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Rheological Approaches of Wheat Flour Dough Enriched with Germinated Soybean and Lentil

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Abstract: Germination is a convenient technique that could be used to enhance the nutritional profile of legumes. Furthermore, consumers' increasing demand for diversification of bakery products represents an opportunity to use such germinated flours in wheat-based products. Thus, this study aimed to underline the effects of soybean germinated flour (SGF) and lentil germinated flour (LGF) on the rheological behavior of dough during different processing stages and to optimize the addition level. For this purpose, flour falling number, dough properties during mixing, extension, fermentation, and dynamic rheological characteristics were evaluated. Response surface methodology (RSM) was used for the optimization of SGF and LGF addition levels in wheat flour, optimal and control samples microstructures being also investigated through epifluorescence light microscopy (EFLM). The results revealed that increased SGF and LGF addition levels led to curve configuration ratio, visco-elastic moduli, and maximum gelatinization temperature rises, while the falling number, water absorption, dough extensibility, and baking strength decreased. The interaction between SGF and LGF significantly influenced ($p < 0.05$) the falling number, dough consistency after 450 s, baking strength, curve configuration ratio, viscous modulus, and maximum gelatinization temperature. The optimal sample was found to contain 5.60% SGF and 3.62% LGF added in wheat flour, with a significantly lower falling number, water absorption, tolerance to kneading, dough consistency, extensibility, and initial gelatinization temperature being observed, while dough tenacity, the maximum height of gaseous production, total CO_2 volume production, the volume of the gas retained in the dough at the end of the test, visco-elastic moduli and maximum gelatinization temperatures were higher compared to the control. These results underlined the effects of SGF and LGF on wheat dough rheological properties and could be helpful for novel bakery products development.

Keywords: germination; lentil; soybean; wheat flour; rheology; microstructure

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1. Introduction

Bread is the most consumed food product in the world, with its contribution to the human diet having the greatest importance [1]. However, because most people prefer white bread, the nutritional benefits are limited because it is obtained from refined wheat flour [2,3]. Moreover, due to the large quality variation of wheat flour used in bread-making, bakery producers usually add different types of additives to wheat flour, especially chemicals, to improve product quality from a technological point of view [4]. Nowadays, one of the trends in bread making is to improve the nutritional quality of bread by substituting wheat flour with other flour types without affecting the quality of the final products. Specialist attempts are made to balance the content of vitamins, minerals, and fibers lost through wheat refining without adding any chemical compound to the bakery products [5]. Moreover, an attempt is made to improve the quality of proteins in baked goods by adding various ingredients which contain essential amino acids that are deficient in wheat flour [6,7]. To correct these nutritional deficiencies, different grain flours can be used in bread making, such as legumes, oilseeds, pseudocereals, etc. [5,7,8].

The substitution of wheat flour with different legumes types improves the nutritional value of bread by decreasing the glycemic index and by increasing the fiber, mineral content, and protein quality [9]. However, the addition of legume flours to wheat flour has the disadvantage that they contain some antinutritive factors, such as tannins, phytates, or trypsin inhibitors which may affect the nutritional value of bread [8]. Furthermore, the addition level in wheat flour is limited due to their gluten dilution effect which affects dough viscoelastic structure and its ability to retain gases during fermentation which can lead to bakery products of poor quality [7]. Thus, it is more convenient to use legume flours in a germinated form, a process that has a positive effect on the nutritional profile of legumes, but also their sensory profile. During legume germination, many positive changes occur, as follows: a decrease in antinutritive factors contained in legumes, an increase in protein content, an improvement in the availability of sodium, magnesium, iron, zinc, a decrease in lipids and carbohydrates content [10–12]. Additionally, the amount of volatile organic compounds, such as 2-methylbutanal and dimethyltrisulfite increases which leads to the intensification of legume grains' flavor. At the same time, their sweetness intensifies [13], improving the sensory characteristics. In addition, during germination the enzymatic activity of legume grains increases which may improve wheat flour quality if it is enzymatically deficient without adding other chemical additives during bread making [14].

Two legumes with a superior nutritional profile that may be used in bread making are lentil and soybean. Lentils boast a significant content of vitamins, minerals, carbohydrates, dietary fiber, and a low glycemic index [15]. Lentils are also rich in proteins (21–31%) which contain all essential amino acids (39.3 g of essential amino acids per 100 g of protein); they are a source of glutamic acid, lysine, arginine, leucine, acid aspartic [16]. Lentils also contain many micronutrients, of which vitamin B9, zinc, and iron have the highest weight. Lentils contain the highest amount of polyphenols, compared to all other vegetables [17]. Regarding health aspects, medical studies have shown that the consumption of lentils has benefits on cardiovascular diseases and cancer prevention [18], but also has implications in promoting slow and moderate postprandial blood glucose increase [19,20]. Soybean is boasted as having a significant amount of high-quality protein (38–55%), essential amino acids, lipids (20%), and carbohydrates (27%) [21–23]. The phytochemicals present in soybean are of great interest for health because studies have shown that they lower the amount of cholesterol, have an anticarcinogenic capacity, and contribute to bone health [24].

Soybean and lentil flour often appear in specialized studies due to the possibility of being used as an ingredient in various bakery products [1,7]. The use of lentil and soybean flours as partial substitutes in wheat flour is justified by both nutritional and sensory aspects. Their use, particularly in the germinated form, may improve the quality of bakery products even from a technological point of view, especially if the wheat flour used has enzymatic deficiencies. According to the results obtained by Zhang et al. [25], native red lentil flour incorporation in wheat dough led to higher water absorption and mechanical weakening, while dough development time, stability and minimum torque, and cooking stability were lower compared to the control and increased with the addition level, due to the influence of the chemical compounds of the ingredient added. In the study of Marchini et al. [26] on the effects of lentil flour in the wheat dough, it is stated that the addition level increase caused water absorption rises, dough stability reduction, delayed protein weakening, and worsening of dough pasting consistency which could be related to the lower swelling power of pulses compared to wheat. The incorporation of germinated lentil flour in Sangak bread determined water absorption, dough development time, and softness degree increases compared to the control, while the stability of dough did not differ significantly. Wheat bread fortification with defatted soybean led to higher water absorption and dough extensibility and lower dough stability compared to the control according to results presented by Mashayekh et al. [27]. The addition of native and germinated soybean flours in wheat dough increased water absorption, maximum consistency time, and dough stability, while dough maximum resistance to extension and extensibility was not significantly affected [28]. The rise of the 7S protein fraction extracted from native and germinated soybean in wheat dough was related to the increment of water absorption and extensographic maximum resistance to extension, especially in the case of protein extracted from native soybeans [29].

This study aimed to optimize the formulation of germinated soybean and lentil flours that can be added to refined wheat flour of low alpha-amylase activity to improve dough rheological properties. For the optimal combination between the soybean and lentil germinated flour, the dough microstructure was analyzed by using epifluorescence light microscopy (EFLM). To our knowledge, no other studies have examined soybean and lentil germinated flour addition to wheat flour in a combined form. The importance of their use in bread making derives from the valuable nutritional composition of these legumes, but also the technological advantages of their use in a germinated form.

2. Materials and Methods

2.1. Materials

Refined wheat flour of the 650 type (harvest 2020) provided by the S.C. Dizing S.R.L. company (Brusturi, Neamt, Romania) was used. Germinated legume flours were obtained from lentil (*Lens culinaris Merr*) and soybean (*Glycine max* L.) which were germinated for 4 days, lyophilized and milled before they were used in the wheat flour according to the method reported in our previous studies [12,14].

The flours were analyzed according to the international ICC standard methods: ash content (ICC 104/1), moisture content (ICC 110/1), fat content (ICC 136), protein content (ICC 105/2) [30]. To be certain that the germinated soybean (SGF) and lentil (LGF) flour may be used in bread making, they were analyzed also from a microbiological point of view according to the following methods: molds and yeast according to SR ISO 7954:2001 [31], mycotoxins by using an ELISA kit (Prognosis Biotech, Larissa, Greece) and *Bacillus cereus* according to the SR EN ISO 7932:2005 [32]. The wheat flour has been also analyzed for its wet gluten content and gluten deformation index according to SR 90:2007 method [33].

Soybean germinated flour (SGF) at ratios of 5, 10, 15, and 20% and lentil germinated flour (LGF) at ratios of 2.5, 5, 7.5, and 15% were mixed with wheat flour and coded as SGF 5, SGF 10, SGF 15, SGF 20 and LGF 2.5, LGF 5, LGF 7.5 and LGF 10, respectively. Wheat flour without SGF or LGF was used as a control (C).

2.2. Dough Rheological Properties

2.2.1. Empirical Dough Rheological Properties during Mixing and Extension

Dough rheological properties during mixing and extension were analyzed by using an Alveo Consistograph (Chopin Technologies, Cedex, France) according to ICC 171 and ICC 121 standards [30] respectively. The Consistograph test was made to determine dough rheological properties during mixing: water absorption capacity (WA), tolerance to kneading (Tol), consistency of the dough after 250 s (D250), and 450 s (D450). The Alveograph test was performed to determine dough rheological properties during extension: maximum pressure (P), dough extensibility (L), baking strength (W), and configuration ratio of the Alveograph curve (P/L).

2.2.2. Empirical Dough Rheological Properties during Fermentation and Falling Number

Empirical dough rheological properties during fermentation were analyzed using the Rheofermentometer device (Chopin Rheo, type F3, Villeneuve-La-GarenneCedex, France) according to the standard method AACC89–01.01 [34]. The Rheofermentometer parameters analyzed for the dough samples obtained by kneading of 250 g mixed flours, 7 g compressed yeast of the *Saccharomyces cerevisiae* type, and 5 g salt according to the Consistograph water absorption value were: the total CO_2 volume production (VT, mL), the maximum height of gaseous production (H′m, mm), volume of the gas retained in the dough at the end of the test (VR, mL) and retention coefficient (CR, %). The falling number values expressed in s were determined by using a Falling number device (FN 1305, Perten Instruments AB, Stockholm, Sweden) according to ICC 107/1 method [30].

2.2.3. Dynamic Dough Rheological Properties

The dynamic dough rheological properties were obtained with a HAAKE MARS 40 device (Termo-HAAKE, Karlsruhe, Germany) with a 2 mm gap and parallel plate geometry of 40 mm diameter, according to previous works [14,35,36]. The dough samples were placed between rheometer plates and analyzed for the storage modulus (G′), loss modulus (G″), and loss tangent (tan δ) at a frequency of 1 Hz. Additionally, the maximum gelatinization temperatures were analyzed for the dough samples during heating from 25 to 100 °C at a rate of 4 °C per min at a fixed strain of 0.001 and a frequency of 1 Hz.

2.3. Dough Microstructure

The epifluorescence light microscopy (EFLM) images of dough with and without the best combination between the soybean and lentil germinated flour addition in wheat flour were analyzed with a Motic AE 31 (Motic, Optic Industrial Group, Xiamen, China) equipped with catadioptric objectives LWD PH 203 (N.A. 0.4). The images and dough samples preparation were obtained according to methods reported in our previous studies [14,37,38]. The dough sample was immersed in a fixing solution made of 1% rhodamine B for protein coloring and 0.5% fluorescein for starch coloring for at least 1 h.

2.4. Statistical Analysis

All the measurements were done in duplicate. Analysis of variance (ANOVA) was applied to compare mean values of the samples with SGF and LGF respectively, at different addition levels. Statistically significant differences were considered at $p < 0.05$ by the Tukey test. For this purpose, XLSTAT for Excel 2021 version (Addinsoft, New York, NY, USA) software was used.

Then, an experimental design was performed to identify single and combined effects of factors on the responses. The study of SGF and LGF addition levels effects on wheat dough characteristics and the optimization were performed on a trial version of Design Expert software (Stat-Ease, Inc., Minneapolis, MN, USA). A full factorial design with two factors varied at five levels, SGF addition at 0, 5, 10, 15, and 20% and LGF addition at 0, 2.5, 5, 7.5, and 10%, and Response Surface Methodology (RSM) with a two-factor interaction (2FI) model were used. The responses considered were the following: FN—falling number, WA—water absorption, Tol—tolerance to kneading, D250—dough consistency after 250 s, D450—dough consistency after 450 s, P—dough tenacity, L—dough extensibility, W—baking strength, P/L—curve configuration ratio, H′m—maximum height of gaseous production, VT—total CO_2 volume production, VR—the volume of the gas retained in the dough at the end of the test, CR—retention coefficient, G′—elastic modulus, G″—viscous modulus, tan δ—loss tangent, T_i—initial gelatinization temperature, T_{max}—maximum gelatinization temperature.

The effects of SGF and LGF addition levels on dough properties were evaluated through mathematical modeling. The most suitable model to predict data variation for each response was selected according to *F*-test results, coefficient of determination (R^2), and adjusted coefficients of determination (*Adj.*-R^2). The effects of factors and their interactions were underlined using Analysis of Variance (ANOVA), considering a significance level of 95%.

SGF and LGF addition levels optimization was done by applying the desirability function. The coded and real values of factors are listed in Table 1.

Table 1. Coded vs. real values of factors.

Run	Coded Values		Real Values	
	A	B	SGF (%)	LGF (%)
1	−1.00	−1.00	0.00	0.00
2	−1.00	−0.50	0.00	2.50
3	−1.00	0.00	0.00	5.00
4	−1.00	0.50	0.00	7.50
5	−1.00	1.00	0.00	10.00
6	−0.50	−1.00	5.00	0.00
7	−0.50	−0.50	5.00	2.50
8	−0.50	0.00	5.00	5.00
9	−0.50	0.50	5.00	7.50
10	−0.50	1.00	5.00	10.00
11	0.00	−1.00	10.00	0.00
12	0.00	−0.50	10.00	2.50
13	0.00	0.00	10.00	5.00
14	0.00	0.50	10.00	7.50
15	0.00	1.00	10.00	10.00
16	0.50	−1.00	15.00	0.00
17	0.50	−0.50	15.00	2.50
18	0.50	0.00	15.00	5.00
19	0.50	0.50	15.00	7.50
20	0.50	1.00	15.00	10.00
21	1.00	−1.00	20.00	0.00
22	1.00	−0.50	20.00	2.50
23	1.00	0.00	20.00	5.00
24	1.00	0.50	20.00	7.50
25	1.00	1.00	20.00	10.00

A: SGF—soybean germinated flour (%), B: LGF—lentil germinated flour (%).

The goals established for the factors and responses considered, along with their lower and upper limits are presented in Table 2. The differences among the optimal and control sample were tested using the Student-*t*-test, at a significance level of 95%, by using XLSTAT for Excel 2021 version (Addinsoft, New York, NY, USA) software.

Table 2. Factors and responses goals established for optimization.

Variable	Goal	Lower Limit	Upper Limit	Importance
A: SGF (%)	is in range	0.00	20.00	3
B: LGF (%)	is in range	0.00	10.00	3
WA (%)	is in range	50.70	54.30	3
Tol (s)	maximize	128.00	232.00	3
D250 (mb)	minimize	270.00	644.00	3
D450 (mb)	minimize	819.00	1117.00	3
P (mm)	is in range	88.00	132.00	3
L (mm)	is in range	25.00	75.00	3
W (10^{-4} J)	is in range	141.00	301.00	3
P/L (adim.)	is in range	1.38	5.04	3
G' (Pa)	is in range	29,290.00	72,310.00	3
G'' (Pa)	is in range	10,780.00	31,460.00	3
tan δ (adim.)	minimize	0.34	0.50	3
T_i (°C)	is in range	47.60	53.50	3
T_{max} (°C)	is in range	73.40	77.40	3
FN (s)	minimize	185.00	350.00	3

Table 2. *Cont.*

Variable	Goal	Lower Limit	Upper Limit	Importance
H′m (mL)	maximize	62.60	77.00	3
VT (mL)	maximize	1268.00	1886.00	3
VR (mL)	maximize	1070.00	1369.00	3
CR (%)	maximize	64.30	86.90	3

A: SGF—soybean germinated flour (%), B: LGF—lentil germinated flour (%), FN—falling number, WA—water absorption, Tol—tolerance to kneading, D250—dough consistency after 250 s, D450—dough consistency after 450 s, P—dough tenacity, L—dough extensibility, W—baking strength, P/L—curve configuration ratio, H′m—maximum height of gaseous production, VT—total CO_2 volume production, VR—the volume of the gas retained in the dough at the end of the test, CR—retention coefficient, G′—elastic modulus, G″—viscous modulus, tan δ—loss tangent, T_i—initial gelatinization temperature, T_{max}—maximum gelatinization temperature.

3. Results

3.1. Flour Characteristics

The wheat flour used in this study presented the following characteristics: moisture content of 14.6%, an ash content of 0.66%, the protein content of 12.3%, the fat content of 1.12%, wet gluten content of 30.4%, and gluten deformation index of 3 mm. The falling number value of the wheat flour was 356 s which indicates that it has a low α amylase activity [39]. The germinated lentil (LGF) presented 19.5% protein, 1.0% fat, 3.1% ash, 8.8% moisture, whereas the germinated soybean (SGF) presented 40.2% protein, 17.9% fat, 5.1% ash and 10.5% moisture. From a microbiological point of view, the germinated and lyophilized legumes samples were free of Bacillus cereus and presented 1 UFC/g yeast and molds. Mycotoxins values for SGF and LGF were the following: for zearalenone of 28.18 and 63.02 ppb respectively, for ochratoxin of 24.64 ppb and 18.53 ppb, and for aflatoxin less than 1.4 ppb. The microbiological data obtained recommend the use of germinated legume flours as ingredients in food products [40,41].

3.2. Effects of SGF and LGF Levels on Falling Number and Dough Rheology

SGF addition to wheat flour resulted in a significant decrease ($p < 0.05$) of Falling Number values as the level was higher and compared to the control, a similar trend was observed for LGF incorporation (Table 3). Dough mixing behavior in terms of water absorption, and tolerance to kneading showed significant reduction as the amount of SGF raised, while dough consistency parameters varied irregularly. Similar reduction trends of water absorption and tolerance to kneading were observed for LGF samples, while dough consistency parameters increased proportionally. Significant decreases ($p < 0.05$) of dough extensibility and baking strength were obtained as the levels of SGF or LGF were higher compared to the control (Table 3). Dough tenacity increased as the amount of SGF was raised, while LGF determined an opposite change, except for LGF 5. The curve configuration ratio values also increased in proportion with the SGF or LGF addition level, except for LGF 7.5. All the parameters listed above were influenced significantly ($p < 0.05$) by SGF or LGF incorporation.

Dough rheological parameters during fermentation, viscoelastic moduli and gelatinization temperatures were affected significantly ($p < 0.05$) by SGF or LGF level (Table 4).

The maximum height of gaseous production, total CO_2 volume production, and volume of the gas retained in the dough at the end of the test was reduced as the amount of SGF raised, the retention coefficient being changed irregularly. On the other hand, LGF caused an increase of dough maximum height of gaseous production and total CO_2 volume production, except for LGF 10 and a decrease in retention coefficient values, while the volume of the gas retained in the dough at the end of the test parameter exhibited an irregular trend. The elastic and viscous moduli increased significantly ($p < 0.05$) as the addition levels of SGF or LGF increased, while the loss tangent changes were irregular (Table 4). The maximum gelatinization temperature registered an increasing trend proportional to the SGF or LGF amount, while the initial gelatinization temperature raised only with LGF level, the opposite trend was observed for SGF.

Table 3. Falling number and rheological properties during mixing and extension of wheat dough with different addition levels of soybean germinated flour (SGF) and lentil germinated flour (LGF).

Sample	FN (s)	WA (%)	Tol (s)	D250 (mb)	D450 (mb)	P (mm)	L (mm)	W (10−4 J)	P/L (adim.)
C	350 ± 2.83 ^{aA}	54.3 ± 0.14 ^{aA}	214 ± 2.83 ^{bA}	394 ± 1.41 ^{aD}	943 ± 4.24 ^{aC}	104 ± 1.41 ^{dB}	72 ± 2.83 ^{aA}	301 ± 4.24 ^{aA}	1.44 ± 0.04 ^{dB}
SGF 5	323 ± 2.83 ^{b}	54.0 ± 0.14 ^{ab}	223 ± 5.66 ^{ab}	293 ± 2.83 ^{c}	881 ± 1.41 ^{b}	115 ± 1.41 ^{c}	53 ± 2.83 ^{b}	241 ± 4.24 ^{b}	2.17 ± 0.08 ^{c}
SGF 10	305 ± 2.83 ^{c}	53.7 ± 0.14 ^{ab}	232 ± 2.83 ^{a}	270 ± 5.66 ^{d}	819 ± 4.24 ^{d}	119.5 ± 0.71 ^{bc}	46 ± 1.41 ^{b}	219 ± 4.24 ^{c}	2.59 ± 0.09 ^{c}
SGF 15	275 ± 4.24 $_{c}$	53.4 ± 0.28 $_{bc}$	217 ± 4.24 $_{ab}$	272 ± 2.83 $_{d}$	858 ± 4.24 $_{c}$	124 ± 2.83 $_{ab}$	35 ± 2.83 $_{c}$	186 ± 2.83 $_{d}$	3.54 ± 0.21 $_{b}$
SGF 20	243 ± 1.41 $_{e}$	52.8 ± 0.14 $_{c}$	191 ± 5.66 $_{c}$	319 ± 2.83 $_{b}$	878 ± 1.41 $_{b}$	128 ± 2.83 $_{a}$	31 ± 1.41 $_{c}$	170 ± 4.24 $_{d}$	4.15 ± 0.07 $_{a}$
One-way ANOVA p values									
	$p < 0.0001$	$p < 0.003$	$p < 0.002$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
LGF 2.5	295 ± 4.24 ^{B}	53.7 ± 0.14 ^{B}	191 ± 5.66 ^{B}	406 ± 7.07 ^{CD}	1000 ± 7.07 ^{B}	109 ± 0.00 ^{B}	75 ± 1.41 ^{A}	285 ± 2.83 ^{B}	1.45 ± 0.03 ^{B}
LGF 5	274 ± 4.24 ^{C}	53.1 ± 0.14 ^{C}	177 ± 4.24 ^{BC}	418 ± 5.66 ^{BC}	1015 ± 7.07 ^{AB}	115 ± 1.41 ^{A}	68 ± 1.41 ^{AB}	269 ± 4.24 ^{C}	1.69 ± 0.01 ^{A}
LGF 7.5	252 ± 2.83 ^{D}	52.6 ± 0.14 ^{CD}	166 ± 5.66 ^{C}	435 ± 4.24 ^{B}	1020 ± 4.24 ^{AB}	91 ± 1.41 ^{C}	63 ± 1.41 ^{B}	183 ± 2.83 ^{D}	1.44 ± 0.06 ^{B}
LGF 10	229 ± 4.24 ^{E}	52.2 ± 0.00 ^{D}	161 ± 2.83 ^{C}	571 ± 4.24 ^{A}	1029 ± 5.66 ^{A}	88 ± 1.41 ^{C}	50 ± 1.41 ^{C}	173 ± 2.83 ^{D}	1.76 ± 0.08 ^{A}
One-way ANOVA p values									
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.002$

FN—falling number, WA—water absorption, Tol—tolerance to kneading, D250—dough consistency after 250 s, D450—dough consistency after 450 s, P—dough tenacity, L—dough extensibility, W—baking strength, P/L—curve configuration ratio. Soybean germinated flour (SGF) containing samples: a–e, mean values in the same column followed by different letters are significantly different ($p < 0.05$); Lentil germinated flour (LGF) containing samples: A–E, mean values in the same column followed by different letters are significantly different ($p < 0.05$).

Table 4. Empirical and dynamic rheological properties of wheat dough with different addition levels of soybean germinated flour (SGF) and lentil germinated flour (LGF).

Sample	H′m (mL)	VT (mL)	VR (mL)	CR (%)	G′ (Pa)	G″ (Pa)	tan δ (adim.)	T_i (°C)	T_{max} (°C)
C	65.9 ± 0.14 ^{cE}	1532 ± 4.24 ^{cE}	1228 ± 2.83 ^{bC}	80.15 ± 0.07 ^{bA}	29,290 ± 5.66 ^{eE}	10,780 ± 4.24 ^{eE}	0.368 ± 0.00 ^{bD}	51.9 ± 0.14 ^{aB}	73.4 ±0.28 ^{dD}
SGF 5	68.7 ± 0.14 ^{a}	1665 ± 5.66 ^{a}	1335 ± 1.41 ^{a}	80.15 ± 0.21 ^{b}	39,190 ± 4.24 ^{d}	13,440 ± 2.83 ^{d}	0.343 ± 0.00 ^{e}	51.4 ± 0.14 ^{a}	74.1 ± 0.28 ^{cd}
SGF 10	67.3 ± 0.14 ^{b}	1567 ± 2.83 ^{b}	1200 ± 2.83 ^{c}	76.55 ± 0.07 ^{c}	44,120 ± 5.66 ^{c}	16,670 ± 2.83 ^{c}	0.378 ± 0.00 ^{a}	49.7 ± 0.14 ^{b}	74.8 ± 0.14 ^{bc}
SGF 15	65.9 ± 0.14 ^{c}	1534 ± 4.24 ^{c}	1235 ± 2.83 ^{b}	80.45 ± 0.07 ^{b}	55,060 ± 2.83 ^{b}	19,750 ± 2.83 ^{b}	0.359 ± 0.00 ^{c}	48.9 ± 0.28 ^{c}	75.5 ± 0.14 ^{ab}
SGF 20	62.6 ± 0.14 ^{d}	1360 ± 7.07 ^{d}	1176 ± 2.83 ^{d}	86.45 ± 0.21 ^{a}	64,920 ± 2.83 ^{a}	23,050 ± 4.24 ^{a}	0.355 ± 0.00 ^{d}	47.6 ± 0.14 ^{d}	76.3 ± 0.14 ^{a}
One-way ANOVA *p* values									
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
LGF 2.5	69.1 ± 0.14 ^{D}	1631 ± 2.83 ^{D}	1281 ± 4.24 ^{B}	78.55 ± 0.35 ^{B}	31,480 ± 2.83 ^{D}	11,810 ± 4.24 ^{D}	0.375 ± 0.00 ^{B}	50.3 ± 0.00 ^{C}	74.6 ± 0.14 ^{C}
LGF 5	73.3 ± 0.14 ^{B}	1836 ± 5.66 ^{B}	1369 ± 5.66 ^{A}	74.50 ± 0.57 ^{C}	32,160 ± 5.66 ^{C}	12,500 ± 2.83 ^{C}	0.389 ± 0.00 ^{A}	52.3 ± 0.14 ^{B}	75.3 ± 0.14 ^{B}
LGF 7.5	77.0 ± 0.28 ^{A}	1886 ± 4.24 ^{A}	1282 ± 4.24 ^{B}	67.95 ± 0.35 ^{D}	40,600 ± 2.83 ^{A}	14,700 ± 5.66 ^{A}	0.362 ± 0.00 ^{E}	52.7 ±0.28 ^{AB}	75.8 ± 0.00 ^{B}
LGF 10	70.7 ± 0.14 ^{C}	1799 ± 4.24 ^{C}	1172 ± 5.66 ^{D}	65.10 ± 0.14 ^{E}	38,100 ± 2.83 ^{B}	14,130 ± 4.24 ^{B}	0.371 ± 0.00 ^{C}	53.5 ± 0.28 ^{A}	76.9 ± 0.00 ^{A}
One-way ANOVA *p* values									
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

H′m—maximum height of gaseous production, VT—total CO_2 volume production, VR—volume of the gas retained in the dough at the end of the test, CR—retention coefficient, G′—elastic modulus, G″—viscous modulus, tan δ—loss tangent, T_i—initial gelatinization temperature, T_{max}—maximum gelatinization temperature. Soybean germinated flour (SGF) containing samples: a–e, mean values in the same column followed by different letters are significantly different ($p < 0.05$); Lentil germinated flour (LGF) containing samples: A–E, mean values in the same column followed by different letters are significantly different ($p < 0.05$).

3.3. Optimization of LGF and SGF Addition Levels

3.3.1. Diagnostic Checking of the Models

The data for falling number (FN), dough tolerance to kneading (Tol), dough consistency after 250 s (D250), dough consistency after 450 s (D450), baking strength (W), and curve configuration ratio (P/L) properties were successfully fitted ($p < 0.05$) to the quadratic model which explained 96, 74, 66, 75, 86 and 74% respectively of the variations, as the ANOVA results showed (Table 5). The 2FI mathematical model chosen for water absorption (WA) and dough extensibility (L) data fitting explained 74 and 87% respectively of the variation and it was significant at $p < 0.05$ in both cases. Dough tenacity (P) alveographic results were fitted to the cubic model which was significant at $p < 0.05$ and explained 77% of data variation.

Table 5. ANOVA results of the models fitted for FN and dough rheological properties during mixing and extension data.

Factors	Parameters								
	FN (s)	WA (%)	Tol (s)	D250 (mb)	D450 (mb)	P (mm)	L (mm)	W (10^{-4} J)	P/L (adim.)
Constant	254.50	52.72	191.11	372.23	933.41	114.85	46.72	196.66	2.49
A	−27.20 ***	−0.64 ***	−7.48	−18.12	−19.36	−10.66	−17.84 ***	−46.28 ***	1.09 ***
B	−47.40 ***	−0.92 ***	−34.60 ***	116.88 ***	77.04 ***	−7.93	−4.96 **	−30.56 ***	0.48 *
A × B	11.48 **	−0.12	−6.44	42.60	37.64 *	3.00	2.56	26.08 **	0.65 *
A^2	−0.6857		−13.31	4.97	37.03	−3.37		1.20	0.08
B^2	−2.80		−5.31	75.77 *	13.60	2.23		−0.9143	0.19
A^2B						−5.54			
AB^2						12.06 *			
A^3						114.85 *			
B^3						−10.66			
Model evaluation									
R^2	0.96	0.74	0.74	0.66	0.75	0.77	0.87	0.86	0.74
Adj.-R^2	0.95	0.70	0.67	0.57	0.69	0.64	0.85	0.82	0.68
p-value	0.0001	0.0001	0. 0001	0.0005	0.0001	0.0017	0.0001	0.0001	0.0001

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, A—soybean germinated flour (%), B—lentil germinated flour (%), R^2, *Adj.-R^2*—measures of model fit, FN—falling number, WA—water absorption, Tol—tolerance to kneading, D250—dough consistency after 250 s, D450—dough consistency after 450 s, P—dough tenacity, L—dough extensibility, W—baking strength, P/L—curve configuration ratio.

The quadratic model successfully fitted ($p < 0.05$) the data for the maximum height of gaseous production (H′m), the volume of gas retained in the dough at the end of the test (VR), elastic modulus (G′), loss tangent (tan δ) and initial gelatinization temperature (T_i), the variations were explained in proportions of 62 to 98% (Table 6). For total CO_2 volume production (VT) and maximum gelatinization temperature (T_{max}) data prediction, the cubic model was found to be adequate ($p < 0.05$) with an explained variation of 78 and 96% respectively, while the retention coefficient (CR) values were fitted to the modified cubic model which was significant at $p < 0.05$ and explained 61% of data variation (Table 6).

Table 6. ANOVA results of the mathematic models fitted for dough empirical and dynamic rheological properties data.

Factors	Parameters								
	H′m (mL)	VT (mL)	VR (mL)	CR (%)	G′ (Pa)	G″ (Pa)	tan δ (adim.)	T_i (°C)	T_{max} (°C)
Constant	69.57	1609.22	1283.41	79.77	49460.86	20268.40	0.4143	50.77	75.57
A	−3.22 ***	−151.59	−68.28 ***	−0.3634	16590.80 ***	7117.60 ***	0.0104	−1.65 ***	1.94 ***
B	0.50	−104.84	−19.32	3.84	4064.80 ***	3569.60 ***	0.0391 ***	0.92 ***	0.51 *
A × B	−1.06	−66.40	−3.52	2.55	−688.80	1040.00 **	0.0115	0.15	−0.44 ***
A^2	0.21	41.77	−50.57 *	79.77 *	1932.00 *		−0.0288 *	−0.33	0.45 **
B^2	−3.29 *	−101.66 *	−43.60	−0.3634	194.29		−0.0008	0.12	−0.09
A^2B		185.14 **		3.84 ***					0.39 *
AB^2		−39.43		2.55					75.57
A^3		36.40							1.94 ***
B^3		11.47							0.51
Model evaluation									

Table 6. *Cont.*

Factors	Parameters								
	H′m (mL)	**VT (mL)**	**VR (mL)**	**CR (%)**	**G′ (Pa)**	**G″ (Pa)**	**tan δ (adim.)**	**T_i (°C)**	**T_{max} (°C)**
R^2	0.62	0.78	0.62	0.61	0.98	0.98	0.71	0.83	0.96
Adj.-R^2	0.53	0.66	0.53	0.45	0.98	0.98	0.64	0.79	0.93
p-value	0.0013	0.0010	0.0012	0.0113	<0.0001	<0.0001	0.0001	<0.0001	<0.0001

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, A—soybean germinated flour (%), B—lentil germinated flour (%), R^2, *Adj.-*R^2—measures of model fit, H′m—maximum height of gaseous production, VT—total CO_2 volume production, VR—volume of the gas retained in the dough at the end of the test, CR—retention coefficient, G′—elastic modulus, G″—viscous modulus, tan δ—loss tangent, T_i—initial gelatinization temperature, T_{max}—maximum gelatinization temperature.

3.3.2. Effects of SGF and LGF on Falling Number and Dough Rheological Properties during Mixing and Extension

Flour properties and dough behavior during processing stages are influenced by the ingredients added, depending on their proportions and chemical composition. Falling number (FN) values decreased significantly ($p < 0.05$) when the SGF addition level increased (Figure 1), a similar trend was observed for LGF (Table 5). The interaction between factors significantly affected flour FN variation in a positive way.

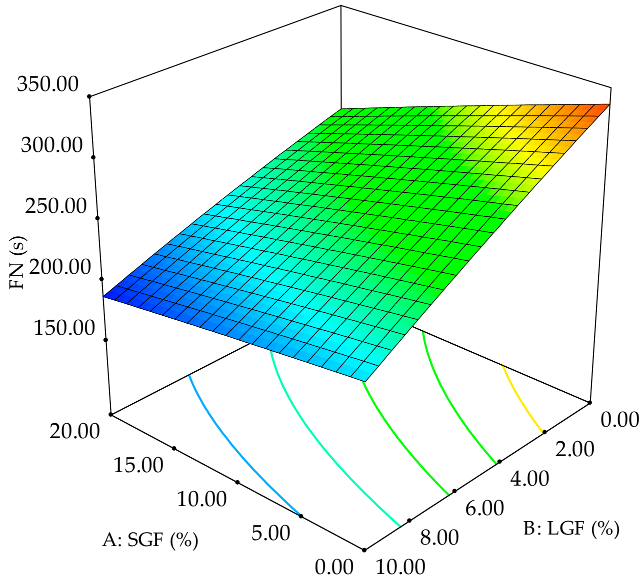


Figure 1. Three-dimensional response surface graphic presenting the interaction between SGF and LGF addition levels on flour falling number (FN).

Dough behavior during mixing was influenced by SGF and LGF addition in wheat flour. Water absorption registered a significant ($p < 0.05$) decrease (Figure 2a) as SGF and LGF addition levels raised, the interaction between factors had a non-significant ($p > 0.05$) effect (Table 5). Dough kneading tolerance showed significant ($p < 0.05$) decreases (Figure 2b) with LGF addition level increase, with SGF showing a non-significant effect ($p > 0.05$).

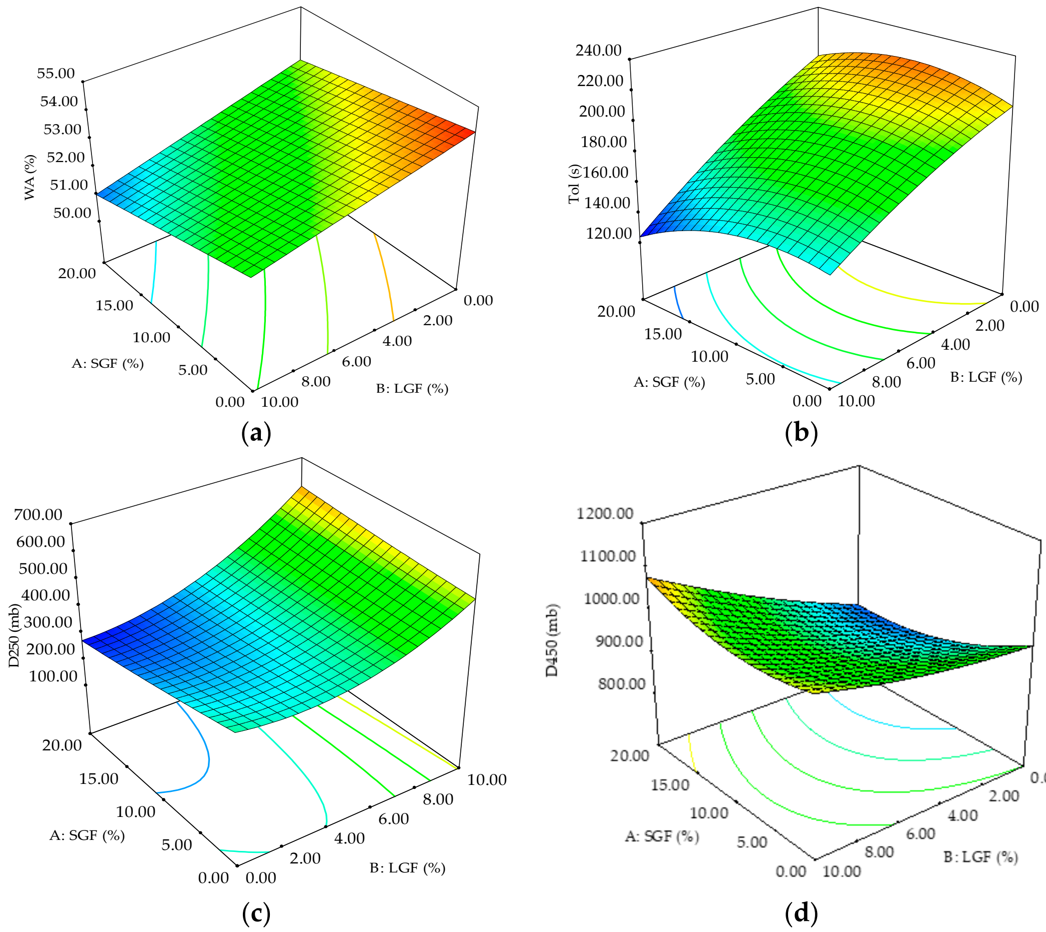


Figure 2. Three-dimensional response surface graphic presenting the interaction between SGF and LGF addition levels on dough properties during mixing: (**a**) water absorption (WA), (**b**) tolerance to kneading (Tol), (**c**) dough consistency after 250 s (D250), and (**d**) dough consistency after 450 s (D450).

Dough consistencies after 250 and 450 s respectively were significantly (p < 0.05) affected by the LGF factor (Table 5), while SGF and the interaction between factors had significant influence only on the D450 parameter. The rise in LGF amounts led to proportionally higher dough consistency parameters (Figure 2c,d).

The effects of factors on dough extension properties are presented in Figure 3.

Dough tenacity showed an irregular trend, rising with SGF levels and decreasing with LGF at levels higher than 2% (Figure 3), the effects being significant for the interaction between SGF and the quadratic term of LGF and for the cubic term on SGF. On the other hand, SGF increases in wheat flour caused a strong decrease (p < 0.001) of dough extensibility, LGF and SGF presented a significant effect (Figure 3b). Dough baking strength was significantly (p < 0.05) influenced by both factors and their interaction (Table 5), with decreases of its values being observed with the addition levels of germinated legumes (Figure 3c). The curve configuration ratio showed increases with raised SGF and LGF amounts (Figure 3d), with both factors and their interaction being significant (p < 0.05).

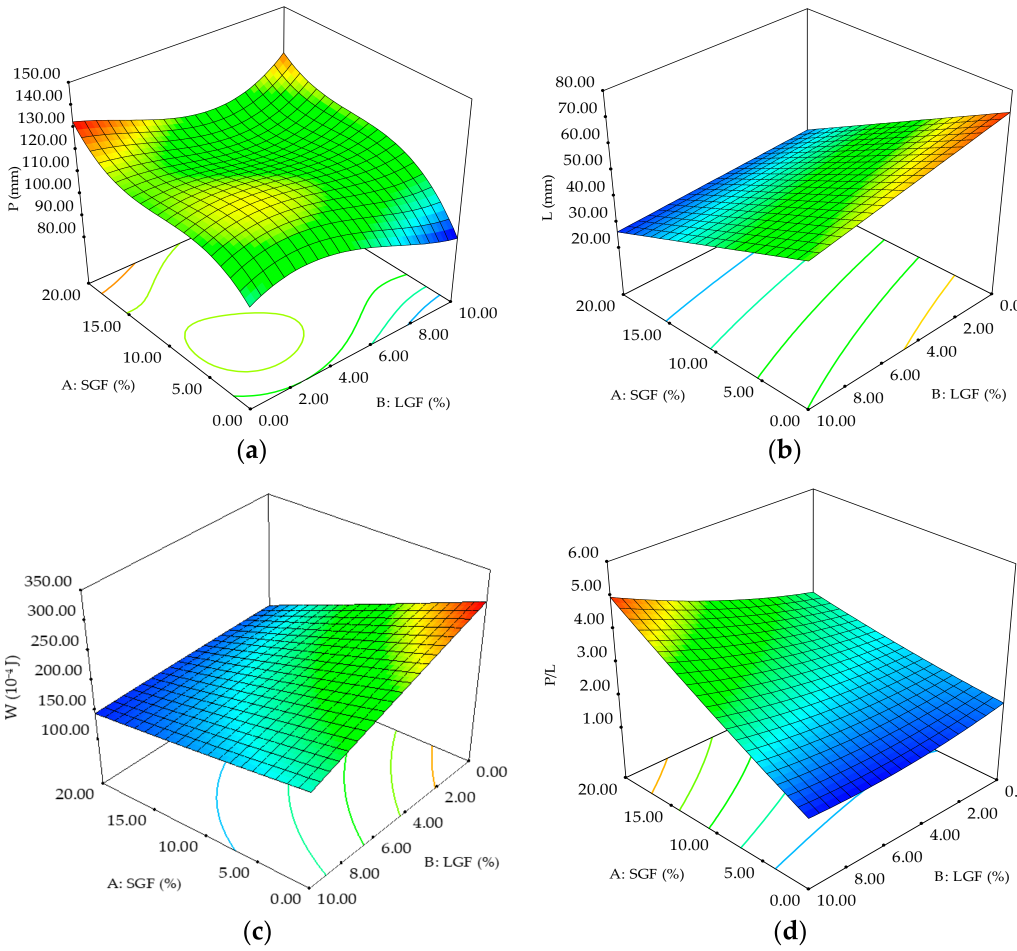


Figure 3. Three-dimensional response surface graphic presenting the interaction between SGF and LGF addition levels on dough properties during extension: (**a**) dough tenacity (P), (**b**) dough extensibility (L), (**c**) baking strength (W) and (**d**) curve configuration ratio (P/L).

3.3.3. Effects of SGF and LGF on Dough Fermentation and Dynamic Rheological Properties

Dough rheological properties are important for baked product processing optimization since they could predict dough behavior during mixing, fermentation, and handling. The maximum height of gaseous production showed a significant ($p < 0.05$) decrease as the addition level of SGF was higher, while LGF and the interaction between factors did not exert significant effects (Figure 4a). Only the interactions between the quadratic term of SGF with LGF factor and the quadratic term of LGF presented significant ($p < 0.05$) influence on the total CO_2 volume production (Table 6), while the volume of gas retained in the dough at the end of the test significantly decreased with increased SGF addition (Figure 4c). SGF quadratic term and the interaction between SGF quadratic term and LGF factor had significant ($p < 0.05$) effects (Table 6) on the dough retention coefficient, with a slightly decreasing trend being observed with LGF amount raise (Figure 4d), while in the case of SGF a reduction of up to 10% was observed, then the values increased.

The dynamic rheological properties in terms of elastic modulus, viscous modulus, and loss tangent were influenced by SGF and LGF addition in the wheat dough as follows: G' increased significantly ($p < 0.05$) as the amounts of SGF and LGF were higher (Figure 5a), while the interactions between them presented a non-significant ($p > 0.05$) effect (Table 6); G'' was affected by both factors and their interaction ($p < 0.05$), an increasing trend was observed with the increasing addition levels (Figure 5b); the loss tangent rose with SGF levels, increased up to 10% and increased as the amount of LGF was higher (Figure 5c), but only the quadratic term of SGF exerted a significant effect.

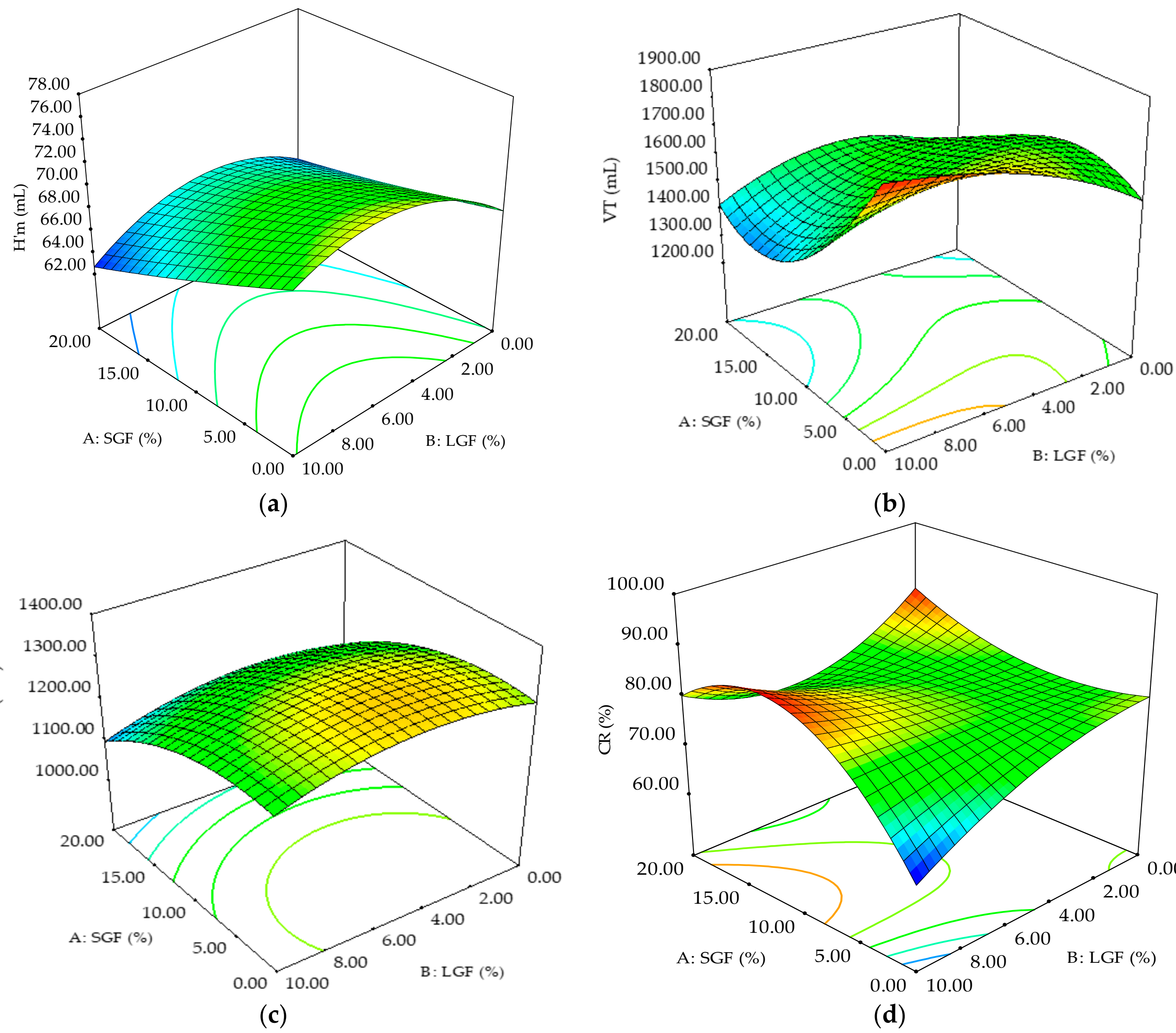


Figure 4. Three-dimensional response surface graphic presenting the interaction between SGF and LGF addition levels on dough rheological properties during fermentation: (**a**) maximum height of gaseous production (H′m), (**b**) total CO_2 volume production (VT), (**c**) volume of the gas retained in the dough at the end of the test (VR) and (**d**) retention coefficient (CR).

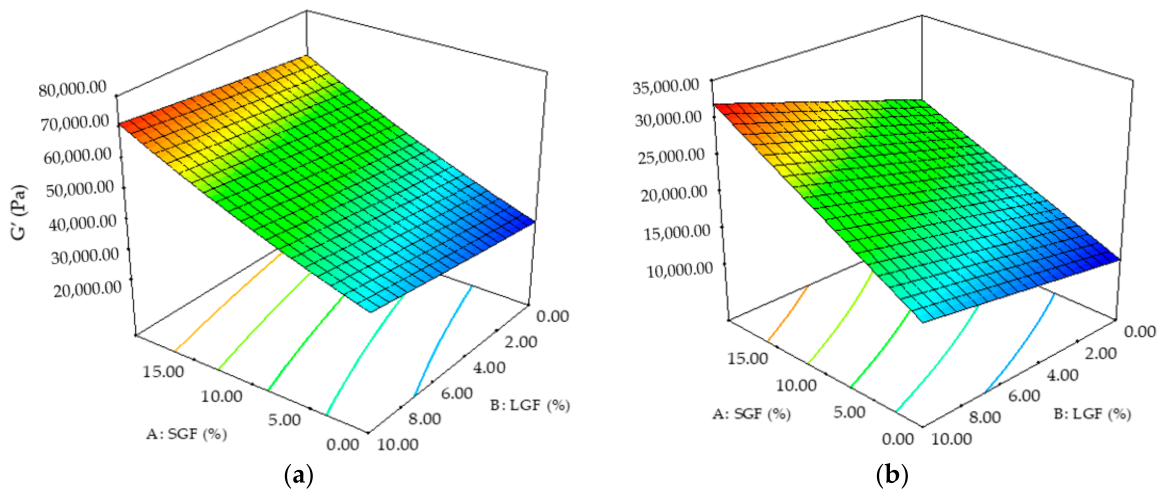
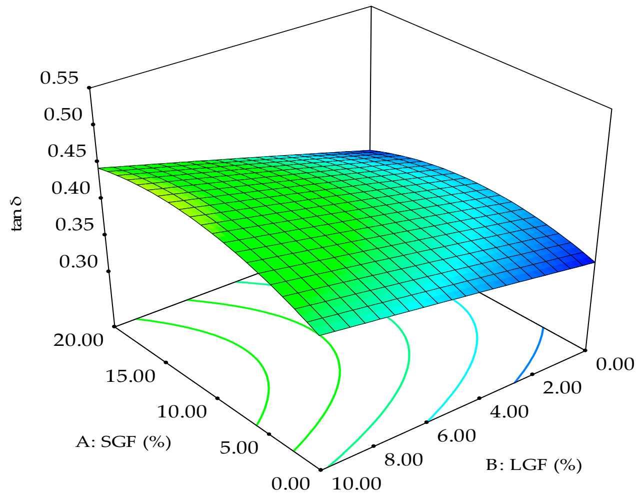


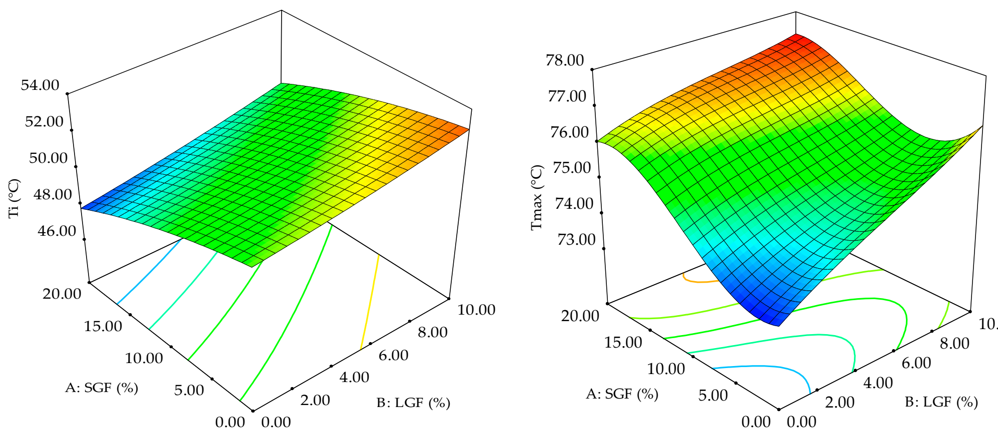
Figure 5. *Cont.*



(**c**)

Figure 5. Three-dimensional response surface graphic presenting the interaction between SGF and LGF addition levels on dough dynamic rheological properties: (**a**) elastic modulus (G′), (**b**) viscous modulus (G″) and (**c**) loss tangent (tan δ).

Composite flour dough elastic and viscous moduli variations during heating provide valuable information on starch gelatinization which could be helpful in the prediction of dough behavior in the baking stage. The initial gelatinization temperature (T_i) is determined at the minimum value of G′, while the maximum gelatinization temperature (T_{max}) is considered at the maximum value of G″ [42]. The initial gelatinization temperature was significantly ($p < 0.05$) affected by SGF and LGF addition (Table 6), a decreasing trend being observed for SGF and the opposite trend for LGF as the addition level was higher (Figure 6a), while LGF and the interaction between factors showed a non-significant influence ($p > 0.05$). SGF and LGF terms exerted significant effects on the maximum gelatinization temperature, an increasing tendency being obtained as the germinated legume flour amounts increased (Figure 6b). A reverse trend on the maximum gelatinization temperature was given by the interaction of SGF with LGF.



(**a**) (**b**)

Figure 6. Three-dimensional response surface graphic presenting the interaction between SGF and LGF addition levels on dough rheological properties during heating: (**a**) initial gelatinization temperature (T_i), (**b**) maximum gelatinization temperature (T_{max}).

3.3.4. Optimal and Control Samples Properties

The optimal addition levels of SGF and LGF in wheat flour and the predicted values of the responses are presented in Table 7. The results of the optimization of the considered response revealed that the optimal formulation contains 5.60% SGF, 3.62% LGF, and 90.76%

wheat flour. The falling number and the rheological properties of the optimal sample showed significantly different ($p < 0.05$) values compared to the control, except for the loss tangent (Table 7). The FN, WA, Tol, D250, D450, L, W, CR, and T_i values of the optimal sample were lower compared to the control, while in the case of P, P/L, H′m, VT, VR, G′, G″ and T_{max} higher values were obtained.

Table 7. Optimal vs. control sample properties.

Variable	Optimal Sample	Control
A: SGF (%)	5.60	0.00
B: LGF (%)	3.62	0.00
FN (s)	280.51 ^{b}	350.00 ^{a}
WA (%)	53.25 ^{b}	54.30 ^{a}
Tol (s)	200.19 ^{b}	214.00 ^{a}
D250 (mb)	359.80 ^{b}	394.00 ^{a}
D450 (mb)	933.32 ^{b}	943.00 ^{a}
P (mm)	119.95 ^{a}	104.00 ^{b}
L (mm)	56.20 ^{b}	72.00 ^{a}
W (10^{-4} J)	228.64 ^{b}	301.00 ^{a}
P/L (adim.)	1.99 ^{a}	1.43 ^{b}
H′m (mL)	70.50 ^{a}	65.90 ^{b}
VT (mL)	1684.98 ^{a}	1532.00 ^{b}
VR (mL)	1305.19 ^{a}	1228.00 ^{b}
CR (%)	78.78 ^{b}	80.10 ^{a}
G′ (Pa)	41,384.57 ^{a}	29,290.00 ^{b}
G″ (Pa)	16,296.27 ^{a}	10,780.00 ^{b}
tan δ (adim.)	0.39 ^{a}	0.37 ^{a}
T_i (°C)	51.20 ^{b}	51.90 ^{a}
T_{max} (°C)	74.67 ^{a}	73.40 ^{b}

A: SGF—soybean germinated flour (%), B: LGF—lentil germinated flour (%), R^2, FN—falling number, WA—water absorption, Tol—tolerance to kneading, D250—dough consistency after 250 s, D450—dough consistency after 450 s, P—dough tenacity, L—dough extensibility, W—baking strength, P/L—curve configuration ratio, H′m—maximum height of gaseous production, VT—total CO_2 volume production, VR—the volume of the gas retained in the dough at the end of the test, CR—retention coefficient, G′—elastic modulus, G″—viscous modulus, tan δ—loss tangent, T_i—initial gelatinization temperature, T_{max}—maximum gelatinization temperature, ^{a,b} values followed by distinct letters in the same row are significantly different ($p < 0.05$).

3.4. Optimal and Control Dough Microstructure

Dough microstructures obtained for dough samples with and without germinated soybean and lentil addition are shown in Figure 7.

The images obtained show a dough structure with red areas interconnected with green areas in a homogeneous and continuous matrix. The red-colored areas indicate the presence of protein, whereas the green-colored areas depict the presence of starch. These different colors for dough compounds were determined by the two fluorochromes used in the EFLM technique namely rhodamine B and fluorescein. Rhodamin B is labeling in red the protein present in the dough system, whereas the fluorescein is labeling the starch granules in green [43]. From both images obtained it may be seen that starch granules are surrounded by a continuous protein network forming a fine dough matrix structure. For the optimal dough sample, it may be seen a slightly higher red area compared to the control due to the high protein content of this dough sample.

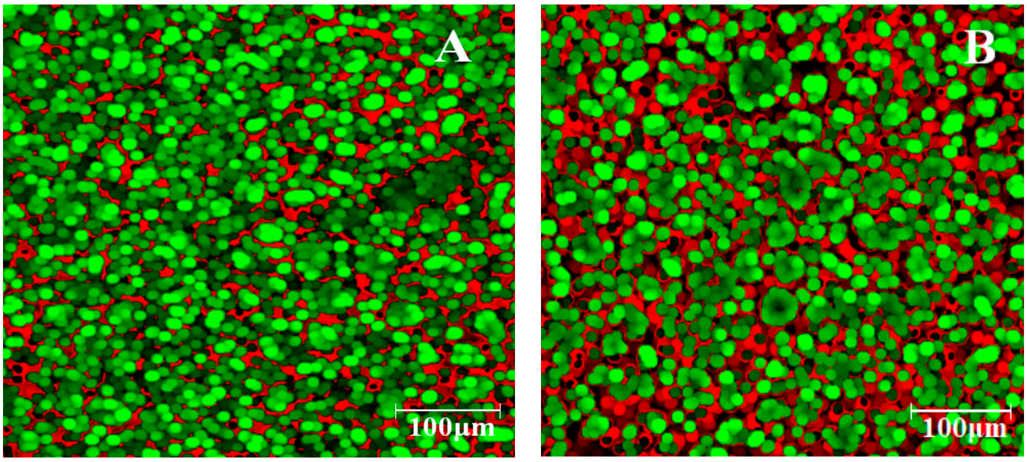


Figure 7. Microstructure taken by EFLM of wheat dough: (**A**) control sample and (**B**) optimal sample. Red is protein and green are starch granules.

4. Discussion

4.1. Effects of SGF and LGF on Falling Number and Dough Rheology

The rheological properties of dough could provide information about its behavior during mixing, extension, and fermentation, underlying also the influence of the ingredients added to the chemical composition that can inhibit or promote molecular interactions in the dough matrix [44]. The falling number is a measure of the α-amylase activity of flour and could be defined as the time necessary to stir and to allow the viscometer stirrer to fall an established distance through the aqueous flour gel undergoing liquefaction [43]. The decrease of falling number values with increased LGF and SGF addition indicated an increase of the α-amylase activity which could be related to the intake of calcium that stabilizes α-amylase [45]. Furthermore, these changes could be due to the enhanced activities of endogenous amylases found in the germinated legume flours which can promptly denature starch grains during heating along with wheat flour amylases, explaining the decrease in falling number values.

Water absorption was expressed as the quantity of water necessary to center the highest part of the mixing curve on the arbitrary 500 BU (Brabender units) [46]. The addition of SGF and LGF caused a reduction of water absorption probably due to the germ enzyme activities on starch grains, causing their hydrolyzation to dextrins which presents low water binding capacity and/or to the proteins de-polymerization as a result of the intense protease activity in germinated flours, similar findings were reported by Hejri-Zarifi et al. [46] and by Marti et al. [47]. Water absorption decrease could be related to the lower falling number values since it is known that high α-amylase activity could give lower water absorption [48]. The decrease in water absorption could be due to protein de-polymerization as a consequence of the intense protease activity in germinated wheat [48]. Dough kneading tolerance showed significant decreases ($p < 0.05$) as the addition level of LGF was higher, similar results being reported by Eissa et al. [49] for Egyptian Balady Bread and biscuits supplemented with germinated legume seeds flours. Dough kneading tolerance increased with increases of SGF up to 10%. Shorter stability of the wheat flour supplemented with germinated soy flour was found by Rosales-Juarez [28], while Sadowska et al. [50] reported dough stability prolongation for the wheat flour with different doses of germinated pea flour added. The decrease of kneading tolerance can be caused by peptidase formed during germination which determines the advanced disruption of the protein network. Kneading tolerance reduction showed a weakening of the gluten matrix structure that could be attributed to a noticeable incompatibility between the protein spectrum of legume flours and wheat gluten protein [51]. It is supposed that with the increase of germinated legumes quantity in the composite flour, the energy required for the optimal development of dough consistency raised, which was related to an increased mechanical agitation need, caused by the non-gluten proteins from the dough system [52]. Dough consistency increases proportionally to the LGF amounts and could be explained by the chemical composition of LGF [14], these results underlying their positive effects on dough rheological properties and confirming the possibility to improve low-protein wheat flours for breadmaking. Similar results were reported by Mohammed et al. [53] for wheat flour enriched with chickpea. The presence of fiber and proteins of germinated legume flours could lead to more intense interactions with water which will contribute to the formation of a more consistent gluten network [54]. Dough consistency changes could be related to the hydrolysis products resulting from germination [55].

The addition of SGF in wheat flour caused the increase of dough tenacity, while LGF determined the decrease of this parameter at levels higher than 10%. The increase of dough tenacity with SGF addition can be related to the ascorbic acid content which increases during germination [56]. Dough extensibility was reduced as SGF and LGF addition levels rose, while the P/L ratio registered an opposite trend, similar to the results reported by Hernandez-Aguilar et al. [57] for wheat dough supplemented with germinated lentil flour. These results supported the data obtained for the viscoelastic moduli which increased as the amounts of SGF and LGF were higher, indicating a dough with greater rigidity which is not easy to handle due to its low extensibility. SGF and LGF factors and their interaction determined the decrease of dough baking strength, suggesting a weakening of the gluten matrix. The decrease of the alveographic parameters could be due to the dietary fiber compositions of SGF and LGF which led to dough strength and stability changes, probably as a result of the small numbers of hydroxyl groups of fiber that can interact with water through hydrogen bonding, which will impact gluten network compactness [58]. The decrease in dough compactness could be related to the disruption of the well-defined protein–starch complex in wheat flour dough by the exogenous proteins, as previously stated [58].

Dough behavior during fermentation can be evaluated using rheofermentometer parameters. Gluten networks developed through mixing properties are essential for gas retention and the final structure of bread [1]. The maximum height of gaseous production and volume of gas retained in the dough at the end of the test decreased with the increase of SGF added in wheat flour, except for the sample with 5% SGF. This behavior could suggest the collapse of dough structures due to the reduction in the ability of the gluten network to withstand the physical stresses as a result of proteolytic activity [47]. When LGF was incorporated into wheat flour, the maximum height of gaseous production increased significantly, up to 7.5% and then decreased, but the value remained higher compared to the control. Total CO_2 volume production was higher in the dough with LGF compared to dough with SGF. On the other hand, the retention coefficient registered higher values in samples with SGF and the best final bread volume could be expected. The addition of SGF led to the decrease of the maximum height of gaseous production and volume of gas retained in the dough at the end of the test as the amount was higher, probably as a result of the gluten matrix dilution effect [59]. The gas retention coefficient expressed as the ratio between the volume of gas retained by the dough and the total volume of gas produced during the test decreased with SGF up to 10% and LGF addition levels increased. Gas retention reduction led to higher dough permeability due to the gluten matrix weakening caused by amylose and amylopectin hydrolysis and could be affected by the enzyme's activities during germination [60]. Furthermore, protease enzymes could hydrolyze peptide bonds, which could promote the partial denaturation of the protein network and thus reduce the dough's ability to enclose air [47]. Our results were in agreement with those reported by Suarez-Estrella et al. [61] for wheat dough enriched with germinated quinoa flour. Probably, the increasing availability of mono- and disaccharides as substrates for yeast due to legume germination enhanced the carbon dioxide produced during fermentation [47].

All the samples included in this study showed a solid-like behavior since G′ > G″, the visco-elastic moduli increased with frequency. Dough elastic and viscous moduli presented higher values as the SGF and LGF amounts raised. The loss tangent increased at SGF levels up to 10%, then it was reduced and raised as the amount of LGF was higher, confirming the positive effects of germinated legume flours for wheat flour since dough from stronger flour has G′ values higher compared to weaker ones [62]. The proteins found in legumes can influence water distribution within the dough matrix with significant implications in components interactions [63]. The incorporation of higher amounts of germinated flours could alter the starch-gluten matrix, influencing the viscoelastic behavior of dough and cumbering its handling, similar observations being made by Hernandez-Aguilar et al. [57] for wheat dough enriched with germinated lentil. Germination causes the decrease of wheat starch crystallinity, the enzymes activated during germination preferentially hydrolyze the amorphous starch areas which led to the raised double-helical ordered structure, contributing to the increase in the formation of the gel structure [64]. The loss tangent could be a measure of the structural order (molecular interactions) of dough, with low tan δ values suggesting a rigid and stiff mass, while higher values led to a moist and slack dough [65,66]. Loss tangent increase indicated the depletion of the elastic character of dough, probably as a result of the incorporation of non-gluten flours, such as SGF and LGF. These changes could be possibly due to the presence of low molecular mass molecules caused by de-polymerization during germination of soybean and lentil which will contribute to the increase of the viscous character of dough samples [63]. Legume flours led to the increase of dough fiber proportion, the effect on the rheological behavior of dough being possibly also attributed to interactions between the fiber structure and wheat proteins [1].

The decrease of the initial gelatinization temperature T_i with SGF addition level increase and the rise of T_i in the case of LGF and T_{max} in the case of SGF could be related to the starch structure which was proven to influence dough behavior during heating [67]. Furthermore, the amylose and amylopectin ratio, the degree of heterogeneity, and the amounts of amylase-lipid complexes could have been impacted the gelatinization temperatures [68]. Probably, these results could be also explained by the activation of enzymes during germination, increasing the α-amylase, proteolytic and lipolytic activities [55]. The decrease of the maximum gelatinization temperature could suggest that germination altered soybean and lentil starch granule surface, determining higher resistance to temperature changes, complying with findings reported by Frias et al. [69].

4.2. Optimal Addition Levels of SGF and LGF

The optimization of SGF and LGF addition in wheat flour resulted in an optimal combination of 5.60% SGF, 3.62% LGF, and 90.76% wheat flour. The differences regarding the falling number and rheological properties between the optimal and control sample could be related to the intake of proteins, lipids, carbohydrates, and minerals of the germinated flours and their interactions within the dough matrix [8]. According to the obtained results, both optimal and control flours are considered strong ones, the enriched sample presenting moderate extension properties (P/L > 0.5) and higher α-amylase activity (lower FN) [48]. Higher dough total CO_2 volume production and volume of the gas retained in the dough at the end of the test of the optimal sample compared to the control could be due to the increased amount of fermentable sugars along with the activation of amylase during germination of legumes, leading to enhanced CO_2 production [14].

The images obtained of the dough microstructure showed slight differences among the structures of dough samples. The addition of germinated soybean and lentil led to a lower green area and a higher red one compared to the control due to the higher level of protein from the enriched dough. This ratio color change from the dough structure is due to the high level of protein from the LGF and SGF compared with wheat flour which is partially replaced by them. Both dough samples' structures appeared compact, homogenous, in which starch granules were enveloped by proteins, being glued together. No black regions are present in these images, meaning that LGF and SGF at these addition levels did not affect in a negative way dough structure which presents good rheological characteristics, such as elasticity, gas holding capacity, and extensibility.

5. Conclusions

Legume flour potential can be increased by applying a germination process, allowing the attainment of high nutritive bakery products, with minimum impairment of quality attributes. The results obtained in this study revealed that SGF and LGF influenced dough behavior during mixing, extension, and fermentation. The mathematical modeling of data allowed the interpretation of the effects of SGF and LGF factors, along with their interactions, on flour and dough properties, the explanation rate of the models proposed varied between 61 and 96%.

LGF led to the decrease of falling number, water absorption, kneading tolerance, dough extensibility, and baking strength, while dough consistency, configuration ratio of the Alveograph curve, visco-elastic moduli, loss tangent, initial and maximum gelatinization temperature increased proportionally with the amount used. SGF increases induced lower values of falling number, water absorption, dough extensibility, baking strength, the maximum height of gaseous production, the volume of gas retained in the dough at the end of the test, and initial gelatinization temperature, while the configuration ratio of the Alveograph curve, elastic and viscous moduli and maximum gelatinization temperature was raised with increased SGF. The interactions between SGF and LGF exerted significant ($p < 0.05$) influences on the falling number, dough consistency after 450 s, dough baking strength, viscous modulus, and maximum gelatinization temperature.

The optimal combination of SGF and LGF in wheat flour was found to be 5.60 and 3.62% respectively. Compared to the control, the optimal sample showed lower falling number, water absorption, tolerance to kneading, consistency of dough, extensibility, and initial gelatinization temperature, while for dough tenacity, the maximum height of gaseous production, total CO_2 volume production, the volume of the gas retained in the dough at the end of the test, visco-elastic moduli and maximum gelatinization temperatures higher values were obtained. Dough rheological property variations when germinated legume flours are added to wheat flour knowledge could help producers to optimize the production and recipes of improved bakery products, according to the consumers and technologies requirements. There is a scarcity of papers underlying the effects of germinated legumes, such as soybean and lentil on wheat dough rheological behavior and a lack of information regarding their combined effects. Thus, the results presented in this work bring useful information about the simultaneous effects of two germinated legume flours in the wheat dough, fulfilling the state of art regarding germination application and incorporation of legume flours in wheat bread production. Further research regarding bread quality parameters as influenced by SGF and LGF should be performed.

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