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The effect of laccase enzyme addition to soft Syrian wheat flour or blending with durum flour on the rheological properties of dough

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ABSTRACT

Wheat is one of the most important crops in the world, its products, especially fermented baked goods, take a major role in the diet. These products require strong flour. In Syria, about 20 – 25% of improved durum wheat is blended with soft wheat to increase the strength of the flour. Laccase enzyme, is one of the important oxidative enzymes, which is added to increase the strength of the dough. Several devices have been used to evaluate flour and measure the properties of dough, but none of them is able to predict all the properties in a single test one. A new instrument Mixolab dough characterizer has been developed to measure the dough properties and the pasting behavior of flour at the same time. Hence, in the present research Mixolab dough characterizer was used to assess the effect of the addition of laccase enzyme and blending of durum and soft Syrian wheat on the rheological properties of wheat flour (80% extraction). The results showed that the values of C_2 (decrease in dough consistency due to excessive mixing), water absorption, and dough development time increase after the addition of laccase enzyme and blending durum and soft wheat, as well as the dough stability increases too, therefore, it can be concluded that, both the addition of laccase and blending durum and soft wheat increase dough strength, but the addition of laccase treatment has higher values of dough stability and gluten index compared to blend flour. The effect of the two treatments on the properties of the starch differed; the values of C_3 (starch gelatinisation), C_4 (gel stability), and C_5 (starch retrogradation) are increasing with laccase addition, while these values are reducing with the blending of durum and soft wheat.

Keywords: rheological properties, Mixolab dough characterizer, laccase, Syrian durum wheat, Syrian soft wheat

INTRODUCTION

Rheology is one of the cereal techniques that has spread widely as a tool for assessing the quality of the final product (Dobraszczyk and Morgenstern, 2003; Hadnađev et al., 2011). The dough is a viscoelastic system that shows a variable rheological behavior. Despite the importance of the protein content in the flour, the quality of the protein has a significant impact on the rheological properties (VanLonkhuijsen et al., 1992; Dhaka and Khatkar, 2013). About 80 – 85% of wheat protein is gluten, which consists of two types of proteins, glutenin and gliadin (Veraverbeke and Delcour, 2002). Gliadin is responsible for gluten extensibility, while the glutenin is related to the elasticity of gluten (Falac˜o-Rodrigues et al., 2005). Brandner etal., (2022) reported that, wheat dough formation depends on the quality and quantity of flour components especially gluten and starch, where the interface between starch particles and gluten polymers effects on mechanical dough properties. Megusar et al. (2022) pointed out the importance of gluten in making baked goods, where gluten containing product has higher shelf-life and desired texture comparable to the gluten free product. A study by Janssen et al. (1996) reported that dough made of poor flour is less elastic than dough made of good flour, because of this it cannot hold gas properly, resulting in a flat loaf. In addition, dough made of very strong flour will not expand well during fermentation, resulting in a small loaf (Lundgren, 2011). Hence, it can be said that the strength of the flour is the state of balance between the elasticity and viscosity of its dough. It is known that soft wheat flour contains less gluten than durum wheat flour, and its dough is more expandable, while the dough made of durum wheat flour has a low ability to expand due to its high gluten strength (Sapirsteina et al., 2007). Blending of durum and soft wheat has a positive effect on the rheological properties of the dough. Some studies indicated that using durum wheat flour at 10 – 30% with weak wheat flour has improved the properties of the bread (Rinaldi et al., 2015). Torbica et al. (2011) studied the possibility of using durum wheat flour as an improvement agent for regular wheat flour with a damaged protein complex. However, the rheological properties can also be improved by the addition of laccase enzyme, one of the important oxidative enzymes, which has recently attracted the attention of researchers. Laccase has an important effect on gluten network development by increasing the cross-linked and disulfide bonds in the protein structure through the oxidation of free and bound ferulic acid; this makes the dough stronger and more stable (Labat et al., 2000; Selinheimo et al., 2008).

Rheological properties are measured using Brabender devices, such as farinograph, extensograph, and amylograph; these devices do not evaluate the flour comprehensively (Dapčević et al., 2009). Mixolab dough characterizer was launched in 2005 by the French company Tripette & Renaud Chopin to evaluate protein, starch, and enzymes in a single test one with a sample of less than 50 g of flour (Dapčević et al., 2009). Most studies have focused on the examination of the effect of the laccase enzyme on the rheological properties of the white flour using amylograph and farinograph devices, while few researchers have studied the effect of blending durum and soft wheat on the rheological properties of the dough. Therefore, the aim of this study to determine the rheological changes of wheat flour (80% extraction) caused by the addition of laccase enzyme and blending of durum and soft wheat using Mixolab dough characterizer.

MATERIAL AND METHODS

Material

Raw material included two improved varieties of certified Syrian wheat: *Triticum durum*: $Sham_5$, and *Triticum aestivum*: $Sham_4$. Samples were obtained from the General Commission for Scientific Agricultural Research, Damascus, Syria, in the harvest year 2019.

Laccase enzyme (isolated from *Trametes Versicolor*) was obtained from Sigma Aldrich.

Methods

Preparation of samples

Wheat samples were sifted and milled according to the method of (AACC, 2000) No. 26 – 50 using by Brabender mill (Brabender Co. Duisburg, Germany) to produce two types of flour (80% extraction): soft wheat flour ($Sham_4$ flour), the proximate composition of flour (%) were as follows: crude protein (12.38), crude fiber (0.83), ash (0.68), moisture content (13.01) and blend flour (flour made of 80% $Sham_4$, and 20% $Sham_5$), the proximate composition of flour (%) were as follows: crude protein (12.8), crude fiber (0.84), ash (0.72), moisture content (13.35).

Protein content

The Kjeldahl method was carried out according to the (AOAC, 2000) Method. The first step is the hydrolysis of a flour sample (1 g) with 15 mL concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets at 420 °C for 2 h to form ammonium sulfate from the total organic nitrogen, the next step is the distillation of formed ammonia into boric acid solution under alkaline conditions. The borate anions formed are titrated with standardized hydrochloric acid as the last step, by which is calculated the content of nitrogen. The nitrogen content in the flour sample is multiplied with a conversion factor of 5.7 to determine the total protein content.

Wet gluten content

The wet gluten content of flour was measured by hand washing using dilute salt (2% NaCl) according to (the AACC, 2000) Method. A 10 gram of flour is weighed and mixed with water to obtain the dough that is washed with a salt solution (2% NaCl). The gluten obtained was pressed as dry as possible between the hands, rolled into a ball, and weighed; the mass was recorded as wet gluten.

Mixolab dough characterizer Test

The Mixolab dough characterizer (Chopin Technologies, Villeneuve La Garenne, France) can be considered an empirical method that records the dough changes when subjected to large deformations and temperature sweeps (Rosell et al., 2010). For the analysis of the mixing and pasting behavior, the standard "Chopin+" protocol was followed according to the standard method (ICC, No173, 2006): initial equilibrium at 30 °C for 8 min, heating to 90 °C over 15 min (at a rate of 4 °C/min), holding at 90 °C for 7 min, cooling to 50 °C over 5 min (at a rate of 4 °C/min) and holding at 50 °C for 5 min. Instrumental settings defined in the Mixolab dough characterizer for running the samples are presented in Table 1 below.

Table 1. Instrumental settings defined in the Mixolab dough characterizer for running the samples

Parameters	Flour Wheat
Dough Mass	75 g
Kneading Speed	80 rpm
Target Torque (C_1)	1.1± 0.05 Nm
Tank Temperature	30 °C
Temperature, 1st Level	30 °C
Duration, 1st Level	8 min
Temperature, 2nd Level	90 °C
1st Temperature Gradient	4 °C/min
Duration, 2nd Level	7 min
2nd Temperature Gradient	- 4 °C/min
Temperature, 3rd Level	50 °C
Duration, 3rd Level	5 min
Total Analysis Time	45 min

Laccase (17nkat/g flour) was added to the water just before mixing with soft wheat flour. The parameters that are obtained from the curve are: water absorption (%) or the percentage of water required for the dough to produce a torque of 1.1±0.05 Nm; dough development time (min) or the time to reach the maximum torque at 30 °C; dough stability (min) or the elapsed time at which the torque produced is kept at 1.1 Nm; C_2 i.e. decrease in dough consistency due to excessive mixing; starch gelatinization (C_3) i.e. starch granules swell and absorb water and amylose molecules leach out resulting in an increase in the viscosity; amylase activity (C_4) i.e. reduction in viscosity of the starch granules; and starch retrogradation (C_5) as a result of the decrease in the temperature (Rosell et al., 2010; Hadnađev et al., 2011; Dhaka and Khatkar, 2013).

Statistical analysis

The data were statistically analyzed using (Gen state 10) software for one-way analysis of variance (ANOVA). Means with a significant difference (*P*<0.05) were compared using the Duncan test. All the analysis were performed in triplicate.

RESULTS AND DISCUSSION

Protein and wet gluten

As shown in Table 2 it can be observed that blend flour has considerable higher protein and gluten content compared with soft wheat flour; this is due to the fact that protein and gluten content in durum wheat is higher than in soft wheat. The amount of wet gluten also increased after the addition of laccase, this may be attributed to the binding of some compounds to the gluten network as a result of laccase action.

Table 2. Protein and wet gluten of studied samples

Sample	Wet Gluten Content (%)	Protein Content (%)
SWF	43.63±0.12^a	12.38±0.11^a
BF	46.53±0.15^c	12.8±0.1b
SWF + LACC	44.3±0.13^b	12.39±0.1a

SWF: Soft wheat flour; BF: blend flour; LACC: laccase enzyme
Values are expressed as means±SD, n=3, values in the same raw followed by different letters are significantly different (*P*<0.05)

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Mixolab dough characterizer Test

The curves of Mixolab dough characterizer for samples are presented in Figure 1. Water absorption (WA), dough development time (DDT), dough stability (DS), and decrease in dough consistency due to excessive mixing (C_2) are parameters that refer to dough characteristics during mixing at a constant temperature of 30 °C, describing the dough behavior during the processing stage (Hadnađev et al., 2011).

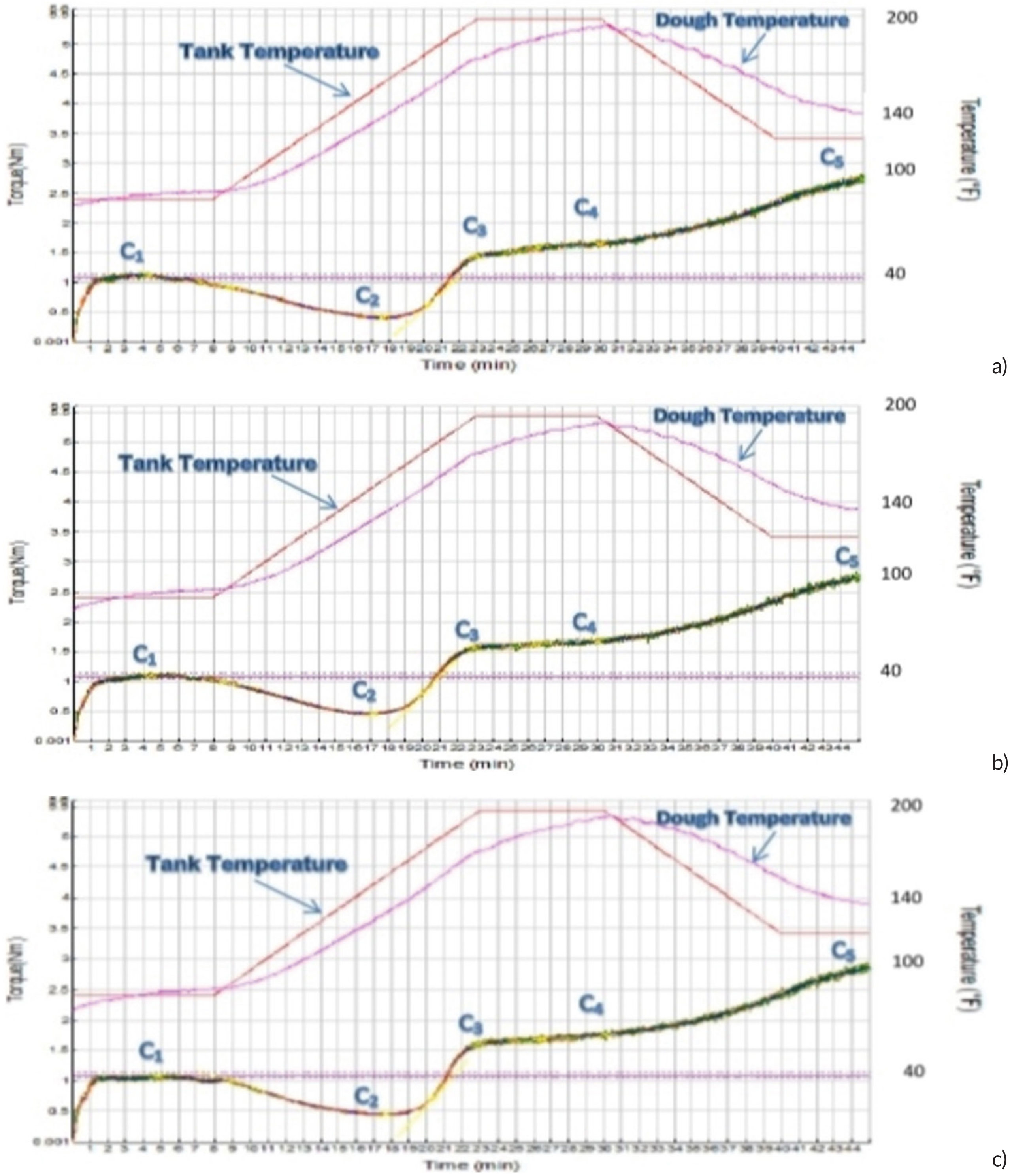


Figure 1. Curves of Mixolab dough characterizer for studied samples: (a) Curve of Mixolab dough characterizer for soft wheat flour; (b) Curve of Mixolab dough characterizer for blend flour; (c) Curve of Mixolab dough characterizer for soft wheat flour + laccase

The strength of each treatment could be adjudged from the data of Mixolab dough characterizer based on dough development time, dough stability, and C_2 (Dhaka and Khatkar, 2013). The rheological properties of flour are summarized in Table 3, it can be seen from the data an increase in the values of C_2, dough development time, dough stability after the two treatments. Blend flour has higher dough development time and water absorption compared to soft wheat flour, indicating that this flour needs longer time to hydrate all compounds than soft wheat flour, and it is required a considerably larger amount of water to achieve a torque of 1.1 Nm compared to wheat soft flour, the value of C_2 is increasingly related to the amount of gluten (Codina et al., 2008), this explains the increase of C_2 and dough stability after blending durum and soft wheat. The addition of laccase treatment has higher values of dough development time, dough stability, and C_2 compared to blend flour, this could be ascribed to the modifications of the bonds in the protein structure during the development of the gluten network. The bonds in the protein structure (physical and chemical forces) especially disulfide bonds play an important role in the improvement of gluten stability (Banu et al., 2012), water absorption also increased after laccase treatment because of the increase in the water holding capacity of the dough.

The values of C_3, C_4, and C_5 decreased with the blend flour compared to the soft wheat flour due to the increase in the content of damaged starch, (Dhaka and Khatkar, 2013; Singh et al., 2019), and thus the lowest starch retrogradation, while C_3, C_4, and C_5 increased after the addition of laccase, probably because of the impact of laccase on the water absorption of the dough. The profile of Mixolab dough characterizer converts the standard curve into a set of six scores graduated from 0 to 9 to characterize a flour by six fundamental criteria. Figure 2 shows profiles of Mixolab dough characterizer: Absorption index is dependent on the flour composition and the higher the index, the more water is absorbed by the flour; mixing index represents the properties of the flour during mixing at 30 °C and the higher the index, the greater the stability of the flour during mixing; gluten index represents the properties of the gluten when the dough is heated and the higher the index, the higher the resistance of the gluten to heating; viscosity index represents the increase in viscosity during the heating phase and the higher the index, the higher the viscosity of the dough when hot; amylase index is dependent on the ability of the starch to resist amylolysis and the higher the index, the lower the amylase activity; retrogradation index is dependent on the characteristics of the starch and its hydrolysis during the test and the higher the index, the shorter the product shelf-life. Index scores determined by the profile of Mixolab dough characterizer are shown in Table 4.

It is clear that two treatments had a similar increase in absorption and mixing index, gluten and amylase index were not affected by the blending of durum and soft wheat, while increased after laccase treatment, no change was observed in the viscosity and retrogradation after two treatments.

Table 3. Parameters of Mixolab dough characterizer for studied samples

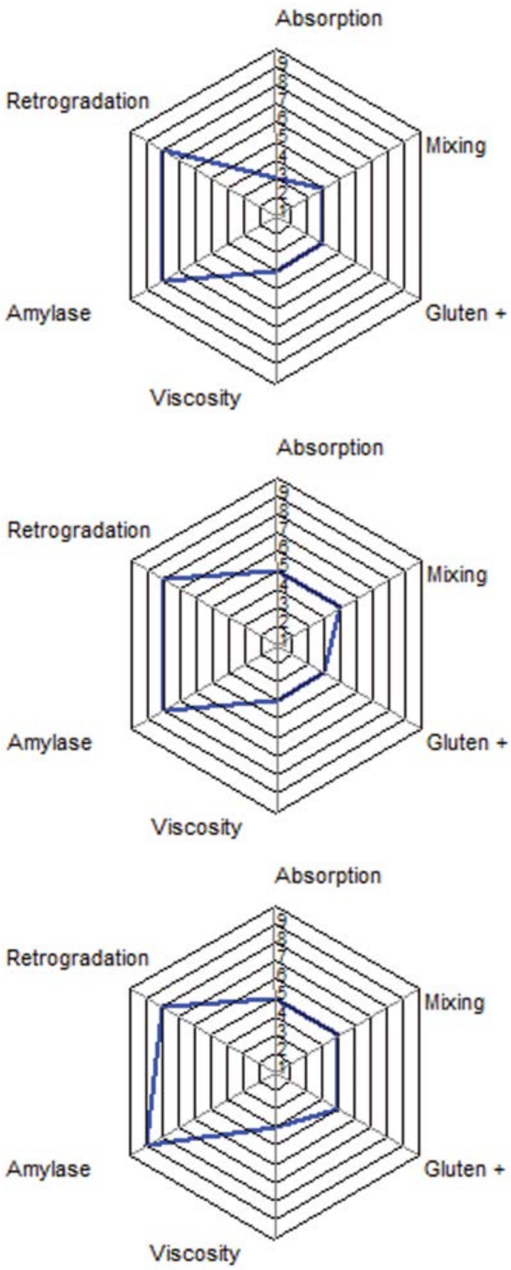
Sample	WA (%)	DDT (min)	DS (min)	C_2 (Nm)	C_3 (Nm)	C_4 (Nm)	C_5 (Nm)
SWF	55±0[a]	4.25±0.05[a]	6.6±0.03[a]	0.41±0.01[a]	1.56±0.01[b]	1.67±0.01[a]	2.76±0.01[b]
BF	57.2±0.2[b]	4.41±0.01[b]	7.07±0.03[b]	0.44±0.01[b]	1.45±0.03[a]	1.66±0.01[a]	2.71±0.02[a]
SWF + LACC	57.33±0.06[b]	4.8±0.01[c]	8.73±0.06[c]	0.47±0.01[c]	1.6±0.02[c]	1.74±0.02[b]	2.81±0.02[c]

SWF: Soft wheat flour; BF: blend flour; LACC: laccase enzyme; WA: water absorption; DDT: dough development time; DS: dough stability; C_2: decrease in dough consistency due to excessive mixing; C_3: starch gelatinisation; C_4: amylase activity (gel stability); C_5: starch retrogradation.
Values are expressed as means±SD, n=3, values in the same raw followed by different letters are significantly different (*P*<0.05)

Table 4. Index scores determined by the profile of Mixolab dough characterizer

Samples	I_1	I_2	I_3	I_4	I_5	I_6
SWF	2	3	3	3	7	7
BF	4	4	3	3	7	7
SWF + LACC	4	4	4	3	8	7

SWF: Soft wheat flour; BF: blend flour; LACC; laccase enzyme; I_1: absorption index; I_2: mixing index; I_3: gluten index; I_4: viscosity index; I_5: amylase index; I_6: retrogradation index



a) b) c)

Figure 2. Profile of Mixolab dough characterizer for studied samples: (a) Profile of Mixolab dough characterizer for soft wheat flour; (b) Profile of Mixolab dough characterizer for blend flour; (c) Profile of Mixolab dough characterizer for soft wheat flour + laccase

CONCLUSION

The results of this study indicate that the addition of laccase and blending of durum and soft wheat changes the thermo mechanical properties of wheat flour. According to result obtained by Mixolab dough characterizer, the addition of laccase treatment has higher values of dough stability and gluten index compared to blend flour, on the other hand, the effect of the two treatments on the starch differed, the starch became faster gelling, and it had greater cooking stability after the addition of laccase while blend flour had the lowest starch retrogradation.

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