Detoxification of Jojoba Meal Simmondsin by Production of Its Protein Isolates as a Protein Source in Food Applications

Rasha K. Mohamed [a,1,*], Dorria M.M. Ahmed [b], Ferial M. Abu-Salem [a]
[a] Department of Food Technology, National Research Centre, Dokki, Giza, Egypt
[b] Department of Pomology, National Research Centre, Dokki, Giza, Egypt
[1] rk.abdel-naby@nrc.sci.eg
* corresponding author

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ABSTRACT

Jojoba meal is a byproduct after oil extraction and serves as an excellent source of protein. Nevertheless, the meal's presence of toxic factor simmondsin as a cyanogenic glycoside restricted its application and made the researchers seek alternative methods for its detoxification. Jojoba protein isolates were prepared by alkaline extraction at different pHs 8, 9, and 10 (JPI 8, JPI 9, JPI 10) followed by isoelectric precipitation as a procedure for detoxification of jojoba meal from simmondsin and produce safe food-grade functional ingredient (jojoba protein isolate JPI) available to use in many industrial applications. The highest protein yield (83.21%) was recorded in protein isolates extracted at pH 9 (JPI 9) with the highest protein content at 91.15%.Simmondsin content (mg /kg) in jojoba meal is 278.79, 5.64 in JPI8, 2.27 in JPI9, and 1.97 in JPI 10. JPI 9 achieved high levels of isoleucine, lysine, cysteine, phenylalanine, tyrosine, and threonine. High chemical protein scores in JPI 9 at 156.98 and 109.01 for threonine, phenylalanine, and tyrosine.The results revealed that JPI9 achieved the highest level and the yield of essential and nonessential amino acids it was used in enriched the biscuit with different levels (5- 10 and 15%) to produce high protein biscuit functional product as one of industrial applications

1. Introduction

Simmondsia chinensis (Link) C.K. Schneider, often known as Jojoba, is an adapted shrub that can grow successfully in adverse conditions and is grown in numerous nations worldwide. The importance of this plant comes from the uniquely oil that makes up over 50% of its seeds. Jojoba oil possesses significant economic importance, particularly in the cosmetic business. The residual meal, abundant in proteins, serves as an excellent source for animal feeding. Nevertheless, the meal's presence of antinutritional factors restricted its application and made the researchers seek alternative methods for its detoxification. Jojoba meal could be detoxified through chemical, biological, and physical means as well. Despite the extensive investigation of the oil's phytochemical composition, the chemical composition of the remaining plant has been reported in a limited number of studies [1]. The jojoba plant, scientifically known as Simmondsia chinensis (Link) Schneider, is a member of the Simmondsiaceae family. It is a type of desert shrub that grows in arid and semi-arid areas and is known for its oil production [2]. Jojoba oil possesses excellent lubricating properties. Furthermore, jojoba has significant interest in several sectors like cosmetics, medicines, feeding animals, and landscape as a means of conserving soil [3]. The defatted meal obtained after oil extraction possesses the potential to serve as a valuable supplementary for animal feeds. After the removal of fat, the

primary components of it consist of proteins (31%) and carbohydrates (55%) [4]. The difficulty facing the jojoba business is figuring out how to increase product quality and productivity [5].

However, this meal includes around 15% of toxic cyanoglucosides [6]. Simmonds has been identified as a main factor that suppresses appetites in rodents and chickens,and leading to higher levels of cyanide and thiocyanate in the blood [7].

Methods for deactivating or removing simmondsin from jojoba meal have been reported by several authors. Detoxification techniques using chemical methods [8, 9], microbiological methods [10], and solvent extraction methods. It has been Performed an analysis on different solvents, such as water, methanol, and 90% ethanol, and found that water effectively extracted all the simmondsin components. Simmondsins could not be completely removed, however, despite repeated extractions using methanol or 90% ethanol [11].

Verbiscar et al [9] performed experiments to evaluate the effectiveness of different solvents, including acetone, isopropanol, water, methanol, and a mixture of 85% dichloromethane and 15% methanol, in extracting simmondsin from the defatted meal. It was discovered that water and methanol were able to remove simmondsin completely.

It has been demonstrated that water may extract simmondsins from jojoba meal. However, only chloroform, methanol, acetonitrile: water, and acetone extracts were found to be appropriate for chromatographic separations [12].

A one-step method that involved repeating the extraction with water at 90 °C was shown to be effective in extracting most of the simmondsin and oil from crushed jojoba seeds [13]. In addition, they stated that the optimal duration for extraction was 1.5 hours, while the ideal temperature was 90 °C. The worldwide market for plant-based protein is rapidly growing, mainly because of its positive impact on the environment, economy, health, as well as society [14]

Nowadays, there is a growing interest in developing bakery products that possess enhanced nutritional content and reduced caloric content. As a result, researchers and food manufacturers are investigating the potential of enhancing baked goods with functional ingredients that can offer further nutritional advantages. Using protein isolates to enhance the protein content is now considered one of the most promising approaches for producing customized food [15, 16].

For many years, high-protein biscuits have been utilized in emergencies, mainly for meeting the children nutritional requirements. High protein biscuits are food products that are formulated by using high amounts of protein-rich ingredients. Emergency food preparations in the form of high-protein biscuits should have a sufficient amount of nutrients, including calories, based on the recommended daily intake of 2100 kcal/day as per the dietary adequacy rate (RDA) set by the Institute of Medicine in 2005 [17, 18].There is a growing interest in extracting the proteins from jojoba meal for various industrial, cosmetic, and food applications to prevent disposal issues and environmental risks that arise after extracting the oil [19].

Therefore, the present study aimed to produce a detoxified non-traditional source of protein isolate at different pH levels for removing simmondsins from a by-product (jojoba meal) and use it as a food-grade product by enriching the biscuit with protein isolate to improve protein content as a functional food.

2. Materials and Methods

Plant materials Jojoba (Simmondsia chinensis) seeds were harvested from 4-year-old Jojoba trees cultivated in the South Sinai Governorate of Egypt. The Jojoba trees that were chosen were consistent in their level of strength and size, and were planted at a distance of 2-4 meters from each other. Trees were planted in sandy loam soil and were irrigated with a drip irrigation system by saline fresh water. All trees were subjected to identical horticultural procedures.

2.1. Preparation of Jojoba Meal

After the seeds are crushed, they are boiled in a solution of one-part seeds to two parts water for 45 minutes. In this step, a little quantity of hydrochloric acid was used to regulate the pH level at 4.5. A

press operation was performed to obtain the pressed cake (meal). A compressed cake (meal) is obtained from a pressing procedure and subsequently subjected to hexane extraction until the solvent turns colorless. Using a hammer mill, the defatted meal was ground and run through 60-mesh screen. Then dried by spread out in trays overnight at 25°C [20].

2.2. Preparation of Protein Isolate

Jojoba protein isolates are produced using the process of alkaline extraction, then followed by the procedure of isoelectric precipitation (IP) [12, 21].

The process of alkaline extraction involves mixing three equal quantities of jojoba meal with NaOH solutions at pH levels of 8, 9, and 10 for 2 h at 23 °C. The solvent-to-meal ratio used was 10:1 (weight to volume). After centrifuging the mixture for 15 minutes at 4000 g, the supernatant was utilized to start an isoelectric precipitation (IEP) by reducing its pH with 0.5 M HCl. After precipitating overnight at 4°C, the pellet was centrifuged at 4000g for 20 min and dried at 50 ° C.

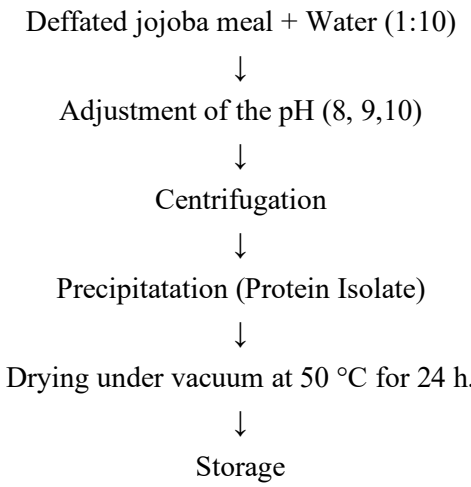

Figure 1. Systematic flow sheet for jojoba protein Isolates

2.2.1. Analytical methods

The whole seed, meal, and protein isolates' moisture content, crude oil content, crude protein (N x 6.25), and total ash had been determined [22].

2.2.3. Isolate Recovery

The weight of the protein isolates obtained during isoelectric precipitation per 100g weight of the corresponding beans was used to calculate the recovery of jojoba protein isolates [23].

2.2.3. Protein Yield

The determination of protein yield carried out according toWang et al [23]

Simmondsin extraction:

The samples (50 g) were treated as follows simmondsins are extracted using methanol (3× 50 mL) through stirring at ambient temperature for 30 minutes. The extracts were concentrated at lower pressures (40–50 ◦C), dissolved in methanol (HPLC) grade, and then adjusted to a final volume of 1 ml [12].

2.3. High-Performance Liquid Chromatography (HPLC)

Simmondsin quantification was performed using reverse-phase high-performance liquid chromatography (HPLC). In brief, the methanolic extract of Simmondin was run through a millipore filter with a pore size of 0.2 µm, and HPLC was carried out using a reverse phase C18 column. A pressure range of 0 to 200 kgf cm–2 was kept. The mobile phase consisted of a water-methanol

combination of HPLC quality, with a ratio of 80% water to 20% methanol (v/v). A flow rate of 0.75 mL min–1 was consistently maintained throughout the run. The eluents were observed at a wavelength of 217 nm. The sample and standard were injected in quantities of 20 µL.

2.3.1. Amino acids composition using HPLC

The amino acid contents of the tested samples were analyzed using the HPLC-Pico-Tag method, following the protocol established by Millipore Cooperative. The protein sample was measured and placed in a hydrolysis tube of 25 × 150 mm. The tube was then heated in an oven set at a temperature of 110ºC for a duration of 24 hours. The HPLC chromatographic analysis was conducted using a gradient of Pico-Tag solvent (Eluent A and B) at a temperature of 38 °C. The flow rate was adjusted to 1 ml/min, and 20 ml sample was injected onto a stainless-steel amino acids C18 column 100x4.6 mm. The PTC derivatives are detected using UV absorption measurements using a Waters detector set at a fixed wavelength of 254nm. The instrument was calibrated by performing two injections of the lysine standards before sample injection. [24, 25, 26].

2.4. Chemical score (CS)

The CS was determined by comparing the essential amino acid (EAA) content in protein to that of a whole egg protein, as stated by the FAO/WHO [27]. The calculation was done using the formula: CS= [EAA in the tested protein (g/100g)/EAA in egg protein (g/100g)] ×100 [28].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein isolates' composition profile was examined using SDS-PAGE. The proteins were resuspended in 50 µl of sample buffer [29] and subsequently subjected to boiling for 10 minutes. Then, the mixture had centrifugation at a speed of 5000 rpm for 5 minutes. The separation of total protein was achieved using a 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technique, specifically employing Mini-gel electrophoresis equipment from BioRad, USA. The protein profile's molecular weight was identified by comparing it to standard molecular weight markers (standard protein markers, 11-245 kDa; Sigma, USA). The protein bands made visible with the application of Coomassie Brilliant Blue R-250 stain (Sigma, USA) [30].

2.5. Biscuits Formulation and Preparation

The biscuits were made using the basic formulation, as follows: The composition consists of 49.5% wheat flour, 20% butter, 20% sugar, 10% beaten whole egg, and 0.5% baking powder [31]. Jojoba protein isolate was added to wheat flour dough at concentrations of 5%, 10%, and 15% through the process of combining with wheat flour and baking powder.

The final dough was shaped to a thickness of 19 mm using a sheeting board. and then cut to a width of 69 mm. It was then baked on oiled pans for 15 minutes at a temperature of 160◦C in an air oven. The biscuits were cooled to a temperature of 30±2◦C and then sealed in high-density polyethylene bags.

2.5.1. Physical Characteristics of Formulated Biscuits

The biscuit was evaluated for the following parameters using the method described by Bala et al. [32]

Thickness: The thickness of the biscuits was measured by using a vernier caliper to determine the diameter of six biscuit samples put next to each other. Subsequently, the reading was divided by a factor of 6 to obtain the precise thickness of a single biscuit. The mean thickness value was provided in centimeters.

Diameter: Six biscuit samples were arranged edge to edge, and the diameter of each biscuit was measured using a vernier caliper. With each set of samples, an average was calculated. The diameter average was expressed in centimeters.

Spread Ratio The spread ratio was determined by dividing the diameter by the thickness.

Spread factor (SF)

The spread factor (SF) is calculated using the formula SF = D/T × CF × 10, where SF represents the spread factor, D represents the diameter, T represents the thickness, and CF is a correction factor at constant atmospheric pressure. The numerical value in this particular instance was 1.0. [33]

2.5.2. Color properties of biscuits

The color of baked biscuits was measured as L*, a*, and b* using a Hunter Lab Colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) according to the CIE L*, a*, b* color system. According to, L* values represent black to white (0–100), a* values represent redness when positive, and b* values represent yellowness when positive [34].

2.6. Sensory Evaluation

A sensory evaluation was performed at the Food Technology Department of the National Research Centre in Egypt. A group of twenty panelists, ranging in age from thirty to fifty, were chosen for participation in the sensory evaluation.

The sensory evaluation was performed using the hedonic scale [35]. The food samples were prepared in standardized sample containers, marked with random letters, and each sample was linked to a unique letter. Each panelist was sequentially presented with the sample in a randomized order. The panelists were directed to evaluate the coded samples for each sensory characteristic, such as color, aroma, texture, flavor, and overall acceptance, based on their personal choice, which may range from 1 to 9.

2.7. Statistical Analysis

The results were presented as means ± standard error after the samples were evaluated three times. The evaluation was performed using the SAS (Statistical Analysis System) program for Windows [36]. A one-way analysis of variance (ANOVA) was employed to evaluate the statistical significance of the differences in mean values. Following that, Duncan's multiple range test was performed with a significance level set at $p < 0.05$..

3. Results and Discussion

3.1. Proximate Composition and Simmondsin Content of The Jojoba Seeds, Meal, and Protein Isolates

The data presented in Table 1 showed the proximate composition of jojoba seeds, meal, and protein isolates (at different pH 8, 9, and 10 of extraction). The moisture content was 2.36, 4.33, 5.82 .4.32, and 6.90%, respectively. The protein isolate (pH 9) showed the highest protein content, reaching 91.15%.

Table 1. Proximate composition of jojoba seeds, meal, and protein isolates g / 100 g sample (dry weight basis)

Components	Jojoba seed	DJM	JPI 8	JPI 9	JPI 10
Moisture	2.36^{d} ± 0.04	4.33^{c} ± 0.03	5.82^{b} ± 0.02	4.32^{c} ± 0.06	6.90^{a} ± 0.10
Crude Protein	14.24^{e} ±0.25	21.43^{d} ± 0.45	86.81^{b} ± 0.04	91.15^{a} ± 0.30	85.49^{c} ± 0.04
Crude oil	47.60^{a} ± 2.40	3.55^{b} ± 0.05	0.54^{c} ± 0.06	0.50^{c} ± 0.02	0.49^{c} ± 0.10
Crude Fiber	10.82^{b} ± 0.22	16.32^{a} ± 0.32	1.52^{c} ± 0.03	1.01^{d} ± 0.01	0.93^{d} ± 0.03
Ash	2.27^{b} ± 0.03	5.42^{a} ± 0.04	1.78^{cd} ± 0.04	1.66^{d} ± 0.10	2.0^{c} ± 0.30
Available carbohydrates**	23.24^{b} ± 2.43	48.95^{a} ± 0.43	3.53^{d} ± 0.10	1.36^{e} ± 0.33	4.19^{c} ± 0.16

Mean values followed by the standard error (±).
Numbers in the same row followed by the same letter are not significantly different ($p < 0.05$).

The seeds had a crude oil content of 47.60%, while protein isolates oil content was the lowest ranging from 0.49% to 0.54%. The jojoba meal indicated the higher concentration of crude fiber, around 16.32%, and protein isolates had the lowest level ranging from 0.93 -1.52 %. In addition, jojoba meal had the highest level of ash 5.42%.

Jojoba meal contains 21.43% protein, 3.55% crude oil, 16.32% crude fiber, 5.42% ash. Our finding of jojoba meal was slightly higher in crude oil, crude fiber, and ash than those reported by El-Anany [37] who reported that Jojoba meal contains 22.9% protein, 1.2% crude oil, 15.4% crude fiber, and 4.1% ash. Our results also agree with those demonstrated by Sobhy et al [38] with sight higher protein and oil content of their jojoba meal.

The chemical composition of the protein isolate extracted at pH 9 (JPI 9) was 91.15% protein, 0.50% crude oil, 1.01% crude fiber, and 1.66% ash which is in the similar range to that reported [38] with a slight decrease in protein and oil content reported by El-Anany who demonstrated that the isolate's chemical composition contained 92.35% protein, 1.2% crude oil, 0.76% crude fiber, and 1.13% ash [37].

3.2. Simmondsin Analysis

The Simmonds was determined by HPLC as presented in Fig 2. The simmondsin content in jojoba meal is 278.79 mg /kg, 5.64 mg/kg of JPI8, 2.27mg / Kg of JPI9, and 1.97 mg/ Kg of JPI 10. Our result was lower than that found that the Simmondsin content of jojoba meal was 0.43% and the lowest concentration of simmondsin, measuring 0.10% in protein isolate. [37].

The result revealed that the simmondsin content was reduced with an increase in pH of protein extraction at 1.97 mg/kg which indicated that producing protein isolate eliminated simmondsin to 1.97 and produced detoxified protein isolate available to use in many industrial applications. The LD50 for rats of simmondsins is 4 g/kg [39]. Several biochemical parameters were employed to determine that a 5-day administration of 250 mg of simmondsin/kg of body weight did not have any toxicological effects on the liver, pancreas, or kidneys.

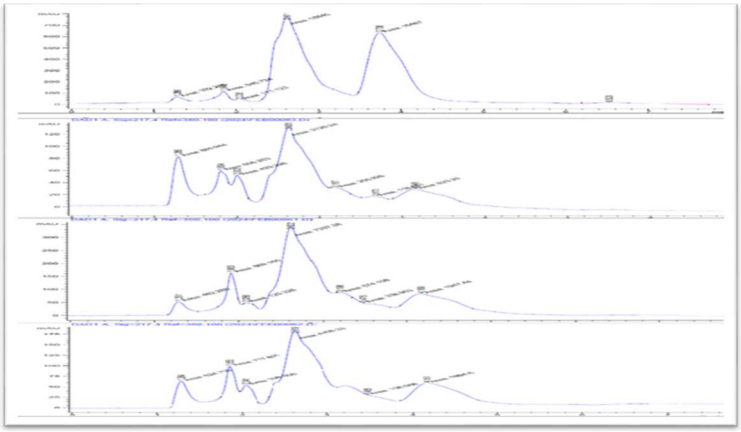

Figure 2. HPLC chromatograms of Simmonds in DJM, JPI 8, JPI 9, and JPI 10

3.3. Protein Isolates Recovery and Yield

Table 2 illustrates the mean values for protein recovery and yield. The protein isolate extraction process resulted in the highest recovery of JPI 10 (13.5%) with protein content (85.49%) and protein yield (81.05%) based on the protein content of jojoba seeds and protein isolate.

Comparatively lower protein isolates recovery (12.5%) and lower protein yield (76.20%) were in JPI 8, similarly protein content was (86.81%). The highest protein yield (83.21%) was recorded in protein isolates extracted at pH 9 with the highest protein content 91.15 %. results indicated that the increase of the extraction pH to 9 led to an increase in protein yield and protein content.

The protein extractability and recovery were shown to be positively correlated with higher pH values. It also indicates that higher concentrations of alkaline medium can improve the ability to extract protein [40].

Table 2. Protein isolates recovery and yield at different pH of extraction

Protein isolates	Protein isolates recovery (g/100g meal)	Crude Protein (%)	Protein yield (%protein)
JPI 8	12.5± 0.20c	86.81± 0.04b	76.20 ± 0.02c
JPI 9	13 ± 0.35b	91.15 ± 0.30 a	83.21± 0.07a
JPI 10	13.5± 0.20 a	85.49 ± 0.04c	81.05± 0.07b

Mean values followed by the standard error (±).
Numbers in the same column followed by the same letter are not significantly different ($p < 0.05$).

Both the extraction pH and precipitation pH have an impact on the protein recovery yield [41]. The cause for this is the dependency of both procedure stages: only the protein that becomes soluble in the initial stage may be precipitated in the next stage since the protein that remains insoluble is retained in the waste solids fraction [42].

3.4. Amino Acids Composition of Jojoba Protein Isolates

The data shown in Table 3 represented the essential amino acid composition of jojoba protein isolate extracted at different pH 8, 9, and 10 compared with the provisional amino acid content of the FAO/WHO standard [27] and the content of non-essential amino acids.

Results in Tables 3 indicated that protein isolate extracted at pH8 achieved the highest level of essential amino acids at 45.73 g / 100 g sample and the lowest level of non-essential amino acids was 50.73, While the protein isolate extracted at pH 9 achieved a high level of both essential and non-essential amino acids 45.32 and 53.28 g/ 100 gm respectively. Jojoba protein isolate extracted at pH 10 had a low level of essential amino acids 41.13 and the highest level of non-essential amino acids 55.05 g /100g respectively

Protein isolate extracted at pH 8 (JPI 8) scored the highest level of methionine and valine 4.58 and 7.67 gm/100gm respectively, while protein isolate extracted at pH 9 achieved high levels of isoleucine, lysine, cysteine, phenylalanine, tyrosine and threonine. Protein isolate extracted at pH 10 had the highest level of histidine 4.41g/100g.

Results revealed that protein isolate extraction at pH 9 achieved the highest level and yield of essential and nonessential amino acids.

3.5. Chemical Score of Essential Amino Acid

The chemical score results for jojoba protein isolates, as reported in Table 4, indicate that methionine and cysteine achieved the greatest chemical protein score of 222.86 in JPI8, followed by JPI9. The data also showed significantly elevated chemical protein scores in JPI 9, with threonine, phenylalanine, and tyrosine scoring 156.98, 109.01, and 109.01 respectively. Conversely, lysine and leucine exhibited the most minimal ratings in all samples, indicating that they are the amino acids that limit the availability of the jojoba protein isolate.

Table 3. Essential amino acid composition of jojoba protein isolate compared with the provisional A.A content of the FAO/WHO standard (1989)

Essential amino acid	g/100 g sample			FAO/WHO
	JPI 8	JPI 9	JPI 10	
Histdine	3.10	2.31	4.41	2.60
Isoleucine	3.63	3.84	3.72	4.60
Leucine	5.45	6.72	5.80	9.30
Lysine	4.10	4.56	4.43	6.60
Methonine + Cysteine	9.36	8.65	7.85	4.20
Phenylalanine + Tyrosine	6.36	7.74	5.65	7.10
Threonine	4.89	6.75	4.45	4.30
Valine	7.76	4.75	5.03	5.50
Tryptophan	ND	ND	ND	1.7
Total essential amino acid	44.65	45.32	41.34	46.00
Aspartic acid	7.35	8.97	5.65	
Glutamic acid	12.19	13.07	10.79	
Serine	3.48	2.48	5.12	
Glycine	4.48	3.02	6.19	
Arginine	7.73	6.90	5.63	
Alanine	3.09	6.14	5.31	
Proline	3.84	5.25	5.46	
Total non-essential amino acid	42.16	45.83	44.15	

Table 4. Chemical scores for the essential amino acids of the jojoba protein isolate compared with the provisional values for the amino acids of the FAO/WHO/UNU standard (1989).

Essential amino acid	JPI 8	JPI 9	JPI 10
Histdine	119.23	88.85	169.62
Isoleucine	78.91	83.48	80.87
Leucine	58.60	72.26	62.37
Lysine	62.12	69.09	67.12
Methonine + Cysteine	222.86	205.95	186.90
Phenylalanine + Tyrosine	89.58	109.01	79.58
Threonine	113.72	156.98	103.49
Valine	141.09	86.36	91.45

3.6. Gel Electrophoresis (SDS-PAGE)

Figure 3 shows the electrophorograph of SDS-PAGE for three samples of jojoba protein isolates, including the standard. The data indicated that the bands of jojoba protein isolate ranged from 20 to 52 kDa . There are two bands in pH 8 found at 22 and 52 KDa while in pH 9 showed two bands at 21 and 52 kDa. Protein isolate extracted at pH 10 showed 3 bands at 20, 33, and 52 kDa.

The main constituents of jojoba protein are albumin (79%) and globulin (21%). The SDS-PAGE analysis revealed the presence of two prominent proteins with molecular weights of 50 kDa and 25 kDa in both the albumins and the globulins [19]. There is a lack of in-depth study and characterization of the proteins found in jojoba meal.

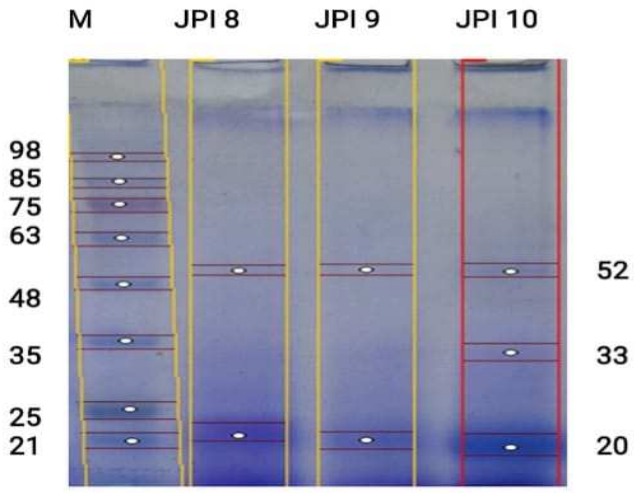

Figure 3. Electrograph of protein isolates pattern by one-dimensional SDS-PAGE showing the change of protein bands in response to different pH 8,9,10

3.7. Physical Characteristics of Formulated Biscuits

Previous results revealed that protein isolate extraction at pH 9 achieved the highest level and yield of essential and nonessential amino acids we enriched the biscuits with protein isolate extracted at pH 9 with different levels of 5, 10, and 15 %, and physical characteristics were examined. The physical properties of the biscuits, including their diameter, thickness, and spread ratio, are presented in Table 5.

Table 5. Physical analysis of jojoba protein biscuit's

Samples	Diameter (cm)	Thickness (cm)	Spread ratio	Spread factor
Control	5.8 ± 0.17^{c}	1.02 ± 0.03^{a}	5.71 ± 0.30^{b}	56.86
JPI 5 %	5.93 ± 0.10^{b}	1 ± 0.02^{a}	5.93 ± 0.20^{a}	59.30
JPI 10 %	6.10 ± 0.06^{bc}	1 ± 0.00^{a}	5.93 ± 0.06^{b}	61
JPI 15%	6.47 ± 0.12^{a}	0.92 ± 0.03^{b}	7.06 ± 3.88^{a}	59.52

Mean values followed by the standard error (±).
Numbers in the same column followed by the same letter are not significantly different ($p < 0.05$).

It was noticed that there was a significant increase in diameter with an increasing level of jojoba protein isolate and the maximum diameter was 6.47cm in biscuits with 15 % jojoba protein isolate. There are non-significant differences in the thickness of control and 5 %, and 10% while there is a significant reduction in thickness has been noticed in jp15 %.

The spread ratio or diameter of biscuits has traditionally been applied as a means of assessing the flour's quality for biscuit production. [43, 44]. The biscuit spread ratio refers to the ratio of the diameter to the height of the biscuit. Biscuits with a higher spread ratio are considered more desirable [45, 46]. The results indicate that as the addition level of jojoba protein isolate increased, the spread ratio of the biscuits also increased. Cookies' thickness and diameter affect their spread factor, which is further dependent on the gluten network [47,48].

Results reveal increasing the spread factor with increasing the level of protein isolate in biscuits Therefore, it can be concluded that the addition of jojoba protein isolate enhances the spreading of biscuits which is desirable for consumers.

3.8. Color Properties of Biscuits

The color is a significant factor that influences the acceptability of biscuits. The incorporation of jojoba protein isolate in the biscuit formulation had significant effects on the surface color of the biscuits represented in Table 6 and Figure 4.

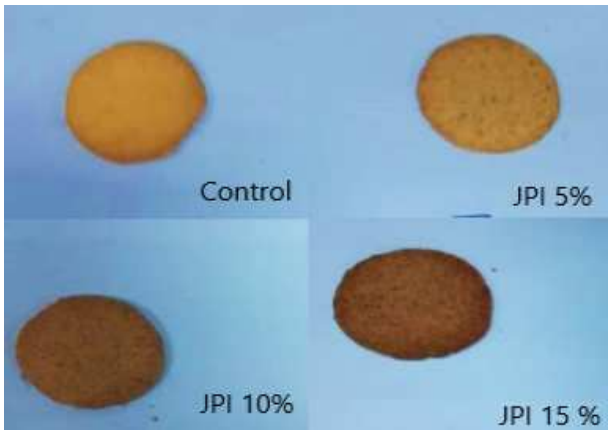

Figure 4. Biscuits with different levels of jojoba protein isolate

Table 6. Color properties of jojoba protein biscuits

Samples	L*	a*	b*	ΔE
Control	78.71 ± 0.45[a]	6.84± 0.10[d]	44.21 ± 0.10[a]	
JPI 5 %	69.21± 0.09 [b]	7.44 ± 0.25[c]	33.89 ± 0.04[b]	14.03967
JPI 10 %	60.14± 0.16[c]	10.22± 0.02[b]	28.80 ± 0.06[c]	24.36673
JPI 15%	54.34± 0.01[d]	10.75± 0.01[a]	25.96 ± 0.02[d]	30.69605

Mean values followed by the standard error (±).
Numbers in the same column followed by the same letter are not significantly different ($p < 0.05$).

The lightness decreased significantly as the added level of jojoba protein isolate increased (69.21, 60.14, and 54.34) compared to the control (78.71). The reduction in L* values reveals that the biscuits have a darker shade at the high level of replacement. A negative correlation between protein content and the lightness of biscuits was observed, indicating that the Maillard reaction had a significant impact in development the color of the biscuits. The process of Maillard browning and caramelization of sugar during baking results in the formation of brown pigments [49, 50]. Many variables, such as water activity, temperature, pH, sugars, and type of amino compounds, influence these browning reactions [51].

The*a color value indicates the redness of biscuits. As the addition level of the jojoba protein isolate increased (7.44, 10.22, and 10.75) in comparison to the control (6.84), there was a noticeably increasing trend of redness.

The b* value in Table 5 reveals the significant differences in surface yellowness values among the four biscuit formulations. The substitution with jojoba protein isolate resulted in significantly lowered b* values with increasing addition levels of jojoba protein isolate (33.89, 28.80, and 25.96) from the control (44.21). The reduction yellowness of composite biscuits may be due to the lower b* value of jojoba protein isolates than the refined flour.

In addition, the total color difference (ΔE) was significantly increased with increasing the level of replacement. Biscuits incorporated with JPI 15% showed the highest dissimilar in color (30.70).

3.9. Sensory Evaluation of Biscuits

The sensory evaluation of the biscuits Table 7 revealed that there were significant differences ($p<0.05$) between the sensory attributes color, aroma, texture, and flavor and overall acceptances as the level of jojoba protein isolates increased from 5 to 15 %.

Table 7. Sensory Evaluation of Biscuits

Sample	Color	Odor	Texture	taste	Overall acceptance
Control	9 ± 0.20 [a]	9 ± 0.18 [a]	9 ± 0.18 [a]	9 ± 0.28 [a]	9 ± 0.20 [a]
JPI 5%	8.43 ± 0.20[b]	8.57 ± 0.37 [a]	8.71 ± 0.18 [a]	8.93 ± 0.28 [a]	9.00 ± 0.22 [a]
JPI 10%	7.50 ± 0.19 [c]	7.36 ± 0.39 [b]	7.86 ± 0.24 [b]	7.43 ± 0.28 [b]	7.64 ± 0.24 [b]
JPI 15%	6.93 ± 0.28 [d]	7.43 ± 0.48 [b]	6.79 ± 0.31 c	7.64 ± 0.24 [b]	6.71 ± 0.18 [c]

Mean values followed by the standard error (±).
Numbers in the same column followed by the same letter are not significantly different ($p < 0.05$).

Control treatment and biscuits with 5 % protein isolate showed the highest score in sensory attributes and there were non-significant differences between sensory attributes for both biscuits. There was a reduction in the rating of sensory parametres specially color and texture with increasing substitution level of jojoba protein isolate with10 and 15 % may be due to the color of protein isolate which lowered the yellowness of biscuits. Texture score decrease to 6.79 in the highest level of protein substitution compared to control 9. The overall acceptability of b 5% JPI was similar to control while the biscuit with 10 %JPI was acceptable at 84.9 % and biscuit with 15 %JPI recorded the lowest acceptability at 74.5%. Generally increasing the level of jojoba protein isolate up to 10 % in the biscuits led to a significant reduction in the sensory attributes..

4. Conclusion

The process of extracting jojoba protein from defatted meals through alkaline extraction and acid precipitation is an effective strategy in simmondsin detoxification. The optimal process parameters for this strategy are an alkaline extraction at pH 9.0 followed by an acid precipitation at pH 4.5. These parameters result in the highest yield of structurally safe proteins, as well as removing of simmondsin, and led to the production of detoxified protein isolate safe and available to use in many industrial applications. A significant increase in protein content can be obtained by incorporating 5 to 15% of jojoba protein isolate in biscuits. This could have nutritional benefits for developing countries, where protein malnutrition is a common issue and jojoba protein can partially address this problem since many people cannot afford expensive, high-protein foods. In addition, protein isolate could be used in many industrial applications.

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