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Study of 'the utilization of pumpkin seed protein isolate for the production of protein rich cookies'

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Abstract

Pumpkin seeds are edible, flat, oval-shaped green seeds. When removed from the flesh of a pumpkin, they can be rinsed and roasted, either plain or with other flavours such as oils and spices, to create a delicious, crunchy snack. They may be small, but pumpkin seeds are little powerhouses of nutrients and health benefits. The seed is an excellent source of protein (39.35g/100g) and unsaturated fats, including omega-3 and omega-6 fatty acids. They also contain a good range of nutrients, including iron, calcium, B2, folate and beta-carotene, which the body converts into vitamin A. The seeds are nutrient rich, especially containing higher content of good quality protein than chia or flax seed. The present study was carried out with the objective to prepare protein enriched cookies by incorporating isolated protein from dehulled pumpkin seed in wheat flour and oats powder in ratio of (*0:50:50,10:40:50,20:40:40 and 30:40:30)* respectively. Isolated protein has a good foaming property and protein purity is 90.94%. Cookies were analyzed for proximate composition, using the AOAC method along with physical parameters (width, thickness and spread factor), antioxidant content (total phenol assay, DPPH (2, 2-diphenyl-1-picrylhydrazyl), oryzanol and carotenoid content) and sensory evaluation. The results proved that the ratio of 30:40:30 (pumpkin seed protein isolate: oats powder: wheat flour) contains the highest amount of protein (24.7g/100g). The cookies were highly acceptable with a maximum hedonic rating score of 9.00. Pumpkin seed cookies are the perfect fall snack or side for soups and used in bakery sectors. So this study is undertaken with an aim at effective utilization of inexpensive and under-utilized plant proteins for nutritional and functional purposes.

Keywords: pumpkin seed, protein isolate, cookies

Introduction

Like nuts, pumpkin seed (pepita) are a great source of protein and unsaturated fats (omega-3 and omega-6 fatty acids). They also contain a good range of nutrients including zinc, iron, calcium, vitamin B2, folate and beta-carotene. Pumpkin seeds are a very good source of powerful lipid soluble antioxidant Vitamin E, containing about 35.10 mg of tocopherol-gamma per 100gm. Pepitas can be enjoyed as a snack, added in desserts and in savory dishes. The seeds, used as plant-byproducts, are affiliated to the family of Cucurbitaceae and produce a number of proteins and peptides that manage hunger and help in weight loss. A sufficient amount of protein in daily diet is important because protein comprises amino acids which can be used in thousands of enzymatic and metabolic processes. Without those amino acids and protein, the body would quit being functional and we would become weak.

Research indicates that adding pumpkin seeds to diet can improve overall health and heart health. It is a good protein source to replace less healthy food choices such as processed and fatty foods. Addition of these seeds can be considered a good substitute for nutritional enhancement of food products as stated in (Gorgonio *et al*. 2011) [1]. Pumpkin seeds are a rich natural source of protein with the range of 37% to 45% and are renowned as valuable oil seeds loaded with protein for human consumption **(**Milovanoic *et al*.2008) [2]. Moreover, pumpkin seeds are loaded with amino acids like tryptophan, lysine, methionine, tyrosine and are also rich in iron. Therefore, these seeds are beneficial to adolescents to cure anaemia caused due to iron deficiency **(**E1-Adaway *et al*. 2001) [3] and (Patel, S.2013) [4]. The use of fruit industrial waste in the processing of new foods represents an important new step for the food industry. This study is aimed at the development of cookie recipes using different amounts of guava peel flour (GPF) levels (30%, 50%, and 70%) to evaluate the proximate composition, the phenolic compound, lycopene, and β-carotene levels in the cookies and flour and also to evaluate the cookie's sensory acceptance. GPF can be used to partially replace the wheat flour in the preparation of cookies to improve its nutritional quality without affecting the product sensory quality **(**Silvana Maria *et al*.2014) [5]

Pumpkin seed (Cucurbita pepo.) has received considerable attention in recent years because of the nutritional and health protective values of seeds. The seed is an excellent source of protein and also has pharmacological activities such as antidiabetic, antifungal, antibacterial, anti-inflammation activities and antioxidant effects **(**Nkosi *et al*. 2006) [6] Recently, several research projects show that supplementary foods are used to reduce the malnutrition and supplementation could be applied in ready to eat bakery products and drinks **(**Opawale *et al*.2011) [7] Such efforts are often aimed at effective utilization of inexpensive and under-utilized plant proteins for nutritional and functional

purposes. Pumpkin seeds could be utilized successfully as an excellent quality source protein for human consumption **(**ES Lazos 1986a) [8]
Therefore the purpose of present investigation is to study the isolation of de-hulled and defatted pumpkin seed protein and to incorporate it in cookies to enrich its nutritional property.

Materials and Methods

Raw materials

Pumpkin seed, Wheat flour (refined), Oats powder, sugar powder, Rice bran oil, Milk powder, Salt, Vanilla essence were obtained from the local market.
All the other chemicals, reagents and solvents used in the study were of analytical grade and obtained from Merck Pvt Ltd**.** Distilled water was used in all of the experiments throughout the study.

Preparation of dehulled pumpkin seed flour

Pumpkin seeds were collected from the nearby market, cleaned and sundried. The seeds were dehulled manually; ground to a powder by a domestic grinder and finally sample powder was ready for analysis**.**

Preparation of dehulled and defatted pumpkin seed protein isolate

Pumpkin seed oil was hexane extracted and then the deoiled cake (DOC) of pumpkin seed was ready for isolation of protein. After that, the DOC was mixed with 5 volumes of 1 M NaCl solution and stirred for 15 minutes at ambient temperature. Then, the pH was adjusted to 9.5 by using 1 M NaOH with continuous stirring for 30 mins. After extraction, the suspension was centrifuged, supernatant was filtered and adjusted to iso-electric pH 4.0 with 1 N HCl solution to precipitate the protein. Then, the precipitate was dispersed in 50% alcohol in 1:5 ratio and stirred for 30 mins. After that, it was centrifuged for 20 mins at an ambient temperature. Finally, the precipitate (protein isolate) was washed several times with distilled water and air dried. Now, the dehulled and defatted pumpkin seed protein isolate was ready for incorporation in cookies and subsequent analysis.

Fig 1: Protein isolate of dehulled Pumpkin seed

Proximate composition analysis

The recommended methods of the Association of Official Analytical chemists (AOAC, 2005) **[9]** were used to determine the proximate composition (carbohydrate, crude lipid, crude fibre, moisture and ash content) of the pumpkin seed powder. Protein was estimated by following the method of Lowry.

Determination of functional properties of dehulled and defatted pumpkin seed protein isolate

Bulk density was determined using the procedure of (Wang and Kinsella, 1976) **[10.11].** as modified by (Narayana and Narasinga Rao, 1984) [12]. Water absorption capacity was determined by using the procedure of (Solsulski, 1962) [13] as modified by (Sathe *et al*.1982) [14]. Oil absorption capacity (OAC) was determined by replacing distilled water with executive vegetable oil. Foam capacity & Foam stability were determined by the procedure of (Coffman and Garcia, 1977) [15]. Protein content or protein purity was described by Folin Lowry method. Isolate recovery was described by the method of (Sheng Wang *et al*. 2011) [16]. Protein yield was described by the method of (Gurpeet and Sogi, 2007) [17].

Preparation of protein rich cookies by incorporating pumpkin seed isolated protein

Sample preparation

Four different sample formulations were prepared using pumpkin seed isolatedprotein powder, oats powder and wheat flour in proportions of 0+50+50 (control sample), 10+40+50, 20+40+40 and 30+40+30 gm respectively.

Formulation of cookies by utilizing pumpkin seed protein powder

Cookies are prepared as per the standard method (AOAC, 2000) **[18]** with some modifications. The ingredients were arranged and weighed accurately. The dry ingredients for making biscuits by utilizing pumpkin seed protein powder were various proportions of oats powder, pumpkin seed isolated protein powder, wheat flour, milk powder, NaCl, NaHCO3, NH4HCO3. They were thoroughly mixed in a bowl by hand. Then, creaming of rice bran oil and crushed sugar powder was done in a mixer till foaming occured. The flour mixture was added to the creamy mass and mixed well. Water was added as per the requirement for making a dough. Then the dough was rested for 30 minutes and rolled into sheets. After that, the sheets were cut into the desired shape of uniform thickness. The dough pieces was placed on baking trays leaving 25mm space in between and baked at 150 °C for 30 min in the baking oven. After baking, the cookies were cooled to ambient temperature and packed in airtight polythene bags. Finally the cookies were ready for subsequent analysis - physical and functional properties, proximate composition analysis, antioxidant properties and sensory evaluation.

Table 1: Ingredients and their amount for pumpkin seed isolated protein rich cookies

Sample	Composition(gm)									
	Pumpkin seed isolated protein powder	Oats powder	Wheat flour	sugar powder	Rice bran oil	Milk powder	Salt	NaHCO3	NH4HCO3	Vanila
Control	0	50	50	30	20	2	1	0.5	1	Few Drops
Sample 1	10	40	50	30	20	2	1	0.5	1	Few Drops
Sample 2	20	40	40	30	20	2	1	0.5	1	Few Drops
Sample 3	30	40	30	30	20	2	1	0.5	1	Few drops

Analysis of products

Physical attributes

Cookies were analysed for width, thickness and spread factor by following procedure of (AOAC 2005) [9]. Width (W) was measured by placing the cookie samples horizontally in a row and their average diameter was measured using a vernier caliper with 0.01 mm accuracy. Thickness of the samples was measured by taking their average thickness using vernier caliper with 0.01 mm accuracy. The spread factor (SF) were calculated using relationship between W, T and correlation factor CF as shown in the formula

$SF = (W/T \times CF) \times 10$

The color of cookie samples was assessed using a Konica Minolta color reader CR 10 (Japan) using an aperture of 1.2 cm diameter. In the Minolta colorimeter, the color of a sample is represented by the three color parameters: L, a and b which were recorded for each sample, where [L = lightness or darkness, +a = redness or greenness, +b = yellowness or blueness]. Wettability is the ability of baked food such as the cookie sample to absorb moisture during a controlled period of time. The higher values indicate the moistness and softness of cookies indicating better baking than the other. Wettability of cookies was analyzed, following the method of (B. Srilakshmi, Food Science, 2006) [19]

Proximate composition analysis

Proximate composition (moisture, fat, ash content) were determined by using (AOAC 2005) [9] methods. Protein was determined by method of Lowry.

Determination of antioxidant properties

Antioxidant properties of the products in terms of total phenolic content, radical scavenging activity, carotenoid content and gamma oryzanol content were determined by following methods. To measure the total phenolic compound of the pumpkin seed oil the modified version of the FCR assay **(**B. Matthaus, 2002) [20] was used. Radical Scavenging Assay by 1, 1-Dipheny l-2-Picrylhydrazyl (DPPH) was carried out by slight modification of the method proposed by (Shetty *et al* 1995) [21]. Carotenoid content was measured according to the method described by (M Isabel Minguez *et al*., 1991) [22]**.** Gamma oryzanol content (%) of oils extracted from protein rich cookies were determined by the method of (Khatoon *et al*. 2004) [23]

Sensory evaluation of the product

Consumer acceptance test of the cookies was evaluated in terms of taste, appearance, odor, texture and overall acceptability by 10 semi-trained panelists using a 9 point hedonic scale.

Results and discussion

Proximate composition analysis of dehulled pumpkin seed

Proximate composition of dehulled pumpkin seed shows that it is high in protein content and fat content. High protein content shows that the seed can serve as a source of protein considering the level of protein deficiency in the society. Lipids are essential because they provide the body with maximum energy. Seed is low in fiber content and moisture content that is 3.5% and 7.5% respectively. The lower moisture content of the seed will give it a storage advantage. The sample could not be considered as a potential source of carbohydrate that is 17.15%. The values are listed in table 2.

Table 2: Proximate composition of dehulled pumpkin seed powder (g/100g)

Parameters	Dehulled pumpkin seed powder
Moisture	7.5 ±0.1
Protein	39.35±0.02
Fat	31 ±0.51
Carbohydrate	17.15±0.01
Fibre	3.5±0.08
Ash	2±0.25

Data expressed in mean ±SD

Study of physical and functional properties of pumpkin seed isolated protein

Bulk density of the protein isolate was found to be 0.13g/cm^3. The reason for this lesser bulk density in protein isolates might be due to particle size. This report was much less as observed by (Suliman *et al*., 2006) [24]. They reported that the bulk density of protein isolates ranged from 0.61g/cm^3 to 1.14 g/cm^3. Foaming capacity was found to be 22.22%, which indicates a fair quality foam-forming ability indicating good quality protein. Water absorption capacity (WAC) was found to be 211%**.** The literature supports the WAC values observed in the present study. Overall, the WAC of peanut protein isolates varies from 203 to 222% **(**Aguilera *et al*., 2009; El-tayeb *et al*., 2011; Li, *et al*., 2010).[25,26,27].

The present results of oil absorption capacity (OAC) are in conformity with (Aguilera *et al*., 2009**)** [25]. The results regarding recovery of protein isolates are in agreement with the findings of (Khan *et al*., 2011) [28] showing 26.84 ± 0.22 to 28.32 ± 0.29 % recovery of protein isolates. Crude protein content of isolate was 90.94g/100g. The results for crude protein are much higher than the findings of (Suliman *et al*., 2006) [24]. The present results of protein isolate yields are in accordance with the findings of other researchers as they observed 77% protein yield. The values are listed in table 3.

Table 3: Physical and Functional properties of dehulled and defatted pumpkin seed protein isolate

Properties	Dehulled and defatted pumpkin seed protein isolate
Protein purity (g/100g)	90.94±0.03
Protein Yield (%)	70.04±1.95
Protein recovery (%)	25.57±0.56
Bulk Density(g/cm3)	0.13±0.01
Foaming Capacity (%)	22.22±0.01
Water Absorption Capacity (%)	211±0.5
Oil Absorption Capacity (%)	750±0.5

Data expressed in mean± S.D.

Study of protein rich cookies made from pumpkin seed isolated protein

Analysis of physical and functional properties

Cookies that are made by utilizing dehulled pumpkin seed protein isolate were tested for their physicochemical and functional properties. The width of cookies was decreased from 59 to 52mm by incorporation with an increasing level of pumpkin seed protein isolate. Cookie thickness increases from 13 to 14mm. The spread factor is maximum in the control sample and it decreases from 45.38 to 37.14 with an increase in the amount of protein isolate incorporation. But in sample 3, the spread factor is slightly increased. Colour reading shows that sample 1 has the highest L value, indicates lightest in color among all the biscuit samples. The $^{+}$a value is increased by increasing the incorporation of pumpkin seed protein isolate. The highest $^{+}$a value was found in sample 3 which indicates more redness and highest $^{+}$b value was found in sample 3, which indicates more yellowness. More wettability indicates better baking character. Wettability percentage of the product increases from (1.0 to 1.4) % which indicates better baking property of cookies. Values are listed in table 4.

Table 4: Physical parameters of cookies

Sample	Width(mm)	Thickness(mm)	Spread factor	Weight of cookies	Wet ability percentage (%)
Control	59±0.1	13±0.1	45.38±0.64	10.15±0.48	110±0.50
Sample 1	54±0.15	14±0.10	38.57±0.29	10.90±0.35	110±0.50
Sample 2	52±0.1	14±0.15	37.14±0.1	10.15±0.43	120±0.50
Sample 3	52±0.1	13±0.05	40±0.76	10.92±0.46	140±0.5

Table 5

Sample	Color values		
	L	$^{+}$a	$^{+}$b
Control	62.75±0.38	6.6±0.34	27.05±0.05
Sample 1	64.73±0.37	7.4±0.07	22.63±0.25
Sample 2	56.65±0.45	7.75±0.07	26.8±0.4
Sample 3	60.6±0.32	8.4±0.1	27.95±0.25

[L=Lightness or darkness, $^{+}$a=redness or greenness, $^{+}$b=yellowness or blueness]

Data is expressed in mean ± S.D

Proximate composition analysis

Proximate analysis of cookies made from pumpkin seed isolated protein shows that the protein content increased by increasing incorporation of pumpkin seed protein isolate and ranged between 8.98-24.7g/100gm. The highest amount of protein was present in sample 3. Control sample has higher carbohydrate content than the other sample and its value is decreased by increasing the incorporation of pumpkin seed protein isolate.

Fat content ranged between 14.86-15.2g/100g. Fiber content is high in control samples and its value is decreased likewise carbohydrate with increasing the incorporation of protein isolate. Energy value increases gradually by increasing incorporation of pumpkin seed protein isolate in cookies than control sample. The moisture content of cookie sample varied between 3.01-3.32%.

Table 6: Proximate values of cookies (g/100gm)

Sample	Protein	Carbohydrate	Fat	Fiber	Moisture	Ash	Energy (kcal)
Control	8.98± 0.09	66.7±0.2	15.2±0.05	6.85±0.05	3.32±0.09	2.23±0.04	422.84±0.06
Sample 1	14.04±0.07	62.78±0.12	14.98±0.14	3.84±0.03	3.14±0.05	2.14±0.02	426.4±0.26
Sample 2	19.37±0.13	58.10±0.1	14.92±0.03	3.68±0.04	3.05±0.03	2.09±0.03	444.16±0.04
Sample 3	24.7± 0.25	53.43±0.09	14.86±0.04	3.51±0.08	3.01±0.03	2.05±0.03	432.9±0.1

Data is expressed in mean ± S.D

Antioxidant content

Antioxidant properties of cookies made from pumpkin seed isolated protein in terms of total phenolic content showed that the control sample has the lowest value but addition of pumpkin seed protein isolate has remarkably increased the polyphenolic content in the products. Radical scavenging power was gradually increased by incorporation of pumpkin seed protein isolate in cookies and ranged between 25.4% to 50.15%. Carotenoid content also increased from control sample by incorporation of pumpkin seed isolated protein in biscuits and ranged between 0.23 ppm to 1.36 ppm. Oryzanol content of the product is ranged between from 1.22 to 1.31.

Table 7: Determination of total phenol assay, DPPH, Oryzanol content and Carotenoid content of cookies

Sample	Value of Total phenol assay(mg of gallic acid equivalent/100gm)	Value of DPPH-free radical scavenging activity (%)	Oryzanolcontent (%)	Carotenoid content(ppm)
Control	1133±0.5	25.4±0.10	1.31±0.01	0.23±0.03
Sample 1	2033 ±1.5	32.65±0.01	1.26±0.02	1.11±0.02
Sample 2	3057±1.5	44.21±0.04	1.22±0.01	1.19±0.01
Sample 3	4096±2	50.15±0.06	1.23±0.01	1.36±0.02

Data is expressed in mean± S.D

Sensory evaluation of the products

Sensory evaluation of the cookies made from pumpkin seed isolated protein showed that the taste and texture were increased in the cookie than the control sample by increasing the amount of protein isolate incorporated cookies. Incorporation of isolated protein also increased the odor of cookies. Sample 2 shows the highest overall acceptability of 8.66 score

Table 8: Sensory evaluation of the cookies

Sample	Taste	Apperance	Odor	Texture	Overall acceptability
Control	6.32±0.02	5.22±0.02	6.2±0.07	7.1±0.10	6±0.1
Sample 1	8.45±0.05	7.06±0.05	7.5±0.1	7.3±0.1	8±0.1
Sample 2	8.33±0.02	7.66±0.03	8±0.1	7.16±0.04	8.66±0.03
Sample 3	7.33±0.03	8±0.1	8.73±0.02	7.16±0.04	8.50±0.15

Data is expressed in mean±S.D

Previous study was carried out on formulation and nutritional evaluation of cookies supplemented with pumpkin seed (Cucurbita Moschata) flour. Cookies supplemented with 30% pumpkin seed flour both in raw or roasted form were highly accepted. Protein content of control sample (6.02%) was significantly lower than the sample treated with raw pumpkin seed flour (8.29%) and the sample treated with roasted pumpkin seed flour (8.32%) (Kaur Manpreet *et al*., 2017) [29]

Another study was carried out that utilization of wheat-pumpkin seed composite flour for biscuit preparation. The results of chemical composition of both samples wheat and pumpkin flour revealed that the pumpkin flour was significantly higher in all proximate composition parameters and minerals profile. The increasing of the level of substitution from 5-10% of full fat pumpkin seeds flour to wheat flour significantly increased protein(from 9.42-10.52, 10.95-11.22%)content respectively. Amino acids result for both samples showed that pumpkin flour protein had better amino acids profile compared to wheat flour (Inas M. Yhia *et al*, 2020) [30]

There is another study which was carried out that preparation and nutritional properties of cookies from the partial replacement of wheat flour using pumpkin seeds powder. Chemical composition of wheat flour 72% extraction, pumpkin seeds powder, and its blends at 5, 10, and 15% levels of pumpkin seeds were determined. Results showed that the blend at 15% fortified pumpkin seeds powder had the highest protein, fat, ash, and crude fiber (13.75, 7.13, 1.62 and 1.95% respectively).The highest results may be due to greater the chemical composition of pumpkin seeds than the wheat flour 72% extraction (Alshehry Garsa Ali *et al*.,2014) [31]

Our study shows that protein enriched cookies are made by incorporating isolated protein from dehulled pumpkin seed in wheat flour and oats powder in different ratio like *0:50:50, 10:40:50, 20:40:40 and 30:40:30* respectively. Purity of isolated protein is 90.94%.Proximate analysis of cookies made from pumpkin seed isolated protein shows that the protein content increased by increasing incorporation of pumpkin seed protein isolate and ranged between 8.98-24.7g/100gm. The ratio of 30:40:30 (pumpkin seed protein isolate: oats powder: wheat flour) contains the highest amount of protein that is 24.7g/100g. This type of pumpkin seed cookies are considered to be a very high protein snack.

Conclusions

It may be concluded from the above findings that pumpkin seeds, being rich in protein, can be incorporated as de-hulled pumpkin seed isolated protein in bakery products (COOKIES) to increase its protein content. By increasing the addition of isolated protein proportionally increases protein content of cookies. Therefore the study was undertaken to utilize this lesser known nutrition rich seed flour for superior quality cookies. Cookies are the all-time favourites of all those who love tasty and healthy snacks and it is a good alternative for any other unhealthy snack choices. Cookies contain macronutrients that provide energy for the body like proteins, carbohydrates and fats. This type of pumpkin seed cookies are considered to be a very high protein snack. There are no added preservatives, artificial sweeteners and colours. So this is safe for children as well. It is also good for people with lifestyle based disorders including diabetes, obesity and also helpful in reducing the risk of cardiovascular diseases. So, if someone feels that they are lacking protein in their diet, this protein rich healthy cookie is sure to make up for it.

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References

1. Gorgonio CMS, Pumar M, Mothe CG. Macroscopic and physiochemical characterization of a sugarless and gluten-free cake enrich with fibers made from pumpkin seed (Cucurbita maxima L)flour and cornstarch. Cienc Technol Aliment Campinas,2011:31:109-118.
2. Milovanoic MM, Vucelic-Radovic BV. Sources, nutritional and health value of omega-3 and omega-6 fatty acids J Agric Sci,2008:53:203-13.
3. E1-Adaway TA, Taha KM. characteristics and composition of different seed oils and flours. Food chem,2001:74:47-54.
4. Patel S. Pumpkin (Cucurbita sp.) Seeds as neutraceutic:A review on status quo and Scopes. Mediterr J Nutr Metab,2013:6:183-89.
5. Silvana Maria Michelin Bertagnolli, Marcia Liliane Rippel Silveira,Aline de Oliveira Fogaca, Liziane Umann, Neidi Garcia Penna: Bioactive compounds and acceptance of cookies made with Guava peel flour, Food Science and technology,2014:34(2):303-308.
6. Nkosi CZ, Opoku AR, Terblanche SE. Antiooxidant effects of pumpkin seeds (Cucurbita pepo) protein isolate in CCl -induced liver injury in 4 low-protein fed rats. Phytotherapy Res,2006:20:935-940.
7. Opawale BO, Giwa OE, Jose OR, Aladejimokun OA, Aladenika AK. Physical, sensory and microbial attributes of taro flour supplemental cookies. National Conf. Proc. Interdisciplinary Cooperation. A Panacea for Food Security, 2011, 1-6.
8. ES Lazos. Nutritional, fatty acid and oil characteristics of pumpkin and melón seeds, Journal of Food Science,1986a, b.
9. Official Methods of Analysis of AOAC International, Association of Official Analytical chemists (AOAC, 2005)
10. Kinsella JE. Functional properties of proteins in foods. Crit. Rev. Food Sci. Nutr,1976:1:219-280.
11. Wang JC, Kinsella JE. Functional properties of novel proteins: Alfalfa leaf protein, Journal of Food Science, 1976.

12. Narayana K, MS Narasinga Rao. Effect of partial proteolysis on the functional properties of Winged bean (Phosphorcapustetragonolobus) Flour. J Food Sci,1984:49:944-947.
13. Sosulski FW. The centrifuge method for determining flour absorption in hard red spring wheats Cereal chem,1962:39:344-350.
14. Sathe SK, Desphande SS, Sathe DK. Functional properties of winged Bean (Phosphocarpustetragonolobus LDC) Proteins J Food,1982b:47:503-509
15. Coffman CW, Garcia VV. Functional properties and amino acid content of a protein isolate from mung bean flour J Food Technol,1977:12:473.
16. Sheng Wang, Gary G. Adams, Shahwar Imran *et al*. Thehypoglycaemic effect of pumpkins as anti-diabetic and functional medicines, Food Research International,2011:44:862-867.
17. Gurpeet KC, Sogi DS. Functional properties of rice bran protein concentrate. Journal of Food Engineering,2007:79:592-597.
18. AOAC. Official methods of Analysis, Association of Official Analytical, 2000.
19. Srilakshmi B. Food Science, New Age International (P) limited, Publishers, India, 2006, 3, 303.
20. Matthäus B. Journal of Agricultural Food Chemistry,2002:50:3444-3452.
21. Shetty K, Curtis OF, Levin RE, Witkowsky R, Anf W. Prevention of vitrification associated with *in vitro* shoot culture of oregano (Origanumvulgare) by Pseudomonas spp. Journal of Plant Physiology,1995:147:447-451.
22. M Isabel Minguez–Mosquera, Luis Rejano-Navarro, Beatriz Gandul-Rojas, Antonio Higinio Sanchez Gomez, Juan Garrido-Fernandez. Color- pigment correlation in virgin olive oil, Journal of the American Oil Chemists' Society,199:68(5):332-336.
23. Khatoon S, Gopalakrishna AG. "Fat-soluble nutraceuticals and fatty acid composition of selected Indian rice varieties". Journal of the American Oil Chemists' Society,2004:81:939-943.
24. Suliman MA, El-Tinay AH, Elkhalifa AEO, Babiker EE, Elkhalil EAI. Solubility as influenced by pH and NaCl concentration and functional properties of lentil proteins isolate. Pakistan Journal of Nutrition,2006:5(6):589-593.
25. Aguilera Y, Esteban RM, Benítez V, Mollá E, Martín-Cabrejas MA. Starch, functional properties, and microstructural characteristics in chickpea and lentil as affected by thermal processing. Journal of Agriculture and Food Chemistry,2009:57(22):10682-10688.
26. El-tayeb ARSM, Ali AO, Arab AAA, Salem FMA. Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut (Vigna subterranean). African Journal of Food Science,2011:5(2):82-90.
27. Li W, Shu C, Yan S, Shen Q. Characteristics of sixteen mung bean cultivars and their protein isolates. International Journal of Food Science and Technology,2010:45(6):1205-1211.
28. Khan SH, Butt MS, Anjum FM, Sameen A. Quality evaluation of rice bran protein isolates based weaning food for preschoolers. International Journal of Food Science and Nutrition,2011:62(3):280-288.
29. Kaur Manpreet, Sharma Sonika. Formulation and Nutritional Evaluation of Cookies Supplemented With Pumpkin Seed (Cucurbita Moschata) Flour, Chemical Science Review and Letters,2017:6(24):2236-2241.
30. Inas M Yhia, Waheeba E Ahmed, Asmahan A Ali, Sara E Mustafa, Amir M Awadel Kareem. Utilization of Wheat-Pumpkin Seed Composite Flour for Biscuit, Pakistan Journal of Nutrition,2020:19(3):117-121.
31. Alshehry Garsa Ali. Preparation And Nutritional Properties Of Cookies From The Partial Replacement Of Wheat Flour Using Pumpkin Seeds Powder, World Journal of Environmental Biosciences,2014:9(2):48-56.