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STUDYINGTHE EFFECTOF FLOURAND LUPINE PROTEIN CONCENTRATE MCORPORATIONON PHYSICAL CHEMICALAND SENSORY PROPERTIESOF BISCUIT

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ABSTRACT

The purpose of this study was to determine the effect of lupine flour (L.f) and lupine protein concentrate (L.P.C) incorporation on chemical, nutritional and sensual qualities characteristics of biscuit (L.P.C) was prepared by isoelectric precipitation method. A standard recipe for biscuit preparation by wheat patent flour used as the control. Wheat flour in the control treatment was replaced with (L.f) and (L.P.C) at levels 10, 20& 30%. Chemical composition of (L.f), (L.P.C) and biscuit treatments were studied. Results showed that protein contents were 35.35& 75.80% for (L.F) and (L.P.C), respectively. While they amounted to 14.70, 16.16& 18.61% for (L.f) incorporated biscuits and 15.20, 18.09 & 21.08% for (L.P.C) incorporated biscuits at the substitution levels studied, respectively compared 12.43% control. Results also indicated contents of total dietary fibers and tannins in (L.f),(L.P.C) and biscuits prepared. Sensory evaluation of biscuit treatments revealed that there was significant decrease at substitution level up to 30% of (L.f) except color score, while all scores of sensory properties were improved significantly at all substitution levels of (L.P.C). Spread ratio was affected adversely by incorporation of (L.f) and slightly when (L.P.C) used. Results showed a reduction in biscuit tenderness during storage. Reverse to the above statement with 30% incorporation of (L.f) while increased at (L.P.C) treatments. The study demonstrated that (L.f) and (L.P.C) can be incorporated into biscuits formulation by replacing up to 20, & 30% of wheat flour control 12.43%, respectively to increase dietary fiber and protein contents.

Keywords: Lupine flour, protein concentrate, chemical nutritional, sensory properties.

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دراسة تأثير الطحين والمركز البروتيني لبذور الترمس على الخصائص الفيزيائية والكيميائية والحسية للبسكويت

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الخلاصة

حدد تأثير دمج دقيق الترمس (Lf) وتركيز بروتين الترمس (L.P.C) على الصفات الكيميائية والغذائية والحسية للبسكويت المحضر بطريقة الترسيب الكهروضوئي من خلال وصفة قياسية لتحضير البسكويت بدقيق القمح المستخدم كعنصر اساسي، اذ تم استبدال دقيق القمح بـ (Lf) و(L.P.C) عند المستويات 10 و20 و30٪، وتمت دراسة التركيب الكيميائي لكل من (Lf) و(C.L.P) ومعاملات البسكويت، وأظهرت النتائج أن محتوى البروتين كان 35.35 و75.80٪ لكل من (Lf) و(L.P.C) على التوالي، بينما بلغت 14.70 و16.16 و18.61٪ للبسكويت المضاف له(L.f)و15.20 و18.09 و21.08٪ للبسكويت المضاف له (L.P.C)، كما أشارت النتائج إلى محتويات إجمالي الألياف الغذائية والعفص في (Lf) و(L.P.C) والبسكويت المحضر، وأظهر التقييم الحسي معاملات البسكويت وجود انخفاض معنوي عند مستوى ابدال حتى 30٪ من (Lf) باستثناء درجة اللون، بينما تحسنت جميع درجات الخصائص الحسية معنوياً في جميع مستويات ابدال لـ (L.P.C)، وتأثرت نسبة الانتشار سلبًا بدمج (Lf) وقليلًا عند استخدام (L.P.C)، وأظهرت النتائج انخفاضًا في طراوة البسكويت أثناء التخزين، وأوضحت الدراسة أن (Lf) و (L.P.C) يمكن إدخالهما في تركيبة البسكويت عن طريق استبدال ما يصل إلى 20 و30٪ من دقيق القمح بنسبة 12.43٪ على التوالي لزيادة محتوى الألياف الغذائية والبروتين.

الكلمات المفتاحية: طحين الترمس، مركز البروتين، الخصائص التغذوية الكيميائية، الخصائص الحسية.

INTRODUCTION

Legumes are considered of a great importance for human nutrition because of their high protein content .And due to their technological characteristics, their use has been carried out not only by consumption by their whole grains but they are used for production flour, protein concentrate and protein isolate which improve nutritional properties that are added to them. **(Polit *et al.*, 2019)**. Among the famous nutritional pulses in the world, lupine is considered as an important source of vegetarian protein. Lupine is planted for improvement of soil structure, animal feed as well as human food **(Maghaydah *et al.*, 2013)**. Many studies, reported the use of lupine flour (L.f) and lupine protein concentrate (C.L.P) in production of pastries, like noodles, bakeries like bread, cake, biscuit and sausage. In addition to its content of about 40% protein and essential amino acids, it is also considered a good source of lipids richened with unsaturated fatty acids, as well as fibers, minerals, vitamins and antioxidants which assist in protection of human from cardiac diseases, hypertention, diabetes, osteoporosis and cancer**(Kohajdova *et al.*, 2011; Mattila*et al.*, 2018)**. Studies proved that both protein and fibers of lupine had physiological advantages of body, but lupine use became limited because of presence of antinutritional factors like phytic acid, alkaloids, saponins and enzyme inhibitors which affected protein digestibility. So production of protein concentrates or isolates is considered a good method to decrease these matters. Incorporation of lupine flour or protein to wheat based foods such as biscuits, has a potential to increase dietary fiber or protein contents, respectively. The amount of high protein and high dietary lupine flour that can substitute wheat flour represent a compromise between nutritional improvement and achievement of satisfactory and physical properties of dough & its many prodded **(Jayasena&Nasar,2011)**.

Commercially, the world market of biscuit was projected to reach $43 billion by the year 2015, and may be multiplied at 2025, and this is primarily driven by the changing consumer trends toward healthy food options & supplemented products with many differ

natural heady plants**(Anonymous, 2010)**.Lupine seeds contain a percentage of tannins and antioxidants that benefit human health in eliminating oxidative stress in the human body . This research aimed to prepare(L.f) and(L.P.C) incorporate them in biscuit making and study some its chemical, nutritional and organoleptic characteristics.

MATERIALS AND METHODS

Preparing the raw materials for biscuit production

Sweet lupine seeds were purchased from AL-Shorja Market, Baghdad, imported from Egypt. Patent wheat flour (Kuwait flour mills and bakeries Co.) sugar, milk, shortening (Turkish origin) baking powder(Arab food industries Co. Amman, Jordan). All chemical and reagents are chemical grade.

Preparation of lupine flour

To prepare (L.f) 2.5 kg of seeds were ground by metal mortar then by coffee mill and sieved to pass 60 mesh sieve. Flour sample were stored by using plastic containers in freezer until utilization in different tests.

Preparation of lupine protein concentrate:

Lupine protein concentrate was prepared by isoelectric precipitation as reported by **Mune*et al.*(2013**) with some modification of sample weight and reagent volumes a sample of 100 gm (L.f) was mixed with 1 liter of 0.15 M sodium chloride solution and stirred for 120 min at 35°C. pH was adjusted to 9.91and mixture was further stirred for 30 min at 4°C. The slurry which was resulted was centrifuged at 2000 g for 30 min. The precipitate obtained after recovering supernatant, was dissolved in the initial sodium chloride solution at the above liquid to solid ratio under stirring. The ph was a digested to the initial value and slurry stirred for 30 min at 4°C and then centrifuged as explained previously.Supernatant of the two alkaine extraction were collected and one part volume of ethanol (95% v/v) added. The pH was adjusted to 4.5 during stirring. Precipitated proteins were recovered under vacuum using filter paper (Whatman No:1). The protein concentrate was dried at 50°C for 48 h in an air over and then ground. The resultant powder was kept in plastic containers until using, (Figure 1) shows the production scheme for lupine seed protein concentrate.

Biscuit making

Biscuit was made according to this recipe as flour basis: wheat patent flour: 100%, sugar: 47.5%, shortening 28.4%, salt 0.9%, baking powder 2%, milk 10%, water 25% which its amount was changed as replacement of (L.f) and (C.L.P) levels were changed, replacement levels of (L.f) and (C.L.P)were 10, 20, and 30% of wheat flour. Water absorption of wheat patent flour was 58% as flour basis. Absorption of (L.f) and (C.L.P) were 4.1, 3.05 gm/gm, respectively. Biscuit formula components were mixed by electrical mixer to get a creamy appearance. The resultant dough was spread, thickness of 7 mm using a hand roller and cut to peaces of 30 mm diameter. The pieces were placed on trays greased with shortening, baked at electrical oven on 200°C for 10 min. Biscuits were cooled at room temperature, kept in polyethylene bags and stored for analysis. Treatment were denoted as follows: control, T1, T2, T3 substitution levels, (L.f), T4, T5, T6: substitution levels of (C.L.P), respectively**(Lopezm*etal.*, 2019)**.

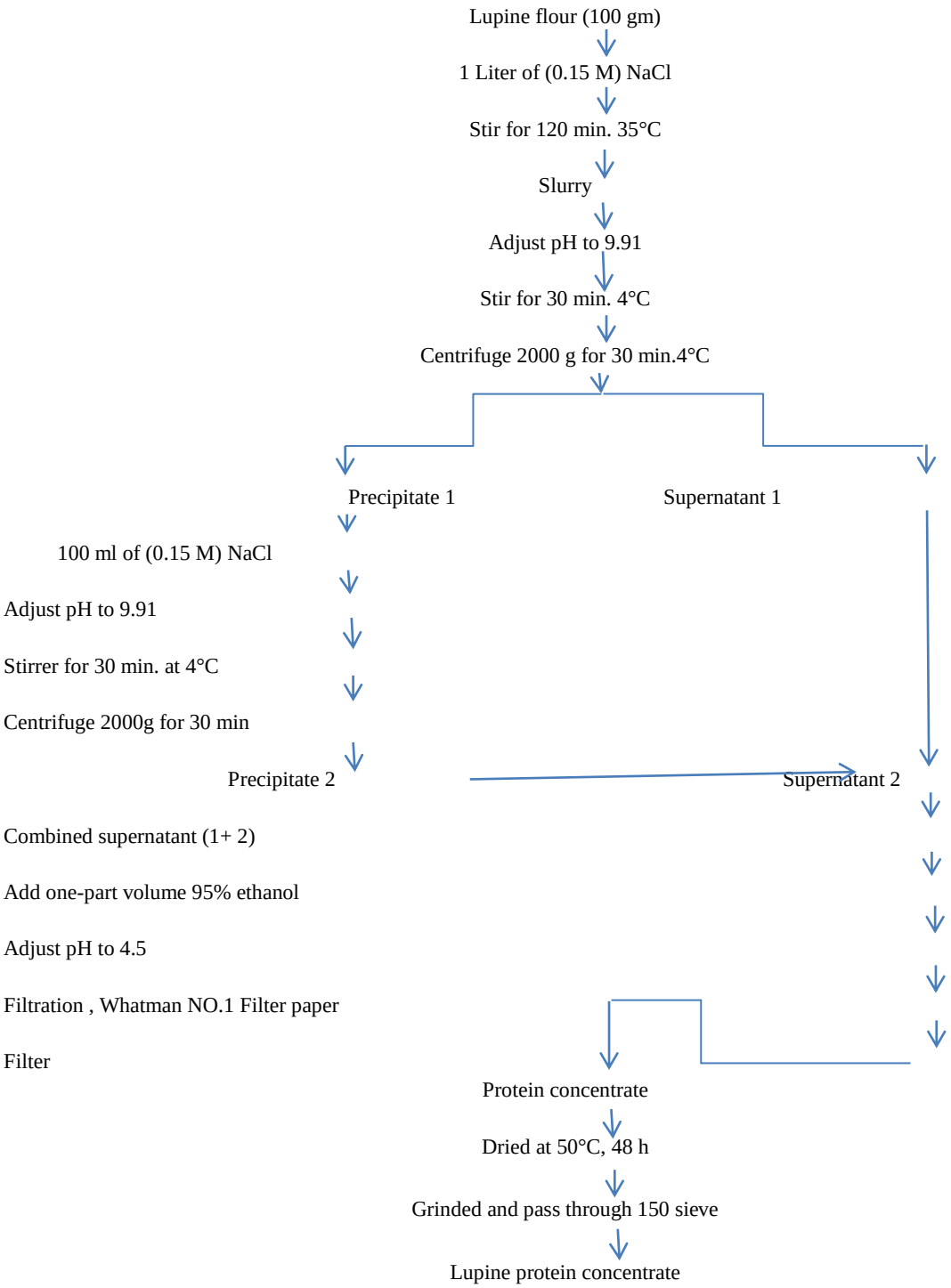

Figure (1): Schematic of lupine concentrate normal.

Spread ratio calculation

Spread ratio was measured according to (AACC 10-50.2000) by estimation diameter and thickness as average of seven pieces of biscuit by vernier. Spread ratio was determined by the equation:

$$\text{spread ratio} = \frac{\text{diameter (width)}}{\text{thickness}}$$

Tenderness of biscuit

Tenderness of biscuit was measured by using a local instrument contained a metal cone which can go down over the piece of biscuit , the depth of the hole formed was measured as millimeters by a ruler fixed inside the instrument.

Sensory evaluation

Fifteen panelists were asked to record their assessment of biscuits color, texture, flavor and appearance scored as: 20, 30, 30 and 20, respectively, Oneway analysis of variance (ANOVA) was performed to test differences between treatment followed by Duncan's analysis (**Kefale&Yetenayet, 2020)**.

Chemical analysis

Moisture was determined by air-oven method at 105°C/ min. Lipids content was estimated by using petroleum ether extraction in soxhlet instrument. Total nitrogen was determined by Kjeldahl method, protein content was calculated as N×5.7 for wheat flour and N×6.25 for lupine flour and its products, respectively. Ash was determined by incineration sample in a muffle. All these determination were methods conducted to (AOAC).Carbohydrates were estimated by difference. Total dietary fiber was quantified by chemical method described by AOAC. Tannins was determined by the method described by**Van-Burden & Robinson (1981)**.

RESULT AND DISCUSSION

Chemical composition of lupine flour and lupine protein concentrate

The results are shown in the (Table 1). chemical composition of control. The moisture content of whole meal flour was 10.93%, which is a high percentage compared to legume flour, which has a lower moisture content. The proportion of ash and fat in whole wheat flour was less than that of lupine seed flour1.64 and 1.60%, respectively, which is close to its percentage in the protein concentrate of lupine seeds. While the protein content of whole meal flour is 12.50%, and carbohydrates its proportion of 73.33%, which is higher than that of lupine seed flour**(Lin *etal.*, 2019)**. (L.f) and (L.P.C). The decrease of moisture, fat and ash in (L.P.C) compared to (L.f) was due to the partial removal of them during protein extraction. Protein content of (L.f) 35.35% was at the range shown by **Monteiro*et al.* (2014**). When comparing (L.f) in our study with other legumes, it had higher protein content than lentils, peas and chickpeas but lower than soybean**(Maphosa & Jideani, 2017)**.

It was for (C.L.P) revealed that its content of moisture, fat, ash and protein were in suppler4.30, 1.33, 1.18 and 75.80%, respectively. The protein content of (L.P.C) was higher compared with previous studies (**Evangelista *et al.*,2004**; **Sujak*et al.*, 2006**). The method of protein extraction is important to obtain a good protein concentrate. Many papers indicated that to prepare concentrate of higher protein contents from lupine it is necessary further to process flours to remove some of low molecular weight components. Fat contents of (L.f) and (L.P.C) were 8.57 and 1.33%, respectively. Content of fat in current study was at the range reported. In general, lupine oil is characterized by a balance fatty acid composition with total saturated fatty acids of 10% and total unsaturated fatty acids of 90% of which 32 to 50% is oleic acid, 17-47%is linoleic acid and 3-11% is linolenic acid**(Kohasdova *et al.*, 2011)**. Various researchers measured carbohydrates with fibers, while others reported their content alone. Author links open overlay pane **Maphosa & Jideani (2017)** found carbohydrate content equivalent to 71% of the seed weight, and its content of raw fiber was 1.7%. Lupine seed contained little quantities of starch 5-12% and higher levels of soluble non-starch polysaccharides 30-40%.

Mierlita*et al.*(2018)production of (L.P.C) normally decreased amount of available carbohydrates, it reached 17.39%.

Table (1): Chemical composition of lupine flour and its protein concentrate

Component (%)	Wheat flour	Lupine flour	Lupine protein concentrate
Moisture	10.93	5.51	4.30
Ash	1.64	2.92	1.18
Protein	12.50	35.35	75.80
Fat	1.60	8.57	1.33
Carbohydrates	73.33	47.65	17.39

Values are average of duplicate

Chemical composition of biscuits

The chemical analysis of biscuit & different incorporation (or supplemented) with L.f and L.P.C was presented in (Table 2). Results indicate low increase in moisture and ash of biscuits prepared with the results indicate that the percentage of humidity in baskets was close in its proportions and that the highest averages wereT3, T6 and reached 2.99 and 2.98% respectively, as for the means significantly, they were in the standard treatment 2.64% **(Polit *et al.*, 2019)**.

The percentage of ash in the samples of biscuit varied, the mean was the most high transaction T3, 3.75% as for the lowest means, it was for the transaction C 2.10%. The percentage of protein in biscuits had different averages, as the highest of those averages were significant differences T6 21.08%, as for the lowest averages, they were C 12.43%. The increase of protein quantity due to lupine incorporation emphasized the potential to use it as a source of protein in biscuit and other pastries. Lupine represented a good balance of essential amino acids **(Drakos*et al.*, 2007; Naumann *et al.*, 2017)**. It was considered to be a good source of lysine. But **Monteiro *et al.* (2014)** reported that cultivars they studied had lower protein quality in relation to the presence of essential amino acids, but with good digestibility when compared to other legumes. The fat percentage of biscuits had high averages and the highest averages were at T3 25.01, while the lowest averages were significantly lower C21.65%.Biscuits prepared with (L.f) and (C.L.P) represented a good source of energy due to its high content of oil ranged 18.5-19.10% which contained high content of C18:1 and C18:2 fatty acids **(AL-Hamdani, 2017)**.No problem in storage of biscuits may be expected due to high content of oil, because of low moisture content of the product as well as vacuumed packing and low acidity of oil extracted, indicating no danger of rancidity in biscuits **(Rutkowski*et al.*, 2016)**. The averages of carbohydrates were different, as the results indicated that the highest averages increased in the mean C 61.18%, while the lowest averages were treatedT3 49.64%.

Table (2): Chemical analysis of biscuit treatment.

Treatment	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrates (%)
C	2.64 d	2.10 f	12.43 f	21.65 f	61.18 a
T1	2.77 c	2.25 e	14.17 e	22.63 c	58.18 b
T2	2.89 b	2.76 b	16.16 c	23.75 b	54.44 e
T3	2.99 a	3.75 a	18.61 b	25.01 a	49.64 f
T4	2.79 c	2.33 d	15.20 d	21.75 e	57.93 c
T5	2.86 b	2.45 c	18.09 b	21.90 d	54.70 d
T6	2.98 a	2.55 c	21.08 a	21.99 d	51.40 e

Values are the average of duplicates Similar letters indicate that there are no significant differences between the parameters ($p< 0.05$) according to Duncan s test.

Fibers and tannins in lupine flour, lupine protein concentrate and biscuit treatments

The results in (Table, 3) show the percentage of fibers and tines in lupine seed flour, the protein concentrate of lupine and the biscuit made from them. as it was noticed that the fiber content of lupine seed flour was the highest of the average protein concentration of lupine and reached 10.62 mg/g, while the average percentage of protein concentrate in lupine seeds was 0.22 mg/g, this percentage of fiber in the protein center is very small compared with flour of lupine seeds, because of high content and good quality of lupine fiber, lupine flour is usually added to many foods such as bakeries. good quality of lupine fiber came from its white color and its high water absorption as well as the nutritional value **(Monteiro*et al.*, 2014)**.

In the (Table 3), the percentage of fiber in the produced biscuit, The results showed that there were significant differences in their averages, as the most high were in T1, T2, and T3. T3 and it ranged between 4.23-6.84 mg/g, while the least studied averages for biscuits were low, it was C 3.01mg/g, whereas the mean protein concentrations ranged between 3.25-3.79 mg/g.The high fiber content is due to the bits of the husks of the lupine seeds, also, a portion of the fiber is found in the endosperm of the seed. and he indicated **Pisarkova&Zraly (2010)** that the extraction rate for flour may be inefficient, which increases the percentage of fiber in the flour, and that the fiber is part dissolved in water and the other part is insoluble, which makes its ratio different between mixtures.

The results showed in the ratio of tannins to lupine seed flour and the protein concentrate of lupine seeds and biscuits produced from different mixtures of them significant differences in their averages, as it was observed that the averages of the tannins for the protein concentrate were higher and reached 0.95 mg/g, while the mean of lupine seed flour was lower than that and reached 0.83 mg/g, as for the results of tannins in the produced biscuits, the highest averages were higher in treatment T6, while the lowest averages were in C, while in lupine seed flour biscuits, they were between that, as they ranged between 0.212-0.076 mg/g. Tannin was considered one of phytochemicals which have antioxidant capacity due to their classification as polyphenols. So lupine products may be source of tannins as antioxidants as well as flavonoids **(Kohajdova*et al.*, 2011; Mattila*et al.*, 2018)**. According to results in (Table 4), there was no adverse effects for higher content of tannins on sensory evaluation, and this may consider a superior property for lupine addition to biscuits formulations.

Table (3): Total dietary fiber and tannins of lupine flour , its protein concentrate and biscuits treatments.

Treatment	Total dietary fiber (mg/g)	Tannins (mg/g)
L.f	10.62	0.83
C.L.P	0.22	0.95
Biscuit C	3.01 g	0.076 g
T1	4.23 c	0.149 f
T2	5.32 b	0.199 d
T3	6.84 a	0.212 c
T4	3.25 f	0.166 e
T5	3.55 e	0.266 b
T6	3.79 d	0.332 a

Values are the average of duplicates Similar letters indicate that there are no significant differences between the parameters ($p< 0.05$) according to Duncan s test.

Sensory evaluation of biscuit

The results of the sensory evaluation in (Table 4), which was conducted by ten evaluators from the college of agriculture, Tikrituniversity, showed that the averages had significant differences in the studied sensory characteristics of the produced biscuits, so the color trait had the highest mean increases in treatment C and T6 and reached 19, or

less.Averages of significant differences in T3 and 14, this may be due to yellowness caused by natural yellow pigments present in (L.f). This finding was agreed with**Jayasena&Nasar(2011)** who reported that there was an improvement in color with (L.f) substitution, it was found that the color of the biscuit was linked to the high level of proteins. The color of the biscuit becomes darker as the levels of proteins in the composition increase due to the interaction of amino acids of proteins with reducing sugars during baking in the Maillard reaction **Mahgoub *et al.* (2015)**.

The texture characteristic of biscuits had the highest averages for treatment C and T6 and reached 28, while the lowest averages for produced biscuits were for treatment T3 and reached 19. This may referred to whiteness of the lupine protein concentrate. These results were in agreed with observations found by **Eman& Ahmad (2012)** who used lupine flour and its protein isolate in cake making. Generally speaking, the incorporation of protein concentrate, and isolates of legumes were better than their flours in respect of protein fortification of certain foods. Taste is the primary factor that determines the acceptability of any product which has the highest impact as far as market success of product is concerned (**Yilma& Admassu, 2019)**.

As for the flavor characteristic of the produced biscuits, the highest averages were in the C and T6 treatment, reaching 28, while the lowest averages were in the decrease in the T3 treatment, reaching 20. The decrease of spread ratio probably caused by the formation of an elastic network, that causes shrinkage after baking due to the higher content of fibers in (L. f) and due to the higher content of protein in (L.P.C) These factors reduced diameter of biscuit, there for reduced spread ratio. the flavor of food products with added legumes can be improved by using traditional processing techniques such as soaking, fermentation, roasting, boiling and other processes **(Joshi &Awasthi2020)**.

The outward appearance of the produced biscuit had its averages showed significant differences, as the results showed that the highest averages were in treatment C and reached 19, while the lowest averages were significantly lower in treatment T3 and reached 11. The general appearance is important for the consumer to accept the product, and the shape of the biscuit produced is affected by the addition of flour in different proportions due to the presence of fibers in the flour, and the Glutennetwork is important in showing the desired shape of the product**(Adbelgadir*et al.*, 2019)**.

The prevalence rate in the produced biscuit was the highest mean significantly in treatment C, reaching 4, while the lowest mean significant decrease was for treatment T3, reaching 2.90.The approximation of the diffusion ratio in the processed biscuits due to the consistency of the dough and the strength of the gluten network in the dough, and the shape of the biscuit cutting is consistent as a result of the correct cutting of the pieces, and the fermentation period is short, which helps not to damage the shape of the pieces **(Ahmad *et al.*, 2019).**

Table (4): Sensory evaluation and spread ratio of biscuit.

Treatment	Color (20)	Texture (30)	Flavor (30)	Appearance (20)	Spread ratio
C	19 a	28 a	28 a	19 a	4.00 a
T1	17 b	26 c	26 b	16 d	3.57 c
T2	18 b	26 c	25 c	17 c	3.32 d
T3	14 c	19 e	20 d	11 e	2.90 g
T4	18 b	25 d	24 c	17 c	3.60 b
T5	18 b	27 b	27 b	18 b	3.12 e
T6	19 a	28 a	28 a	18 b	3.05 f

Values are the average of duplicates Similar letters indicate that there are no significant differences between the parameters ($p< 0.05$) according to Duncan s test.

Tenderness of biscuits

Results in (Table 5) revealed tenderness values of biscuits measured as a distance unit (Mm) which represented the depth of the hole formed by the metal cone when goes down over the piece of biscuit. Measurements were followed during four weeks of storage. Results showed three trends: the first: reduction of tenderness during storage (Rows). The second: reduction of tenderness as substituted level of (L.f) increased. The third: its increase as substituted level of (L.P.C) increased.Also it was noticed that values at second and third trends had the same tendency of sensory evaluation of texture scores (Table 5). These results agreed to the conclusion of **Pop *et al.* (2017) &Feyera(2020)** but didn't agree with results of, which indicated that hardness measured with Instronuniversal testing machine was opposite panelists opinion. This difference between current study and that research may be due to the variance of raw materials tested in biscuit preparation.Anyhow, the physical texture of biscuit is related to its moisture content and functional properties. Such as oil binding, water binding capacity, gelation properties, and fat content in the basic formulation **(Xing *et al.* (2021)**.Decreasing of biscuits tenderness during storage as illustrated by previous studies as well as current study-render the products losing brittleness, a desirable property of biscuits.

Table (5): Tenderness of biscuit during storage for four weeks.

Weeks/Treats	0 (mm)	1 (mm)	2 (mm)	3 (mm)	4 (mm)
C	20	20	16	12	10
T1	16	12	12	10	8
T2	21	15	12	9	6
T3	13	13	10	8	3
T4	25	19	15	9	6
T5	28	19	15	7	7
T6	30	14	14	8	7

Zero time: (after 2 h of baking).

CONCLUSIONS

Biscuits of high dietary fiber and protein can be produced by using lupine flour, while high protein biscuit with good quality can be made by incorporation of lupine protein concentrate. This study showed that lupine flour can be substituted 20% of wheat patent flour in biscuit without adverse effects. Also it indicated that lupine protein concentrate can be substituted 30% of wheat patent flour successfully. This study emphasized that lupine flour, its protein concentrated is lake had superior attributes rendered them a promising alternative of low-cost protein, with nutritional quality comparing with other sources. Further research will be conducted to improve quality of biscuits and then promote the use of lupine to alleviate protein malnutrition problems in world .

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