall results (table -4) emphasized that the short time of farinogrph determination not allow to measure the effect of TGase or GOase on dough rheology. However, adding of TGase and GOase increased of water absorption in white flour more than in Aras flour. The increasing of water absorption is a required property since it increases the amount of dough in the same amount of flour. In general, adding of TGase and GOase didn’t appear any improving effect on the farinograph stability time, mixing tolerance index, Time to breakdown. ***[16]*** studied the effect of TGase on farinograph parameters for oat and wheat composite flour who found a decreasing of flour water absorption, with increasing of TGase levels. Dough development time and stability values initially increase with increasing TGase level between 0.5 to 1%, but it decreased at higher TGase levels from 1 to 1.5%.

Table -4: Effect of TGase and GOase addition on farinograph parameters.

Flour Samples	*Water absorption (correct for 500 FU)*	*Development time min.*	*Stability min.*	*Mixing Tolerance index(MTI) FU*	*Time to break down min.*	*Farinograph quality number*
Aras + 0.03% TGase	*74.9*	*5.2*	*2.4*	*60*	*6.4*	*64*
Aras + 0.07% TGase	*75.8*	*5.0*	*2.5*	*62*	*6.5*	*65*
Aras + 0.15% TGase	*75.6*	*4.9*	*2.4*	*62*	*6.5*	*65*
White+30%Oat+ 0.03% TGase	*72*	*4.8*	*2.5*	*87*	*6.5*	*65*
White+30%Oat+ 0.03% GOase	*72.6*	*4.8*	*3.1*	*97*	*6.0*	*60*
Aras+30%Oat+ 0.03% TGase	*79.6*	*4.0*	*1.6*	*86*	*4.9*	*49*
Aras+30%Oat+ 0.03% GOase	*79.8*	*4.0*	*1.5*	*129*	*4.9*	*49*

D. Baking Test.

Baking test results (table-5) showed that there was insignificant difference in loaf specific volume between white (3.435±0.02) and Aras (3.447±0.02) but there was higher significant difference between these treatments in volume ml/g protein value, which was (48.30±0.28ml/g protein) for white flour and (37.79±0.11mg/ g protein) for Aras. ***[17]***, reported that the quantity and quality of proteins, especially gluten, may be determined loaf volume ml/ g protein characteristic. Adding of oat flour significantly decreased most of the baking test parameters, especially the important criteria, specific volume and volume ml/g protein. ***[18]*** Stated that adding of oat products to wheat flour caused dilution of gluten, which resulted in a loss of dough strength and produces low loaf volume. Results of using TGase and GOase showed that there are different effects related to each enzyme on each type of flour composition. Loaf specific volume and volume/g protein were (2.69ml/g and 36.85ml/g) respectively for white - (30%) oat composite flour. Adding of TGase significantly improved these parameters to (3.01 and 41.43) respectively. In contrast, these values did not change, when GOase had been added and remained at (2.67ml/g and 36.35ml/g) respectively. In the opposite of these actions, the adding of TGase to Aras - (30%) oat composite flour caused insignificant decreases in loaf specific volume and volume per g protein from (2.57ml/g and 30.45ml/g) to (2.52ml/g and 29.18ml/g) respectively whereas the addition of GOase or the mixture of TGase and GOase significantly increased these parameter values to (2.76ml/g and 32.80ml/g) respectively. These results were in agreement with Soulaka and Ilia (2015) ***[19]*** who found that loaf specific volume of wheat barley composite flour increases with addition of TGase.

Table -5: Baking Test Parameters for White, Aras and their composite flours.

Flour samples	*Loaf weight (g)*	*Loaf volume (ml)*	*Specific volume (ml/g)*	*Protein (%)*	*Volume/protein (ml/g)*
White	*140.3 ± 0.06(abcd)*	*482±2.82(a)*	*3.435±0.02(a)*	*9.979*	*48.30±0.28(a)*
Aras	*137.78 ±0.61(cdef)*	*475±1.41(a)*	*3.447±0.02(a)*	*12.57*	*37.79±0.11(d)*
White + 30%Oat	*137.51±0.42(def)*	*370±14.14(def)*	*2.690±0.11(efg)*	*10.039*	*36.86±1.41(de)*
Aras +30% Oat	*140.11±0.41(abcd)*	*361±21.21(ef)*	*2.576±0.14 (g)*	*11.855*	*30.45±1.79(gh)*
White+30%Oat+0.003% (TGase)	*137.78±0.282(cdef)*	*416±2.82 (bc)*	*3.019±0.02 (bcd)*	*10.039*	*41.44±0.28(bc)*
Aras+30%Oat+0.03% (TGase)	*136.82±0.60 (ef)*	*346±39.59 (f)*	*2.529±0.30 (g)*	*11.855*	*29.19±3.34(h)*
White+30%Oat+0.03% (GOase)	*136.39±4.59 (f)*	*365±7.07(def)*	*2.676±0.03(efg)*	*10.039*	*36.36±0.70(def)*
Aras +30%Oat + 0.03% (GOase)	*141.12±0.28 (a)*	*390±14.14(cde)*	*2.763±0.09(defg)*	*11.855*	*32.90±1.19(fg)*
Aras+30%Oat+0.03% (TGase + GOase)	*140.61±0.44 (ab)*	*388±25.45(cde)*	*2.759±0.18(defg)*	*11.855*	*32.73±2.15(gh)*
LSD (P≤0.05)	*2.821*	*41.451*	*0.296*	---	*3.584*
LSD (P≤0.01)	*3.856*	*56.659*	*0.404*	---	*4.899*

* Each value represents the means of three replications ±standard deviation.

** Different letters within a column shows significant differences between the treatment means (P≥ 0.05) & (P≥ 0.01).

E. Sensory evaluation of loaf.

The results of loaf sensory evaluation (table -6) showed that except crumb color, there were no significant differences between white and Aras flour in the other sensory parameters while these two types of flour significantly exceeded on the sensory characteristics of the other treatments. Adding of 30% oat flour caused decreasing of all sensory parameters especially crust uniformity and crust thickness may due to its role in gluten dilution in addition to its content of dietary fiber. Adding of TGase and GOase improved the defects of some sensory parameters that occur by oat addition especially crust color, crust thickness. The improving effect by both enzyme was higher on white oat composite flour than Aras oat composite flour. However, all treatments were acceptable although the existence of significant differences between oat composite flour treatments and wheat flour treatments. In general, these results were agreement with Ngemakwe, (2014) ***[20].***

Table -6: Sensory evaluation test with LSD values for 9-loaf bread treatment.

Samples	**Crust color**	**Crust uniformity**	**Crust thickness**	**Crumb color**	**Crumb texture**	**Crumb cell uniformity**	**Taste**	**Flavor**	**Chewing**	**Over all acceptability**
White	*3.81±0.98*	*4.54±0.68*	*4.36±0.67*	*4.90±0.30*	*4±1.18*	*3.63±1.12*	*4.54±0.82*	*4.36±0.92*	*4.63±0.50*	*4.36±0.80*
Aras	*4.72±0.46*	*4.72±0.46*	*4.27±0.64*	*3.90±0.70*	*4.09±0.94*	*3.90±0.94*	*4±0.63*	*3.54±0.93*	*3.90±0.83*	*4.18±0.98*
White + 30% Oats f.	*3.36±0.50*	*3±0.89*	*2.63±0.67*	*3.54±0.52*	*3.72±0.78*	*3.36±0.67*	*3.63±0.92*	*3.54±0.52*	*3.45±0.68*	*3.63±0.80*
Aras + 30% Oats f.	*2.81±0.75*	*2.54±0.52*	*3.63±0.92*	*3.54±0.68*	*3.36±1.12*	*3.63±0.67*	*3.72±0.78*	*3.36±0.67*	*3.72±0.78*	*3.63±0.67*
White +30%Oatsf. +0.003%TGase	*4±0.44*	*3±0.63*	*3.27±0.46*	*3.63±0.67*	*3.27±0.90*	*3.18±0.75*	*3.18±0.60*	*3.45±0.52*	*3.63±0.67*	*3.54±0.52*
Aras+30%Oats f. + 0.003% TGase	*3.72±0.64*	*3±0.89*	*3.72±0.7*	*3.90±0.53*	*3.63±1.12*	*3.81±0.87*	*3.09±0.83*	*3.63±0.67*	*3.54±0.82*	*3.54±0.68*
White +30%Oats f. + 0.003%GOase	*3.27±0.46*	*2.72±0.90*	*3.09±0.53*	*2.54±0.68*	*2.81±1.16*	*3.63±0.50*	*2.81±0.60*	*3.45±0.68*	*3.09±0.53*	*3.45±0.52*
Aras +30%Oats f.+0.003%GOase	*4.27±1.10*	*3.36±1.02*	*3.36±0.67*	*2.63±0.6*	*3.09±0.70*	*3±0.77*	*2.63±1.02*	*3.18±0.75*	*3.54±1.03*	*3.27±0.64*
Aras +30%Oats f. + 0.003%(TGase+GOase)	*3.72±1.27*	*2.90±0.94*	*3.18±0.87*	*2.45±0.68*	*2.72±0.90*	*3.27±0.46*	*3±0.77*	*3.27±0.64*	*3.27±0.78*	*3.27±0.64*
LSD (P≥ 0.05)	*0.718*	*0.629*	*0.533*	*0.525*	*0.822*	*0.808*	*0.935*	*0.771*	*0.661*	*0.792*
LSD (P≥ 0.01)	*0.981*	*0.860*	*0.729*	*0.718*	*1.124*	*1.105*	*1.277*	*1.053*	*0.903*	*1.083*

* Values mean for three replication of each Backed sample.
** Different letters within a column shows significant differences between the treatment means (P≥ 0.05) & (P≥ 0.01)

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