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Influence of Legumes Flour on Physical and Sensory Attributes of Gluten-free Bread

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Abstract

The aim of this investigation was incorporation of some legume flours into making of gluten-free pan bread (GFPB) and study their impact on the physical properties, staling rate and acceptability of such bread. Three types of legume flours namely**:** defatted soybean (DSB), sweet lupine (SL) and chickpea (CP) were used. The base formula (BF) consisted of 47.5 g rice flour, 47.5 g corn starch, mixed with constant amount of skim milk powder and xanthan gum (XG). The starchy formula (rice flour and corn flour) abbreviated as SF was partially replaced by 10, 20, and 30 g of any legume flour. The results of physical properties indicated that substitution 30g SF by that of CP flour gave highest specific volume of bread than the other gluten-free samples, while replacing 30g of DSB flour decrease significantly the specific volume. However, substitution of 30 g SF by equal amount of DSB flour gave the highest bread yield. Furthermore, substitution 10 g SF by the same amount of DSB flour showed the highest baking loss value. While this value significantly diminished when 20g SF substituted by equal proportion of DSB or SL flours as well as by mixture of 10g DSB and 10g SL. In addition, CP flour at level of 20g was characterized by the lowest significant firmness. Texture profile analysis (TPA) results indicated that hardness and chewiness increased, while resilience, cohesiveness, springiness and staling rate decreased with increasing DSB flour replacement. In contrast, hardness and chewiness significantly decreased at 20g CP flour.

Key words: *Gluten-free, Legume, Staling, TPA, sensory.*

1. Introduction

Gluten-free foods (GFF) are specified for Patients with celiac disease. Celiac disease, a chronic enteropathy caused by gluten intolerance, prolamin, led atrophy of intestinal villi, malabsorption and clinical symptoms in childhood and adulthood **[1, 2].** celiac's people are unable to consume certain gluten proteins from cereals such as wheat, rye, barley and triticale **[3].** Although they just make up a tiny portion of the population (1.4%) **[4].** However, there has been a 64% increase in gluten-free diet (GFD) consumption since 2013 **[5]**, may be due to the popular belief that a gluten-free (GF) diet is a healthier option along with gluten-related disorders driving an increasing number of consumers to opt for a gluten-free diet as a lifestyle choice **[6].** This prompted researchers numerous research have been conducted on GF loaves of bread and foods to improve their technological and nutritional properties **[7, 8].** Nonetheless, other studies have found that consumers are dissatisfied with the quality of gluten-free bread and foods **[9]**.

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Legume aren't contain gluten but are one of the most important sources of protein. Legume playing a big role in the texture and sensory qualities improvement of gluten-free bread. Therefore, efforts should be made to incorporate legumes into gluten-free flour in order to provide nutrient-dense foods with appetizing flavor and texture **[10].** Furthermore, legumes have recently been conspicuous among scientists as texture- and structure-forming agents, as a source of beneficial and bioactive compounds, and as a lowly-glycemic-index ingredient **[11].** In the same trend, **[12]** mentioned that the challenge of gluten-free bread with soybean flour and usual formulations hinges, nevertheless, on combining levels adequate to yield health benefits and safe gluten-free foods to celiac's people, while guaranteeing optimal functional properties and maintaining organoleptic quality. Moreover, Experimentation in standard bread making showed that lupine protein isolate (LPI) addition enhances extensibility and resistance to deformation, as well as bread volume and texture **[13, 14].** Also obtained similar results when using lupine protein isolate replacement ratios with starch in gluten-free bread, which clearly indicates the importance of lupine in improving gluten-free bread. On the other hand, lupine proteins added to gluten-free bread recipes resulted in low acceptance and an overall low opinion of appearance, also, many studies have used chickpea flour to improve gluten-free bread, especially because of its effects on reducing the glycemic rate, based on the fact that there appears to be a high incidence of Type I diabetes among celiac **[15].** Even though adding legume flour to gluten-free mixtures has been shown to have many benefits; but there were drawbacks to using legumes, particularly when using large amounts of them. These mainly relate to a reduction in the bread's sensory qualities **[16]**, and the lack of guidance on the optimal ratios for incorporation.

Bread is well-acknowledged as an important staple food that is high in carbohydrates, fiber, vitamins, and minerals **[17].** Wheat is considered a major food source in some countries, Egypt, for example, consumes more than 160 kg of wheat per person each year, most of which is consumed in the form of bread **[18].** Making bread is a three-step procedure that includes dry ingredient blending, hydration, fermentation, and baking. Wheat, barley, and rye flours are often used in bread-making because gluten proteins have superior functional qualities in terms of their ability to be rehydrated and form a cohesive, visco-elastic dough that entraps gases produced during fermentation **[19].** The development of high-quality gluten-free bread made from components other than wheat flour is a major challenge, in addition, the GFB is poor in protein, minerals, vitamins and dietary fiber. Therefore, this investigation was carried out to incorporate some legumes flour into making of gluten-free pan bread and study their effect on the quality, sensory, staling rate and acceptability of bread with using the optimal combination of base formula recommended by **[20].**

2. MATERIALS AND METHODS

2.1 Materials:

Wheat flour (*Triticum aestivum*), Commercial rice (*Oryza sativa*), Corn starch (*Zea maays amylace*), Sweet lupine (*Lupinus albus*), Chickpea (*Cicer arietinum*) and Skim milk powder were purchased from marketplace in Aswan Governorate, Egypt. Defatted soybean (*Glycine max*) was obtained from a Soy products outlet, Agriculture

Research Center, Giza, Egypt. Basic ingredients for dough making such as instant dry yeast (*Saccharomyces cerevisiae)*, sunflower oil, salt, and sugar were purchased from a local market in Aswan, Egypt. Xanthan gum (XG) was obtained from Loba Chemie PVT. Ltd. Co. India.

2.2 Methods:

2.2.1 Preparation of raw materials:

Defatted soybean, sweet lupine and chickpea were cleaned and roasted at 140˚C for 60 min in a convection oven (Bender heating chambers: Bd 115, Germany). Rice grains and other roasted materials (sweet lupine and chickpea) were milled to flour by using a laboratory mill (Roller mill 4000, Bastaka Turkey), then sieved to obtain fine particles less than 75 μm in diameter (pass through a 200 mesh sieve). Rice flour and legume flours were packed in plastic bags and stored at −20 ˚C until use.

2.2.2 Preparation of gluten-free composite flour:

Gluten free composite flours were prepared by substituting SF (47.5g rice flour and 47.5g cornstarch) with various portions of each legume flour as shown in Table 1. All prepared samples were used for GFPB making.

Table 1: Composite flour formulation.

Treatment Code	Wheat flour (g)	Rice flour (g)	Corn starch (g)	Defatted soybean (g)	Sweet lupine flour (g)	Chickpea flour (g)	Skim milk powder (g)	Xanthan gum (g)
Control	**100**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Mixture of gluten free flours for making GFPB								
T1	-	47.5	47.5	-	-	-	5	2
T2	-	42.5	42.5	10	-	-	5	2
T3	-	37.5	37.5	20	-	-	5	2
T4	-	32.5	32.5	30	-	-	5	2
T5	-	42.5	42.5	-	10	-	5	2
T6	-	37.5	37.5	-	20	-	5	2
T7	-	32.5	32.5	-	30	-	5	2
T8	-	42.5	42.5	-	-	10	5	2
T9	-	37.5	37.5	-	-	20	5	2
T10	-	32.5	32.5	-	-	30	5	2
T11	-	37.5	37.5	10	10	-	5	2
T12	-	37.5	37.5	10	-	10	5	2
T13	-	37.5	37.5	-	10	10	5	2
T14	-	40	40	5	5	5	5	2

2.2.3 Bread making of GFPB:

Gluten-free pan breads were made from different composite flours in Table 1, with 4g oil, 1.2 g salt, 1.5 g instant dry yeast and 6 g sugar as well as 110 ml water, whereas variable water for control bread (baked from wheat flour) was used. All dough samples were put in an aluminum pan. GFPB was made according to a method described by **[20],** while control pan bread was baked according to a method provided by **[21]** with slight modifications where the baking temperature was started with 240 °C for 5 minutes and thereafter decrease to 180 °C for 22 minutes.

2.2.4 Physical properties of GFPB:

Weight of pan bread: This was determined by weighing the bread loaves using a laboratory weighing scale (IIAXIS, CR12, Japanese) after 2 hrs of baking and the values were expressed in grams (g).

Bread volume: Alfalfa seeds (*Medicago sativa*) displacement was used to calculate the volume of each bread loaf after 2 hrs of baking according to the method described by **[22].**

Specific volume: Specific volume of the bread samples calculated by dividing volume (cm^3) by weight (g) of bread, as described by **[23].**

Height/ width ratio: A slice of bread was cut from the middle, the width of the bottom of the bread slice was measured in centimeters, and then the height of the bread slice was measured from the middle of the bottom to the highest point at the top. The height/width ratio was obtained by dividing height/width.

Bread yield: After cooling of bread for two hrs the bread samples were weighed to calculate bread yield according to **[24]**, and based on the following equation: $P_1 = (W_2 / W_1) \times 100$; where, P_1: bread yield, W_2: bread weight and W_1: flour weight.

Baking loss: Baking loss computed as described by **[25]** according to the following equation:

$$\textbf{Baking loss} = \frac{\text{Initial loaf weight before baking} - \text{Loaf weight after 2 h of baking}}{\text{initial loaf weight before baking}}$$

Crumb firmness: Breads were longitudinally cut 2 hours after baking to test crumb firmness, and one 25 mm thick slice was taken from the center of each loaf. Firmness was measured using a Texture Analysis Machine **(BROOKFIELD, Ct3, USA)** equipped with a 33 mm diameter probe **(AACC.Ref: TA-AACC 36)** at a rate of 2 mm/sec and 40% deformation and compression. Firmness was defined as the maximum force obtained at 25% deformation (recorded in either Newton's or g).

2.2.5 Color characteristics:

Chroma Meter CR-400 **(Konica Minolta Co., Ltd., Osaka, Japan)** was used to measure the color of the bread in three replicates for each sample. Three points were used to determine the color of the crust and crumb as described by **[26]**. Using a D65 light source and a 2° observer angle, L* (lightness), a* (+a*=redness, -a*=greenness), b* (+b*=yellowness, -b*=blueness), and ΔE were measured. L*, a*, and b* readings' tristimulus values were calibrated against a standard white plate (Y=85.6; x=0.3149; y=0.3213).

2.2.6 Texture profile analysis (TPA) of loaf:

Textural profile analysis was carried out on three replicates made for each sample using a Texture Analysis Machine (BROOKFIELD, Ct3, USA). Texture profile analysis (TPA) was used to simultaneously quantify hardness, springiness, cohesiveness, chewiness, and resilience of bread slices, as stated in a paper by **AACC Method (74-09)**. Breads were cut using a slicer knife into slices with an approximate height of 25mm (the crust was removed). Force calibration of the instrument was done prior to the start of the experiment to minimize measurement error. Afterward, the height calibration was done with a return distance of 20mm. The bread samples were compressed twice with a 36 mm diameter stainless steel cylinder **(AACC.Ref: TA-AACC 36)** to the strain of 40%, while a 10kg load cell was equipped.

2.2.7 Staling rate of bread crumb:

Hardness of bread crumb measured after 2, 24, 48 and 72 hrs after baking where the bread kept at room temperature. Staling rate of stored bread was calculated according the methods described by **[27].**

2.2.8 Sensory characteristics evaluation:

Sensory evaluation of control and gluten-free breads were carried out as the 9-point Hedonic Scale described by **[26]**. Sensory analysis was performed after 2hrs of baking by ten panelists (5 women and 5 men) from Food Science and Technology Department; Faculty of Agriculture and Natural Resources; Aswan University. Samples were evaluated by measuring seven scales; general appearance, color of crust and crumb, crumb grain distribution, flavor, taste and freshness, with the anchor points 1 extremely dissatisfied and 9 extremely satisfied. The acceptance percentage was expressed as the sum of points for all criteria divided by a total score of 63. Testing took place in a Food Science and Technology laboratory. Drinking water was supplied during the tests to cleanse the palate between each scale.

2.2.9 Statistical analysis:

The Statistical analysis was carried out using IBM SPSS Statistics PC statistical software. LSD Multiple Range Test was applied to assess significant differences between means at 5% levels of probability. Each experiment in triplicate reported and the values presented in terms of means standard error. Means with different letters in the same column different significantly at ≤ 0.05, while those with similar letters are not significant by differences **[28].**

3. RESULTS AND DISCUSSION

3.1 Physical properties:

Specific volume (SV) is one important parameter in GFB making, known to strongly influence consumer's choice, the loaf specific volume, as larger loaves are perceived as more appealing. Likewise, from an economic standpoint, a high ratio of volume per weight is also desirable for the producer **[29].** SV of GFPB was affected by increasing levels of legume flour as partial replacement of SF as shown in Table (2). The increasing proportion replacement of defatted soybean and sweet lupine in gluten-free flour caused a decrease in specific volume of bread compared to T1 (2.53cm^3/g) as a base formula (without substitution legume flour). On the contrary, SV increased with boost substituting quantities in samples containing chickpea flour from 2.2 to 2.53 cm^3/g. Samples containing defatted soybean and sweet lupine flours showed gradual decrease in volume and specific volume with increasing the amount substituted of these legumes, who could be attributed to the loaf's structure being unable to retain gas during the baking and proofing process, which is a result of its higher fiber content **[10, 30].**

Furthermore, a chickpea's as partial replacement of SF in the amount of 30g results in the best values of specific volume (2.53 cm^3/g) which is due to chickpea proteins having higher foam expansion and stability values than SL and soybean proteins due to the specific content of amino acids **[31].** Moreover, samples T12, T13 and T14 containing mixed replacement ratios of legumes indicated that the presence of chickpeas with 10g in sample T13 gave the best SV values (2.37 cm^3/g); supporting the interpretation of chickpea's benefits in raising specific volume. Generally, the results in Table (2) showed that the high specific volume were 2.53, 2.53 and 2.37 cm^3/g for T1, T10 and T13; respectively compared to other samples except the C sample (wheat bread) which had highest value with 3.1 g/cm3. The Height/Width **(**H/W) ratio ranged from 1.08 to 1.74 and there are no significant difference observed between samples.

Table 2: Physical characteristics of pan bread baked from gluten free composite flour.

T	Weight of bread (g)	Volume (cm³)	specific volume (cm³/g)	H/W* ratio	Bread yield %	Baking % loss	Firmness (gf/cm³)
C	143.80 ±2.65^e	530.66 ±8.14^a	3.13 ±0.10^a	1.26 ±0.01^b	143.8 ±2.80^d	16.55 ±1.54^b	200.65 ±2.54^f
T1	175.97 ±0.81^d	445.66 ±1.15^d	2.53 ±0.01^b	1.26 ±0.01^b	185.22 ±0.86^{cd}	13.41 ±1.14^{de}	113.12 ±1.01^i
T2	175.53 ±0.78^d	365.66 ±6.02^h	2.08 ±0.02^e	1.08 ±0.01^{cd}	184.77 ±0.82^{cd}	19.01 ±0.66^a	155.34 ±5.42^g
T3	187.54 ±3.38^c	332.00 ±7.00^j	1.77 ±0.06^f	1.04 ±0.02^{cd}	197.41 ±3.56^{bc}	15.09 ±1.60^c	284.66 ±2.51^d
T4	195.47 ±6.03^{ab}	308.00 ±4.58^k	1.51 ±0.09^h	1.02 ±0.01^{cd}	210.04 ±6.62^a	11.92 ±0.60^e	379.98 ±5.10^a
T5	195.36 ±2.71^{ab}	441.66 ±6.11^d	2.25 ±0.05^d	1.13 ±0.01^c	198.39 ±2.85^{bc}	14.98 ±0.67^c	225.33 ±2.92^e
T6	195.79 ±2.60^{ab}	381.33 ±2.51^g	1.95 ±0.01^f	1.11 ±0.01^c	203.35 ±0.65^{ab}	13.23 ±0.32^{de}	280.00 ±2.00^d
T7	196.97 ±0.77^a	369.33 ±7.63^h	1.87 ±0.04^g	1.10 ±0.01^c	204.85 ±0.81^{ab}	12.14 ±0.53^e	320.91 ±1.66^b
T8	193.28 ±2.01^b	425.66 ±4.04^e	2.20 ±0.01^d	1.24 ±0.01^{bc}	203.45 ±2.11^{ab}	11.86 ±0.48^e	142.00 ±6.24^h
T9	191.40 ±3.40^{bc}	463.33 ±2.88^c	2.42 ±0.04^c	1.30 ±0.01^{ab}	201.47 ±3.58^b	13.01 ±0.90^{de}	87.00 ±1.00^j
T10	190.70 ±1.02^{bc}	484.33 ±5.13^b	2.53 ±0.03^b	1.35 ±0.01^a	200.53 ±0.75^b	13.12 ±0.58^{de}	151.66 ±3.05^g
T11	194.09 ±0.69^{ab}	344.00 ±3.60^i	1.77 ±0.01^f	1.13 ±0.02^c	204.31 ±0.72^{ab}	10.50 ±0.34^f	302.80 ±2.84^c
T12	189.13 ±0.11^{bc}	393.33 ±5.77^f	2.07 ±0.03^e	1.21 ±0.01^{bc}	199.09 ±0.11^b	12.56 ±0.15^e	222.33 ±5.26^e
T13	185.79 ±1.35^{cd}	441.66 ±5.77^d	2.37 ±0.01^c	1.31 ±0.01^{ab}	195.57 ±1.43^c	14.71 ±0.45^{cd}	137.83 ±9.13^h
T14	184.72 ±5.09^{cd}	386.66 ±7.63^{fg}	2.09 ±0.05^e	1.34 ±0.11^a	194.44 ±5.35^c	17.65 ±0.42^b	153.45 ±7.54^g

Means ±SD (standard deviation) with different small letters in the same column differ significantly at $p<0.05$,, as determined by the Duncan's multiple range test. * H/W : Height/Width ratio

Bread yield and baking loss have an inverse correlation and are affected by the weight of the bread (Table 2). Bread yield of GFPB ranged from 184.77 to 210.04% for the samples T2 and T4; respectively which indicating an increase in bread yield with enhancement amount of DSB as partial replacement of SF. The same observation was recorded also for SL, while bread yield was decreased with increasing chickpea flour proportion. These results may suggest that the bread yield and baking loss are related to water holding capacity, it is entirely related to the weight of the final loaf. Similar observations have been previously reported by **[32].** The results of Table 2 showed that the sample T4 with a 30g of DFS reported high significantly (at $p < 0.05$) crumb firmness compared to other loaves. Considering bread with SL, crumb firmness was less reduced with a medium substitution level 20g sample T9. These results are in agreement with **[10].** Additionally, the sample T9 showed the lowest value of crumb firmness (87gf/cm^3) compared to control (200.65 gf/cm^3). Generally, the data in Table (2) are in agreement with that reported by **[11]**.

3.2 Colors characteristics:

Table (3) showed significant difference ($p < 0.05$) between bread samples in color parameters. Since all types of flour had received the same treatment, it may be inferred that the color of the flour used to make bread mostly determines the color of the loaf **[33].** For the crust color, T4 showed a lower L* value (L*=33) than the control (L*=51.88) a significance at ($p < 0.05$), while T1 displayed a higher L* value and no significance with C. Samples T1 and T8 gave similar L*values to the control**.** ΔE values of loaf crust were high in control (ΔE=64.5), T1 (ΔE=65.92) and T8 (ΔE=64.02) samples, on the contrary low ΔE value was found by T7 (ΔE =41.27). On the other hand, loaves characterized with a lighter color of crumb (higher L* values) were samples that contained low levels of SL and CP flour, while the sample with dark color (lowest L* 36.86) was found in T4 which has 30 g DSB flour. In addition, the sample T1 showed lower L* value (73.97) compared to samples (T5, T6, T7 and T8) that recorded higher yellow color than the T1 as shown from b* value (Table 3).

Table 3: Color characteristics of crust and crumb of GFPB.

T	Crust				Crumb			
	L*	a*	b*	ΔE	L*	a*	b*	ΔE
C	51.88 ±0.64^{b}	14.67 ±0.61^{e}	35.34 ±0.66^{a}	64.50 ±0.98^{a}	66.10 ±0.97^{ef}	-1.10 ±0.08^{g}	16.92 ±1.05^{f}	68.25 ±0.89^{g}
T1	54.08 ±0.33^{a}	12.38 ±0.47^{f}	35.61 ±0.39^{a}	65.92 ±0.47^{a}	73.97 ±0.36^{c}	-1.85 ±0.06^{i}	9.88 ±0.34^{h}	74.65 ±0.31^{cd}
T2	44.05 ±0.74^{d}	18.38 ±0.18^{bc}	29.61 ±0.54^{c}	56.17 ±0.35^{c}	66.91 ±0.66^{ef}	1.87 ±0.12^{e}	21.71 ±0.53^{d}	70.37 ±0.73f
T3	40.95 ±0.79^{fg}	18.57 ±0.24^{bc}	28.38 ±0.89^{cd}	53.69 ±0.58^{def}	51.99 ±0.50^{h}	8.59 ±0.13^{b}	28.16 ±0.65^{b}	59.75 ±0.69^{h}
T4	33.00 ±0.60^{h}	18.75 ±0.47^{bc}	26.99 ±0.86^{d}	51.64 ±0.45^{f}	36.86 ±1.49^{i}	15.10 ±0.46^{a}	34.36 ±1.19^{a}	49.10 ±2.26^{i}
T5	47.03 ±0.82^{c}	18.81 ±0.49^{bc}	31.15 ±0.79^{b}	59.46 ±0.19^{b}	77.53 ±0.38^{a}	-2.01 ±0.14^{i}	16.71 ±0.56^{f}	79.34 ±0.26^{a}
T6	42.13 ±0.78^{def}	18.67 ±0.04^{bc}	27.83 ±0.30^{d}	53.84 ±0.63^{de}	76.63 ±0.25^{ab}	-1.67 ±0.05^{hi}	20.83 ±0.43^{d}	79.43 ±0.22^{a}
T7	39.20 ±0.09^{g}	16.73 ±0.11^{d}	18.27 ±0.67^{e}	41.27 ±0.82^{g}	74.04 ±0.61^{c}	-1.11 ±0.07^{g}	21.72 ±0.16^{d}	77.16 ±0.57^{b}
T8	50.18 ±0.78^{b}	19.25 ±0.22^{a}	34.79 ±0.48^{a}	64.02 ±0.89^{a}	75.46 ±0.94^{bc}	-1.82 ±0.39^{i}	13.95 ±0.79^{g}	76.76 ±0.80^{b}
T9	47.70 ±0.36^{c}	18.81 ±0.27^{bc}	32.04 ±0.19^{b}	60.46 ±0.46^{b}	74.38 ±1.85^{c}	-1.72 ±0.04^{h}	17.36 ±0.38^{f}	76.40 ±1.73^{bc}
T10	43.84 ±0.49^{de}	18.20 ±0.36^{c}	28.21 ±0.88^{cd}	55.22 ±0.46^{cd}	70.24 ±1.94^{d}	-1.38 ±0.04^{gh}	18.98 ±0.41^{e}	72.77 ±1.97^{d}
T11	42.44 ±0.94^{def}	19.71 ±0.38^{a}	27.52 ±0.41^{d}	54.29 ±0.74^{cde}	63.93 ±1.29^{g}	3.12 ±0.47^{c}	22.95 ±1.13^{c}	68.01 ±0.92g
T12	41.92 ±0.32^{ef}	18.86 ±0.13^{bc}	27.99 ±0.35^{cd}	53.82 ±0.43^{de}	65.45 ±1.04^{fg}	2.60 ±0.13^{d}	21.90 ±0.30^{c}	69.06 ±1.03^{fg}
T13	41.69 ±0.35^{f}	18.28 ±0.25^{bc}	26.71 ±0.44^{d}	52.78 ±0.58^{ef}	70.59 ±1.19^{d}	-2.56 ±0.01^{j}	16.76 ±0.22^{f}	72.58 ±1.12^{e}
T14	41.63 ±0.88^{f}	17.93 ±0.52^{c}	26.96 ±0.64^{d}	52.74 ±0.71^{ef}	67.95 ±0.98^{e}	-0.04 ±0.14^{f}	17.87 ±0.48^{f}	70.26 ±0.91^{f}

Means ±SD (standard deviation) with different small letters in the same column differ significantly at $p<0.05$, as determined by the Duncan's multiple range test.

This result may be due to enlarge the pores size of crumb in the T1 sample, suggesting affect reflection of light during measurement. On the other hand, T5 and T6 showed high values of ΔE (79.34 and 79.43; respectively), while the lowest values were recorded for T4 and T3 samples (49.10 and 59.75; respectively).

3.3 Texture profile analysis of crumb:

The legume flour as partial replacement of SF formula are significantly affect the crumb of bread textural features, especially in hardness and chewiness as shown in Table (4). However, customers like soft, pliable crumbs and low hardness, making hardness one of the most significant quality characteristics of bread texture **[34]**. The results in Table (4) showed that the hardness of GFPB crumb was significantly increased with increment DSB and SL in flour formula. While the hardness of GFPB crumb baked from flour formula with 20g CP was significantly decreased to 4.7 N. This value is close to the value of sample C (3.06 N) and that of T1 (3.32 N). Besides, the crumb of GFPB baked from flour formula with mixed different legumes samples indicated that presence of CP, except with DSB (T12), led to decrease hardness (T13 and T14 samples 5.14 and 5.61 N; respectively), this may be due to that chickpea protein enhances the crumb's textural qualities **[10, 35].**

Otherwise, the presence of 30 g DSB in sample T4 gave the highest hardness of crumb (17.86 N), this could be related to the lowest specific volume (see Table 2) and therefor highest density of such bread as well as to the high fiber content in DSB used.

Cohesiveness of crumb, a parameter describing the extent to which the food structure can be deformed before it tears **[36].** It can be from Table 4 observed that both samples T3 and T4 resulted in the lowest value of cohesiveness 0.66 mJ with high significant at $p < 0.05$ to control. While the best value of cohesiveness was sample C (0.83 mJ) followed by sample T9 (0.81 mJ).

On the other hand, springiness of GFPB crumb was significantly affected by presence of 30g DSB in flour formula in sample T4 with lowest value (8.63 mm) compared to other samples, which may be due to the higher content of DSB and consequent more fiber content as well as compact crumb. While in the most of samples, springiness values ranged from 9 to 9.5 mm; a slight superiority over the C sample (9.4 mm) in favor of both sample T8 (9.5 mm) and T9 (9.46 mm).

In Table (4) it can noted that the results of the chewiness values of all samples behave the same way as the hardness parameter. In addition, it can be seen from Table 4 that the presence of legume flour as partial replacement of SF effect the crumb chewiness properties. The substitution of 20g CP in flour formula T9 had the GFPB baked from it crumb chewiness with value (35.45mJ) closed of both C and T1 samples (23.98 and 24.93 mJ; respectively), while the substitution of DSB in amount 30g (T4) had a negative impact on crumb chewiness with 101.78 (mJ).

Table 4: Texture profile analysis of GFPB:

T	Hardness (N)	Adhesiveness (mJ)	Resilience (mJ)	Cohesiveness (mJ)	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
C	3.06 ±0.16[g]	0.10 ±0.00[d]	0.87 ±0.04[c]	0.83 ±0.01[a]	9.40 ±0.01[b]	2.55 ±0.16[h]	23.98 ±1.59[h]
T1	3.32 ±0.01[g]	0.16 ±0.05[c]	0.93 ±0.05[ab]	0.79 ±0.01[bc]	9.46 ±0.05[a]	2.63 ±0.03[h]	24.93 ±0.31[h]
T2	6.78 ±0.08[d]	0.10 ±0.01[d]	0.94 ±0.01[ab]	0.74 ±0.03[ef]	9.26 ±0.05[cd]	4.95 ±0.29[e]	45.87 ±2.48[e]
T3	12.52 ±0.50[b]	0.50 ±0.01[a]	0.92 ±0.01[b]	0.66 ±0.01[i]	9.10 ±0.01[f]	8.26 ±0.33[c]	75.21 ±3.01[c]
T4	17.86 ±2.44[a]	0.00 ±0.01[e]	0.92 ±0.01[b]	0.66 ±0.01[i]	8.63 ±0.05[g]	11.78 ±1.61[a]	101.78 ±13.88[a]
T5	8.63 ±0.11[c]	0.30 ±0.01[b]	0.97 ±0.01[a]	0.79 ±0.01[c]	9.20 ±0.01[e]	6.81 ±0.08[d]	62.73 ±0.79[d]
T6	11.80 ±0.03[b]	0.20 ±0.01[c]	0.94 ±0.01[ab]	0.77 ±0.01[d]	9.40 ±0.01[b]	9.08 ±0.02[b]	85.42 ±0.26[b]
T7	12.91 ±0.12[b]	0.10 ±0.01[d]	0.94 ±0.01[ab]	0.74 ±0.01[efg]	9.10 ±0.01[f]	9.55 ±0.08[b]	86.98 ±0.81[b]
T8	5.14 ±0.06[ef]	0.20 ±0.01[c]	0.95 ±0.01[ab]	0.79 ±0.01[c]	9.50 ±0.01[a]	4.06 ±0.04[fg]	38.57 ±0.46[fg]
T9	4.70 ±0.04[f]	0.10 ±0.01[d]	0.95 ±0.01[ab]	0.81 ±0.01[b]	9.30 ±0.01[c]	3.81 ±0.03g	35.45 ±0.37[g]
T10	6.25 ±0.20[de]	0.10 ±0.01[d]	0.95 ±0.01[ab]	0.75 ±0.01[d]	9.06 ±0.05[f]	4.73 ±0.11[er]	42.91 ±0.78[ef]
T11	12.00 ±0.74[b]	0.00 ±0.01[e]	0.92 ±0.01[b]	0.68 ±0.01[h]	9.23 ±0.05[de]	8.16 ±0.50[c]	75.40 ±5.13[c]
T12	8.80 ±0.20[c]	0.06 ±0.11[d]	0.94 ±0.01[ab]	0.73 ±0.01[fg]	9.40 ±0.01[b]	6.45 ±0.06[d]	60.69 ±0.63[d]
T13	5.14 ±0.06[ef]	0.00 ±0.01[e]	0.94 ±0.01[ab]	0.75 ±0.01[e]	9.40 ±0.01[b]	3.89 ±0.02[g]	36.58 ±0.25[fg]
T14	5.61 ±0.23[def]	0.00 ±0.01[e]	0.94 ±0.01[ab]	0.72 ±0.01[g]	9.40 ±0.01[b]	4.06 ±0.20[fg]	38.17 ±1.88[fg]

Means ±SD (standard deviation) with different small letters in the same column differ significantly at $p<0.05$,, as determined by the Duncan's multiple range test

3.4 Staling rate:

Storage of bread, especially dependent on starch, that causes several structural changes. These are mainly caused by the migration of water from crumb to crust and hardening of the crumb related to starch retrogradation **[37].** However, several additives and components that regulate water retention or slow down starch recrystallization could be used to reduce these undesirable occurrences **[38, 39, 40]**. The results in Fig. 1 indicated that a decreasing in staling of bread after 24 hrs of storage at room temperature as affected by substitution of SF with 30g of any legume. It can be seen that the DSB (sample T4) was more effect for decreasing bread staling rate (30 %) followed by SL (T7) 82 % and CP (T10) 169 %. The same trend for staling rate by substituting with 20g legume flour showed 61%, 100% and 294% for T3, T6 and T9; respectively. While after continues storage for 48 hrs the samples T4, T12, T13 and T14, were crumbled during hardness measuring may be due to their high fiber content. It was impossible to perform staling test since the bread crumb was very brittle and the fibers showed crumbling, this interpretation is similar to that described by **[41].** In addition, the value of staling rate at 48 hrs storage for any sample was higher than that corresponding value at 24 hrs storage. This effect would not only be related to a decrease in amylopectin retrogradation but more likely to a reduction in moisture loss during storage, which retards staling phenomena **[42, 43].** Furthermore, the sample (T1) that consisted of a considerable of starch, indicted a high staling rate (390%) after 48 hrs storage compared to other samples, due to high amylopectin content **[44].** Generally, these results are in agreement with other investigation reported by **[11].**

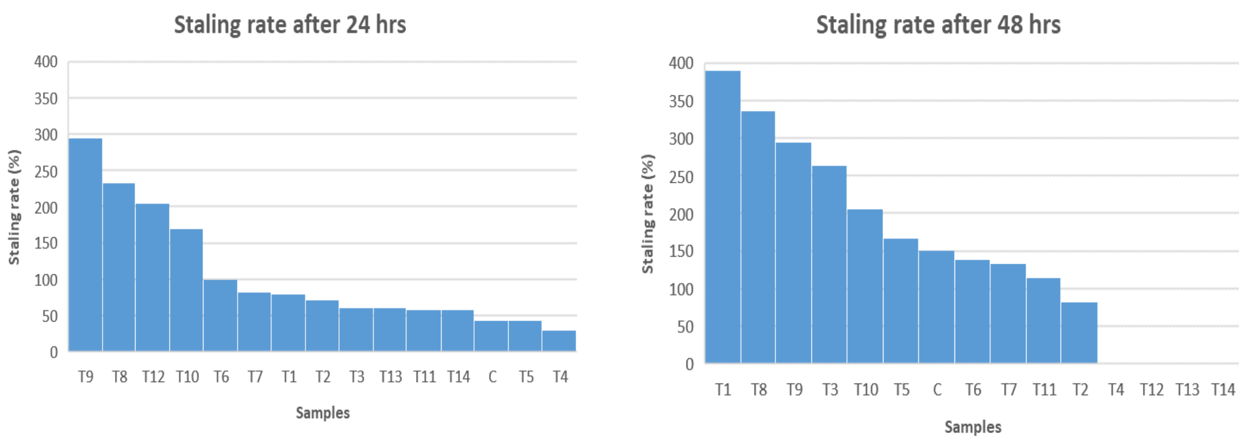

Fig 1. Staling rate of GFPB during storage at room temperature for 24 and 48 hrs.

3.5 Sensory characteristics of GFPB:

Table (5) shows the results of seven sensory attributes for GFPB. In sensory evaluation, it is important to compare samples of gluten-free bread with wheat bread to show which is better. Concerning general acceptance, sample C was found to be the most acceptable with value of 88.8%, followed by samples T5 (83.6%), T6 (77.7%) and T13 (76.5%); respectively, while T4 and T3 samples were recorded less acceptable (49.8% and 44.6%; respectively) with highly significantly ($p < 0.05$) to sample C. Furthermore, the overall acceptance scores for the other samples were fairly similar from 63.1% to 73.1%. It is worth noting that Sample T5 outperformed to C in terms of general appearance with 8.4 score while 7.7 for C. This is because the committee evaluated the general appearance of the loaf from the outside, but it can be seen that the slices of samples containing chickpeas were better as shown in Figure 2. Generally, acceptance decreases with an increasing proportion of legume flour due to low scores for the characteristics of general appearance, color and taste, except for samples containing chick pea flour (T8, T9 and T10) which recorded increases in taste and total acceptance values with enhancing of CP flour. These findings are agreement with that reported previous by **[16].**

The crumb grain distribution scores were diminished significantly at ($p < 0.05$) in samples T3, T4 and T11 compared to C with increasing DSB flour, which may be due to the high content of fiber in DSB causing impedes the extent of gas during fermentation, proofing and baking. The lack of crumb grain distribution was observed by **[45]** with high soybean dose. Moreover, sample T6 baked with 20g SL had the highest scores for flavor and taste (6.9 and 7.6) followed by sample T10 (6.66 and 7); respectively with no difference significant observed at ($p < 0.05$) than C. No statistically significant difference was recorded in freshness parameter except T4 and T3. The control C recorded the highest freshness (8.2) followed by T5 (7.9) and T6 (7.2). These results are gut in agreement with **[10, 46, 47].**

Table 5: Sensory characteristics of GFPB:

Samples	General Appearance	Color of crust	Color of crumb	Crumb grain distribution	Flavor	Taste	Freshness	Total acceptance %
C	7.7^{ab}	6.7^{ab}	8.2^{a}	8.4^{a}	8.4^{a}	8.4^{a}	8.2^{a}	88.8
T1	7^{ab}	7.6^{a}	6.6^{b}	6.9^{b}	4.2^{d}	4.5^{d}	6^{bc}	67.9
T2	6.5^{b}	5.3^{b}	5.2^{c}	6.5^{bc}	4.2^{d}	5.3^{c}	6.8^{b}	63.1
T3	4^{cd}	3.3^{c}	5.4^{c}	5.1^{d}	3.9^{e}	4.5^{d}	5.2^{d}	49.8
T4	2.5^{d}	1.9^{d}	5.1^{d}	4.6^{d}	4^{d}	4.6^{d}	5.4^{c}	44.6
T5	8.4^{a}	7.7^{a}	7.7^{ab}	7.7^{ab}	6.7^{b}	6.6^{b}	7.9^{ab}	83.6
T6	6.7^{b}	6.4^{ab}	7.7^{ab}	6.5^{bc}	6.9^{b}	7.6^{ab}	7.2^{ab}	77.7
T7	5.8^{c}	5.4^{b}	7.1^{ab}	6.9^{b}	5.8^{c}	5.5^{c}	5.9^{bc}	67.3
T8	7.91^{ab}	6.16^{ab}	7^{ab}	5.08^{c}	6^{b}	6^{bc}	6.33^{b}	69.9
T9	8^{bc}	6.25^{ab}	6.08^{b}	4.91^{c}	6.5^{b}	6.75^{ab}	6.33^{b}	71.1
T10	6.25^{bc}	6.58^{ab}	7.08^{ab}	6.41^{bc}	6.66^{b}	7^{ab}	6.08^{bc}	73.1
T11	6.2^{bc}	6.5^{ab}	4.9^{e}	4.8^{d}	5.8^{c}	5.8^{bc}	6^{bc}	63.4
T12	6.5^{b}	6.5^{ab}	5.4^{c}	5.3^{cd}	5.7^{c}	5.8^{bc}	7.1^{ab}	67.1
T13	7.2^{ab}	6.7^{ab}	7.7^{ab}	6.3^{bc}	6.2^{b}	6.6^{b}	7.5^{ab}	76.5
T14	7.1^{ab}	6.9^{ab}	6.4^{b}	5.8^{c}	6.4^{b}	6.6^{b}	6.5^{b}	72.5

Means ±SD (standard deviation) with different small letters in the same column differ significantly at p<0.05, as determined by the Duncan's multiple range test.

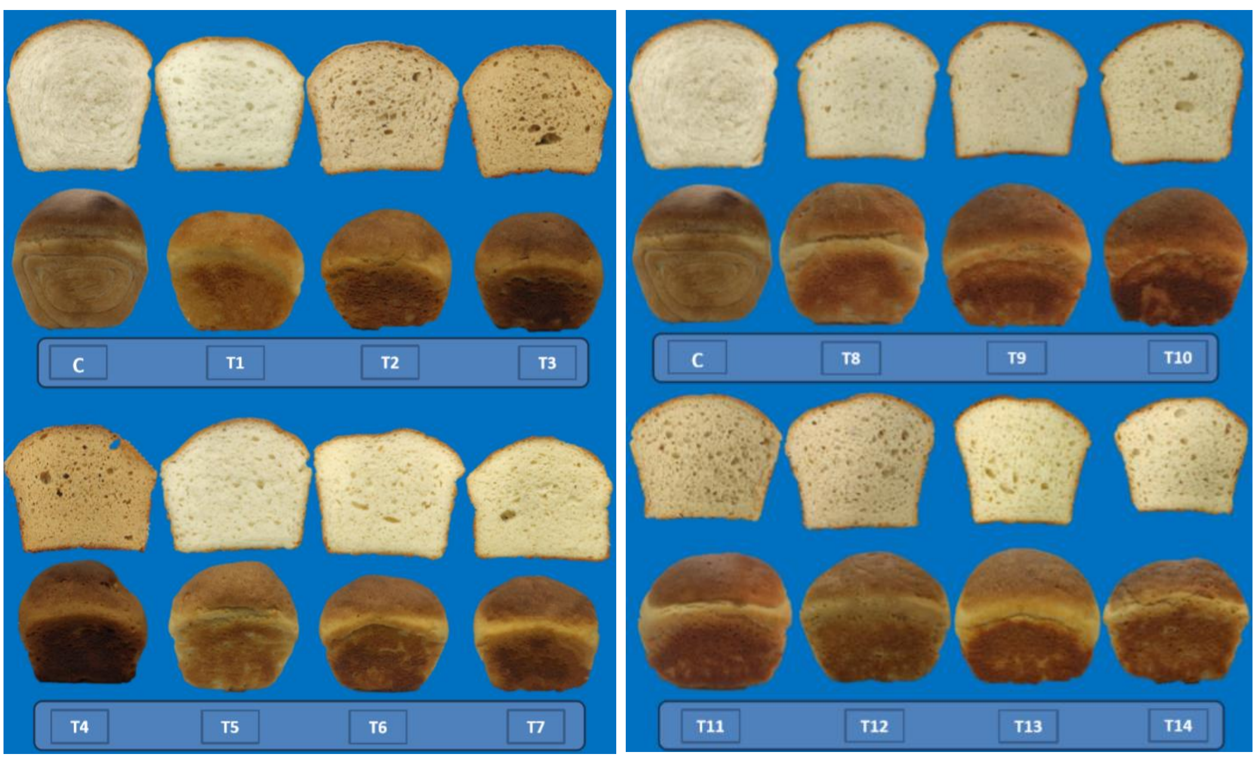

Figure 2: Wheat (C) and Gluten-free pan bread (T1-T14 see Table 1).

4. Conclusion:

Results described previously revealed that the chickpea (30g) as partial replacement of starchy formula (rice flour and corn starch), in gluten-free pan bread produced loaves with higher specific volume, while a sample containing 20g of chickpea resulted in a significant decrease in hardness and chewiness of bread crumb compared to other substitution levels and other legume flours. The chickpea flour has a significant impact in improving the physical and crumb textural attributes, even when combined with other additives (sweet lupine and defatted soy bean)**.** The acceptability of gluten-free pan bread showed decreasing with increment of defatted soy bean or sweet lupine flours in bread. In contrary, the samples containing CP flour, showed highly acceptable by panelists.

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