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STUDY OF PHYSICO-CHEMICAL AND FUNCTIONAL PROPERTIES OF SOYA BEAN PROTEIN ISOLATE (LABORATORY-PRODUCED) AND ITS USE IN CAKE MAKING

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ABSTRACT

This study was conducted to prepare soybean protein isolate (SPI) by using soybean (Glycin max Lee) and studying its physicochemical functional and sensory properties. The results of the chemical analysis showed that soybeans and isolated soybean protein contained moisture 2.5%, 3.25%, protein, 38%, 81% fat, 22%, 1. 5% carbohydrates, 31.34%,10.75% and 6.16 %,3.5% ash, respectively. The functional characteristics included water absorption, ligation, foam, viscosity, jelly coloring, emulsion, and solubility. The results of the sensory assessment of these qualities showed that there is an odorless product with yellow cream, solid strength, high storage capacity, desired bait, and no flavor of beans. The results showed that it could be used in different diets. Thus, isolated soybean protein has been used in the cake industry, and the results of increased protein content have shown moral growth by increasing the replacement rate from 10.50 to 15 %. There has also been a decrease in ash content by increasing the replacement ratio from 1.80 to 1.40 %. The fat ratio in mixtures has increased by an increase in the addition ratio from 0.80 to 90.1 %. There has also been a decrease in carbohydrate content by increasing the replacement ratio from 75.70 to 71.00 %. As for the physical properties of the cake, the results varied by increasing the replacement ratio. The shrinkage factor and volume factor are increased by an increase in the replacement ratio compared with the ordered mix. In contrast, The increase reduces both the symmetry factor and the regularity factor in the replacement ratio. As for the sensory calendar, the treatment (B) had a 5% replacement score of 47.76% compared to the treatment (A) of 0% (manufacturing mix) of 86.51% and the treatment (C) of 10% of 65.24%. While treatment (D) received a score of 54.51% overall, the transactions (C) and (B) can be considered the best of the selected transactions, taking into account the fact that with high nutritional value, qualitative and sensory characteristics of the resulting cake are taken.

Keywords: Soybean protein isolate, Functional property, physicochemical, Cake

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INTRODUCTION

Most of the world's population is suffering from food shortages and malnutrition problems. This problem has emerged in residences of developing countries, particularly the poor countries, and has affected some social groups in industrialized countries. Thus, specialized food agencies and organizations have conducted research and studies to develop food products of high nutritional value at relatively low cost, including those baked and subsidized by various protein sources. (WHO 2000). Insulated soybean protein is one of the soybean products subject to many of the necessary manufacturing methods, and it contains 90% protein. On about 20 types of basic and non-essential amino acids required by the human body, the protein efficiency ratio (protein efficiency ratio) is 2.56. (Douglas 2014). Smith, 1981, pointed out the need to support wheat flour with soybean enzymes, such as soybean flour concentrated soybean protein, or soybean. Insulated soybean protein is important for increasing nutritional value because it contains 7.0 g/16 g nitrogen, and the daily per capita need of nitrogen, as defined by WHO and FAO, is 5.8 g/ 16 g nitrogen. Soybean products are used in the world at present to fortify many foods and food products, especially baked products, to increase their nutritional value. Soybean flour is used by 20% of wheat flour production, which has been subsidized by 70% and 20% fat-free soybeans and 10% corn flour and has received a high degree of sensitivity from adults and children. (Dhilal 2009). Also, Thabet (2000) found that the reinforcement of biscuits with various protein sources, including soy flour, casinos, and fully dried eggs of 50%, 40%, and 10% each, respectively, led to a rise in protein and improved sensory product evaluation. The American Soybean Society (2002) stated that using 2.5% fat-free soybean flour in the biscuits industry would produce a fragile product. He referred to the possibility of using 63% concentrated soybean protein in bread-making mixes to increase the content of essential amino acids to 10% without affecting the qualitative characteristics of the resulting bread. This study aimed to replace wheat flour with different proportions of the insulated soybean protein (80%) and to study its impact on the chemical composition and physical and sensory properties of the mixtures used to manufacture cake and its qualitative and sensory properties.

MATERIALS AND METHODS

Materials

1- Soybean seeds (*Glycine max(lee*) were obtained from the Agricultural Research Center, grade Lee 75.

2- Standard gluten - sodium hydroxide - hydrochloric acid - hexane imported from BHD company, and all of them are materials designated for analysis.

3- Flour - sugar - eggs - oil - low-fat milk - baking powder from the local markets of Babylon.

METHODS

First: Chemical Estimation

I. **Moisture determination:** use of electric (oven) at 105 °C temperature to estimate humidity as explained in (AACC, 2010)

II. **Ash determination:** estimated using incineration ovens at 550 m, as shown in (AACC, 2010).

III. Protein determination: pre-existing semi-micro Kjeldahl method AACC, 2010).

IV. **Fat determination**: Fat value by soxhlet, depending on the method used (AOAC, 2008).

V. **Carbohydrate determination** is calculated by comparing the different components as reported (AOAC, 2008).

Second: Functional characteristics

- Water absorption characteristic: The potential of protein to absorb and carry water was estimated according to Butt and Battol (2010).

- Fat ligation: The potential of protein for fat ligation was assessed according to Butt& Battol, (2010).
- Foam property: The ability of protein color foam and its persistence was measured according to Ahmed *et al.* (2011)
- protein metabolism: The metabolism of protein was estimated (Fekria *et al.* (2012).
- Viscosity, gelatinization, and melting properties were determined according to Butt & Battol, (2010).

III. Manufacture of isolated soybean protein (SPI)

Soy protein was isolated according to the method used by (Makri *et al.*, 2005) with some conversions, as shown in Figure (1).

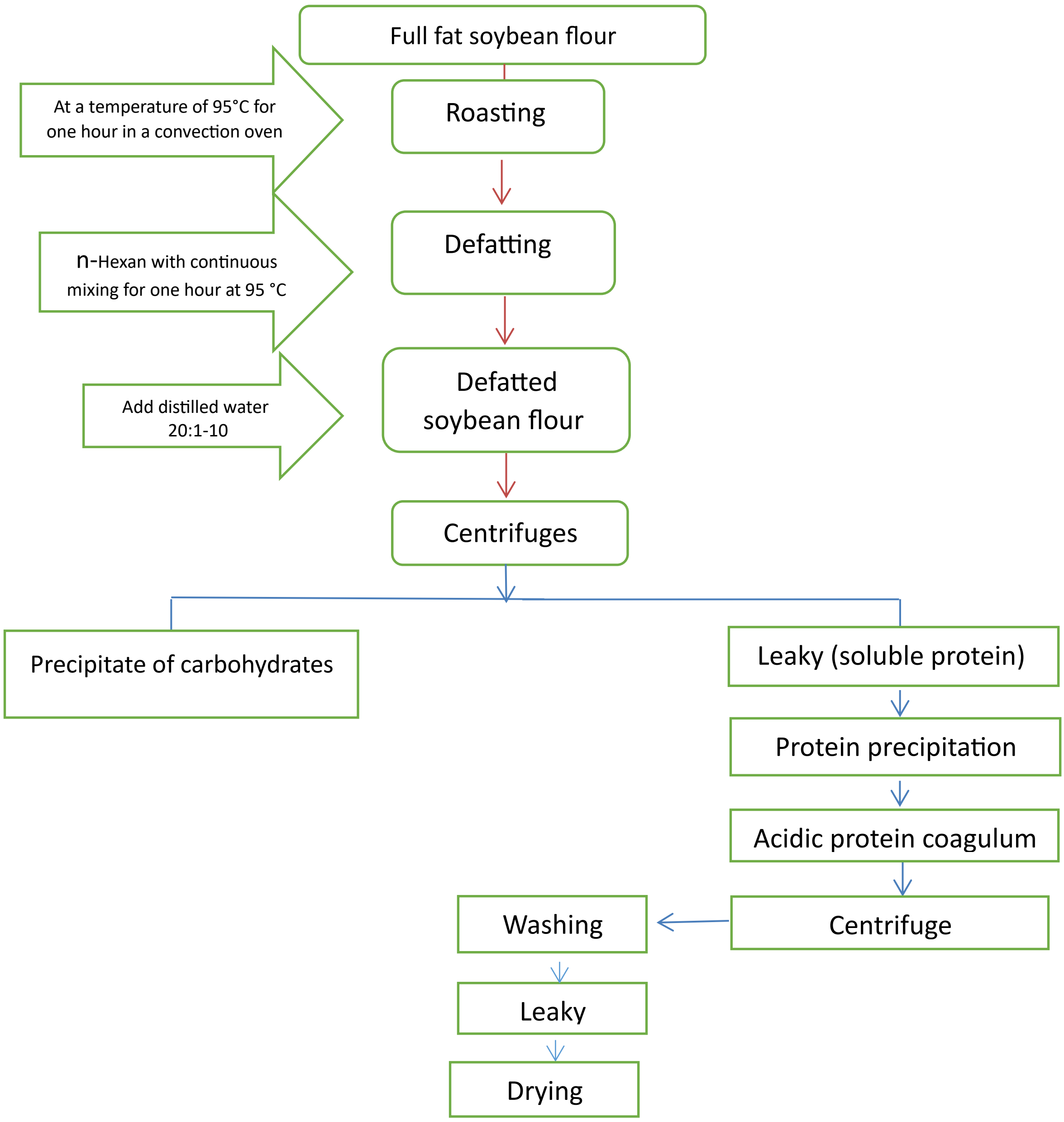

Figure (1) diagram for manufacturing laboratory isolated soybean protein

IV. Manufacturing cakes

Wheat flour was replaced by soybean protein (0, 5, 10, 15 g), and the composition of the mixture to prepare cakes of materials and quantities: Wheat flour 100 g, sugar 75 g, eggs 56 g, oil 31.25 g, fat dilution 62.5 g, and Sodium hydrogen carbonate 3.2 g.

Eggs were scrambled for 2 minutes, sugar was added to it, and the fluke lasted for 5 minutes oil was added to the mixture. Then, flour was added, and the bakery atoms with milk were in rotation with the previous mixture. The mixture is placed in a greased mold and sprayed with flour.

Depending on the method (AACC, 2008), these samples were placed into the oven at 175 C for 40 minutes.

The cake was evaluated after being removed from the oven by two repeaters per treatment, the physical and sensory properties of the cake produced by 10 residents of the Food Science and Technology Department were estimated according to the calendar form of 25-10. (AACC, 2008).

Statistical Analysis
The Triplicate of each sample was statistically analyzed (ANOVA) using the XLSTAT (2016.02.28451) program. The least significant differences (Duncan) test was determined to separate the means at (0.05) significant level. Weight gain and blood parameters were statistically analyzed by (ANOVA), using SPSS version 24 programs for window, and data were expressed as Mean ± SEM. P-value ≤ 0.05 was considered significant.

RESULTS

Chemical Analysis
Table 1 shows the chemical composition of essential substances. Remarkably, a high moisture in the isolated soybean protein compared to soybean beans of 3.5% and 2.5%, respectively. The proportion of ash was within acceptable limits at 3.5% and 6.6%. While the low-fat content in isolated soybean protein compared to soybean beans is observed, the low-fat ratio has been close to the usual range. Carbohydrates were higher than some researchers got because of the incompetence of the separation process, which led to a fraction of carbohydrates coming out with other components.

Table 1: Chemical composition of essential substances.

Components %	Wheat flour	Isolated soybean protein	Soybean beans
Moisture	11.2 a	3.25 b	2.5 c
Ash	1.8 c	3.5 b	6.16 a
Protein	10.5 c	81 a	38 b
Fat	0.8 c	1.5 b	22 a
Carbohydrates	75.7 a	10.75 c	31.34 b

* Different letters indicate significant differences between averages.

Table (2), shows the chemical composition of the various mixtures (A) (B) (C) (D) where the moisture ratio in the mixtures (C) and (D) is low, where it was 10.40% for the two treatments while the amount for treatment B was 11.00%, and there are no significant differences between it and treatment A which amounted to 11.20%. The highest proportion of ash in mixture A was 1.55%, which has a difference between mixtures B,C and D. As for proteins and fats, the percentage in mixture D is higher, at 15.00% and 1.90%, respectively, due to the amount of soybean protein isolated. The proportion of carbohydrates in mixture D is lower compared to the other mixtures, and there are moral differences between them. The reason is the amount of wheat flour used in the mixtures.

Table 2: Chemical composition of various mixtures.

Components %	Mixtures			
	A	B	C	D
Moisture	11.2a	11a	10.4b	10.4b
Ash	1.8a	1.55b	1.44c	c 1.4
Protein	10.5d	12.5c	13.6b	15a
Fat	0.8d	0.95c	1.65b	1.9a
Carbohydrates	75.7a	74b	73c	71d

* Different letters indicate significant differences between averages.

Functional characteristics

Water absorption and fat ligation

Table (3) shows the estimated water absorption percentage of soybean protein isolate (SPI), which is higher than standard gluten. This heightened water absorption is a good indicator, as different kinds of pulse protein flour lie in this category. The chemical composition of soybean protein increases the water absorption property. Protein composition, correlations, and interactions in dietary formulations play a critical role in water absorption, which is of significant importance. This is because fat enhances the protein's ability to retain flavor compounds and improve overall product quality. There's a combination of factors that affect the ability of the protein to bind fat, including the size and distribution of protein molecules and the forces that hate water. The output in Table (3) shows that the amount of fat absorbed by grams per gram of isolated soybean protein was 1.25 g, which is close to what it found in isolated cowpea protein, soy protein, and comparable to the functional properties of isolated protein from types of legumes.

Table 3: Water and fat properties absorbed for isolated soybean protein (SPl).

Property	SP I	Standard gluten
Water absorption (ml)	4 a with PH 4.6	6 a with PH 5
Hydrogen bonding (g)	1.25 b with PH 4.6	1 b with PH 5

*Different letters indicate moral differences between averages.

Foam capacity

Table (4) indicates the volume of foam that can be obtained and the extent of its stability using a concentration of 1% and at a pH = 5. It is noted that the volume of the foam decreases over time, and it is less stable than standard gluten. This result was similar to other studies that used pepsin and bromelain enzymes in protein concentrates produced from *Pisum sativum*. In general, the character of the foam and its stability are affected by the temperature, concentration, speed, and time of whipping, in addition to the methods of mixing the protein substance and the speed of the mixer (Barac et al., 2010).

Table 4: foam characteristic of isolated soybean protein at 1% and 5 pH.

Time (minute)	Foam volume (cm3)	Standard gluten
0	100 a	275 a
10	50 b	275 a
30	0 c	260 b
60	0 c	250 c

* Different letters indicate significant differences between averages.

Emulsifier

Table (5) shows an increase in water stratification and a decrease in the emulsion layer of isolated soybean protein over time. These results are identical to what was found in the proteins of both isolated soybeans and beans, and this property is affected by factors including the size of the droplets, the type of emulsion, and its movement. The chemical composition of the protein has a major role in the stability of an oil-in-water emulsion because it occupies a place between the surface of the oil and the water, which leads to a reduction in surface tension. Thus, it is attracted to the polar phase (water) and the non-polar phase (oil). The table also notes that there are differences. Significant results were obtained for the emulsifying property of isolated soybean protein.

Table 5: **Emulsifier for Soybean insulated Protein SPI.**

Time (minute)	Water layer (cm3)	Emulsion layer (cm3)
0	0 c	30 a
10	25 b	10 b
30	25 b	10 b
60	27 a	8 c

* Different letters indicate moral differences between averages.

Viscosity property

Table (6) shows the viscosity of isolated soybean protein at a concentration of 1% at a temperature of (30, 40, 50) C, which was 0.7913, 0.628, and 0.5329 cP (centipoises), respectively. It is noted that the value of viscosity decreases with increasing temperature.

Table 6: Soybean Insulated Protein

Quality of functional property	Celsius temperatures			Viscosity of standard gluten at 30°C
	30	**40**	**50**	
Viscosity	0.5239	0.6728	0.7913	0.8381

Gelling feature

Table (7) shows the inability of isolated soybean protein to form a gel at all concentrations used, and this is what the negative sign indicates. The same result was also observed compared to standard gluten. Using the same concentrations, gelation could appear at concentrations as high as 18% when studying this property in bean protein. This property is considered important as it refers to the three-dimensional network with semi-rigid properties and compares it to mechanical stresses.

Table 7: The concentrations used in the estimation of jelly property.

Functional property type	1	2	3	4	5	6	7	8	9	10
Gel formation	-	-	-	-	-	-	-	-	-	-

A negative signal (-) indicated that jelly is not formed.

Melting property.
It is noted from Table (8) that the dissolution property was 70% at pH 5. The solubility property is due to the difference in the solubility of proteins and the amino acid content of these proteins. If the protein contains a large percentage of hydrophilic amino acids, it will be highly soluble in water, but if the protein contains a larger percentage of hydrophobic amino acids, it is poorly soluble in water.

Table 8: **Solubility of isolated soybean protein with a concentration of 1%**

Functional property	Spi at pH 5	Standard gluten at pH 5
Dissolution %	70%	45%

Sensory properties.
From observing results in general, it can be concluded that the isolated soybean protein produced in this study has good properties and qualities, and the possibility of its use in some diets is normal and variable at the same time.

Physical cake properties
The percentage value of the shrinkage factor for the control sample was 0.7, while the shrinkage coefficient for the working samples of 5, 10, and 15% and the isolated soybean protein were as follows (1, 1.1, and 1.1 cm), respectively. These results were close to that found by Roccia et al. (2009).

With regards to the volume factor, an increase in values was observed with an increase in the replacement ratio, compared to the control sample was 12.4 cm, while the highest percentage was 15% treated sample, at a rate of 15.3 cm.

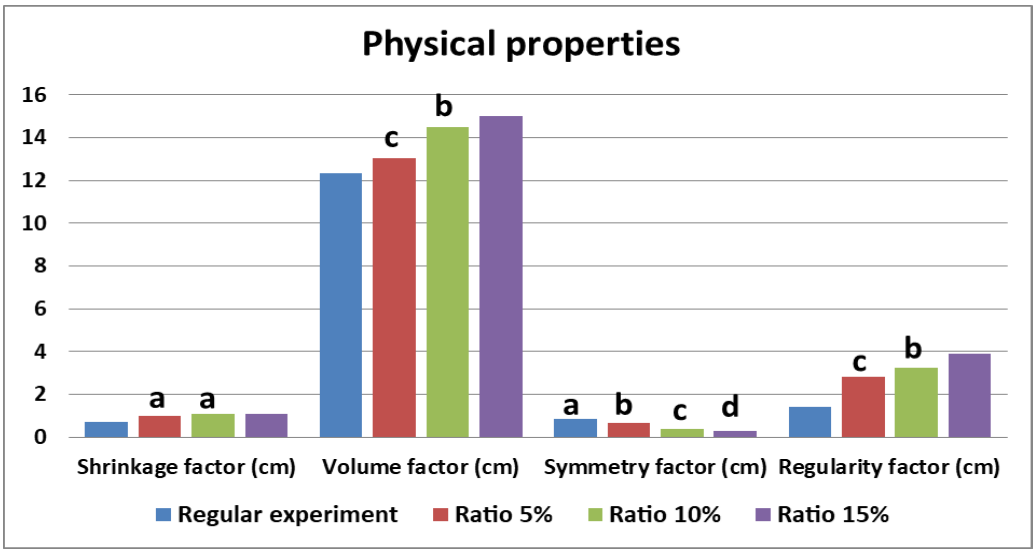

Figure (2) Physical properties of cake

Sensory properties of cake
Figure (3) shows that the cake volume increased with the increase in soybean protein from 540.20 cm3 for the control mixture to 575.70 cm3 for the 15% isolated soybean protein treatment.

The height of the cake increases with the increase in substitution with isolated soybean protein if the control mixture has a height of 4.22 cm to 5.75 cm for a substitution rate of 15%. These results are consistent with the findings of Majzoobl *et al.* 2014.

As for the flavour characteristic, it was found that increasing the addition of (SPI) caused a decrease in acceptance, especially for the 15% replacement, which led to a decrease in the evaluation score of the cake and because of the addition of (SPI), it led to a decrease in the softness of the cake compared to the control mixture, due to the ability of (SPI) to bind Water and reducing free water.

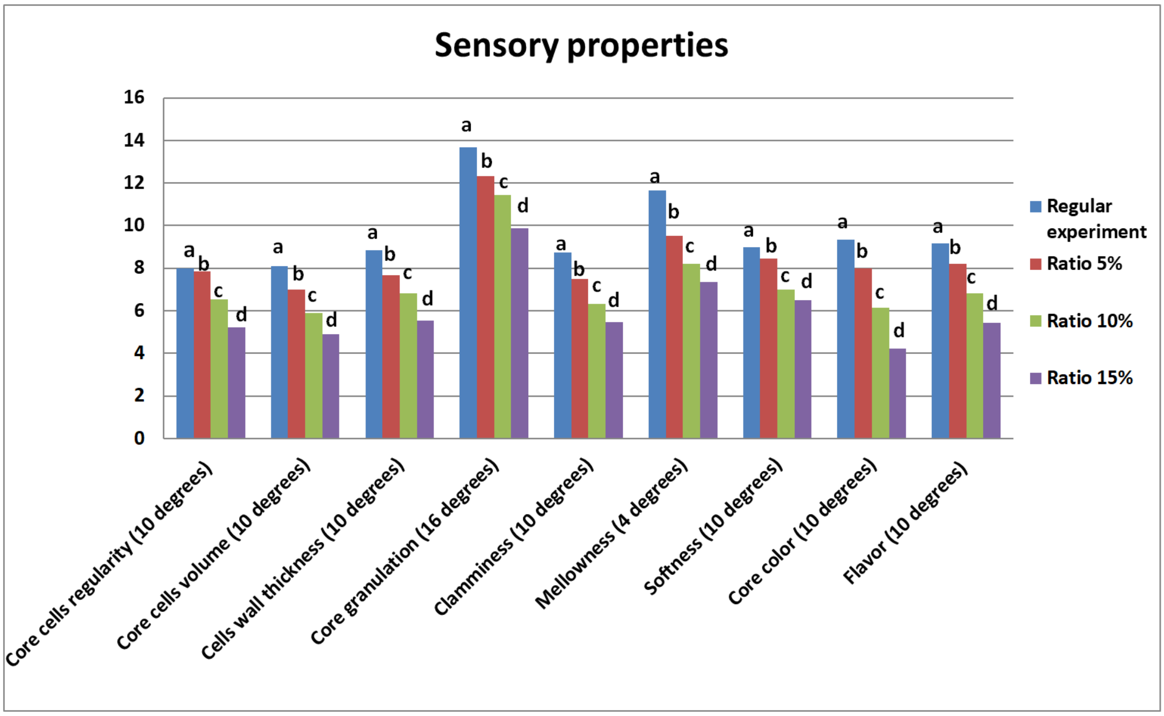

Figure (3) Sensory properties of cake

DISCUSSION

The protein content of SPI is 81%. This percentage may be attributed to the extraction method used, where protein determined by the U.S. soybean protein system was 86% to 92%. (Soy Protein Council 1987). In addition, the speed of the mixture, the temperature used, the mixing rate, and the concentration of the base affect the protein extraction process. (Kuhlman & Bradly 2019).

While the low-fat ratio in isolated soybean protein compared to soybean beans was observed due to the defatting process and increased protein concentration, the low-fat ratio has been close to the usual range, as found (Hu, *et al.,* 2010). Carbohydrates come out with other components, as shown by the evidence (Al-Saraj, 1997). The proportion of ash was within acceptable limits as found by Roesch & Corredige, (2002) at 3.5% and 6.6% of the amount of soybean protein isolated. The proportion of carbohydrates in mixture D was lower compared to the other mixtures, and there are significant differences between them. The reason is the amount of wheat flour used in the mixtures.

Functional characteristics

The amount of water absorption for flour, soy protein, and isolated soybean protein was 2.75, 5, and 4.1 ml, respectively. These results were comparable to research results, and there are many factors affecting the extent to which protein was by water, including pH, temperature, and ion power (Kuhlman & Bradly, 2019). The output in Table 3 shows that the amount of fat absorbed by grams per gram of isolated soybean protein was 1.25 g, which is close to what is found in isolated cowpea protein and soy protein, as well as a comparison with the results when they studied the functional properties of isolated protein from types of legumes.

Foam capacity

Foam capacity is the maximum volume that can be obtained from the spread of protein followed by the introduction of air by whipping, stirring, or aeration, while foam stability means its ability to maintain the maximum volume during a certain period (Butt& Battol, 2010)

Emulsifier

Table (5) shows an increase in water stratification and a decrease in the emulsion layer of isolated soybean protein over time. These results are identical to what was found in the proteins of both isolated soybeans and beans, and this property is affected by factors including the size of the droplets, the type of emulsion, and its movement and vibration of the emulsion. The chemical composition of the protein has a major role in the stability of an oil-in-water emulsion because it occupies a place between the surface of the oil and the water, which leads to a reduction in surface tension. Thus, it is attracted to the polar phase (water) and the non-polar phase (oil). The table also notes that there are differences. Significant results were obtained for the emulsifying property of isolated soybean protein which agreed with that reported by Ahmed *et al.* (2012)

Viscosity property

The viscosity property is affected by pH, temperature, number of molecules, size and shape, interactions between molecules, and the presence of solid materials (salts) (Jasim, 1996).

Gelling feature

This physicochemical property is related to protein interactions, dissolution, association, disintegration, and aggregation (Barac *et al.,* 2010).

Melting property

Melting property is considered as evidence of the possibility of using the protein or not in a specific food. It, in turn, affects some functional properties such as foaming, emulsification, and gelatinization.

Sensory properties

The sensory evaluation of the cake is shown in Figure (3). A creamy yellow color characterizes laboratory-manufactured soybean protein isolate (SPI), odorless, solid consistency, and high storage solubility. It has a desirable taste and is free of the legume flavour.

Physical cake properties

Figure (2) shows the values of some physical properties of the cake produced from wheat flour replaced with soybean protein isolate (SPI) compared to the control mixture. It is noted that the shrinkage coefficient of the control cake mixture did not differ significantly from the samples made from flour replaced with soybean protein isolate (SPI). The regularity coefficient is that isolated soybean protein causes an increase in the cake's components. Soybean protein isolate components have protein, which leads to increased interactions with flour proteins through ionic, covalent, and disulfide bonds. In addition, soybean proteins can absorb water more than wheat flour proteins. Therefore, the increase in water absorption caused homogeneity of the cake components, as it increased the water (Ronda *et al.,* 2011).

Sensory properties of cake

This is because the addition of isolated soy protein led to the preservation of air bubbles resulting from the whipping process or the second. Carbon dioxide resulting from baking powder, and the cohesion of the mixture components are necessary to retain gases during the baking process, which causes an increase in the size of the cake (Sedeuffer, 1990). It has been noted that adding (SPI) has an effect on starch and protein during the baking process, which was necessary because both components affect the size of the final cake, as it was found (Liet *et al.,* 2017) that it can increase the temperature of the starch ring, which allows increasing the size of the cake to delay the transfer of water from the gluten to the starch due to the gelation of the latter. As for the color of the crust (Figure 3) it is affected by Maillard and caramelization reactions, while the color of the pulp is affected by the ingredients used in making cake components. There were significant differences in some properties of the cake. It is noted in Figure (3) that it increases with the addition of (SPI), and the colour of the cake crust is redder and deeper in colour compared to the mixture. The control mixture and these results were consistent with the findings of Singh & Mohamed, (2007). This is due to (SPI), a rich source of lysine, which interacts with sugars during the cake-baking process. It is noted from the results of this research that the mixture made from wheat flour substituted with 5% soybean protein has superior physical and sensory characteristics to the control mixture over other mixtures. The higher the percentage of isolated soybean protein added, the lower the final cake evaluation score, especially in terms of taste, flavor, and colour. And vice versa, although increasing the addition leads to an increase in nutritional value.

CONCLUSIONS

The demand for soy proteins is expected to increase to meet the needs associated with their increased popularity and possible health-promoting effects. Soy protein isolate is a typical commercial product of soybean, widely used as a food ingredient. The physical and flow properties of intact and hydrolyzed Soy protein isolate powders were investigated. There was a slight increase in protein solubility and a decrease in foaming capacity in the modified. It was also noted that adding isolated soy protein increases the nutritional value of the cake and improves its sensory and qualitative qualities.

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دراسة الخواص الفيزيائية والكيميائية والوظيفية لبروتين فول الصويا المعزولة (مختبريا) واستخدامها في صنع الكيك

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تهدف الدراسة الى تحضير بروتين فول الصويا المعزول (SPI) باستخدام حبوب فول الصويا الجافه (clycine max (Lee)) ودراسة خواصه الفيزيائية والكيميائية وخصائصه الوظيفية مختبريا. أظهرت نتائج التحليل الكيميائي أن فول الصويا وبروتين فول الصويا المعزول يحتويان على رطوبة ٥.٢٪ و ٣,٢٥٪ و بروتين ٣٨٪ و ٨١٪ ودهون ٢٢٪ و ١.٥٪ في حين كانت قيمة الكربوهيدرات ٣٤.٣١٪ و ١٠,٧٥٪ اما الرماد فكانت قيمتة ١٦.٦٪ و ٥.٣ ٪ على التوالي. وشملت الخصائص الوظيفية امتصاص الماء وسعة الرغوة ، واللزوجة ، وتكوين الهلام ، والمستحلب ، والذوبانية. وأظهرت نتائج التقييم الحسي لصفاته أن هناك منتجا عديم الرائحة مع كريم أصفر، وقوة صلبة، وسعة تخزين عالية، وطعم مستساغ، وأظهرت النتائج أنه يمكن استخدامه في أنظمة غذائية مختلفة وفي صناعة الكيك، وأظهرت نتائج زيادة محتوى البروتين نموا معنويا عن طريق زيادة معدل الاستبدال من ١٠,٥٠ إلى ١٥٪. كان هناك أيضا انخفاض في محتوى الرماد عن طريق زيادة نسبة الاستبدال من ١,٤٠ إلى ١,٨٠٪. زادت نسبة الدهون في المخاليط بزيادة في نسبة الإضافة من ٠,٨٠ إلى ٩٠,١٪. كان هناك أيضا انخفاض في محتوى الكربوهيدرات عن طريق زيادة نسبة الاستبدال من ٧٥,٧٠ إلى ٧١,٠٠٪. أما بالنسبة للخصائص الفيزيائية للكيك، فقد اختلفت النتائج بزيادة نسبة الاستبدال. يتم زيادة كل من عامل االتقلص وعامل الحجم بزيادة نسبة الاستبدال مقارنة بالخلطة الضابطة ، بينما انخفض كل من عامل التناظر ومعامل الانتظام بزيادة نسبة الاستبدال. أما بالنسبة للتقويم الحسي، فقد حصل المعاملة (B) على درجة استبدال ٥٪ بنسبة ٤٧,٧٦٪ مقارنة بالمعاملة (A) بنسبة ٠٪ (الخلطة الضابطة) بنسبة ٨٦,٥١٪ والمعاملة (C) بنسبة ١٠٪ بنسبة ٦٥,٢٤٪. في حين حصلت المعاملة (D) على درجة ٥٤,٥١٪ بشكل عام لا يمكن اعتبار المعاملة (C) و (B) أفضل المعاملات المختارة ، مع الأخذ في الاعتبار حقيقة أنه مع القيمة الغذائية العالية ، يتم أخذ الخصائص النوعية والحسية للكيك الناتج في الاعتبار.

الكلمات المفتاحية: عزل بروتين فول الصويا ، الخواص الوظيفية ، الفيزيائية والكيميائية ، الكيك