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Possibilities to Use Germinated Lupine Flour as an Ingredient in Breadmaking to Improve the Final Product Quality

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Abstract: This study focuses on the possibility of using germinated lupine flour (GLF) in the bread-making process in order to improve dough rheology and bread characteristics. For this purpose, different levels (0%, 5%, 10%, 15% and 20%) of germinated lupine flour were used, and the influence of its addition in wheat flour was analyzed. On empirical dough rheological properties, GLF addition in wheat flour has the effect of decreasing the water absorption capacity, dough consistency, baking strength, extensibility, tolerance for mixing and of increasing total gas production and falling number value. On fundamental dough rheological properties, GLF addition in wheat flour increased the tan δ and decreased the G' and G'' modules with the increased dough temperature. The microscopic distribution of starch and gluten in the dough system was changed by GLF addition in wheat flour by an increase of the protein area and a decrease of the starch one. Regarding the bread characteristics, the GLF addition improved the specific volume, porosity and elasticity up to 15% GLF addition in wheat flour and decreased the textural properties gumminess and resilience. Regarding the color parameters of the bread, the GLF addition in the dough recipe had a darkening effect on the crumb and bread crust. The sensory data show that the bread samples up to 15% GLF addition in wheat flour were better appreciated than the control sample. According to our data, it is recommended to use a maximum level of 15% of the addition of germinated lupine flour in the dough recipe for making white wheat bread.

Keywords: germinated lupine flour; white wheat flour; bread quality; dough rheology

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1. Introduction

Bakery is one of the most important sectors in the food industry that includes a wide range of products and a considerable place in human nutrition. Therefore, it is desirable that bakery products have a balanced nutritional profile [1]. Among these, the most consumed one is refined wheat flour bread [2]. Unfortunately, its nutrient content is lower than that of whole wheat bread. This is explained by the fact that the lower the degree of flour extraction is, the lower is its nutritional value, because the aleurone layer and the wheat grain coating contain valuable nutrients (minerals, lipids, vitamins, dietary fiber and phytochemicals) [3]. To balance this shortcoming, specialists have tried to find different methods, such as the addition of various ingredients, to bring an additional nutritional intake. Studying the literature, it can be observed that, among these additions can be listed fibers [4], germinated grains [5], different flour types [6], etc. In order to improve white wheat bread from a nutritional point of view, a successful alternative is the addition of various legume flour types in wheat flour. From the category of legumes, lupine has been gaining more and more interest lately [7]. Lupine grains belong to the genus *Lupinus*, family *Fabaceae*. In the past, this legume found applications only as an ornamental plant and for medicinal purposes. However, the significant amount of protein and fiber and the beneficial effects of its consumption on health have made it gain importance in the food field [8]. The intake of legume proteins is desirable to the detriment of those from meat or dairy products, because legume cultivation is more sustainable [9]. As medicinal properties, studies in the field have shown that it has positive properties on glucose and cholesterol levels [10], has a positive effect on lowering triglycerides, blood pressure, has anti-inflammatory effects and an antioxidant capacity [11]. One problem concerned with lupine consumption is related to their alkaloids content (quinolizidine derivatives), which is toxic and harmful for human health [12]. However, there are also varieties of sweet lupine that contain a very small amount of alkaloids that do not endanger health, such as *Lupinusalbus* L. (white lupine), *Lupinus luteus* L. (yellow lupine) and *Lupinus angustifolius* L. (narrow-leaved lupines) that have been used in food consumption, especially in the Mediterranean area [13,14]. In general, the use of lupine grains in the food industry has similar applications to those of soybeans [9]. In the literature, there have been reported many foods in which lupine flour has been incorporated, such as cheese, sausages, noodles, bread, ketchup, cakes, pizza, spices, jam, etc. Vegetarians often use lupine grains to supplement their diet instead of milk, soy and eggs. At the same time, lupine also has applications in gluten-free products. Lupine has a nutritional profile containing a significant amount of protein (20–48%), with a balanced profile of amino acids, fiber and small amount of lipids [15]. Lupine is also rich in phenolic compounds [16]. However, lupine grains also contain some antinutritional compounds such as saponins and phytates [16], which reduce the nutrient bioavailability. By using different methods such as germination ones, the antinutritional compounds can be reduced. More along the improvement of the nutritional profile of lupine grains, toxic alkaloids can be eliminated from the grains [17,18]. In addition to the fact that germination improves the nutritional profile of lupine, in this process, the enzymes present in legumes are activated, which results in an increase in the digestibility of the constituent compounds. Additionally, these enzymes may have a positive effect on breadmaking from a technological point of view, especially if the wheat flour from which bread is obtained is of a strong quality for breadmaking.

In order to reduce the humidity in the lupine grains subjected to germination and to maintain to high levels in its enzymatic activity, the lyophilization process may be used. Studies have shown that the process of freeze drying is usually desirable, because this process preserves the physical and chemical characteristics of grains. Additionally, the process does not negatively influence the shape and volume of them, and the sensory characteristics are well-preserved [19].

Until today, only a few studies have investigated the use of lupine grains in germinated or nongerminated forms as an addition in breadmaking recipes. According to Wandersleben et al., the use of nongerminated lupine flour up to 10% in wheat flour did not negatively affect the bread quality [11]. The uses of germinated lupine flour (GLF) in combination with germinated chickpea in breadmaking have been previously reported by Atudorei et al. [20], who concluded that germinated legume flour addition leads to a decrease of dough consistency and an increase in dough elasticity. For this reason, the aim of our study is to analyze the effect of GLF on dough rheological properties, microstructure and bread quality. The importance of using GLF in breadmaking derives from its valuable nutritional composition that may improve the bread quality but, also, from its technological advantages it may have in breadmaking due to its enzymatic activity.

2. Materials and Methods

2.1. Materials

White wheat flour, type 650 (minimum content of 10.5% protein and of 26% wet gluten), purchased from S.C. Dizing S.R.L. (Brusturi, Neamț, Romania) was used to prepare the bread samples. To obtain germinated lupine flour, sweet lupine grains with a low alkaloids content, *Lupinus albus* L., were used. The lupine grains were provided from the University of Life Sciences (Faculty of Agriculture), Iași, Romania. After germination, the lupine grains were lyophilized to lower the moisture and then ground. Lupine sprouts were lyophilized without removing the rootlets. The germination process was performed at a constant humidity of 80%, at a temperature of 25 °C in dark conditions only, according to the method previous described [21]. After germination, the lupine grains were freeze-dried using a Biobase BK-FD12 lyophilizer (Jinan, China). Lyophilization was performed at −50 °C at 10 Pa for 24 h. A laboratory Perten Mill 3100 (Hägersten, Sweden) was used to mill the germinated lupine grains.

Both flours from wheat and germinated lupine grains were analyzed according to the ICC standard methods: fat content (ICC 136), ash content (104/1), protein content (ICC 105/2) and moisture content (ICC 110/1). The wheat flour was also analyzed for its falling number value, according to the ICC 107/1, and its gluten content and gluten deformation index, according to the Romanian standard method SR 90:2007. The carbohydrates content was determined by a difference: 100–(protein % + fat % + ash % + moisture %).

2.2. Dough Rheological Properties

2.2.1. Dough Rheological Properties during Mixing and Extension

Dough rheological properties during mixing were performed using the consistograph test (to 14% moisture) from the AlveoConsistograph (Chopin Technologies, CEDEX, Villeneuve-la-Garenne, France) device according to ICC 171. The water absorption capacity (WA), maximum pressure (PrMax), tolerance to mixing (Tol), consistency of the dough after 250 s (D250) and consistency of the dough after 450 s (D450) were determined. The dough rheological properties during extension, determined using the alveograph part of the AlveoConsistograph (Chopin Technologies, CEDEX, France) device according to ICC 121, were the maximum pressure (P), dough extensibility (L), index of swelling (G), baking strength (W) and configuration ratio of the Alveograph curve (P/L).

2.2.2. Dough Rheological Properties during Fermentation and Falling Number Values

Dough rheological properties during fermentation were performed using the Rheofermentometer device (Chopin Rheo, type F4, Villeneuve-La-Garenne CEDEX, France), according to the AACC89-01.01 method. For this test, the dough was prepared by mixing the ingredients in the consistograph cuvette for 8 min. The raw materials for the dough were: 250 g of flour mix (mix of wheat flour and GLF), 7 g of compressed yeast (*Saccharomyces cerevisiae*) and 5 g of salt and water, according to the water absorption capacity obtained through consistograph analysis. The following parameters were determined: the total CO_2 volume production (VT, mL), 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 based on viscosity of the mix flour suspended in water were determined according to the ICC 107/1 method with the Falling Number device (FN 1305 type, Perten Instruments AB, Stockholm, Sweden).

2.2.3. Dough Fundamental Rheological Properties

The dough fundamental rheological properties were analyzed using the HAAKE MARS 40 rheometer device (Termo-HAAKE, Karlsruhe, Germany). For this purpose, a gap of 2 mm and a plate system of 40 mm were used. Taking into account the optimal value of the water absorption capacity, the dough ingredients were mixed in the AlveoConsistograph tank, and then, the sample was placed between rheometer plates and rested before analysis for 5 min for relaxation. The tests performed on the rheometer were: the frequency sweep test (from 1 to 20 Hz at 25 °C in a range of linear viscoelasticity), the storage modulus G', the loss modulus G'' and loss tangent tan δ (at a constant stress of 15 Pa for the frequency sweep tests and during heating from 25 to 100 °C at a heating rate of 4 °C per min at a frequency of 1 Hz and a fixed strain of 0.001).

2.3. Dough Microstructure

The microstructure of the dough samples was determined using Motic AE 31 (Motic, Optic Industrial Group, Xiamen, China) equipped with catadioptric objectives LWD PH 203 (N.A. 0.4). These epifluorescence light microscopy (EFLM) images were captured for the control sample and for samples with different levels of GLF addition at room temperature, according to the method described in our previously study [21].

2.4. Breadmaking

In order to obtain bread, the wheat flour was mixed with GLF (according to the established recipe; the proportion of GLF addition was 5%, 10%, 15% and 20%); 3% compressed yeast (*Saccharomyces cerevisiae* type); 1.5% salt and water, according to the mix water absorption capacity. The ingredients were mixed for 15 min using a Kitchen Aid mixer (Whirlpool Corporation, Benton Harbor, MI, USA). After kneading, the dough was divided into three pieces weighing 400 g each. The obtained samples were fermented for 60 min in a fermentation chamber (PL2008, Piron, Campodarsego, Padova, Italy) at a constant temperature of 30 °C. After fermentation, the samples were baked at a temperature of 220 °C for 30 min using a convection electric oven equipped with a ventilation, humidification and steam production system (PF8004 D, Piron, Italy).

2.5. Bread Quality Evaluation

The quality of the bread samples was determined by analyzing its physical characteristics (loaf volume, porosity and elasticity); the crumb and crust color values; the textural proprieties; crumb structure and sensory characteristics.

2.5.1. Bread Physical Characteristics

The bread physical characteristics, namely loaf volume through seed displacement method, porosity and elasticity, were determined according to the SR 90: 2007 method.

2.5.2. Bread Color Parameters

The bread color characteristics were determined by using the Konica Minolta CR-400 colorimeter (Tokyo, Japan). The analysis was made in the UV–Vis domain based on the CIE Lab* color system, which allowed to determine the values of the parameters L* (darkness/brightness), a* (shade of red/green) and b* (shade of blue/yellow).

2.5.3. Texture Profile Analysis

The textural characteristics of the bread samples were determined by using a TVT-6700 texturometer device, Perten Instruments (Hägersten, Sweden), which was equipped with a 10-kg loading cell. To highlight the textural parameters of the bread samples, they were cut in 50-mm-high slices and then subjected to two compression processes up to 20% of their initial height. For this determination, a cylindrical probe of 45 mm was used at a speed of 1.0 mm/s and a trigger force of 5 g. The recovery period between compressions was 15 s. The textural parameters were springiness, gumminess, chewiness, cohesiveness and resilience.

2.5.4. Crumb Structure

The bread structure was determined by using the Motic SMZ-140 stereo microscope (Motic, Xiamen, China). The images were obtained by using a 20× objective and a resolution of 2048 × 1536 pixels.

2.5.5. Sensory Analysis

The sensory characteristics of bread were determined by using a 9-point hedonic scale. For this purpose, 30 semi trained judges were used. The bread sensory characteristics analyzed were the following: appearance, color, taste, smell, texture, flavor and global acceptability.

2.5.6. Statistical Analysis

The Statistical Package for Social Science statistical package (v.16, SPSS, Chicago, IL, USA) was used for statistical analysis. A one-way analysis of variance (ANOVA) with

Turkey's test was used to highlight the significant differences at the 5% level. All values were expressed as the mean ± standard deviation.

3. Results

3.1. Flour Characteristics

The physicochemical characteristics of the white wheat flour and of the germinated lupine flour used were determined. In the case of white wheat flour, the values were: 71.33% carbohydrates, 14.6% moisture, 0.65% ash content, 12.3% protein, 1.12% fat, 30.4% wet gluten and 3-mm gluten deformation index. The type of flour was 650, one representing the mineral content (ash) expressed as a percentage of the dry matter, multiplied by 1000. The value of the falling number index was 350 s. Therefore, the white wheat flour used was characterized by a low α amylase activity and has a strong quality [22].

In the case of GLF, the physicochemical characteristics were: 3.4% ash, 39.4% protein, 50.3% carbohydrates and 6.9% fat. It can be seen that it has high protein content, a result that is similar to those reported in the literature [23].

3.2. Dough Rheological Properties

3.2.1. Dough Rheological Properties during Mixing and Extension

The Consistograph data of the dough samples prepared with different addition levels of GLF are shown in Table 1. As it may be seen, the GLF addition conducted to a significant decreased ($p < 0.05$) to all the consistographic parameters: water absorption, tolerance to mixing, dough consistency after 250 s and dough consistency after 450 s.

Table 1. Consistograph parameters of the dough samples with different levels of GLF additions.

Dough Samples	WA (%)	Tol (s)	D250 (mb)	D450 (mb)
Control	54.3 ± 0.10^{e}	214 ± 1.00^{e}	394 ± 3.00^{e}	943 ± 1.00^{e}
GLF_5	53.7 ± 0.16^{d}	181 ± 1.63^{d}	339 ± 3.27^{d}	878 ± 1.63^{d}
GLF_10	53.2 ± 0.00^{c}	167 ± 0.62^{c}	185 ± 2.05^{c}	762 ± 3.68^{c}
GLF_15	52.6 ± 0.08^{b}	149 ± 2.45^{b}	174 ± 1.63^{b}	624 ± 3.27^{b}
GLF_20	52.1 ± 0.08^{a}	126 ± 2.45^{a}	163 ± 2.45^{a}	569 ± 1.63^{a}

WA, water absorption; Tol, tolerance to mixing; D250, dough consistency after 250 s; D450, dough consistency after 450 s. The results are the mean ± standard deviation ($n = 3$). Dough samples contain germinated lupine flour, GLF: $^{a–e}$, mean values in the same column followed by different letters are significantly different ($p < 0.05$).

The Alveograph parameters of the dough samples are presented in Table 2. Compared to the control sample, it was observed that the addition of GLF decreased the values of the parameters: dough extensibility, index of swelling and baking strength. On the other hand, the GLF addition in wheat flour led to a significant ($p < 0.05$) increase in the value of the maximum pressure index and the value of the configuration ratio (P/L) of the Alveograph curve.

Table 2. Alveograph parameters of the dough samples with different levels of GLF additions.

Dough Samples	P (mm)	L (mm)	G (mm)	W (10^{-4} J)	P/L
Control	104 ± 2.51^{a}	72 ± 1.15^{d}	19.4 ± 0.28^{d}	301 ± 5.13^{d}	1.43 ± 0.05^{a}
GLF_5	113 ± 0.47^{b}	46 ± 0.82^{c}	15.0 ± 0.12^{c}	204 ± 2.45^{c}	2.45 ± 0.05^{b}
GLF_10	122 ± 0.63^{c}	40 ± 2.05^{b}	14.0 ± 0.37^{b}	198 ± 2.45^{c}	3.02 ± 0.11^{c}
GLF_15	127 ± 0.82^{d}	33 ± 1.63^{a}	12.7 ± 0.33^{a}	174 ± 2.45^{b}	3.85 ± 0.17^{d}
GLF_20	133 ± 0.82^{e}	29 ± 0.82^{a}	11.9 ± 0.16^{a}	164 ± 1.63^{a}	4.59 ± 0.10^{e}

P, maximum pressure; L, dough extensibility; G, index of swelling; W, baking strength; P/L, configuration ratio of the Alveograph curve. The results are the mean ± standard deviation ($n = 3$). Dough samples containing germinated lupine flour, GLF: $^{a–e}$, mean values in the same column followed by different letters are significantly different ($p < 0.05$).

3.2.2. Dough Rheological Properties during Fermentation and Falling Number Values

In the proofing stage of the dough, carbon dioxide is released, which leads to the expansion of the gas cells that were previously formed in the mixing stage of the dough by incorporating air into the dough network [24]. Monitoring the behavior of the dough in the leavening stage is essential, because this technological stage has decisive effects on the quality of the finished product: volume, porosity, texture, etc. Table 3 shows the effect of the GLF addition on the dough rheological properties during fermentation on the Rheofermentometer device and the Falling Number values of the mixed flours compared to the control sample without any GLF addition. From Table 3, it can be seen that the values of the parameters H′m and VT were significantly higher ($p < 0.05$) compared to the control sample, up to a maximum GLF addition level of 15% in wheat flour. At an additional level of 20% GLF in wheat flour, the values of the two parameters were significantly lower ($p < 0.05$) than the control sample. The value of the VR parameter was significantly higher ($p < 0.05$) than the control sample only in the case of a 5% GLF addition in wheat flour, and then, this value significantly decreased ($p < 0.05$). The CR parameter decreased due to the GLF addition. Only at a GLF addition of 20% in wheat flour, the value of this parameter was higher than in the case of the sample without any GLF addition. Regarding the Falling Number values, these significantly decreased ($p < 0.05$) by the GLF addition in wheat flour.

Table 3. Rheofermentometer parameters and a falling number values of dough samples with different levels of GLF additions.

Dough Samples	H′m (mm)	VT (mL)	VR (mL)	CR (%)	FN (s)
Control	65.9 ± 0.30^{b}	1532 ± 2.51^{b}	1228 ± 2.51^{d}	80.1 ± 0.50^{b}	350 ± 3.29^{e}
GLF_5	74.6 ± 0.08^{e}	1651 ± 2.45^{e}	1239 ± 1.63^{e}	75.0 ± 0.21^{a}	320 ± 1.63^{d}
GLF_10	70.5 ± 0.08^{d}	1621 ± 3.27^{d}	1214 ± 1.63^{c}	74.9 ± 0.08^{a}	302 ± 1.63^{c}
GLF_15	68.1 ± 0.08^{c}	1550 ± 1.63^{c}	1179 ± 2.45^{b}	76.0 ± 0.12^{a}	277 ± 1.63^{b}
GLF_20	64.3 ± 0.16^{a}	1425 ± 2.45^{a}	1147 ± 2.45^{a}	80.5 ± 0.05^{b}	247 ± 2.45^{a}

H′m, maximum height of gaseous production; VT, total CO_2volume production; VR, volume of the gas retained in the dough at the end of the test; CR, retention coefficient; FN, falling number value. The results are the mean ± standard deviation ($n = 3$). Dough samples containing germinated lupine flour, GLF: $^{a\text{–}e}$, mean values in the same column followed by different letters are significantly different ($p < 0.05$).

3.2.3. Dough Fundamental Rheological Properties

Figure 1 shows the diagrams for frequency sweep tests. From the figure, it can be seen that the storage modulus, loss modulus and loss tangent depend very much on the frequency. It can be seen that, in all the frequency ranges, the storage modulus was higher than the loss modulus. G' and G'' increased when high levels of GLF were added in wheat flour. Tan δ increased in a frequency-dependent manner. The ratio of the viscous and elastic components of the dough (tan δ) was less than 1 for all dough samples.

Figure 2 shows the evaluation with the temperature of the G', G'' and tan δ values for the samples with different levels of GLF addition in wheat flour. It can be seen that, up to a certain temperature, G' and G'' decreased, followed by an increase in their values. During heating, the G' and G'' values were higher for the control sample compared to the samples with different levels of GLF addition in wheat flour.

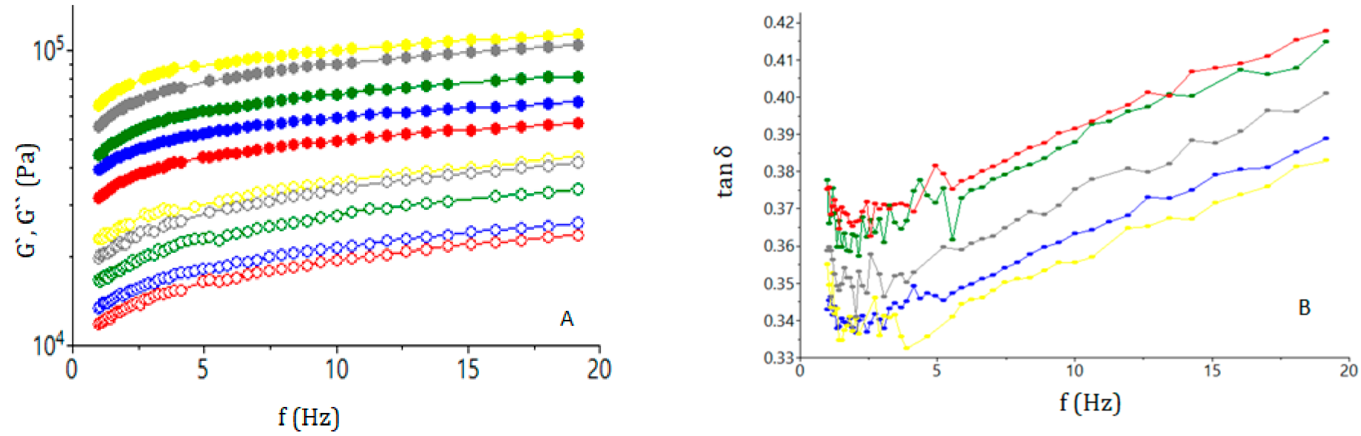


Figure 1. Evaluation with a frequency of G′ (open symbols), G″ (solid symbols) (**A**) and tan δ (**B**) for the samples with different levels (-●-0%, -●-5%, -●-10%, -●-15% and -●-20%) of germinated lupine flour (GLF) additions in wheat flour.

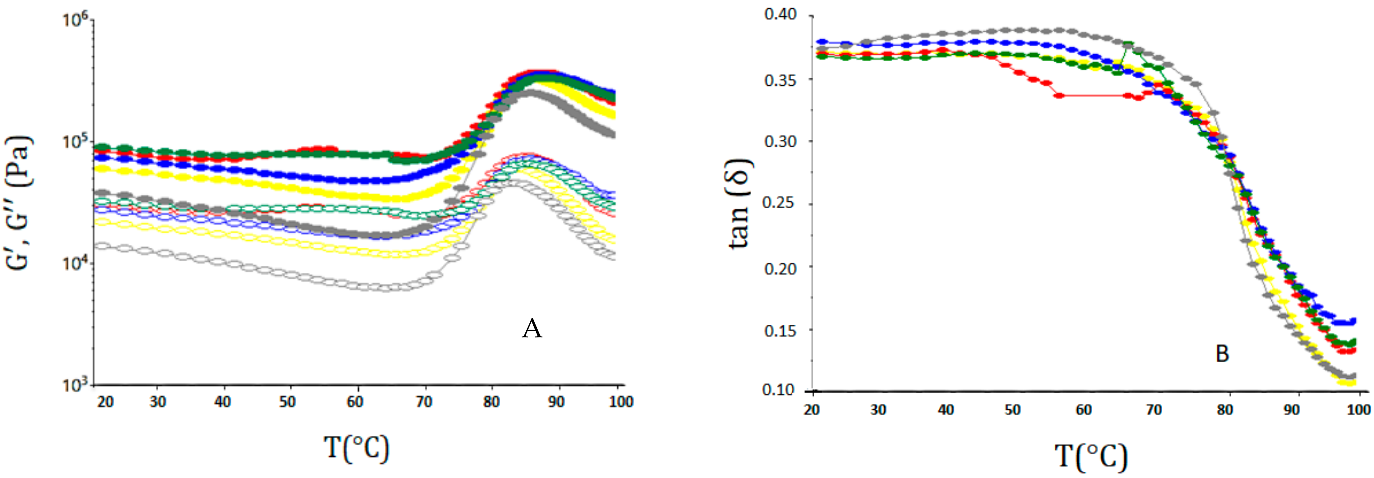


Figure 2. Evaluation of the temperature of the G′ values (open symbols), G″ (solid symbols) (**A**) and tan δ (**B**) for the samples with different levels (-●-0%, -●-5%, -●-10%, -●-15% and -●-20%) of germinated lupine flour (GLF) additions in wheat flour.

3.3. Dough Microstructure

The addition of GLF on the dough microstructure is shown in Figure 3. As it may be seen, with the increased level of GLF addition in wheat flour, the red area increased, whereas the starch area decreased. This is due to the fluorochromes used for the EFLM analysis, namely rhodamine B and fluorescein, which stain proteins in red and starch in green [21].

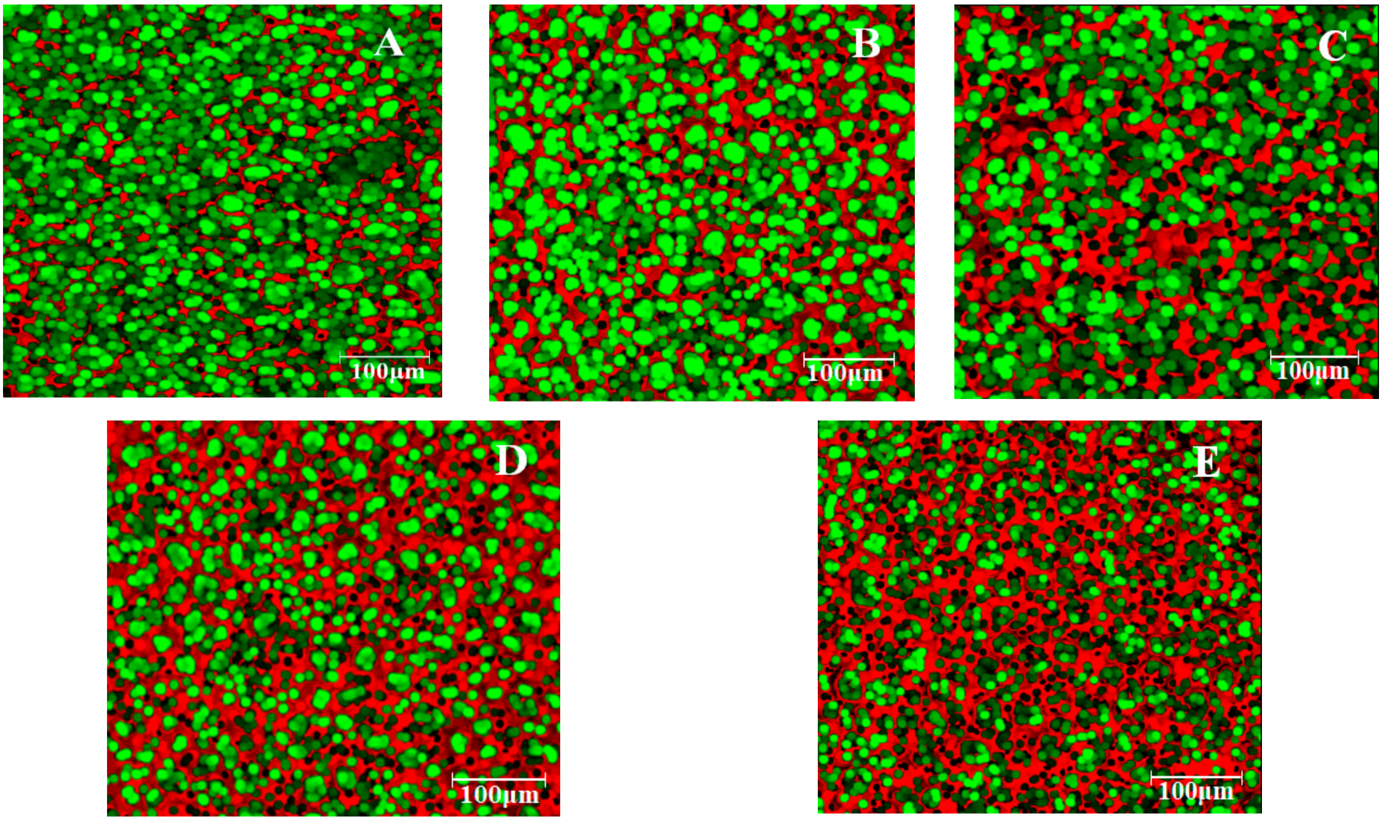


Figure 3. Microstructure taken by EFLM of wheat dough with germinated lupin flour (GLF) at different levels: 0% (**A**), 5% (**B**), 10% (**C**), 15% (**D**) and 20% (**E**). Red, protein; Green, starch granules.

3.4. Bread Quality Evaluation

3.4.1. Bread Physical Characteristics

Highlighting the influence of the addition of germinated lupine (GLF) flour on the physical characteristics of bread samples is important, because they play an important role in consumers' perception of quality. Consumers usually prefer bread with a high volume, uniform porosity and superior elasticity.

The physical characteristics of the bread samples are shown in Table 4. From this table, it can be seen that the addition of GLF had an influence on all three physical characteristics of the bread samples. Thus, the addition of GLF significantly improved ($p < 0.05$) the specific volume of the bread, up to a maximum of 15%. The addition of 20% resulted in a smaller sample volume compared to the control sample. Regarding the porosity parameter, it was observed that the addition had a similar influence in the sense that an addition of 5%, 10% and 15% had a positive impact on the porosity, while a higher addition led to a decrease in the porosity value. The elasticity also had an upward trend due to the addition, the value of the elasticity decreasing to an addition of 20%. Thus, it can be concluded that, if an improvement of the physical characteristics of the bread samples is desired, an addition of a maximum of 15% GLF is recommended.

3.4.2. Color Parameters of Breads Samples

Table 5 shows how the values of the parameters L*, a* and b* varied due to the addition of GLF in the recipe for breadmaking of white wheat bread. The color determinations were made both for the crust of the bread samples and for their crumbs. The color parameters are important, because they have a major impact on the visual acceptability of consumers.

Table 4. Physical characteristics of the bread samples with different levels of GLF additions.

Bread Samples	Specific Volume (cm^3/100 g)	Porosity (%)	Elasticity (%)
Control	331.5 ± 0.74^{b}	67.4 ± 0.86^{b}	91.3 ± 0.57^{b}
GLF_5	345.4 ± 1.18^{c}	70.8 ± 0.67^{c}	92.2 ± 0.17^{bc}
GLF_10	355.6 ± 0.98^{d}	72.6 ± 0.48^{cd}	92.4 ± 0.88^{c}
GLF_15	362.8 ± 0.73^{e}	74.2 ± 0.32^{d}	94.9 ± 0.19^{d}
GLF_20	318.3 ± 0.85^{a}	63.8 ± 0.63^{a}	87.0 ± 0.72^{a}

The results are the mean ± standard deviation (n = 3). Bread samples containing germinated lupine flour; GLF: $^{a–e}$, mean values in the same column followed by different letters are significantly different ($p < 0.05$).

Table 5. Color parameters of the bread samples with different levels of GLF additions.

Bread Samples	Crust Color			Crumb Color		
	L*	a*	b*	L*	a*	b*
Control	76.25 ± 0.94^{e}	3.44 ± 0.27^{a}	3.14 ± 0.43^{a}	66.37 ± 0.88^{e}	-4.62 ± 0.32^{a}	1.69 ± 0.22^{a}
GLF_5	74.22 ± 0.24^{d}	5.89 ± 0.16^{b}	4.41 ± 0.24^{b}	63.13 ± 0.61^{d}	-3.63 ± 0.44^{b}	2.31 ± 0.20^{a}
GLF_10	65.89 ± 0.76^{c}	8.72 ± 0.25^{c}	5.63 ± 0.25^{c}	60.42 ± 0.41^{c}	-2.67 ± 0.04^{c}	3.58 ± 0.33^{b}
GLF_15	61.25 ± 0.55^{b}	10.08 ± 0.12^{d}	6.78 ± 0.29^{d}	58.58 ± 0.35^{b}	-1.43 ± 0.04^{d}	4.61 ± 0.26^{c}
GLF_20	57.42 ± 0.55^{a}	10.95 ± 0.15^{e}	8.84 ± 0.20^{e}	56.28 ± 016^{a}	-0.86 ± 0.02^{e}	5.52 ± 0.37^{c}

The results are the mean ± standard deviation (n = 10). L*: lightness; a*: green–red (−a = green and +a = red) opponent colors; b*: blue–yellow (−b = blue and +b = yellow) opponent colors. Bread samples containing germinated lupine flour, GLF: $^{a–e}$, mean values in the same column followed by different letters are significantly different ($p < 0.05$).

As can be seen from Table 5, the value of the parameter L* (luminosity), both in the case of crumb and crust, decreased as the amount of GLF addition increased. Therefore, they became darker in color due to the addition of GLF. The value of the parameter a* (chromaticity on a green (−) to red (+) axis) increased due to the addition both in the case of the bread crumb and in the case of the bread crust. Thus, the GLF addition led to obtaining samples of bread with a more reddish color. Regarding the value of the parameter b* (chromaticity on a blue (−) to yellow (+) axis), it can be seen from the data obtained that the numerical value increased, which means that the yellow hue of the samples intensified. Therefore, both crumb and bread crust had similar variations of the color parameters values depending on the GLF addition level in the sense that the value of the parameters L* decreased and the value of the parameters a* and b* increased with the increased level of the GLF addition in wheat flour.

3.4.3. Texture Profile Analysis of Breads Samples

Table 6 shows the variation of the texture properties of the bread samples, depending on the addition level of GLF, compared to the control sample. The evaluation of these parameters is an important factor in breadmaking, because they are indicators of the bread quality. All four texture parameters determined (firmness, gumminess, cohesiveness and resilience) were influenced by the GLF addition in wheat flour. According to our data, the GLF addition led to a decrease of the gumminess and cohesiveness values up to a maximum addition level of 15% GLF in wheat flour. An increased level of 20% GLF addition led to an increase in the values of these parameters. In the case of resilience values, they decreased with the increased level of GLF addition in wheat flour.

3.4.4. Crumb Structure of Breads Samples

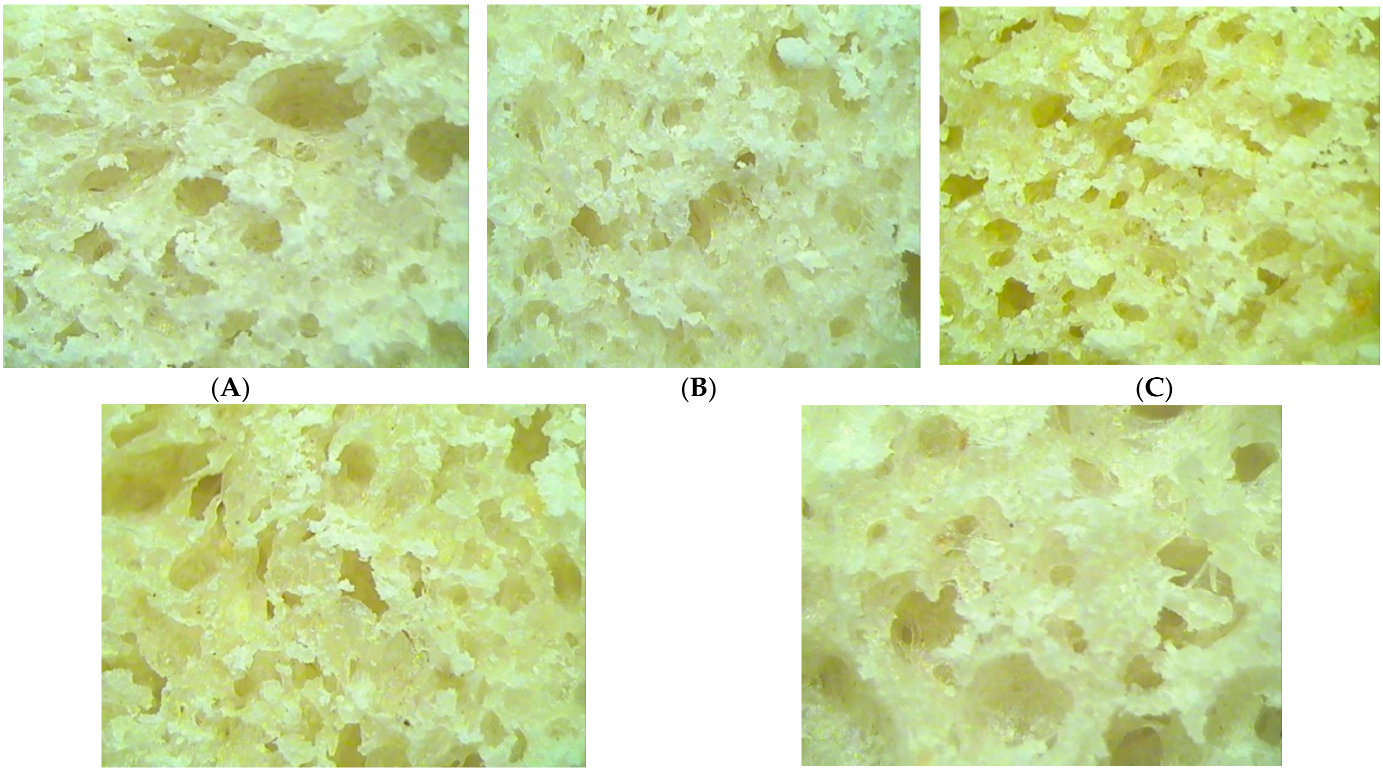
The crumb structure of the bread is an indicator of its quality, because it has an influence on the consumers' choice, being at the same time an indicator of the porosity of the bakery products. Consumers prefer well-grown bread with fine pores. The structure of the bread refers to the profile of the pores formed due to the carbon dioxide that is released during the proofing stage of the dough and which is retained in the dough network during the proofing and baking stage.

Table 6. Texture parameters of the bread samples with different levels of GLF additions.

Bread Samples	Firmness (*N*)	Gumminess (*N*)	Cohesiveness (Adimensional)	Resilience (Adimensional)
Control	9.01 ± 3.06^{a}	7.23 ± 1.73^{bc}	0.82 ± 0.03^{c}	1.72 ± 0.04^{e}
GLF_5	13.19 ± 0.02^{b}	7.62 ± 0.04^{c}	0.85 ± 0.03^{c}	1.78 ± 0.01^{d}
GLF_10	14.77 ± 0.04^{b}	5.41 ± 0.04^{ab}	0.76 ± 0.01^{a}	1.63 ± 0.01^{c}
GLF_15	16.36 ± 0.06^{bc}	4.06 ± 0.04^{a}	0.63 ± 0.02^{b}	1.32 ± 0.01^{b}
GLF_20	19.33 ± 0.05^{c}	6.16 ± 0.03^{bc}	0.52 ± 0.01^{a}	1.06 ± 0.04^{a}

The results are the mean ± standard deviation (n = 3). Bread samples containing germinated lupine flour, GLF: $^{a–e}$, mean values in the same column followed by different letters are significantly different ($p < 0.05$).

Figure 4 shows that the addition of GLF resulted in bread samples with larger pores and a lower pore density. Thus, with the increase in the percentage of GLF addition, the pore diameter increased, and their density decreased. However, as it resulted from the sensory determinations, the addition did not negatively influence, beyond the limit of acceptability, the visual perception of the consumers.



(**A**) (**B**) (**C**) (**D**) (**E**)

Figure 4. Structure of wheat dough with germinated lupin flour (GLF) at different levels: 0% (**A**), 5% (**B**), 10% (**C**), 15% (**D**) and 20% (**E**).

3.4.5. Sensory Analysis of Breads Samples

After all, consumer opinion is the most important. Thus, the aspects related to the sensory analysis of bread samples (appearance, color, taste, smell, texture and global acceptability) provided valuable information regarding the quality of the samples and how the addition of GLF in the breadmaking recipe influences the perception of consumers regarding bread samples. These sensory determinations help to determine the optimal addition, so that the bread obtained fully meets the preferences of consumers also in terms of sensory aspects.

Figure 5 show that the addition of GLF in the breadmaking recipe had an influence on all sensory characteristics. Thus, a maximum of 10% of the GLF addition in wheat flour had the effect of improving the sensory characteristics of the bread samples. On the other hand, at an increased level of 20% GLF addition in the bread recipe, these characteristics were assessed less than in the case of bread samples without any GLF addition. Thus, it can be concluded that it is not recommended to exceed 15% of the GLF addition in wheat flour, so that the sensory attributes are not negatively influenced.

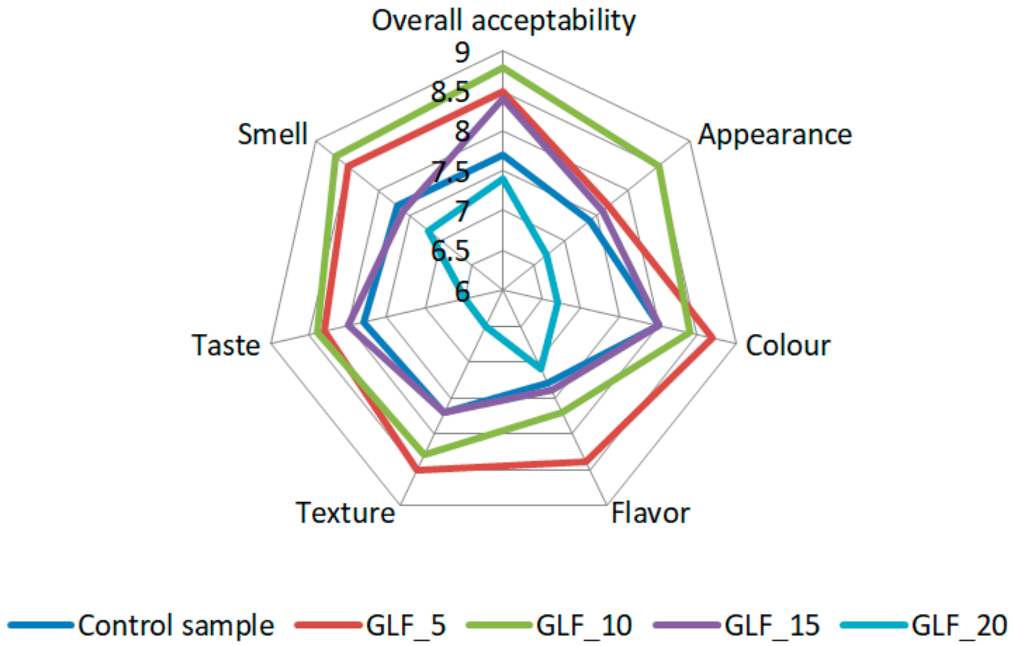


Figure 5. Sensory analysis for the bread samples.

4. Discussion

4.1. Dough Rheological Properties

4.1.1. Dough Rheological Properties during Mixing and Extension

According to our data, the addition of GLF decreased the value of the water absorption parameter. Thus, for a maximum value of the 20% GLF addition in wheat flour, the value of this parameter decreased up with 4.03%. The decrease in the water absorption capacity can be explained by several considerations that are attributed to the changes brought about by the germination process on the components of the lupine bean. Thus, knowing that, during germination, there is an activation of the enzymes of lupine, it is clear that these enzymes will act on proteins, resulting in amino acids and peptides. Additionally, during germination, part of the amount of protein and starch is used to develop the component parts specific to the sprout. At the same time, the starch is also broken down into dextrins. Thus, these compounds will have a lower water absorption capacity. The decrease in the amount of starch also has the effect of reducing the emulsifying and foaming capacity of lupine flour, leading to a decrease in water absorption. Our data were similar with those reported by other studies that have also noticed a decrease of the water absorption capacity with the increased level of GLF addition in wheat flour [25,26]. The decrease in the value of the tolerance to the mixing index with the increased level of GLF addition in wheat flour may be explained by the fact that, during the germination stage, the enzymes from lupine grains were activated. Amylases had the effect of hydrolyzing starch, which led to an increase in the amount of maltose. At the same time, the partial substitution of the white wheat flour with GLF led to a decrease in the amount of gluten, which had the effect of decreasing the stability of the dough network. The decrease in the value of the dough consistency parameters after 250 s and 450 s may be explained due to GLF composition. GLF contains a higher amount of dietary fiber and protein, which influences the viscosity of the dough. The dietary fiber in the composition of lupine grains interferes with the gluten in wheat flour, which had the effect of increasing the viscosity and decreasing the values of the consistency parameters values. Studies in the field have also shown that the addition of germinated legumes in wheat flour has, in general, the effect of decreasing the dough consistency [27].

Regarding wheat flour dough rheological properties during extension, it may be seen that an increase in the value of the dough tenacity with the increase level of GLF addition in wheat flour indicates that the dough becomes firmer. The highest value of the P (maximum pressure) parameter was recorded for the dough samples with an addition level of 20% GLF in wheat flour. The increase in the value of the P index (dough tenacity) may be attributed to the increase in the amount of protein in the dough network [28] due to the GLF addition in the dough recipe. Dough extensibility significant decreased ($p < 0.05$) with the increase level of GLF addition in wheat flour. The decrease of this value may be due to the water competition between the proteins and fibers from the GLF composition and the proteins from wheat flour [29]. The results of this study were similar with other studies, which also have been reported that the addition of leguminous fiber in wheat flour dough led to a decrease in dough extensibility [30]. Additionally, other studies have explained the decrease of dough extensibility due to the increase in the sulfhydryl and thiol groups from sprouts flour, which may produce an oxidation of dough during mechanical actions [31]. Cappelli et al. highlighted that the values of the parameters' dough extensibility (L) and index of swelling (G) depend on the amount of gluten and starch in the dough network [32]. More, the dough rheological properties during extension were determined as constant hydration for all the dough samples analyzed. According to the Consistograph data by GLF addition in wheat flour, the water absorption significant decreased ($p < 0.05$) with the increased level of GLF addition in the dough recipe. This will lead to a water excess in the dough system for the Alveograph evaluation that will conduct a lower value of Alveograph dough baking strength (W).The fact that the value of the two parameters decreased with the increase level of GLF addition in wheat flour can be explained by the fact that the amount of gluten in the dough network has decreased. Similar results were reported by Cappelli et al., which concluded that dough extensibility decreased due to a replacement of more than 10% of wheat flour with chickpea flour [33]. The decrease in the value of the W parameter (deformation energy) with the increase level of GLF addition in wheat flour may be due to the gluten dilution but, also, due to the fact that the proteins from GLF compete with the proteins from wheat flour, which delays the formation of gluten [34]. Similar data has also been reported by others for dough samples with different levels of legume flour addition in wheat flour [35]. The value of the P/L significantly increased ($p < 0.05$) with the increased level of GLF addition in wheat flour. This increase may be due to a higher amount of fiber from the dough system due to the GLF addition in wheat flour. The increase in p-value, along with the decrease in the L, G and W values, can be explained by the increased amount of cellulose present in the composition of lupine in dough, which is able to form strong interactions with proteins from wheat flour [11].

4.1.2. Dough Rheological Properties during Fermentation and Falling Number Values

The results recorded with the Rheofermentometer device showed that, to a GLF addition level between 5% and 15% in wheat flour, the maximum height of gaseous production (H'm) increased compared to the control sample. This parameter had a lower value at an addition level of 20% GLF in wheat flour. The increase in the value of this parameter can be explained by the activity of amylolytic enzymes, which were activated during lupine germination and which had the effect of increasing the amount of carbon dioxide released during the fermentation phase of dough, because these enzymes acted on starch, resulting in maltose, which the yeast needs during fermentation. Thus, there was an extension of the dough, depending on the amount of the gas released and the ability of the dough to retain it in the system, which corresponded to the increase in the value of the H'm parameter. The decrease in the value of this parameter to an addition of 20% GLF in wheat flour may be explained by the weakening of the dough network when high levels of GLF were incorporated in the dough recipe due to a decrease in the amount of gluten produced by GLF addition. This had led to a decrease in the capacity of the dough to retain the gas released in the system during the proofing stage of the dough [36]. A similar trend evolution with the H'm value may also be noticed for the VR and CR parameters, which increased up to a 15% GLF addition in wheat flour and decreased when 20% of GLF was incorporated in the dough recipe. These data are in agreement with other studies that have reported an increase in gas production during the leavening stage when different germinated flours were added in wheat flour [37]. Regarding the Falling Number index, it can be stated that the wheat flour used in this study was one with a low α-amylase activity, because the Falling Number value was higher than 330 s [38]. The GLF addition led to a decrease in Falling Number values up to 270 s due to the amylases enzymes from its content. This value indicates a normal α-amylase activity in the mixed flours [38,39].

4.1.3. Dough Fundamental Rheological Properties

The storage modulus (G') characterizes the elasticity of the dough, and the loss modulus (G'') is an indicator of the viscoelastic properties of the dough [40]. Tan δ is related to the general viscoelastic response of the dough [41]. The fact that, in all frequency ranges, the storage modulus was higher than the loss modulus indicated that the viscous properties of the dough samples were less prominent than the elastic ones [25]. The fact that the values of G' and G'' increased as the additional level of GLF increased shows that this addition improved the viscoelasticity of the dough. These data were similar to the results reported by others when germinated peas [25] or roasted chickpea flour [42] were incorporated in dough recipes. The fact that the value of the parameter tan δ is less than 1 indicates that the dough had a solid-like behavior. The increase in the value of this parameter due to the addition of GLF is in correlation with the increase in the ratio of elastic structure.

The decrease in G', G'' and tan δ values with the increase of the dough temperature was caused by denaturation of the proteins. Their increase once the temperature was higher was due to the gelatinization process of the starch. Hydrolysis of starch after the gelatinization stage reduced the consistency of the dough. The process of gelatinization of starch led to an increase in the viscoelasticity of the dough, and when this process was intensified, the viscoelasticity began to decrease. The gelatinization process of starch was influenced by the activity of the amylase enzymes, especially the α-amylase one. This enzyme was activated in lupine beans during the germination process. As the level of GLF addition in the dough recipe increased, the activity of the enzyme in the dough was higher, and thus, the process of the gelatinization of starch was intensified, which led to a decrease in viscoelastic modules. After gelatinization, the hydrolysis of starch becomes very evident due to the α-amylase activity [43]. Therefore, as the GLF addition in wheat flour increased, the G' and G'' values were lower, and the tan δ values were higher compared to the sample without GLF addition in the dough recipe. When the inactivation of α-amylase occurred, the hydrolysis of the starch stopped, which led to the beginning of the increase of the dough consistency.

4.2. Dough Microstructure

The images obtained for the dough microstructure showed that, at low levels of GLF addition in wheat flour, starch granules appeared to be linked, forming groups in some areas. The effect of GLF addition is more evident with the increase level of GLF added in the dough system. The protein content became higher, and as expected, more red area was seen in the dough structure. GLF seemed to act as a filler of the dough matrix that exhibited a quite homogeneous and continuous network in the dough. As GLF increased, the starch granules become more enveloped by protein. This fact is explainable, since lupine flour contains a high amount of protein compared to the wheat flour. Therefore, by wheat flour substitution with GLF flour, the amount of protein from the dough system increased whereas the amount of starch decreased.

4.3. Bread Quality Evaluation

4.3.1. Bread Physical Characteristics

The influence of the addition of GLF in wheat flour on the increased value of the specific volume of the bread samples may be related to many factors such as: activation of lupin enzymes during germination, change in the process of protein aggregation and starch gelatinization, change in the protein solubility and the ability to foam and emulsify of the lupine flour and change in the amount of fermentable sugars. Activation of amylases during GLF germination leads to an increase in the amount of fermentable sugars, to an increase in the yeast capacity and, implicitly, to an increase in the release of carbon dioxide. Partial degradation of starch granules also results in an increased expansion of gas cells [44]. The improvement in the bread-specific volume may also be due to the fact that lupine has a higher fat content. This is explained by the fact that lipid monolayers are formed at the gas/liquid interphase of the gas cells but also by the fact that lipids increase the capacity of the dough to retain gases and to stabilize gas cells [45]. Moreover, during germination, the foaming and emulsifying activity of the GLF improved [25,46]. Similar results have been presented in other studies, which have reported that the addition of lupine flour in wheat flour at low levels improved the loaf volume of bread samples [11]. However, when high levels of GLF were added in bread recipes, its value decreased. Given the fact that lupine does not contain gluten, it is understandable that, as the amount of GLF added increased, the amount of gluten in the dough network decreased. Gluten is a protein that has a special importance in breadmaking due to its ability to expand during the proofing and baking process but, also, in its ability to retain gases. The decrease in the value of the specific volume of bread samples to an addition of more than 15% GLF in wheat flour may be explained by the fact that, in the dough system, a disruption of the gluten matrix by the nonelastic lupine proteins from GLF incorporated in bread recipe may occur [47]. At the same time, the decrease in the loaf volume value may be a consequence of the increase in the viscosity of the dough and its resistance to deformation, which leads to a decrease in its expansion capacity during the proofing and baking stages [42]. The lowest loaf volume value for the bread sample with the highest level of GLF addition in the bread recipe compared to the control one is in accordance with the Alveograph data that presented, for this sample, the highest tenacity and the lowest extensibility and baking strength values. However, a worsening of the dough rheological properties during extension was noticed, and when low levels of GLF were added in wheat flour, values that are in contradiction with bread quality characteristics improve the data. This fact may be explainable, since the Alveograph characteristics were determined for constant hydration, whereas the bread samples were made for the optimum mix water absorption capacity. Adding the same amount of water in the Alveograph mixer will lead to water excess for the mixed dough, which will have lower dough baking strength (W) values. However, this value does not vary in a significant way ($p < 0.05$) for dough samples with low levels of GLF addition in wheat flour. When bread is obtained and other ingredients are used such as yeast, the fermentation period is higher, and therefore, the bread quality data are more related with the Rheofermentometer values, which were also improved for dough samples up to 15% GLF addition in wheat flour. Moreover, the elasticity of the dough decreased due to the gluten dilution as a result of the wheat flour substitution by GLF addition in wheat flour [11]. The decrease in the loaf volume of the bread can also be attributed to the insoluble fibers from the composition of lupine (interaction between fibers and gluten) and to the reducing amount of starch from the dough system [25].

According to our data, the porosity of the bread samples increased up to a 15% GLF addition in wheat flour. This may be due to the enzyme activity from the GLF system, especially amylases, which act on starch, leading to an increase in the amount of fermentable sugars metabolized by yeasts, which increased the amount of carbon dioxide released. Consequently, the porosity of the bread samples increased. The fact that the porosity value of the bread samples was not adversely affected up to a level of 15% GLF addition in wheat flour indicates that the gluten network is still able to retain in it structure the gas released during the fermentation and baking processes of the bread samples [26,39]. Of course, with the incorporation of lupine flour in the breadmaking recipe, the amount of gluten in the mix flour decreased and, as has been pointed out in various studies, the porosity value decreased [48]. This decrease has been recorded in our study only at high levels of 20% GLF addition in wheat flour.

The improvement of the bread elasticity up to 15% GLF addition in wheat flour can be explained in terms of amylases that were activated during the germination stage of lupine grains and were incorporated in wheat flour [49,50]. Studies in the literature have already shown that bread with the addition of α-amylase has a higher elasticity. This is due to the accumulation of dextrins in the crumb but, also, to the better gelatinization of the starch left unhydrolyzed, which retrogrades more slowly [51].

4.3.2. Color Analysis of Breads Samples

Regarding the parameter L*, its value decreased with the increased level of GLF addition in wheat flour, both in the case of the crumb and the crust of the bread. One explanation for this decrease is due to the increased amount of protein from the dough system due to the GLF addition in wheat flour [52]. This can be explained in terms of the Maillard reaction. A higher amount of amino acids, due to the higher amount of proteins, will lead to an increase of the intensity of the Maillard reaction and to the specific browning compounds formed [53]. Additionally, by GLF addition in wheat flour, the enzymatic activity in the dough system increased, leading to a release of the reducing sugars and amino acids, which, during baking, participate in the Maillard reaction, leading to the darkening of the bread crust [54]. Regarding the decrease of the brightness value due to the addition of GLF in wheat flour, a similar trend was observed in other food products in which different legume flours or powders were incorporated into their recipes [55]. The increase in the value of the parameter a* can be explained in terms of a higher amount of ash (and, thus, of minerals) due to the addition of GLF in wheat flour [56]. The increase in the value of parameter b* can be attributed to the fact that GLF contains a large amount of yellow pigments (carotenoids) [56] but also a high amount of proteins and enzymes that favor the Maillard reaction, leading to a specific shade of yellow of the bread samples [57].

4.3.3. Texture Profile Analysis of Breads Samples

The influence of the addition of GLF on the textural characteristics of the bread samples can be explained by the fact that lupine flours are characterized by a good water- and oil-binding capacity and specific emulsifying and foaming properties. At the same time, the increased level of GLF in wheat flour will lead to an increase of the α-amylase activity, which will increase the low-molecular-weight dextrin and will conduct better gelatinization of the nonhydrolyzed starch in the presence of water released by the coagulating proteins [22,51]. Therefore, the cohesiveness and resilience increased when low levels of GLF were added in wheat flour. However, due to the gluten dilution, these values begin to decrease. Additionally, the firmness value increased with the increased level of GLF addition in wheat flour [58]. Other studies have reported that the firmness increase is due to the increase of fiber content from the legume composition, which has the ability to form films and bind and hold water throughout the baking process [59].

4.3.4. Crumb Structure of Breads Samples

The characteristics of the bread structure are influenced primarily by the amount of carbon dioxide, resulting in the proofing stage of the dough, but also by the ability of the dough structure to retain gases in the proofing and baking stages of breadmaking. Due to the fact that, during germination, there was an activation of the enzymes from lupine grains, these enzymes will have an effect on the breakdown of starch into fermentable sugars, necessary for the activity of yeast with an increasing amount of carbon dioxide released and the improvement of the bread porosity. Thus, bread with a crumb with uniform pores in size will result [60]. The uneven distribution of pores can be explained by the fact that, due to the GLF addition in wheat flour, the amount of gluten in the dough network decreased, gluten having a significant role in retaining the gas formed during the proofing and the baking stages of breadmaking.

4.3.5. Sensory Analysis of the Bread Samples

In general, the bread sensory characteristics have been improved by GLF addition in wheat flour. This may be due to the germination process that lupine grains were subjected that improved its sensory profile and changed its composition. This fact and, also, the increased level of enzymes from GLF, especially the amylases and proteases ones, increase the amount of simple sugars and aminoacids from the dough system. These components are the precursors of the aromatic compounds resulting from the Maillard reaction, which has an effect on improving the sensory profile of the bread samples [61]. Moreover, some studies have shown that germination also has the effect of reducing the specific bitter taste of the grains subjected to this process [62]. It has also been reported in other studies that the samples of bread with the addition of sprouted legumes have a special sweetness. This is due to the fact that, during germination, activated enzymes convert starch into oligosaccharides and sugars that give a specific taste of sweet. Germination also leads to the formation of compounds with a specific taste and smell of caramel. Similar results were also reported by Perri et al. for bread samples with different germinated legume types incorporated in the bread recipe [54]. More, the sensory characteristics appearance and texture are related to the bread physical and textural characteristics, of which the values were improved with the increased level of GLF addition up to 15% in wheat flour. Additionally, the bread samples with the highest level of GLF addition in wheat flour were less appreciated by consumers, probably due to its low specific volume, porosity and elasticity. This may be due to the fact that, to high levels of GLF addition in wheat flour, the dough network was no longer able to retain carbon dioxide in its structure, which had a negative effect on the bread quality. Additionally, a higher level of GLF addition in wheat flour may lead to a bread sample with a specific lupine-like smell, aroma and taste, which they may not like.

5. Conclusions

The addition of germinated lupine flour to the breadmaking recipe affected both the rheological properties of dough and the qualitative characteristics of bread. Regarding the rheological aspects of the dough, it was observed that the consistency of the dough, baking strength and extensibility decreased with the increased level of GLF addition in wheat flour. The dough samples with GLF addition presented an increase in the value of tan δ, indicating a less-elastic structure. As the temperature increased, the dynamic modules decreased with the increased level of GLF addition in the dough recipe. The decrease in the value of the Falling Number index with the increase level of GLF addition showed an increase of the α- amylase activity in the mixed flours up to a normal value for breadmaking. Dough rheological property values during the fermentation maximum height of the gaseous production and total CO_2 volume production increased when low levels of GLF were incorporated in the dough recipe, mainly due to a more intense amylase activity. However, at a high GLF level addition in wheat flour, their value decreased due to the gluten dilution from the dough system.

The addition of GLF in wheat flour up to a maximum of a 15% value led to an improvement in the quality characteristics of the bread samples. An addition of 15% of GLF can be used to obtain a good quality of bread only for wheat flour, which is characterized by a strong baking value and a low α-amylase activity, because a level of addition higher than 10% has a significant influence on lowering the quality of the flour. The samples with GLF were darker in color, as shown by the values of the L*, a* and b* parameters. The GLF addition in the dough recipes in low levels conducted in bread samples with uniform pore sizes that become higher when high levels of GLF were added in bread. The sensory analysis showed that the bread samples with GLF addition in wheat flour were better appreciated by the consumers compared with the control one.

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