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PREPARATION AND EVALUATION OF FUNCTIONAL CORN SNACKS USING *SPIRULINA* PROTEIN ISOLATE

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Abstract

Extruded grain-based foods have a high starch content, which makes them very energising, but they typically lack a number of nutrients, especially proteins, which are vital for developing children who are energetic and active. So, in this current study, corn snacks were enriched with different levels of *Spirulina* protein isolates (SpPI) (0, 5, 8, and 10%). Then the nutritional, physicochemical, and functional properties of the snacks were assessed. The obtained results showed that the major essential amino acids in SpPI were leucine and valine. Cysteine, histidine, and methionine were the least abundant amino acids. There was an increase in the moisture, fat, crude fibre, and ash content and a decrease in total carbohydrate content in the snacks as a function of increasing SpPI addition levels. Also, there was an increase in bulk density, water absorbance index, and hardness as well as a decrease in water solubility index and expansion ratio, in the snacks as a function of increasing SpPI addition levels. According to our findings, SpPI supplementation reduced the lightness, yellowness, and redness of snacks but increased their content of amino acids. The sensory evaluation results revealed that all snack samples enriched with SpPI were accepted.

Keywords: Algea; Physical ; Snacks; Spirulina; Protien

1. Introduction

A broad variety of puffed products are produced using the extrusion method of cereal grains (rice and maize) with great productivity, low cost, and little energy consumption **[1,2]**. Extruded items are bad because the extrusion process occurs quickly at high temperatures **[3]**. Although there are many other types of snack foods on the market and they all have their own unique appeal to customers, puffed and expanded foods are the most well-liked by kids and travellers. Extruded grain-based foods have a high starch content, which makes them very energising, but they typically lack a number of nutrients, including zinc, iron, vitamin A, and protein, which are vital for developing children who are energetic and active **[4]**. Because of their deficiency of nutrients, customers find them to be unhealthy and undesirable **[5]**. In order to increase their nutritional content, several research investigations studied supplementing them with diverse sources of animal, plant, and insect proteins, such as fish **[6]** and legume proteins **[7]**. A macronutrient found in food called protein has a variety of structural and physiological functions in the body. Protein is crucial for building muscle mass, upholding bones, and avoiding malnutrition by providing both energy and protein **[8]**. Protein is essential for life and has a significant impact on the sensory qualities of processed meals from the industrial standpoint.

However, producing protein from animals uses up a lot of land, nitrogen, and water, as well as it emits a lot of greenhouse gases since it uses fossil fuels for both manufacturing and transportation **[9]**. As a result, it was essential to develop more sustainable food systems and find alternate sources of proteins. Assessments regarding alternative sources of proteins, including insects **[10]**, plant foods **[11]**, lab meats **[12]**, and micro-algae **[13]**, are being developed as part of this environmental and dietary initiative.

One of the most well-known microalgae, *Spirulina platensis*, is a blue-green algae that is a member of the *Oscillatoraceae* family **[14]**. It has

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been sold and consumed as food for people, and numerous governments, including the Egyptian health agencies and associations, have approved it as such. It is referred to as a "superfood" because of its high nutritional value and percentage of beneficial proteins (55–70%), which is compared to the protein percentage in meat products (71–76%) and soybeans (approximately 40%), as well as its high levels of vitamins, long-chain polyunsaturated fatty acids,and minerals, as well as carbohydrates (15–18%) with high physiological activity **[15].**

Spirulina protein is also simple to digest and is thought to be more suitable for malnourished kids **[16,17]**. *Spirulina* also has a special combination of nutrients that can't be found in a single plant source. It provides 18 amino acids, including all of the necessary ones in an unbalanced proportion, and has a high protein content (60–70% on dry weight) **[18,19].**

As far as the author's knowledge is concerned, there is no information about fortifying corn snacks with SpPI. Therefore, the goal of this research was to create a new class of value-added extruded corn snacks by integrating varying amounts of SpPI (0, 5, 8, and 10%), which might provide customers with a variety of functional advantages. The nutritional, physicochemical, and functional properties of the snacks were assessed.

2. Materials and Methods

Spirulina was purchased from the Aquaculture Research Centre at the Arab Academy for Science, Technology, and Maritime Transport, Alexandria, Egypt.

Corn grits were employed as a bulk component in the present investigation and were purchased from the Egypt Food Factory in Minoufiya, Egypt. They included 126.5 g of moisture kg-1, 6.5 g of ash kg-1, 86.7 g of protein kg-1, 35.4 g of fat kg-1, and 22.4 g of crude fibre kg-1.Corn oil was bought from Tanta Company for Oils and Soaps, Tanta, Egypt. El-Gomhoria Company for Chemicals and Drugs, Tanta City, Egypt, provided all of the chemicals that were utilised, which were all of HPLC quality.

2.1 Proximate Analysis

The moisture, protein, crude fibre, ash, and lipid contents of *Spirulina*, SpPI, and snacks were determined using the AOAC procedures 935.29, 920.87, 985.29, 923.03, and 920.85 **[20]**. The total percentage of carbohydrates was estimated by deducting 100 from the sum of the other ingredients.

2.2 Protein Extraction

An alkaline solution was used to extract the *spirulina* protein isolate (SpPI), which was then isoelectrically precipitated. The defatted powder of *spirulina* was disseminated in a NaOH solution (0.5 N). Then pH had been adjusted to 10 with HCl (0.1 M) in a 1:30 ratio. The microalgae dispersal was then agitated and heated using the CimarecTM Series water bath (Thermo Fisher, Massachusetts, USA) at 45°C for 60 minutes. The mixture was spun in a centrifuge (Kubota KN-70 Centrifuge, Japan) for 25 min at 6000 rpm at ambient temperature, and its supernatant had been acidified to pH 3 with HCl (1 M) for the protein participating and was then centrifuged shortly thereafter. The obtained proteins were oven-dried at 40°C and crushed up to pass through a sieve (100 mesh). The powdered protein specimens were stored at ambient temperature in sealed plastic containers until usage.

2.3 Determination of amino acids:

Using a Beckmann Amino Acids Analyzer apparatus (Beakmann, model 119 CL-USA), amino acid content was assessed as described by **Vijayakumari *et al.* [21]**. Amino acids score (AAS) was calculated using the following equation: AAS%= [g amino acids of sample] / [g same amino acids of FAO/WHO reference protein] x100

2.4 Extrusion process:

Four groups of corn snacks were prepared. These groups were as follows: the first group did not have SpPI added (the control group), while the other three groups were enriched with SpPI at three different concentrations of 5, 8, and 10%. A Barabender Laboratory twin-screw extruder with serial number 2150511, a feeding screw of type HAAKE-RHEODRIVE, a speed control of the feeding die, temperature regulators for the extrusion zones, and a die head were used to carry out the extrusion operation. The extrusion process settings were 90, 130, and 200°C zone temperatures, 249 min-1 screw speed, and 160 min-1 feeder screw speed. The initial phase of processing was conditioning the evaluated materials by adding a commendable quantity of oil and water (2:1 ratio v/v) to be set at 18%. The water and oil amounts were gradually added while stirring with a mechanical stirrer. In the following phase, the conditioned materials were extruded under the aforementioned circumstances. In the third stage of production, the species was dispersed in oils and sprayed on the surface of the extruded products. After manufacturing, the snacks were left to cool to room temperature (25±1°C) before being packaged in plastic bags.

2.5. Physical Properties

2.5.1. Bulk density:

The average of 20 pieces from each treatment was used to determine the weight, diameter, and length of the snacks. Then, using the following equation, the bulk density (g/cm 3) of the snacks was determined as described by **Huang and Ma [22]**:

$$\text{Bulk density (g/cm 3)} = \frac{4 \times \textit{sample weight}}{\pi \times \text{snacks diameter} \times \text{snacks length}}$$

2.5.2. Water solubility index (WSI) and absorption index (WAI):

The extrudate samples were ground into fine powders in order to calculate the water solubility index and water absorption index. The powdered samples (2.5g) were suspended in distilled water (30 ml) in a capped 50-ml centrifuge vessel and agitated for 30 s to estimate the water absorption index and water solubility index. Following that, the suspension was centrifuged for 10 min at 3,000 × g. After centrifuging, the supernatant was moved to aluminium cups that had been covered with tar and dried for two hours at 135°C to eliminate moisture. WSI and WAI were then calculated in accordance with the recommendations of **Singh and Muthukumarappan [23]**.

2.5.3. Expansion ratio:

The extrudates' expansion ratio (%) was estimated by dividing the extrudates' radial diameter by the die nozzle diameter. The average of 10 measurements of the radial diameter was recorded.

2.5.4. Texture analysis:

The texture parameters of the snacks were assessed in a texture analyzer TA-XT plus (Stable Micro Systems, England) using The Exponent 32 software and a 5 kg charged cell, at a speed of about 1 mm/s, utilising a Warner-Bratzler shear blade coupled to a "V" probe. In this cutting test, specimens were cut parallel to the snack's primary axis, and the force (g) required for penetration of the extruded sample was calculated.

2.5.5. Color anaylsis:

The specimens that were extruded were ground. In order to achieve a homogenous sample, ground materials were sieved using a 60-mesh sieve. Chromameter (CR-400, Konica Minolta) measurements were taken five times for each sample **[24]**. a- value indicated the extent of the colour green in the range from 100 to zero and the colour red in the range from zero to 100. The L-value in the colour system represented lightness, with zero for darkness and 100 for lightness; the b-value quantifies blue in the range from 100 to zero and yellow in the range from zero to 100.

2.6. Sensory Evaluation of Products

Using a 9-point hedonic scale (1 = strongly dislike, 9 = extremely like), a sensory evaluation was conducted to determine consumer acceptance of the 5%, 8%, and 10% SpPI-incorporated snacks relative to the control treats. According to the methodology outlined by **Al-Okbi *et al.* [25]**, 30 panellists from the Food Technology Department, Faculty of Agriculture, Kafrelshiekh University rated appearance, taste, colour, odour, crispness, and overall acceptability.

2.7. Statistical Analysis

The SPSS10 software was used to conduct the statistical analysis. The statistical analysis was carried out using one-way analysis of variance followed by Duncan's tests, and the data were reported as means± SD **[26]**.

3 Results and discussion

3.1 Chemical composition of *Spirulina* and SpPI:

Depending on the growth environment (fresh water versus sea water), *spirulina's* chemical composition is likely to change. Table 1 illustrates the proximate chemical composition of dry *spirulina* as well as SpPI.

The acquired results indicated that the *spirulina* included 9.43% moisture, 53.46% crude proteins, 3.52% ether extract, 16.93% ash, 0.79% crude fibre, and 25.30% carbohydrate (by difference). While the SpPI contained 6.83% moisture, 94.5 % crude proteins, 0.24% ether extract, 2.21% ash, and 3.05% carbohydrate (by difference), the findings attained were comparable to those of **Kumar *et al.* [17]**. Based on the preceding information, one may infer that *spirulina* is an excellent and alternate source of protein.

Table (1):

Proximate composition of *spirulina* and *spirulina* protein isolated (SpPI)

Component	***Spirulina***	***spirulina* protein isolated (SpPI)**
Moisture%	9.43	6.83
Crude protein(N x 6.25)%	53.46	94.5
Ether extract%	3.52	0.24
Ash%	16.93	2.21
Crud fiber%	0.79	ND
***Total carbohydrates (%)**	25.30	3.05

*All data are the mean±SD of three replicates

3.2 Amino acids profile of SpPI:

Protein quality is often influenced by its overall and, in particular, by its important amino acids composition. Table 2 compares the obtained amino acids profile for (*Spirulina* and SpPI) with the actual EAA needs for adults.

Table (2):

Amino acids composition (g/100g) of *spirulina* and *siprulina* protein isolates (SpPI).

Amino acid	Spirulina (g/100g)	SpPI (g/100g)	EAA* RDA (Adults)
Essential amino acids			
Histidine	0.92	1.71	1.5
Lysine	2.75	5.15	4.5
Isoleucine	2.95	5.51	3.0
Leucine	4.98	9.35	5.9
Phenylalanine	2.70	5.04	---
Tyrosine	2.49	4.65	--
Phenylalanine + tyrosine	5.19	9.69	3.8
Methionine	1.39	2.59	-
Cysteine	0.52	0.97	-
Methionine + cysteine	1.91	3.56	2.2
Threonine	2.68	5.01	2.3
Valine	3.41	6.38	3.9
Sum essential AA	24.79	46.36	27.1
Nonessential amino acids			
Serine	3.18	5.94	
Glycine	2.78	5.20	
Alanine	4.07	7.60	
Proline	2.05	3.83	
Arginine	3.62	6.77	
Aspartic	5.11	9.56	
Glutamic	6.86	12.83	
Sum non-essential AA	24.04	51.73	
EAAI	0.91	1.82	1.0

Results are expressed as g of amino acid per100g of protein (g/100g).

EEA = Essential amino acid requirements for adults. * RDA = Recommended Dietary Allowances [27].

EAAI: essential amino acid index. Sum of essential AA, sum of non-essential AA, and EAAI are reported in bold

The amino acids composition of (*Spirulina* and SpPI), which were shown to be excellent sources of essential amino acids, is shown in Table 2. It can also be found that in both *Spirulina* and SpPI, glutamic acid, aspartic acid, leucine, and alanine were the most predominant amino acids. The major essential amino acids in spirulina and SpPI were leucine and valine.

Cysteine, histidine, and methionine were the least abundant amino acids in both Spirulina and SpPI.

All SpPI essential amino acids were also present in quantities greater than those required to satisfy human demands. In this instance, the total amount of essential amino acids (46.36 g/100 g) was less than twice the minimal amount that was suggested for human needs (minimum 27.1 g/100 g) **[27]**. The total amount of necessary amino acids was also greater than what **Bashir *et al.* [28]** and **Menegotto *et al.* [29]** had estimated. Essential amino acid index (EAAI) calculations showed that its value was 1.82. The value is higher than that reported for proteins from unconventional sources, including those extracted from insects like the superworm (*Zophobas morio*) (1.66), small mealworm *(Alphitobius diaperinus)* (1.65), yellowfin mealworm (*Tenebrio molitor)* (1.60), and edible cockroaches *(Acheta localus)* (1.39), as well as soybean proteins (1.56) and casein (1.93) **[30]**.

3.3 Chemical composition of corn snacks blends:

The results shown in Table 3 indicate that there was an increase in the percentage of moisture, fat, crude fibre, and ash in the snacks, with an increase in the percentage of SpPI added from 5 to 10%. While there was a decrease in the percentage of total carbohydrates in the prepared snacks as a function of increasing the percentage of added SpPI.

Table (3):

Chemical composition of the produced snacks integrated with SpP

Treatment	Control	Snacks with 5% SP	Snacks with 8% SP	Snacks with 10% SP
Moisture	8.90±0.15	9.24±0.39	9.45±0.17	9.58±0.74
Crude fat (%)	0.74±0.13	0.75±0.07	0.76±0.12	0.76±0.08
Protein (%)	8.52±0.41	13.25±0.81	16.17±0.79	17.93±0.85
Crude fiber (%)	0.84±0.33	0.84±0.05	0.85±0.11	0.85±0.09
Ash (%)	1.12±0.25	1.23±0.36	1.30±0.23	1.34±0.11
***Total carbohydrates (%)**	88.73±1.02[a]	87.01±1.10	80.92±0.97	79.12±1.05

*All data are the mean±SD of three replicates.

Mean followed by different letters in the same row differs significantly (P≤0.05).

*Calculated on dry weight basis as 100 – Ash + Protein + Fiber + Fat.

3.4 Physical Properties of Corn Snacks enriched with SpPI:

3.4.1 Bulk Density

The bulk density enables evaluation of the extrusion quality, process variables, and raw material effect on the technical quality of the snack. This metric takes all expansions into account. After adding SpPI up to 10%, there was a statistically significant change in the bulk density of the snacks between snacks that contained 8% or 10% SpPI and the control (Table 4). Snacks containing 10% SpPI have the highest bulk density value (0.380 g/cm^3) compared with others and control (0.358 g/cm^3) Due to their high protein concentration, the SpPI samples in this investigation had the highest bulk density, suggesting that addition had an impact on this attribute.

This happens as a result of the hydrophilicity of certain proteins, which tend to compete with water for starch granule hydration, increasing bulk density. These data were comparable to those proposed by **Lazou *et al.* [32]**.

3.4.2 Snacks WSI and WAI:

Table 4 displays the mean (and the standard deviation) values for the WSI and WAI. Water absorption measures the quantity of water immobilised by the extrudate, whereas water solubility measures the number of tiny molecules solubilized in water **[33]**. The WSI and WAI are capable of making educated guesses about the functional properties of foods and making predictions about how the materials will respond to subsequent processing **[34]**.

These indices provide details on the physicochemical modifications made to the biopolymers by the extrusion process **[35]**. The ranges of the WSI and WAI values (Table 4) are consistent with previous extrudates reported in other research **[36]**. SpPI reduced the solubilization of matrix components during extrusion, resulting in lower WSI values compared to the control. The WAI values showed the opposite behaviour. According to data in Table 4, the control snacks had WSI (17.96%) and WAI (6.3 g gel/g), whereas the snacks integrated with 10% SpPI had WSI (14.87) and WAI (7.44). The WSI reduced by 17.20% and the WAI increased by 18.1% as the percentages of SpPI integration climbed from 0 to 10%. The acquired results might be attributed to the protein-water interface's higher concentration of polar amino acids.

3.4.3 Expansion ratio:

The most crucial physical characteristic of snacks is expansion **[37]**. This attribute, which affects the crushing characteristics and the technical quality of extrusions, is closely connected to the microstructure. The elasticity index did not significantly change across samples when compared (Table 4), indicating that the incorporation of SpPI had no impact on the snack's physical quality. Data in Table 4 showed that as the incorporation ratio of SpPI rose from 0 to 10%, the expansion ratio reduced by 5.47%. Additionally, the rise in protein content, which results in cracking the cell wall before the gas bubbles expand to their full capacity, may be responsible for the reduced expansion ratio of the snacks.

Table (4):

Physical Properties of corn Snacks integrated with *siprulina* protein isolate.

Properties	Treatments			
	Control	Snacks with 5% SpPI	Snacks with 8% SpPI	Snacks with 10% SpPI
Bulk Density (g/cm^3)	0.358±0.09^b	0.364±0.09^b	0.373±0.04^a	0.380±0.03^a
Water Solubility Index (%)	17.96±0.52^a	15.51±0.59^b	15.01±0.61^b	14.87±0.50^c
Water Absorption Index (g gel/g)	6.3++7±0.33^b	7.29±0.62^a	7.38±0.58^a	7.44±0.71^a
Expansion Ratio (%)	2.74±0.11^a	2.71±0.19^a	2.65±0.20a^a	2.59±0.23^a
Hardness (N)	5.98±0.30^c	6.41±0.27^b	6.83±0.35^b	7.24±0.29^a
Colour				
L*	85.9±0.18^a	78.13±0.42^b	74.91±0.53^c	72.11±0.76^d
a*	3.8±0.24^a	2.97±0.18^b	2.64±0.40^{bc}	2.36±0.33^c
b*	23.8±0.28^a	20.82±0.59^b	19.05±0.24^{bc}	17.89±0.69^c

Different letters in the same line mean significant differences between samples (p < .05).

3.4.4 Snacks texture analysis:

The extrudate texture is an essential physical quality for the snacks, and it is heavily influenced by the mix's raw material composition **[38]**. According to the data in Table 4, the investigated snacks' hardness values for the control and enriched snacks with 5, 8, and 10% SpPI were 5.98, 6.41, 6.83, and 7.24 N, respectively. By the way, it was found that the hardness and fracture characteristics of the snacks enriched with the SpPI increased in comparison to the control sample. This action may be connected to the protein content in this section. Furthermore, protein and starch compete for water during extrusion, which reduces gelatinization and results in less product expansion while maintaining a higher hardness **[38]**.

3.4.5 Colour analysis of the snacks:

Consumer acceptance of food in general and extruded items in particular is significantly influenced by the colour of food products, particularly in the case of youngsters. The colour changes of enriched snacks at different SpPI concentrations (5, 8, and 10%) are shown in Table 4. As expected, there was an increase in darkness (L*) in the colour of the enhanced snack products. These findings provide an image of the products. Comparing the SpPI-added extruded specimens to the control (0% SpPI) specimen, the control specimen had the greatest values for L* (85.9), b* (23.8), and a* (3.8). According to these findings, SpPI supplementation reduced the lightness, yellowness, and redness of snacks. **Lucas *et al.* [39]** found similar findings in gluten-free bread enhanced with *spirulina*. Additionally, in extruded items created for kids, the colour offered by the use of SpPI may be an attraction.

3.5 Amino acids profile:

Table 5 shows the final amino acid profile of the extruded snacks (control and enriched with SpPI). Data in Table 5 showed that there was an increase in all amino acid amounts as a function of the increase in the addition percentage of SpPI. The highest amount of amino acids was found in snacks enriched with 10%, where the total amount of essential and non-essential amino acids was 8.26 and 9.97%, respectively. Every specimen under investigation includes both essential and non-essential amino acids, which generally satisfy an individual's demand for these acids.

Table (5):

Amino acid composition (g/100g protein) of corn Snacks integrated with *siprulina* protein isolate.

Amino acid	Control	Snacks with SpPI 5%		**Snacks with** SpPI 8%	**Snacks with** SpPI 10%
		Essential amino acids			
Histidine	0.36	0.44	0.49	0.53	0.53
Lysine	0.19	0.45	0.60	0.70	0.70
Isoleucine	0.22	0.49	0.66	0.77	0.77
Leucine	1.10	1.57	1.85	2.03	2.03
Phenylalanine + tyrosine	0.82	1.30	1.59	1.79	1.79
Methionine + cysteine	0.15	0.35	0.47	0.55	0.55
Threonine	0.28	0.53	0.69	0.79	0.79
Valine	0.46	0.78	0.97	1.10	1.10
Sum essential AA	3.58	5.91	7.32	8.26	8.26
		Nonessential amino acids			
Serine	0.42	0.72	0.89	1.01	1.01
Glycine	0.23	0.49	0.65	0.75	0.75
Alanine	0.72	1.10	1.33	1.48	1.48
Proline	0.97	1.16	1.27	1.35	1.35
Arginine	0.40	0.74	0.94	1.08	1.08
Aspartic	0.47	0.94	1.23	1.42	1.42
Glutamic	1.6	2.24	2.63	2.88	2.88
Sum non-essential AA	4.81	39		8.94	9.97

3.6. Sensory evaluation:

The added ingredients may boost the nutritional content, but they also alter the finished product significantly from the original in many ways. The consumer attractiveness of a product can be greatly impacted by different physicochemical qualities and a colour shift **[40]**. According to the sensory analysis findings, which are shown in Figure 1, respondents are wholly in agreement with the inclusion of SpPI in the range of 5–10%. The scores that were kept did not statistically vary from the scores that were provided with the control snacks. The results of the instrumental colour measurement agreed with the ocular evaluation of the colour change. Generally, with the increase in SpPI addition levels, there was an increase in the scores of mouth feel, appearance, texture, odour, and taste, but there was a decrease in flavour and colour scores.

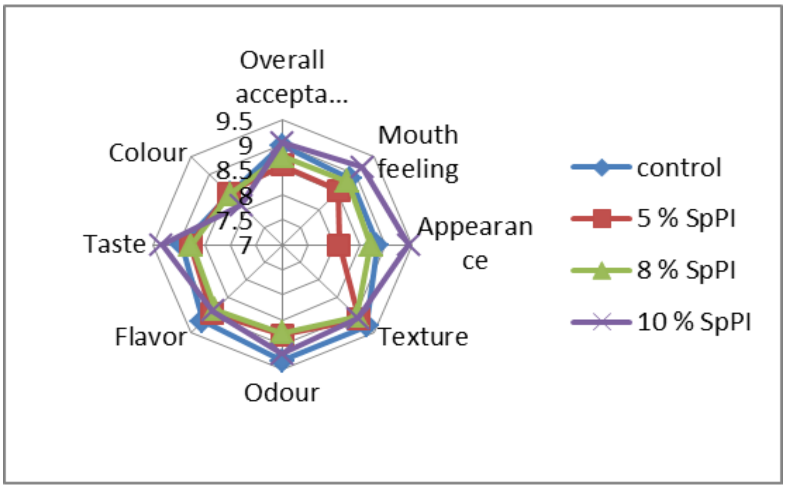

Fig.1. Effect of adding different percentages of SpPI on the sensory properties of snacks

4. Conclusion:

Spirulina is considered an untraditional source of protein because it contains 53.46% protein. SpPI is an excellent source of essential amino acids. The major essential amino acids in SpPI were leucine and valine, while cysteine, histidine, and methionine were the least abundant amino acids. SpPI was used in order to prepare high-protein snacks. The addition of SpPI to the snacks up to 10% caused an enhancement in their physical properties (bulk density, water absorbance index, and hardness) and nutritional value (chemical composition and amino acids content). All snack samples containing SpPI (5, 8, and 10%) were accepted by the consumer.

5. References

5. Refrences

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