Use of response surface methodology to investigate the effects of brown and golden flaxseed on wheat flour dough microstructure and rheological properties

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Abstract The aim of this study was to assess the effects of brown flaxseed and golden flaxseed on the water absorption and the rheological parameters of wheat flour. The rheological properties were evaluated by the Mixolab. The optimum parameters were identified for high quality flaxseed-wheat composite flour bread. Each predictor variable was tested at five levels. The microstructure of dough was assessed by epifluorescence light microscopy. The substitution of 4.81/100 g for brown flaxseed and 5.07/100 g for golden flaxseed was found optimal. At the optimum level of brown and golden flaxseed, the water absorption of 55.07/100 g, dough development time of 1.28 min and dough stability of 10.04 min was observed. In terms of protein quality the optimum values were 0.41 N m for C2 and 0.69 for C1–2, and in terms of starch characteristics they were 1.56 N m for C3, 1.14 for C3–2, 1.33 N m for C4, 0.20 for C3–4, 1.97 N m for C5 and 0.60 for C5–4.

Keywords Wheat flour · Flaxseed flour · Rheological properties · Epifluorescence light microscopy · Optimization

Introduction

There are more than 200 species of flaxseed (*Linum usitatissimum* L.) known as linseed, varying in color from golden to deep brown (Oliveira et al. 2014; Kajla et al. 2015). The flaxseed color is determined by the amount of pigment presence in the seed, the higher, the darker the seed is (Ganorkar and Jain 2013). It contains almost 40–50% oil, 23–34% protein, 5% mucilage and 4% ash (Touré and Xueming 2010). Flaxseed is considered a functional food or a functional ingredient due to the fact that it contains phytochemicals such as lignans, especially secoisolariciresinol diglucoside (SDG), alpha-linolenic acid (omega 3-fatty acid) and dietary fibers (Hao and Beta 2012; Rao et al. 2013). Flaxseed is also a good source of high quality proteins, flavonoids and other phenolics (Cameron and Hosseini 2013). Its lipid composition is high in polyunsaturated fatty acids (73% of total fatty acids), moderate in the amount of monosaturated fatty acids (18%) and low in the total content of saturated fatty acids (9%) (Morris 2001). The oil fatty acid profile consists of linolenic acid (52%), linoleic acid (17%), oleic acid (20%), palmitic acid (6%) and stearic acid (4%). It also contains monoacylglycerides, diacylglycerides, sterol-esters, sterols, tocopherols, waxes, phospholipids, CLs, free fatty acids (FFAs), chlorophyll, carotenoids and other compounds (Shim et al. 2014). The high content in flaxseed of the essential omega 3-fatty acids makes it the leading plant source (Cameron and Hosseini 2013) flaxseed content in alpha-linolenic acid (ALA) being five times higher than in walnuts and canola oil (Oliveira et al. 2014). During the heating process fatty acids' bioavailability of flaxseed is not affected (Cunnane et al. 1995), the alpha-linolenic acids remaining stable in the food in which the flaxseed is incorporated (Chen et al. 1992). Of the two

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varieties, the golden flaxseed contains a higher amount of omega 3 and omega 6 fatty acids than the brown one (Sargi et al. 2013). Regarding the amino acid composition of flaxseed, it is comparable with that of soy, both oilseeds having high levels of glutamic acid, leucine, aspartic acid and arginine. From the point of view of amino acids, there are no significant differences between golden and brown flaxseed, the golden variety presenting only slightly higher values than the brown one (Oomah 2001; Friedman and Levin 1989). Flaxseeds contain almost 30% more dietary fibers, twice than beans (Bernacchia et al. 2014) with a proportion ratio of soluble to insoluble fiber that varies between 20:80 and 40:60. The soluble fiber consists in mucilage associated with flaxseed hull, forming a gum composed from acidic and neutral polysaccharides (Ganorkar and Jain 2013). The insoluble fiber content is composed of lignin, cellulose and acid detergent fiber (Cui et al. 1994). Of the two varieties of flaxseed, the brown one contains a higher amount of fiber and a lower amount of carbohydrates than the brown variety (Epaminondas et al. 2011). Flavonoids, lignans and phenolic acids are other bioactive compounds present in flaxseed (Basavaraj 2009). Flavones-C and *O*-glycosides are the most important flavonoids in flaxseed and ferulic acid, chlorogenic acid, gallic acid and 4-hydroxybenzoic acid are the most important phenolic acids (Siger et al. 2008). Lignans, compounds with a high antioxidant activity compounds, are the richest dietary source in flaxseed. Also some bio-active flaxseed substances are Vitamins (A, C, F and E) and minerals (P, Mg, K, Na, Fe, Cu, Mn and Zn) are bioactive substances present in flaxseeds as well (Goyal et al. 2014). Flaxseed provides health benefits due to the presence of various bioactive compounds by decreasing the risk of prostate cancer and mammary cancer, reducing cardiovascular diseases, having a laxative effect and anti-inflammatory activity, and by alleviating osteoporosis and menopausal symptoms (Goyal et al. 2014).

Nowadays flaxseed is increasingly used in bakery products in order to enrich them with a healthy ingredient. Its incorporation in bread product has been the subject of many studies (Hussain et al. 2012; Marpalle et al. 2014). To our knowledge no studies have been made yet to compare the effect of the two flaxseed varieties, the brown and golden one in wheat flour addition. Therefore, in the present study response surface methodology coupled with the desirability function approach was applied to optimize the formulation of brown and golden flaxseed that can be added in wheat flour thus improving the flaxseed–wheat dough rheological properties. For the best combination between the brown and golden flaxseed found, the dough microstructure was analyzed by using the technique of epifluorescence light microscopy (EFLM).

Materials and methods

Materials

A commercial wheat flour 650 type of 13.9/100 g moisture (ICC methods 110/1), 0.65/100 g ash (ICC 104/1), 12.2/100 g protein (ICC 105/2), 27.5/100 g wet gluten (ICC 106/1), 3 mm gluten deformation index (SR 90:2007), 325 s falling number (ICC 107/1) was used. The golden and brown flaxseed varieties used were purchased from SC DECO ITALIA SRL Cluj-Napoca, Romania. The flaxseeds grains were ground in a domestic blender and were incorporated in the wheat flour at different substitution levels. Flaxseed varieties were analyzed for their chemical characteristics (moisture, protein, fat, ash) according to ICC methods (2010). The golden flaxseed presents 5.6/10 g moisture content, 20.85/100 g protein content 41.12/100 g fat content and 3.41/100 g ash content, whereas the brown flaxseed presents 6.2/100 g moisture content, 19.74/100 g protein content 42.25/100 g fat content and 3.5/100 g ash content. The values obtained are the average expression of the analyses made in duplicate.

Experimental design and statistical methods

The effect of two factors, brown flaxseed flour and golden flaxseed flour at five levels for each factor on water absorption of flaxseed-wheat composite flour dough and Mixolab rheological parameters (responses) were investigated using full factorial design and response surface methodology. A full factorial design is used because relatively few experimental combinations of the factors are adequate to estimate response functions. The matrix design of the coded and real values of factors in order to establish the optimum addition of flaxseed in wheat flour is shown in Table 1. The levels of 0, 5, 10, 15, and 20% used in this study for the formulation factors namely brown and golden flaxseed flour to be incorporated in wheat flour were chosen taking into account the results obtained by different researchers in some previous studies (Marpalle et al. 2014; Xu et al. 2014; Koca and Anil 2007; Roozegar et al. 2015a, b).

Multiple linear regression analysis was used to fit the data obtained for each response variable to linear, quadratic and cubic models. The most accurate model was chosen through sequential F-test and other adequacy measures such as, coefficients of determination (R^2), adjusted coefficients of determination (Adj.- R^2) and significant probabilities. Statistical significance of the coefficients in the model was found by analysis of variance (ANOVA) for each response. The coefficients of each model were given as coded values of the independent variables. For the

Table 1 Coded and real values of formulation factors used in full factorial design

Run	Coded value		Real value	
	X ₁	X ₂	BFs (g/100 g)	GFs (g/100 g)
1	0.50	0.50	15.00	15.00
2	1.00	−0.50	20.00	5.00
3	0.00	1.00	10.00	20.00
4	1.00	1.00	20.00	20.00
5	−1.00	0.00	0.00	10.00
6	−1.00	−0.50	0.00	5.00
7	0.00	0.50	10.00	15.00
8	0.00	−1.00	10.00	0.00
9	1.00	0.00	20.00	10.00
10	0.00	1.00	10.00	20.00
11	−1.00	−1.00	0.00	0.00
12	1.00	−1.00	20.00	0.00
13	−0.50	1.00	5.00	20.00
14	−0.50	0.00	5.00	10.00
15	1.00	0.50	20.00	15.00
16	−0.50	0.50	5.00	15.00
17	−0.50	−1.00	5.00	0.00
18	0.50	−0.50	15.00	5.00
19	0.00	0.00	10.00	10.00
20	−1.00	0.50	0.00	15.00
21	−1.00	1.00	0.00	20.00
22	0.50	0.00	15.00	10.00
23	0.50	−1.00	15.00	0.00
24	0.00	−0.50	10.00	5.00
25	−0.50	−0.50	5.00	5.00

BFs brown flaxseed flour, GFs golden flaxseed flour

analysis of the fitted mathematical models, only terms that were statistically significant ($p < 0.05$) were used. The combined effect of the factors, brown flaxseed flour and golden flaxseed flour on the responses, water absorption of flaxseed-wheat composite flour dough and Mixolab parameters was modeled using a polynomial response surface.

The software, state-ease design expert 7.0.0 (trial version) was used for model generation, test of model adequacy, contour plot generation for each response variable, and to generate the optimum levels of brown flaxseed flour and golden flaxseed flours which may be added in wheat flour. The optimal values of the factors were obtained by the desirability function proposed by Derringer and Suich (1980). The desirability function is an efficient method which can optimize multiple responses simultaneously at one best setting of factors. The approach is to first transform each response variable into an individual desirability

function, d_n that ranges between 0, for a completely undesired response, and 1, for a response that is at its goal or target. The individual desirability functions are then combined into a single composite response, which gives the total desirability function, D ($0 < D < 1$) defined as the geometric mean of the individual desirability function d_n , expressed as:

$$D = (d_1 \times d_2 \times \dots \times d_k)^{(1/k)} \quad (1)$$

where d_n , $n = 1, 2, \dots, k$ is the individual desirability function and k is the number of the responses.

Determination of rheological behavior of flaxseed-wheat composite flour by using the Mixolab device

Mixing and pasting behavior of flaxseed-wheat composite flour were studied using the Mixolab (Chopin, Tripette et Renaud, Paris, France) which measures the dough rheological behavior subjected to dual mixing and temperature constraints. Mixolab characteristics were determined according to ICC standard method no. 173 (ICC 2010). The device was set for the following parameters: mixing temperature 30 °C, heating rate 4 °C/min and total time to run the analysis 45 min. From the Mixolab curves the following mixing parameters were determined: water absorption (the percentage of water required to reach the maximum consistency torque C1 of 1.1 ± 0.07 N m), development time (time elapsed before maximum consistency torque C1 reached during the mixing) and stability (time in min until the loss of consistency does not exceeds 11% of the C1 torque). When the dough temperature increases due to the protein weakening the minimum consistency during stage 2 (C2 in N m) was recorded. When the dough temperature increases to certain values, the starch characteristics were recorded through the values of maximum consistency during stage 3, C3 torque (N m), minimum consistency during stage 4, C4 torque (N m) and maximum consistency during stage 5, C5 torque (N m). Also the difference between the C1 and C2 torques (N m) representing the protein weakening, the difference between the C2 and C3 torques (N m) representing the starch gelatinization, the difference between the C3 and C4 torques (N m) representing starch breakdown and the difference between the C5 and C4 torques (N m) representing the starch recrystallization during paste cooling were recorded.

Epifluorescence light microscopy (EFLM)

The control sample and the sample with BFS at 4.81/100 and GFs at 5.07/100 g addition was analyzed by using EFLM and they were prepared and processed according to Peighambardoust et al. (2010), Codină and Mironeasa

(2013), Mironeasa et al. (2016). The samples were stained in a solution of 0.5/100 g fluorescein and 1/100 g rhodamine B in 2-methoxyethanol for at least 1 h. Fluorescein will preferentially stain starch and rhodamine B will preferentially stain proteins. EFLM images acquired in 1280×1024 pixels (300 dpi) were recorded at Motic AE 31 Epifluorescence Light Microscope (Motic, Optic Industrial Group Co. Ltd., Xiamen, People's Republic of China). The ImageJ processing program (version 1.45, National Institutes of Health, Bethesda, MD, USA) was used to quantify the images obtained.

Results and discussion

Model fitting

Twenty-five experiments were performed according to the experimental design to determine the combined effect of two independent variables, brown flaxseed flour and golden flaxseed flour on water absorption and Mixolab rheological properties of the dough. The quadratic and cubic regression models respectively were highly significant for the response variables with a satisfactory coefficient of determination (R^2) that varied from 0.61 to 0.99 (Table 2). The models fitted for the dough water absorption and Mixolab rheological parameters are suitable with significant regression and satisfactory R^2 and $Adj.-R^2$ values. The results of the analysis of variance (ANOVA), including significantly regression coefficients ($p < 0.05$) expressed in term of coded values, R^2 , $Adj.-R^2$ and p values are shown in Table 2.

Based on the mathematical equations obtained, the response can be predicted within the experimental area and showed as a response surface.

Effects of formulation samples on dough water absorption

Water absorption (CH) of flaxseed-wheat composite flour dough ranged from 51.40 to 56.00/100 g. Table 2 shows the effects of formulation factors, brown flaxseed flour (BFs) and golden flaxseed flour (GFs) on CH of flaxseed-wheat composite flour dough exhibited as their corresponding regression coefficients in the quadratic model. The accuracy test of the model (Table 2) indicates that the quadratic model can predict adequately the CH as a function of the formulation factors. The equation indicated that BFs and GFs had significant ($p < 0.05$) effects on CH. By ANOVA, the correlation coefficient value (R^2) of this model was determined to be 0.95, while the adjusted determination coefficient value ($Adj.-R^2$) was 0.94. These values showed that the regression model defined well the

real behavior of the dough among water absorption. The negative coefficient of BFs and GFs indicated that the water absorption of flaxseed-wheat composite flour dough increased with decrease levels of BFs and GFs addition in wheat flour.

The effects of different levels addition of BFs and GFs in wheat flour on CH of flaxseed-wheat composite flour dough is shown in Fig. 1a. The contour plot indicates that with the increased level of BFs and GFs addition in wheat flour, the CH decreased. These results are in disagreement with the results obtained by Xu et al. (2014), Koca and Anil (2007), Marpalle et al. (2014). However, Najila (2015) found no significant changes in water absorption with the increase level of flaxseed and Roozegar et al. (2015a, b) found a decrease in water absorption with the increase of flaxseed addition similar with results obtained by us. It seems that water absorption value depends on flaxseed particle size, lower size increasing the values of dough water absorption (Hall et al. 2008; Tulbek and Hall 2006). This may be due to the larger particle size phenomenon (therefore lower surface area) of the flaxseed flour in comparison to that of wheat flour. Also according to Kundu et al. (2014) a decrease in CH may be attributed to the gluten dilution which requires less hydration and, therefore, a lower quantity of water added.

CH was not significantly correlated ($p > 0.05$) with quadratic effect of golden flaxseed (Table 2), while the quadratic effect of brown flaxseed on CH observed in the present study was significant ($p < 0.05$) and highlights the importance of adding an optimal amount of brown flaxseed to achieve desirable flaxseed-wheat composite flour.

Effects of formulation samples on the Mixolab rheological parameters

Effects of formulation samples on DT

The effect of BFs and GFs addition in wheat flour on the dough development time (DT) exhibited as their corresponding regression coefficients in the quadratic models are shown in Table 2. The test for the precision of the model indicates that the equations can be suitable to predict the DT as a function of the formulation factors. The R^2 and $Adj.-R^2$ values were satisfactory to confirm the significance of the model. The quadratic model showed that BFs and GFs had significant ($p < 0.05$) effects on DT of flaxseed-wheat composite flours which ranged from 0.57 to 1.57 min. The statistical analysis of the coefficients of the model showed that linear terms were significant while the interaction coefficients were non-significant ($p > 0.05$) (Table 2). It indicates that each factor individually, BFs and GFs level addition affected the response variable.

Fig. 1 The response surface plots of: **a** water absorption (CH); **b** development time (DT); **c** stability (ST); **d** C2 torque (C2); **e** difference between torques C1 and C2 (C1–2); **f** C3 torque (C3); **g** difference between torques C3 and C2 (C3–2); **h** C4 torque (C4); **i** difference between torques C31 and C4 (C3–4); **j** C5 torque (C5); **k** difference between torques C5 and C4 (C5–4) as affected by the levels of brown flaxseed (BFs) and golden flaxseed (GFs) incorporated in wheat flour

Figure 1b is a response surface plot showing the effect of BF_s and GF_s addition in wheat flour on DT. The levels of BF_s and GF_s addition in wheat flour have a negative linear effect on DT, while the interaction effect between BF_s and GF_s was insignificant ($p > 0.05$) on DT (Table 2). Compared to the level of GF_s addition in wheat flour, BF_s (−0.240) has a higher influence on DT of the composite flours. According to the response surface plot (Fig. 1b), the DT decreased as the BF_s and GF_s levels increased. The decrease in DT may be correlated with the decrease of water absorption which indicates that dough absorbs less water and, therefore, it requires less mixing time. Also, the addition of a non-gluten flour leads to a dilution of gluten which decreases its quantity and quality and, therefore, the DT value which is a measure of dough strength (the lower it is the weaker the dough is).

Effects of formulation samples on ST

The stability of the dough (ST) was significantly influenced ($p < 0.0001$) by the levels of BF_s and GF_s addition in wheat flour. Regression model (Table 2) for ST showed a significant effect in linear term of BF_s, GF_s, and of the interaction terms between BF_s and GF_s. Also, the model showed a significant effect of quadratic term of BF_s while the quadratic term of GF_s were non-significant ($p > 0.05$). The statistical significance of the quadratic term of brown flaxseed level addition in wheat flour as well of its linear term highlights a higher effect of this factor on the dough stability. Major negative effects on dough stability were given by BF_s and interaction between BF_s and GF_s. The result of the F-test for ANOVA showed that the regression model for ST is statistically significant ($p < 0.0001$) and the R^2 value showed that the model explains 86% of the obtained data variation.

A contour plot for the stability (Fig. 1c) showed that the dough stability significantly decreased with an increase in BF_s and GF_s levels of composite flours. This behavior was probably due to the gluten dilution as a result of an addition of non-gluten flour which reduces the dough viscoelastic properties (Mohammed et al. 2014). At high doses of flaxseed addition the gluten matrix may present physical interruption that can cause the dough weakening. A decrease of dough stability with the increase dose of flaxseed addition was reported by Koca and Anil (2007), Xu et al. (2014). However at low doses of flaxseed addition, the dough stability increases in agreement with results obtained by Roozegar et al. (2015a, b). This is probably due to flaxseed gum which causes a continuous phase of the dough gluten that appears more uniform and dense than the dough without flaxseed addition. Meral and Dogan (2013) also reported that flaxseed addition may increase or decrease dough stability depending on the level in which it was incorporated.

Effects of formulation samples on C2

The ANOVA results (Table 2) indicated that BF_s, GF_s and the interaction between these variables (BF_s × GF_s) have significant negative effects on the C2 torque. Non-significant quadratic effect of BF_s and GF_s were observed on C2 torque. As for C2 torque, a high coefficient of determination ($R^2 = 0.91$) was obtained for the quadratic model, statistically significant at $p < 0.0001$, showed that this model is a predictive one for this variable. A negative effect on C2 torque was provided by the linear and interaction regression coefficients, suggesting that the increased levels of BF_s and GF_s addition in wheat flour induced a decrease in C2 torque (Fig. 1c). The decrease in C2 with the increase of factors levels may be due to lower water availability in the dough system due to the fact that the water absorption capacity of the dough decreases with the increase level of BF_s and GF_s addition. Due to the increase temperature of the dough the proteins begin to denature and to lose their capacity to hold water which is retained by the starch granules. Because the starch gelatinization process does not coincide with protein denaturation phenomenon dough consistency decreases proportionally with the increase levels of BF_s and GF_s addition in wheat flour (Fig. 1d). The results were similar to those obtained by Tulbek and Hall (2006).

Effects of formulation samples on C1–2

The protein network strength under increased heating, represented by the difference between torques C1 and C2 (C1–2), indicated that the quadratic model fitted to the experimental results of C1–2, having a high coefficient of determination ($R^2 = 0.91$). The BF_s and GF_s variables have significant effect on the C1–2, while the quadratic terms of these variables were not significant. The results (Table 2) showed an increase in C1–2 as the BF_s, GF_s values and their interaction (BF_s × GF_s) increased. Figure 1e showed the effect of BF_s and GF_s levels added in wheat flour on protein network strength under increasing heating.

Effects of formulation samples on C3

Through ANOVA, the cubic model was found to fit adequately the experimental data for C3 torque. The independent variables, taken individually, BF_s and GF_s respectively, and the interaction between these ones, exhibited significant effect on C3 torque. Also, the quadratic term of BF_s level addition in wheat flour had a significant effect ($p < 0.05$) on C3 torque, while the quadratic term of GF_s did not show significant effect ($p > 0.05$) on C3 torque. The negative sign of the significant coefficients

included in the regression model showed that C3 decreased with the increase of BF_s and GF_s levels addition to wheat flour. According to Fig. 1f, higher levels of BF_s (20) and GF_s (20%) addition in wheat flour lead to a lower value for C3 torque (1.02 N m). By competing for water, flaxseed addition modified the starch gelatinization, causing some discontinuities in the dough matrix resulting in a decrease of dough viscosity. This may be attributed due to an increase in the protein content from the dough system which decreases the dough viscosity because of their protective effect on starch granular integrity (Kaur et al. 2016). Also due to the flaxseed high content in lipids in the dough system, some complexes protein–lipid or starch–lipid complexes can be formed. If lipids are available when starch begins to gelatinize the starch granule surface may be coated with insoluble inclusion complex, thus preventing the leaching of amylase from the complex. It is known the fact that the gelatinization can take place only if amylase molecule can leach out from the starch granules (Larsson 1982). Therefore by addition of raw materials with high lipid content in wheat flour, starch gelatinization process is inhibited, delaying the swelling of the starch granules.

Effects of formulation samples on C3–2

The effects of brown flaxseed and golden flaxseed levels addition in wheat flour on the difference between torques C3 and C2 (C3–2) expressed as their corresponding regression coefficients in the quadratic regression model are shown in Table 2. The linear term of GF_s level influenced significantly C3–2, while the BF_s level was not significant. The quadratic coefficient of BF_s indicated a significant negative influence on the C3–2. The cubic regression model represents adequately the experimental results of C3–2, the coefficient of determination value ($R^2 = 0.95$) confirming the adequacy of the model. The effect of BF_s and GF_s levels addition in wheat flour can be seen in Fig. 1g, indicating a decrease in C3–2. This can be due to the high content of lipids in flaxseed knowing that starch gelatinization decreased with increasing amounts of lipids (Lauro et al. 2000).

Effects of formulation samples on C4

The effects of formulation factors, brown flaxseed level and golden flaxseed level addition in wheat flour on C4 torque expressed as their corresponding regression coefficients in the quadratic regression model are shown in Table 2. C4 torque was significantly dependent ($p < 0.05$) on the levels of BF_s and GF_s addition in wheat flour. Also, the interaction between BF_s and GF_s variables was found to have a negative influence on the C4 parameter. For C4 torque, the quadratic regression model obtained was an

adequate one, indicating a good R^2 value of 0.82. The C4 torque of flaxseed-wheat composite flour dough ranged from 0.77 to 1.43 N m. The lowest C4 torque value (0.77 N m) was obtained at higher levels of BF_s (15/100) and GF_s (20/100 g) addition in wheat flour, whereas the highest value (1.17 N m) was obtained at lower levels of BF_s (5/100) and GF_s (5/100 g). As can be seen in Fig. 1h, along with the increased levels of BF_s and GF_s, the C4 torque and the stability of the starch gel formed were decreased. This may be due to the from the soluble fiber present in the flaxseed content which binds water by the hydrogen bounds, causing a decrease of available water for the starch granules. Also, the dough network stability decreased due to the high content of oil from flaxseed content and due to the starch dilution, taking into account that flaxseed contain low amount of starch.

Effects of formulation samples on C3–4

The cubic regression model was used to determine the difference between torques C3 and C4 (C3–4). C3–4 was not significantly associated ($p > 0.05$) with either BF_s or GF_s, however the interaction between BF_s or GF_s (BF_s × GF_s) was significant ($p < 0.05$) (Table 2). The cubic term of BF_s had highly significant negative effect, while the interaction term between BF_s and GF_s levels addition in wheat flour had a significant positive effect on C3–4, indicated the ability of the interaction term to increase the response variable C3–4. As for C3–4 parameter the coefficient of determination (R^2) is 0.61 indicating that the model explains only 0.61% of the observed data variation. The effect of BF_s and GF_s levels addition in wheat flour on C3–4 is represented in Fig. 1i.

Effects of formulation samples on C5

Cubic model was fitted for C5 torque (Table 2). Analysis of variance (ANOVA) shows that the selected cubic model is well adjusted to the experiment data obtained for C5 torque. The regression model was highly significant, showing higher coefficient of determination ($R^2 = 0.96$). The negative linear effect of BF_s and GF_s were observed on C5 torque, indicating low retrogradation and recrystallization. The response surface obtained for C5 torque (Fig. 1j) showed that the increasing of BF_s level together with the increasing of GF_s level added in wheat flour decreased C5 torque. The results were according to those obtained by Tulbek and Hall (2006).

Effects of formulation samples on C5–4

The cubic regression model which was obtained for the differences between torques C5 and C4 (C5–4) showed good

correlation coefficient ($R^2 = 0.75$). It is clear from Table 2 that C5–4 was influenced significantly ($p < 0.05$) by the interaction term between quadratic term of BFs and GFs. The effect of BFs and GFs levels addition in wheat flour on C5–4 is shown in Fig. 1k. The response surface plot illustrated that the lowest value for C5–4 (0.44 N m) was obtained at maximum levels of BFs (20/100) and GFs (20/100 g) addition in wheat flour. It seems that flaxseed addition limits starch retrogradation and, therefore, it preserves bread freshness. The delay of bread staling by flaxseed addition was also found by Montes et al. (2008), Khorshid et al. (2011).

Optimization

Multiple response optimizations were based on generating a solution that showed the optimum levels of independent variables to achieve the desired response goals. Dough stability and C3–2 were desired maximal, C2, C3, C4, C4–5 and C5 in the range, whereas C1–2 and C5–4 were specified as minimum desirable. The optimization method called desirability was applied to models fitted in this study to optimize multiple responses. The desirability function incorporated desires and priorities for each of the variables established as the basis of optimization.

The optimal values of variables (Fig. 2) leading to the global desirability value (0.635) for the formulation of flaxseed-wheat composite flour are BFs = 4.81/100 and GFs = 5.07/100 g, with predicted response variables of 55.07/100 g for CH, 1.28 min for DT, 10.04 min for ST, 0.41 N m for C2, 0.69 for C1–2, 1.56 N m for C3, 1.14 for C3–2, 1.33 N m for C4, 0.20 for C3–4, 1.97 N m for C5 and 0.60 for C5–4.

The results for optimization suggested that flaxseed-wheat composite flour containing 4.81/100 g BFs and 5.07/100 g GFs could be a good mixture of these flaxseeds to achieve the best flaxseed-wheat composite flour bread quality.

Fig. 2 The contour plot of optimum levels of brown flaxseed and golden flaxseed added in wheat flour

Effect of flaxseed on the dough microstructure

EFLM method was successfully used to study dough microstructure with and without flaxseed flour addition, showing how protein and starch were distributed. As seen in Fig. 3a, b the high fluorescent intensity in starch granules is due to the main affinity of fluorescein isothiocyanate (FITC) with starch which can be non-covalently labeled. Rhodamine B may also present affinity with starch granules, but it shows more affinity with proteins and in a protein–starch system (like dough) labeling the protein by non-covalent bindings (Peighambardoust et al. 2010). The two different microstructure of dough samples (the control one and those with the BFs 4.81/100 and GFs = 5.07/100 g addition) were

Fig. 3 Microstructure taken by EFLM of wheat dough with **a** A1 0% flaxseed flour, **b** B1 brown flaxseed flour at 4.81/100 g and golden flaxseed flour at 5.07/100 g. Green are starch granules, while red is protein. Images A1 and B1; 1×1 mm, bars 50 μ m are higher magnification images of **a**, **b**; 2×2 mm, bars 100 μ m at position indicated by the squares (color figure online)

Table 2 Effects of formulation factors, expressed as their corresponding coefficients in the predictive models for water absorption of flaxseed-wheat composite flour dough and Mixolab parameters

Factors ^a	Parameters										
	CH (g/100 g)	DT (min)	ST (min)	C2 (N m)	C1–2 (N m)	C3 (N m)	C3–2 (N m)	C4 (N m)	C3–4 (N m)	C5 (N m)	C54 (N m)
Constant	54.010	1.070	9.500	0.38	0.720	1.430	1.060	1.220	0.210	1.82	0.6
BFs	−2.380	−0.240	−0.810	−0.054	0.054	−0.140	ns	−0.130	ns	−0.21	ns
GFs	−1.740	−0.180	−1.130	−0.050	0.050	−0.120	−0.075	−0.130	ns	−0.12	ns
BFs × GFs	−1.040	ns	−0.770	−0.038	0.038	−0.026	ns	−0.082	0.056	−0.061	ns
BFs ²	−1.290	ns	−0.630	ns	ns	−0.034	−0.036	ns	ns	ns	ns
GFs ²	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
BFs ² × GFs	−	−	−	−	−	−0.100	−0.097	−	ns	−0.085	−0.062
BFs × GFs ²	−	−	−	−	−	−0.085	−0.069	−	ns	−0.087	ns
BFs ³	−	−	−	−	−	ns	ns	−	−0.120	0.110	ns
GFs ³	−	−	−	−	−	ns	ns	−	ns	ns	ns
R ²	0.95	0.95	0.86	0.91	0.91	0.97	0.95	0.82	0.61	0.96	0.75
Adj.-R ²	0.94	0.94	0.82	0.89	0.89	0.95	0.92	0.78	0.38	0.93	0.60
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0464	<0.0001	0.0027

Only values of significant coefficients are presented (95% confidence level); ns no significant effect at the 5% level

^a BFs brown flaxseed flour (g/100 g), GFs golden flaxseed flour (g/100 g), R² Adj.-R² are measures of fit of the model

CH water absorption, DT development time, ST stability, C2 minimum torque-measure of protein weakening, C1–2 deference between torques C1 and C2, C3 maximum torque-measure of starch gelatinization, C3–2 deference between torques C3 and C2, C4 minimum torque-measure of starch gel stability, C3–4 deference between torques C3 and C4, C5 maximum torque-measure of starch retrogradation, C5–4 deference between torques C5 and C4

confirmed by EFLM images. The images were taken at two magnifications to observe better the changes in the dough microstructure. At higher magnifications, dough microstructure is more evident. In the control sample (Fig. 3a) the protein domain is interconnected with the starch granules which surround it. Wheat flour dough appears like a continuous and homogeneous matrix with starch granules surrounded by a protein matrix, probably because the wheat gluten matrix is its major determinant. In the presence of flaxseed (Fig. 3b) more areas rich in proteins are visible and the dough microstructure presents a higher non-homogeneity. The protein phase is unevenly distributed and starch granules are separated from the protein matrix. At high magnification (Fig. 3B1) the discontinuous structure of dough seemed to be higher due to black holes in the flaxseed-wheat dough microstructure. This may be due to the flaxseed content which interacts with the wheat gluten and starch. This interaction may interrupt the dough matrix continuity and damages it mechanically. The images showed a decrease in the starch–protein interaction.

Conclusion

Response surface methodology is an efficient statistical tool able to model the influence of golden and brown flaxseed on flaxseed-wheat composite flour dough properties. Wheat flour replacement at different levels with

flaxseed decreases the water absorption and changed the thermo-mechanical properties of flaxseed-wheat dough. The Mixolab parameters of dough stability increased at low levels of flaxseed addition and decreased at high ones. Our results indicated that by flaxseed addition, especially at high doses, the dough became weaker by a decrease of dough development time and C2 torque and it limited starch retrogradation by the C5 torque and the difference between points C5 and C4 decreased.

The wheat dough microstructure analyzed by EFLM for the optimal levels established by response surface methodology was changed significantly by flaxseed addition. A continuous matrix was not found in the flaxseed addition sample as compared with the one obtained without flaxseed addition. This effect is more evident at high magnification where the black holes in the dough microstructure were more evident.

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