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WHEAT GERM BREAD QUALITY AND DOUGH RHEOLOGY AS INFLUENCED BY ADDED ENZYMES AND ASCORBIC ACID

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ABSTRACT. Wheat germs are valuable bread making ingredients, but their addition in bread loaf negatively influences the bread quality by increasing the hardness and decreasing the volume. In the context of modern baking industry, with high demands for bread with superior nutritional and sensorial quality, it clearly appears to be necessary to modulate the rheological properties of dough by using additives. In this study the influence of amylase, xylanase, glucose-oxidase and ascorbic acid on the wheat/ wheat germ flours dough and bread properties was studied. Initially, the assessment of wheat germ flour substitution (0-4%) on rheological properties and bread quality of three commercial wheat flours was determined. A substitution of 4% wheat germ flour was found to be acceptable. Secondly, the effect of improvers on dough rheology and on bread quality obtained by wheat flour/wheat germ flour blends was studied. Xylanase markedly improved the elasticity and porosity. Amylase significantly increased the crust color, the porosity and the bread specific volume. Glucose-oxidase effect is related to a higher bread specific volume, an improvement of the bread shape and crumb porosity. Ascorbic acid caused a significant increment of specific volume. Enzymes and ascorbic acid can be used to improve wheat germ bread quality.

Keywords*: breadmaking improvers, farinographic and alveographic parameters*

INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the most important crops and has been used worldwide as a main ingredient in bread making. The increasing mechanization of the baking industry and the demand for a wide range of bread types have determined the necessity to modulate structure and viscoelastic properties of dough. In order to improve bread making performance,

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chemical compounds and enzymes are usually included in bread formulas [1]. On the other hand, nowadays consumers are aware about the relationship between diet and diseases and from this point of view it is a great challenge for bakers to obtain nutritious bread, rich in bioactive compounds, with high quality in terms of bread specific properties (volume, crumb and crust texture, firmness, color, taste etc). In this context, wheat germs have been proposed as valuable baking ingredients due to their nutritional interest and due to the possibility of using a by-product from the milling industry. The addition of raw wheat germ or its derivate to baking products has been studied using various approaches [2]. Some of those studies were based on the addition of raw wheat germ [3,4], but others incorporate defatted wheat germ [5], heated wheat germ [6], extruded wheat germ [2] or a combination of the two to obtain products with a longer shelf life and better functional properties than raw wheat germ [7]. Wheat germs are rich in bioactive substances such as antioxidants (tocopherols, tocotrienols, phenolics, carotenoids), sterols [8,9], unsaturated fatty acids (oleic, linoleic and α-linoleic acids) and essential amino acids [10]. As for human health benefits, it is reported that the processed wheat germ can be applied in prevention and treatment of cancers [11]. Studies showed that the raw wheat germ loaves had superior nutritive value but also a reduction of quality mainly due to the increase in bread hardness and decrease in bread volume [12]. On the contrary, heat treated and extruded wheat germ addition had a smaller effect on the gluten matrix than raw wheat germ [2,6]. More recently, Sun et al., 2015 [8] reported that the addition of wheat germ flour could be an effective way of producing functional white flour but caution should be paid to addition level of wheat germ because of the adverse increase in solid-like properties of the dough. Many local bakeries are confronted with such issues due to the supplementation of wheat flour with wheat germ flour and the solution could be the adjusting of the flour blends properties by using enzymes and chemical additives. The enzymatic treatment of wheat flours is an interesting alternative to generate changes in the structure of the dough and consequently, for improving functional properties of flours. They are generally recognized as safe (GRAS) and do not remain active in the final product after baking. Therefore, enzymes do not have to appear on the label, which is an additional commercial advantage [13]. Between enzymes used usually as bread-making improvers are gluten cross-linking enzymes and polysaccharides degrading enzymes [14]. Glucose oxidase is an oxidative enzyme that catalyzes the oxidation of β-d-glucose to δ-d-gluconolactona and hydrogen peroxide. Disulfide bond interchange and the gelation of pentosans promoted by hydrogen peroxide action are the most widespread theories to explain the strengthening effect of the glucose oxidase. Furthermore, it has been related with the formation of non-disulfide covalent intermolecular bonds in the gluten proteins [13]. Amylases, xylanases are hydrolytic enzymes able to change physicochemical and structural properties of polysaccharides, making

dough softer and viscous and increasing the availability of fermentable sugars. α-amylase prolongs oven rise and results in an increased loaf volume [15]. An increase in loaf volume and an improvement of bread crumb structure can be pursued with the addition of ascorbic acid that acts on the redox systems of wheat dough. In particular, the improver action of the ascorbic acid is due to its oxidation by gaseous oxygen to dehydroascorbic acid which determines the rapid oxidation of glutathione present in flour thus minimizing the SH/SS interchange reactions of reduced glutathione with intermolecular SS bonds of gluten molecules [16].

To the best of our knowledge the influence of additives (enzymes and ascorbic acid) on wheat flour/ wheat germ flour blends properties has not been studied. This study aimed to test several bread making improvers and evaluated their effects on dough rheology and bread quality of wheat germ supplemented wheat flours by two objectives: (I) assessment of wheat germ flour substitution on rheological properties and bread quality of three commercial wheat flours and (II) evaluation of improvers effect on dough rheology and bread quality obtained by wheat flour/wheat germ flour blends.

RESULTS AND DISCUSSION

Table 1 shows the mean values of psysico-chemical and rheological parameters for the three sets of wheat flours (WF1, WF2, WF3) used in the study. For all analyzed samples the wet gluten content was higher than 22%, minimum value indicated by SR ISO 7970/2001 for the bread making wheat. Zeleny sedimentation value (SDS) ranged between 30-40. This parameters was used to estimate the bread loaf volume [18] and the quality of the gluten. The Falling number (HFN) for the tested flours did not go below 200s. HFN ranged between 290s and 354s meaning that the wheat flour with lower extraction rates obtained by wheat milling most probably needs to be supplemented with amylases during bread making to get high-quality baking products [19]. The alveographic P value is the parameter that can differentiate particular flours and the baking volume could be significantly correlated with flour water absorption and protein content [20]. For the tested WF, P ranged between 84 and 93, with the higher value for WF2. Bread volume is positively correlated with farinograph dough development time and flour water absorption [21]. With respect to the maturograph characteristics, the wheat flour WF2 recorded the longer average proofing time 46 min, while WF3 samples showed shorter average proofing time 40 min. This parameter is important from technological point of view. Generally, shorter proofing time is more suitable for technological purposes [22]. Values of maturograph dough resistance ranged from 424 to 516 BU. Variety with the lowest protein content in the set (WF3)

had technologically optimal time of final proofing (40 min) and low dough resistance (440 BU). By assessing all parameters for the tested wheat flours we concluded their quality to be medium to good for baking purpose.

Table 1. Mean values of wheat flours psysico-chemical and rheological parameters

Parameters	WF1	WF2	WF3
Moisture (%)	14.43	13.89	14.91
Protein (%)	12.29	11.72	10.52
Ash (%)	0.545	0.56	0.554
Wet gluten content, %	28.1	26.6	25.2
Falling Number HFN (s)	351	320	294
Sedimentation value SDS (mL)	38	39	31
FWA (%)	60.8	61.75	57.3
DDT (s)	3.2	3.5	3.1
ST (s)	3.5	3.7	4.12
DS (BU)	61	71	100
P (mm H_2O)	84	93	88
L (mm)	80	80	61
P/L	1.05	1.16	1.44
W (x 10^{-4} J)	200	234	204
Proofing time (min)	42	46	40
Dough resistance (BU)	505	510	440
Baking volume (BU)	420	430	485
Oven rise (BU)	80	80	180

I. Influence of WGF addition on rheological properties

I.1. The Falling number (HFN)

The HFN decreased as the level of WGF increased in blends for all WF used in the study. 329s, 298s and 282s were the HFN average values recorded in blends with 4% WGF, compared to the controls (Tab.1). Results indicated that partial substitution of WF by WGF decreased the HFN. This trend could be explained by the higher α-amylase content founded in germ [8,23]. The presence of amylases increases the level of fermentable and reducing sugars in the flour and dough, thereby promoting yeast fermentation [24], which suggested that α-amylase activity might improve bread quality in terms of volume and crust color.

I.2 Farinograph and Alveograph characteristics

Farinograph characteristics of WF1, WF2, WF3 and those of blends obtained with WGF addition at 0, 2 and 4% level are shown in Table 2. A slight increment (non-significant, $p>0.05$) in FWA with the increased addition of

WGF was found, with a higher value for WF2. Incorporation of WGF had weakening effect on rheological characteristics of the dough (DDT, ST, DS). This tendency was observed for all tested blends.

Results for alveograph characteristics of WF:WGF blends are shown in Table 3. Incorporation of 4% WGF slightly decreased the tenacity value (P) in the case of WF1 samples, while a more marked decrement was observed for samples WF2, WF3.

Table 2. Farinographic properties of dough obtained by blending WGF:WF

Wheat flour	Addition level of WGF, %	FWA, %	DDT, min	ST, min	DS, BU
WF1	0%	60.8(0.01)	3.2(0.1)	3.8(0.2)	61.02(0.2)
	2%	61.0(0.01)	3.1(0.2)	3.71(0.1)	61.4(0.2)
	4%	61.2(0.02)	2.9(0.14)	3.45(0.13)	62 (0.1)
WF2	0%	61.75(0.02)	3.5(0.2)	4.2(0.2)	71(0.15)
	2%	60.8(0.02)	3.2(0.1)	4.09(0.15)	71.3(0.15)
	4%	63.8(0.01)	3.0(0.15)	3.88(0.1)	71.9(0.2)
WF3	0%	57.3(0.01)	3.1(0.2)	5.12(0.2)	100.0(0.2)
	2%	58.12(0.03)	3.1(0.1)	5.04(0.14)	100.45(0.2)
	4%	58.6(0.02)	2.9(0.12)	4.9(0.2)	100.91(0.1)

This tendency could be explained by the presence of pre-gelatinized starch in the heat treated WGF which, increase dough consistency [2] and its ability to retain gas. Dough extensibility (L) decreased with the level of WGF addition. Decrease in values of extensibility (L) caused reduction in energy value of dough. In consequence, dough strength (W) decreased with increasing proportions of WG, for all tested blends. These effects of WGF addition on rheological parameters can be attributed to the dilution of the gluten matrix, responsible for dough extensibility and to a competition to a certain extent with other WF components for water, creating a dough strengthening. Gomez et al., 2012 [2] showed that by measuring dough strength after 3h of resting the value was similar to the initial strength, suggesting that despite of the high level of enzyme activity in this part of the grain, these enzymes do not modify dough rheology, due probably to enzyme inactivation during heating [23,26]. The presence of glutathione- a reducing agent in WGF, was reported as an influencing factor on rheological properties [2,8,23]. These findings suggest that raw wheat germ and heat-treated germ did have significantly different effects on dough stability. By roasting or steaming WG the effect on dough stability is minimized. The type of thermal treatments partially inactivate the glutathione and produce different degrees of starch gelatinization [2,7,25].

I.3. Influence of WGF addition on bread quality and sensory characteristics

Increasing WGF from 0% to 4% in WF:WGF blends, decreased the specific volume (SV) with an average value of 11.20% and increased the elasticity of bread compared to the control sample. The crumb porosity had an opposite trend compared with the elasticity, lowered scores were obtained as the WGF addition increased in the blends. The use of WGF leaded to an intense and appreciated color of the crust, due to a higher α-amylasic activity leading increased amounts of sugars for Maillard reaction and caramelization during baking. All the samples of WGF breads scored marked higher in terms of flavor and dryness than the control, increasing substitution levels increased the flavor and dryness scores. This behavior is due to the special flavor of WGF while the improvement in dryness is due to the higher water absorption of WGF:WF blends than wheat flour. WGF addition caused an improvement of taste and sweetness for all tested bread samples. Results are consisting with [2,8,10].

Table 3. Alveographic properties of dough obtained by blending WGF:WF

Wheat Flour	Addition level of WGF, %	P (mm H_2O)	L (mm)	P/L	W ($x10^{-4}$ J)
WF1	0%	84(0.2)	80(0.14)	1.05(0.1)	200(0.1)
	2%	82(0.25)	74(0.25)	1.10(0.2)	198(0.1)
	4%	81(0.1)	71(0.2)	1.14(0.25)	175(0.15)
WF2	0%	93(0.4)	80(0.1)	1.16(0.2)	234(0.2)
	2%	90(0.3)	78(0.3)	1.15(0.3)	212(0.1)
	4%	87(0.1)	73(0.4)	1.19(0.2)	203(0.1)
WF3	0%	88(0.2)	61(0.1)	1.44(0.1)	204(0.1)
	2%	84(0.2)	58(0.2)	1.45(0.2)	188(0.2)
	4%	81(0.13)	55(0.1)	1.47(0.1)	167(0.2)

Considering the results obtained on the WGF influence on dough rheological parameters and bread quality of wheat flour, we selected blends with 4% WGF as acceptable and it were subjected to the second stage experiment to study the effect of enzymatic and chemical improvers. The codifications B1C, B2C, B3C were used for blends of WF1, WF2, WF3 with 4% WGF. This concentration mimics the percentage of wheat germ in the kernel, while higher concentrations of wheat germ (e.g.,6–8%) favored an excessive sweet taste which seemed to be rather far from the main sensory attributes [10].

II. Influence of additives on dough properties

II.1. Hagberg Falling Number (HFN)

As we expected the addition of AMYL at the levels of 100 and 200 mg/kg caused a significant ($p<0.05$) decrease of HFN for all tested blends. For AMYL2 treatments HFN reached values as for normal content in α amylase. 245s, 232s and 223s were the HFN values recorded for the AMYL 2 treatments of wheat/wheat germs blends. In the case of the other enzymes used no changes in HFN values were recorded.

II.2. Farinograph and alveograph characteristics

Effects of single treatment enzymes and AA on farinographic parameters of dough obtained from blends with 4% WGF addition is shown in Figure 1. The addition of AMYL lowered the FWA of each blends tested with lowest FWA for treatment AMYL2. A value of 59,8% FWA was recorded for B1C blend when AMYL2 treatment was applied, while for the other blends the water absorption recorded about of 62.9% (B2C) and 58.1% (B3C). Both, XYL and GOX increased FWA of each tested blends as compared to the controls. Non-significant differences ($p>0.05$) between the two level of addition of XYL (1,2) and GOX (1,2), respective, were recorded. By comparing the effects of XYL and GOX addition on the water absorption, it could be noticed that both levels of XYL (50 and 60 mg/kg) had the highest influence on increasing the FWA for all tested blends than GOX levels (30 and 60 mg/kg). FWA values changed very little with the addition of either XYL or GOX; our results are consistent with [27]. AA addition had a slight, non-significant ($p>0.05$), increasing effect on FWA for both levels of addition as compared to control blends. Comparing to the GOX effect, AA addition caused smaller increase in FWA for all tested blends. The complex mechanism by which this enzyme increase water absorption is attributed to the drying effect of GOX on dough and explained as being caused by hydrogen peroxide resulted from the oxidation of β-D-glucose catalyzed by glucose oxidase [30-32]. Hydrogen peroxide induces the oxidative gelation of water soluble pentosans and a greater water sequestration that should explain the increase in water absorption [16].

AMYL at both level of addition, significantly ($p<0.05$) decreased the DDT for all dough samples. The lowering effect on DDT was higher for AMYL2 treatments of all samples. The lowest value was recorded in the case of blend B2C (1.7 min) with a control value of 3.0 min. This tendency could be explained by the presence of a low molecular weight dextrin produced from damaged starches by amylase hydrolysis [16]. Similar results

were reported by [28,29]. XYL, GOX and AA induced an increase in DDT, without significant differences ($p>0.05$) between level of addition inside the same enzyme. Addition of XYL at 60 mg/kg caused the highest increment of DDT. XYL prevents the interference of pentosans with gluten formation [33, 34]. Addition of GOX at 30, 60 mg/kg and AA at 40,50 mg/kg led to similar effect on DDT for all tested blends. Similar results were reported by other studies concluding that addition of GOX or XYL increased the DDT [27, 34].

The lowest ST was measured on dough supplemented with AMYL and AA, without significant differences between levels of addition. GOX at both levels of addition significantly ($p<0.05$) increased the dough ST. Our results are consistent with [27,35]. All treatments increased the softening index except GOX. AMYL2 induced the highest increment of DS for all tested blends. The high hydration capacity of starch decreases as the amylolytic attack starts, particularly when water is added to the flour and mixing begins. This resulted in a decrease in dough stability and an increase in the dough softening [36].

With respect to the alveographic parameters, for all tested blends, both oxidative agents (GOX and AA) had strengthening effect, as we expected (Fig.2). By adding GOX at both level, dough tenacity (P) and energy (W) increased while dough extensibility decreased (L) in all tested blends. The effect of GOX is especially clear from the significant decrease in extensibility [37], producing stiffer and less extensible dough [38]. Similar results were obtained by [35, 39]. GOX action is related to the hydrogen peroxide produced which promoted the formation of disulphide linkages in gluten protein [16,40]. Results show (Fig.2) that AA significantly ($p<0.05$) influenced the dough extensibility (L) for all tested blends.

For both treatment with AA, L decreased, while the P and W parameters increased. AA acts by inhibiting the cleavage of the intermolecular SS bonds of the gluten [16]. Addition of AMYL caused a slight reduction in dough tenacity (P) and energy (W) in all blends at both levels of addition. Dough extensibility increased with the level of added AMYL in all tested blends. XYL addition had an opposite effect than amylase on dough alveographic parameters causing increment in P, W value and a slight decrement in extensibility. These enzymes can influence the gluten properties by changing the water distribution in the dough and also by having covalent interactions with gluten [16]. According to literature [15, 27, 28] they are also responsible for several changes in dough properties including decrease of the absorption capacity, slackening of dough consistency and development of a stickier dough. The rate at which these changes occur is directly proportional to the amount of starch damage and α-amylase level of the flour [16].

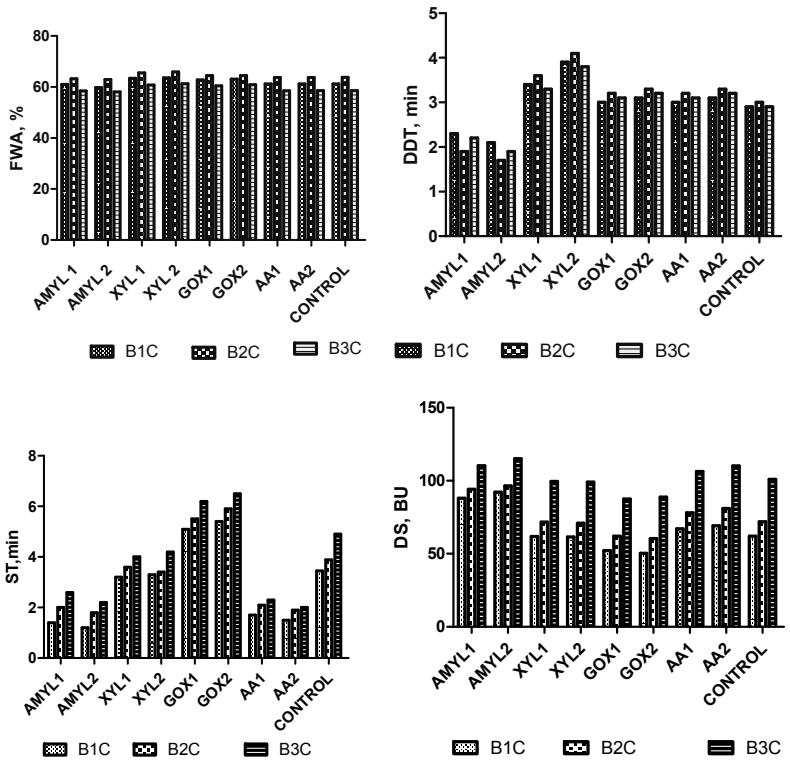

Figure 1. Effects of single treatment enzymes and ascorbic acid on farinographic parameters (FWA, DDT, ST, DS) of dough (4% WGF)

II.3. Influence of additives addition on wheat germ bread quality and sensory characteristics

When the influence of enzyme-supplementation on blends with 4% WGF was analyzed the SV of the control bread (without enzymes) prepared by using blends B1C, B2C, B3C were 3.02, 3.12, 3.09. All tested enzymes lead to bread with higher SV. Nevertheless, in the case of treatment GOX2 the SV was quite similar to the control sample for all tested blends meaning that for good quality flour, high dose of GOX increase dough strength causing an over-reinforcement of dough [28]. This effect hampers expansion during proofing and, consequently, negatively affects bread volume [28,41]. GOX2 treatment effect was moreover in shape improvement. Similar effect was reported by [13]. Lower dose of glucose oxidase (GOX1) lead to higher loaf

height improving the bread loaf volume. Supplementation with AMYL and XYL also caused significant improvement of bread loaf SV for both added doses. Ravi et al., 2000 [42] reported considerable increase in volume of amylase supplemented loaf. Amylase effect is explained by the presence of some deformed starch granules due to the action of α-amylase on long starch chains [27] and a slight leakage of amylose [13]. XYL supplementation, in both doses, lead to higher SV than AMYL supplementation. By scanning electron microscopy studies, it was observed thinning of some protein fibrils and a slight distortion of starch granules in the micrograph of dough with XYL addition [27].

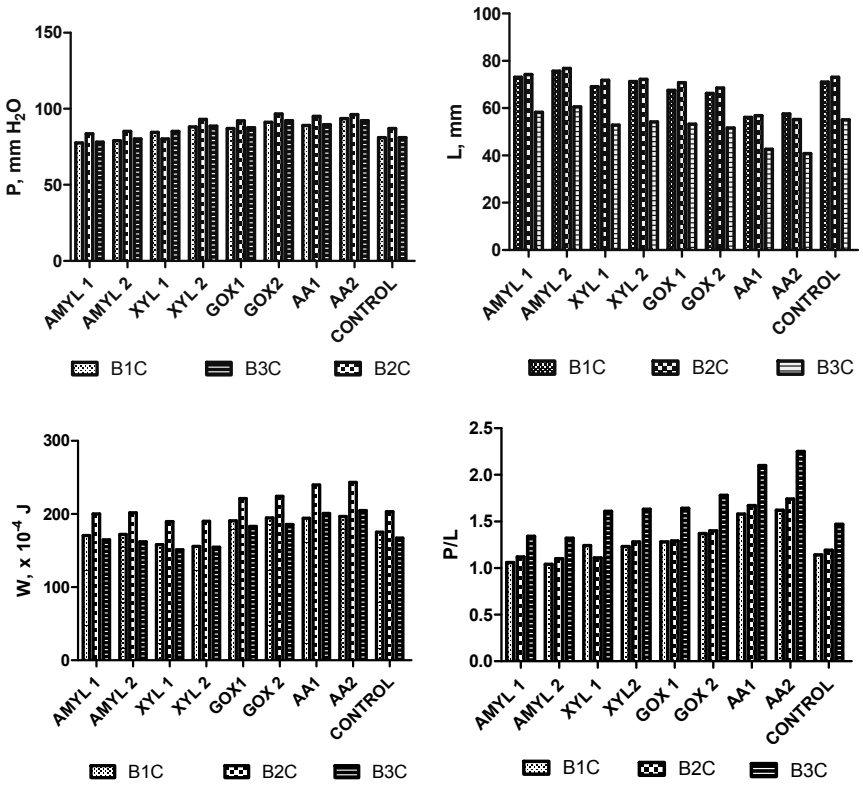

Figure 2. Effects of single treatment enzymes and ascorbic acid on alveographic parameters (P,L, W, P/L) of dough obtained from blends with 4% WGF addition

Also this effect could be explained by a delay in the crumb formation during baking giving better oven spring and larger bread volume and softer crumb [43]. AA addition caused also a significant increment of SV independently

of the added dose. By PCA studies it was showed that the effects of the addition of ascorbic acid is independent of its concentration [16], result consistent with ours.

The sensory scores for elasticity, porosity, color, mouth satisfaction increased with all improvers treatments in case of all tested blended (WGF:WF) breads. The highest improvement in the total overall quality was brought about by XYL2 recording an average value of 37.06, followed by AMYL2 with an average value of 35.63 and GOX 2 with the average value of 31.36 very close to AA2 with average value of 31.13. XYL caused a marked improvement of elasticity and porosity scores. Xylanase have a positive effect on dough properties and bread quality because they stabilize gas cells, improving its expansion capacity during proofing and baking and consequently improving bread characteristics, such as specific volume and firmness [28, 44]. AMYL significantly increased the crust color and the porosity while elasticity was less influenced. Amylases are able to change physicochemical and structural properties of polysaccharides, making dough softer and viscous and increasing the availability of fermentable sugars. α-amylase prolongs oven rise and results in an increased loaf volume [16] while the increased amount of fermentable sugars intensify bread color, taste and aroma. Glucose-oxidase effect is related especially to a greater specific volume, a better shape, an improving effect in the crumb grain and is attributed to the hydrogen peroxide released from the GOX reaction [39].

II.4 Relationship between rheological properties and wheat germ-enzyme supplemented bread quality parameters

Data were subjected to a Pearson correlation analysis in order to determine significant ($p<0.05$) relationships between rheological and bread quality parameters. The coefficients of significance are shown in Tab.4. The alveograph parameters W, P and P/L were found to be positively and significantly ($p< 0.01$) correlated to the SV, while among farinograph parameters only FWA was positively and significantly ($p< 0.01$) correlated to the SV. Dough extensibility L and DDT were negatively correlated to the SV. Flour strength is a measure of the gluten quality whereas tenacity is a predictor of the ability of the dough to retain gas and this lead to a better balance between elasticity and extensibility [16]. The correlation between water absorption and bread specific volume is related to the contribution of the evaporated water to increasing of bread volume [45]. P, P/L and FWA values were negative correlated to the H/W ratio, while L –dough extensibility was positively correlated to the H/W ratio.

Table 4. Coefficients of significant correlations ($p<0.05$) between rheological and bread quality parameters of dough

Rheological Parameter	SV (cm^3/g)	H/W ratio
P (mm H_2O)	0.554	-0.619
L (mm)	-0.501	0.615
W (x 10^{-4} J)	0.621	0.524
P/L	0.561	-0.603
FWA, %	0.756	-0.492
DDT, min	-0.488	-

CONCLUSIONS

Wheat flour replacement at different levels by WGF changed the rheological characteristics of the dough as well as the bread quality (SV, H/W ratio, sensory properties). A decrement of bread volume and H/W ratio was found with the increment of WGF in bread formulation. A substitution of 4% WGF was found as acceptable. The addition of bread making improvers (enzymes and ascorbic acid) improved the WGF:WF dough rheological parameters as well as the quality indices of wheat germ bread. Xylanase caused a marked improvement of elasticity and porosity. Amylase significantly increased the crust color, the porosity and the bread specific volume. Glucose-oxidase effect is related to a higher bread specific volume, an improvement of the bread shape and crumb porosity. Ascorbic acid addition caused also a significant increment of SV independently of the added dose. In the case of WGF:WG dough, the alveograph parameters W, P and P/L were found to be positively and significantly ($p< 0.01$) correlated to the SV, while among farinograph parameters only FWA was positively and significantly ($p<0.01$) correlated to the SV. Dough extensibility L and DDT were negatively correlated to the SV.

EXPERIMENTAL SECTION

Materials

Three types of commercial wheat flour (WF1,WF2,WF3) were used in this study. Wheat flour samples produced by local mills were sold as type 550 according to ash content by Romanian classification. Wheat germs (WG) were brought from a specialized local store and processed after method described by [8]. Dried yeast (Pakmaya) and salt were brought from the local market. Ascorbic acid (AA), fungal α-amylase (AMYL, 50000 SKBU/g), fungal xylanase (XYL, 2700 FXU/g) and glucose oxidase (GOX, 10000 GU/g) were procured from Enzymes & Derivates, Neamt, Romania.

Blends, dough formulations, bread making procedure and optimization

In the first step, blends of wheat flour (WF1,WF2,WF3) and wheat germ flour (WGF) were obtained by substituting the same amount of wheat flour with 0%, 2% and 4% WGF. A straight dough method for bread preparation and the following formula (for control bread) was used: wheat flour 100%, dried yeast 2%, salt 2% (amount of ingredients in reference to flour) and water needed for preparation of dough with farinograph consistency of 500 BU. Wheat germ bread were prepared using blends describe forementioned; a total of nine type breads were baked in triplicate. In the second step, for each type of blends composed by wheat flour (WF1, WF2, WF3) and the selected optimum level of WGF (as found by dough rheological and bread quality evaluation from the first step) four additives were included in the bread formulas one by one. Samples codification, based on additive and amount were: AA1-ascorbic acid 40mg/kg, AA2- ascorbic acid 50 mg/kg, XYL1-xylanase 50 mg/kg, XYL2- xylanase 60 mg/kg, AMYL1- amylase 100 mg/kg, AMYL2- amylase 200 mg/kg, GOX1-glucose oxidase 30 mg/kg, GOX2-glucose oxidase 60 mg/kg. Enzymatic and chemical additives were tested at different levels of addition chosen on the basis of preliminary tests (data not shown) and included in the ranges usually applied in bakery industry. Control bread samples were prepared without using additives. Dough was kneaded using a single spiral mixer (type Hobart) for 12 min; dough with 24˚C temperature was divided into pieces of 1000g and the following steps were used: rounding, first pre-proofing (20 min, 25˚C, relative humidity (RH) 60%), second rounding, second pre-proofing (30 min, 25˚C, 60% RH), final shaping, final proofing (70 min, 30˚C, 80% RH), baking in electrical oven (40 min, 225˚C), cooled and packed. Two hour after baking, the loaves were weighed, the height and width of the central slice was measured and bread volume was determined according to AACC Approved Method 10-05 (American Association of Cereal Chemistry, 2000) procedure. Specific volume (SV) of bread was expressed as the volume ⁄ weight ratio (cm^3/g) of finished bread and H/W ratio was calculated.

Physicochemical and Rheological Characteristics

Wheat flour samples were subjected to physicochemical analyses (moisture, ash, protein content). Also, Hagberg's Falling number (HFN), Zeleny's sedimentation value (SDS) and rheological –farinograph and alveograph-characteristics were determined. The American Association of Cereal Chemists (AACC 2000) methods were used to determine all these parameters. Farinograph (Brabender, Duisburg, Germany) was used and the following results were

expressed as flour water absorption (FWA, %), dough development time (DDT, min), dough stability (ST, min) and the degree of softening (DS, BU). The viscoelastic properties of the dough were assessed using an Alveograph MA 82 (Chopin). The following parameters were automatically recorded: tenacity or resistance to extension (P, mm H_2O), dough extensibility (L, mm), curve configuration ratio (P/L) and the deformation energy (W x 10^{-4} J). In addition, in order to obtain more information about rheological characteristics of the fermented dough the maturograph and oven spring apparatus (Brabender, SRN) was used for recording the proofing time (min), the dough resistance (BU), the baking volume (BU), the oven rise (BU). All measurements were performed in triplicate and the average values were used.

Sensory evaluation

Sensory analysis of bread was carried out according to the method described by [17], with minor modifications. Elasticity, color, porosity, flavor, sweetness, dryness, taste and mouth satisfaction were evaluated using a scale from 0 to 10 points, with 10 being the highest score. The panel group was composed of 25 bread usual consumer volunteers from 20 to 57 years of age and from various socioeconomic backgrounds, consisting of Faculty of Food Science and Technology staff and students from Cluj-Napoca, Romania. The sensory evaluation was performed in both steps of experiment fore mentioned. All breads were analyzed in the same session.

Statistical analysis

The results of three independent assays (performed with replicates each) were expressed as mean value (SD). All data were compared by one-way analysis of variance (ANOVA) followed by Duncan test and Pearson's correlation coefficient. The statistical evaluation was carried out using Graph Prism Version 5.0 (Graph Pad Software Inc., San Diego, CA, USA).

REFERENCES

1. B. Dunnewing, T. van Vliet, R. Orsel, *Journal of Cereal Science*, **2002**, *36*, 357.
2. M. Gómez, J. González, B. Oliete, *Food Bioprocess Technol*, **2012**, *5,* 2409.
3. J.S. Sidhu', S.N. Al-hooti', J.M. Al-Saqer', Amani al-Othmav, *Journal of Food Quality*, **2001**, *2,* 235.
4. S.N. Al-Hooti, J.S. Sidhu, J.M. Al-Saqer, A. Al-Othman, *Nahrung-Food*, **2002**, *46(2),* 68.

5. M.U. Arshad, F.M. Anjum, T. Zahoor, *Food Chem*, **2007**, *102*, 123.
6. Srivastava Alok, K. Sudha, M.L. Baskaran, V. Leelavathi, *European Food Research Technology*, **2007**, *42*, 358.
7. Y. Pomeranz, M.J. Carvajal, M.D. Shogren, R.C. Hoseney, K.F. Finney, *Cereal Chemistry*, **1970**, *47(4),* 429.
8. Sun Ru, Zhengmao Zhanga, Xinjuan Hua, Qinhui Xinga, Wuyan Zhuo, *Journal of Cereal Science*, **2015**, *64*,153.
9. N. Gelmez, N.S. Kıncal, M. E. Yener, *J. Supercrit. Fluid*, **2009**, *48*, 217.
10. C.G. Rizzello, L. Nionelli, R. Coda, R. Di Cagno, M. Gobbetti, *Eur Food Res Technol*, **2010**, *230*, 645.
11. A. Zalatnai, K. Lapis, B. Szende, E. Rásó, A. Telekes, A. Resetár, Hidvégi M.*Carcinogenesis*, **2001,** *22*, 1649.
12. M. Majzoobi, S. Farhoodi, A. Farahnaky, M.J. Taghipour, *J. Agr. Sci. Tech*., **2012**, *14*, 1053.
13. P.A. Caballero, M. Go´mez, C.M. Rosell, *European Food Research and Technology*, **2007**, *224*, 525.
14. V. Stojceska, P. Ainsworth, *Food Chemistry*, **2008**, *110*, 865.
15. P.A Caballero, M. Go´mez, C.M. Rosell, *Journal of Food Engineering*, **2007**, *81*, 42.
16. A. Baiano, C. Terracone, *CyTA - Journal of Food*, **2001**, *9(3)*, 180.
17. Å. Haglund, L. Johansson, L. Dahlstedt, *J. Cereal Sci*., **1998**, *27*, 199.
18. J.L. Ward, K. Poutanen, K. Gebruers, V. Piironen, A.M. Lampi, L. Nystrom, A.A. Andersson, P. Aman, D. Boros, M. Rakszegi, *J Agric Food Chem*., **2008**, *56*, 9699.
19. I. Banu, I. Aprodu, *International Journal of Food Science and Technology*, **2015**, *50*, 1644.
20. I. Konopka, L. Fornal, D. Abramczyk, J. Rothkaehl, D. Rotkiewicz, *International Journal of Food Science and Technology*, **2004**, *39*, 11.
21. M.S. Butt, F.A. Anjum, D.J. van Zuilichem, M. Shaheen, *International Journal of Food Science and Technology*, **2001**, *36*, 433.
22. M. Hrušková, I. Švec, O. Jirsa, *Journal of Food Engineering*, **2006**, *77(3),* 379.
23. D. Every, S. C. Morrison, L. D. Simmons, M. P. Ross, *Cereal Chem.,* **2006**, *83* 57.
24. S. Hamada, K. Suzuki, N. Aoki, Y. Suzuki, *J. Cereal Sci*., ***2013***, *57*, 91.
25. M. Miyazaki, N. Morita, *Food Research International,* **2005**, *38(4),* 369.
26. M.L. Sudha, A.K. Srivastava, K. Leelavathi, *Eur Food Res Technol*, **2007**, *225*, 351.
27. D. Indrani, P. Prabhasankar, J. Rajiv, R. Venkateswara, *Journal of Food Science*, **2003**, *68(9),* 2804.
28. M.E. Steffolani, P.D. Ribotta, G.T. Pérez, A.E. León, *International Journal of Food Science and Technology*, **2012** ,*47*, 525.
29. T. Maeda, T. Hashimoto, M. Minoda, S. Tamagawa, Morita, *Cereal Chemistry*, **2003**, *80*, 722.
30. V. Vemulapalli, K.A. Miller, R.C. Hoseney, *Cereal Chemistry*, **1998**, *75*, 439.
31. H.S. Gujral, C.M. Rosell, *Food Research International*, **2004**, *37*, 75.
32. A.D. Bettge, C.F. Morris, *Cereal Chemistry*, **2007**, *84*, 237.

33. M. Wang, T. van Vliet, R.J. Hamer, *Journal of Cereal Science*, **2004**, *39*, 341.
34. J.C. Pescador-Piedra, A. Garrido-Castro, J. Chanona-Pérez, R. Farrera-Rebollo, G. Gutiérrez-López, G. Calderón-Domínguez, *International Journal of Food Properties*, **2009**, *12(4)*, 748.
35. P. Prabhasankar, D. Indrani, R. Jyotsna, R.G. Venkateswara, *Journal of the Science of Food and Agriculture*, **2004**, *84(15)*, 2128.
36. I.S. Doğan, *International Journal of Food Science and Technology*, **2003**, *38*, 209.
37. C. Primo-Martín, J. Mingwei Wang, W. J. Lichtendonk, J.J. Plijter, R. J. Hamer, *J Sci Food Agric*., **2005**, *85*, 1186.
38. A.F. Dagdelen, D. Gocmen, *Journal of Food Quality*, **2007**, *30*, 1009.
39. C.M. Bonet, P.A. Rosel, M. Caballero, I. Gómez, Pérez-Munuera, M.A. Lluch, *Food Chemistry*, **2006**, *99*, 408.
40. C.H. Poulsen, P.B. Hostrup, *Cereal Chemistry*, **1998**, *75*, 51.
41. C.M. Rosell, J. Wang, S. Aja, S. Bean, G. Lookhart, *Cereal Chemistry*, **2003**, *80*, 52.
42. R. Ravi, R. Sai Manohar, P. Haridas Rao, *Eur Food Res Technol.,* **2000**, *210*, 202.
43. B. Polderman, P. Schoppink, *Cereal Foods World*, **1999**, *44*, 132.
44. T. Jiménez, M.A. Martínez-Anaya, *Food Science and Technology International*, **2001**, *7*, 5.
45. I. Švec, M. Hrušková, *Scientia Agriculturae Bohemica*, **2009**, *40*, 58.