International Journal of Food Science and Nutrition
ISSN: 2455-4898; Impact Factor: RJIF 5.14
Received: 20-12-2020; Accepted: 06-01-2021; Published: 05-02-2021
www.foodsciencejournal.com
Volume 6; Issue 1; 2021; Page No. 63-67

Study of the utilization of "Pumpkin seed" for the production of nutritionally enriched biscuits

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Abstract
Pumpkin seeds (*Cucurbita pepo*) are the edible kernels of fruit Pumpkin that are used as plant by-products. The seed is an excellent source of protein (39.35g/100g). The presented study was carried out with the objective to prepare nutritionally enriched biscuits by blending whole pumpkin seed flour with oats powder in the ratio of 0:100, 10:90, 20:80 and 30:70 respectively. All types of biscuits were analyzed for proximate composition (according to AOAC method), physical parameters (width, thickness and spread factor), antioxidant content [total phenol assay, DPPH (2, 2-diphenyl-1-picrylhydrazyl), oryzanol carotenoid content] and sensory evaluation. The results proved that the ratio of 30:70 for whole pumpkin seed flour and oats powder contained protein 17.50g/100g. The present study showed that the nutritionally enriched biscuits made from whole pumpkin seed flour may be suitable for superior quality food products, thereby promoting the scope of utilization of pumpkin seeds in bakery sectors.

Keywords: pumpkin seed flour, nutrition, biscuit

Introduction

Pumpkin seeds (pepita) are edible kernels of fruit pumpkin. In the central Americas, hulled and gently roasted pumpkin kernels are popularly known as *Pepita.* The seeds are semi-flat features, typically ovoid shape with a conical tip. Inside its kernels feature olive-green colour, sweet, buttery in texture and nutty in flavor and have a white outer hull. The seeds are used as plant by-product and at the same time have been in use as food and to extract pumpkin seed oil since centuries. In some parts of central Europe, pumpkins are being cultivated solely for their-seeds, as-a-major-oil-seed-crop-at-a-commerciall scale.

Pumpkin seeds (Cucurbita Pepo) have received considerable attention in recent years due to its health protective and nutritional benefits. They are earthy in flavour and are used in a variety of dishes like snacks (baking), soups, and salads. They are a good source of calories, proteins, carotenoids, minerals, fibre and phytosterols. They also have omega 3 & omega 6 fatty acids needed for brain function and skin health. Thus the study of incorporating pumpkin seed flour in bakery products aims to create awareness through nutrition education (M N Revathy1*and N Sabitha) [1]

The seeds of plants affiliated to the family of *Cucurbitaceae* 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 builds amino acids, which are used in thousands of enzymatic and metabolic processes. Without those amino acids and protein, the body would quit being functional and result in weakness. The seeds are also concentrated sources of many healthy vitamins, minerals and antioxidants. Pumpkin seeds are high in calories, about 559 calories per 100 gm. Protein and oil content of Pumpkin seeds are 350g/kg and 444g/kg respectively for human consumption and new dietary supplement. The seeds contain better quality protein than chia or flax seed. 100 grams of seeds provides 54 percent of the daily requirement in terms of protein. They are also an excellent source of amino acids such as tryptophan and glutamate. The kernels are rich in monounsaturated fatty acid [MUFA] like oleic acid that helps lower bad LDL cholesterol, increases good HDL cholesterol in the blood and prevents coronary artery diseases. They are also antidiabetic and prevent kidney stone formation. Additionally, they are a very good source of powerful lipid soluble antioxidant vitamin E; containing about 35.10 mg of tocopherol-gamma per 100 g. Pumpkin kernels are also an excellent source of B-complex group of vitamins (work as co-factors for various enzymes during cellular substrate metabolism in the human body) and contain essential minerals like zinc (for immunity) and manganese (co-factor for antioxidant enzyme, superoxide dismutase that scavenge harmful oxygen-free-radicals).

A scientific study was carried out by utilizing the seed flour of two species of *Cucurbitaceae* with wheat flour for biscuits making. The highest spread ratio of the wheat biscuits and composite *Cucurbitaceae* seeds flour biscuits was observed and that provides evidence to support the good nutritional value of seeds flour that reinforced the usefulness and beneficial role of the flour in biscuit-making (Abdeen Elsiddig Eltyeb Elkhedir1* *et al*) [2]

Worldwide, much research has focused on various sources of plant proteins (El-Adawy *et al*., 2001; Rangel *et al*., 2003) [3, 4] that may help in increasing the nutritional value of food products at low cost. Pumpkin (*Cucurbita pepo*) has received considerable attention in recent years because of the nutritional and health protective values of the seeds. Compositionally, pumpkin seeds contain 25-55% oil and 23-35% protein by weight (Bemis *et al*., 1968;Lazos, 1986a, b; Lal *et al*., 1983) [5,6,7].The economic importance of the protein in new and under-utilized oilseed sources is especially vital as many countries have to solve the problem of oil and protein shortage. Pumpkin seeds could be utilized successfully as an excellent quality source protein for human consumption (Lazos, 1986a) [6]. More attention has

been focused on the utilization of pumpkin seed proteins for the production of various new foods, drugs, cosmetic ingredients and even biodegradable packing film(El-Soukkary, F.A.H 2001,E.S.Lazos 1992) [8,9].
Therefore the study was undertaken to utilize this lesser known nutrient-rich seed flour for superior quality biscuits and their analysis.

Materials and Methods

Raw materials

Pumpkin seed, 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 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**.**

Proximate composition analysis

The recommended methods of the Association of Official Analytical chemists (AOAC, 2005) **[10]** 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.

Preparation of nutritionally enriched biscuits by using whole pumpkin seed flour

Sample preparation

Four different sample formulations were prepared using whole pumpkin seed flour and oats powder in proportion of 0+100 (control sample), 10+90, 20+80 and 30+70 gm respectively. Ingredients and their amounts for biscuit preparation are shown in Table 1.

Formulation of biscuits by utilizing whole pumpkin seed flour

Biscuits are prepared as per the standard method (AOAC 2000) **[11]** with some modifications. The ingredients were arranged and weighed accurately. The dry ingredients for making biscuits by using whole pumpkin seed flour were oats powder, whole pumpkin seed flour, milk powder, NaCl, $NaHCO_3$, NH_4HCO_3 and they were thoroughly mixed in a bowl by hand for some time. Then creaming of rice bran oil and crushed sugar powder were 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, we cut the sheets to desired shape of uniform thickness. The dough pieces were placed on baking trays leaving 25mm space in between and baked at 150 degree C for 30 min in the baking oven. After baking, the biscuits were cooled to ambient temperature and packed in airtight polythene bags. Finally the biscuits were ready for subsequent analysis - physical and functional properties, proximate composition analysis, antioxidant properties and sensory evaluation.

Table 1: Ingredients and their amount for nutritionally enriched biscuits

Sample	Composition(gm)								
	Whole pumpkin seed flour	Oats powder	Crushed sugar powder	Rice bran oil	Milk powder	Salt	NaHCO3	NH4HCO3	Vanila
Sample 1	10	90	30	20	2	1	0.5	1	Few Drops
Sample 2	20	80	30	20	2	1	0.5	1	Few Drops
Sample 3	30	70	30	20	2	1	0.5	1	Few Drops
Sample 4 (control)	0	100	30	20	2	1	0.5	1	Few Drops

Analysis of products

Physical-attributes

Biscuits were analyzed for width, thickness and spread factor by following the procedure as described in (AOAC, 2005) [10]Width (W) was measured by placing the biscuit 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 using a vernier caliper with 0.01 mm accuracy. The spread factor (SF) was calculated using the relationship between W, T and correlation factor CF as shown in the formula

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

The color of biscuit 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, 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 biscuit samples to absorb moisture during a controlled period of time. The higher values indicate the moistness and softness of biscuits suggesting better baking than others. Wettability of the biscuits was analyzed according to the method of (B. Srilakshmi, Food Science, 2006) [12]

Proximate-composition-analysis

Proximate composition (moisture, fat, ash content) were determined by using (AOAC 2005) [10] 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) [13] was used. Radical Scavenging Assay by 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) was carried out by slight modification of the method proposed by (Shetty and others 1995) [14] Carotenoid content was measured according to the method described by (Minguez and others 1991) [15] Gamma oryzanol content (%) of oils extracted from protein rich biscuit were determined by the method of (Khatoon and others 2004) [16]

Sensory-evaluation-of-the-product

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

Results and Discussion

Proximate-composition-analysis-of-dehulled-pumpkin-seed

Proximate composition of dehulled pumpkin seeds show 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. The 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 carbohydrates, which only constitutes about 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 nutritionally enriched biscuits made from whole pumpkin seed flour

Analysis of physical and functional properties

Biscuits that are made by utilizing whole pumpkin seed flour were tested. By decreasing the amount of pumpkin seed powder in biscuits, the width of biscuits decreases from 57 to 50 mm, the thickness increases from 11 to 15 mm and the spread factor of biscuits decreases from 51.81 to 33.5. The highest value of spread factor was found in the control sample. The color reading of biscuit shows that sample 1 and 3 have the highest L value that is these samples are the lightest in color among all biscuits. L value was increased by decreasing the amount of pumpkin seed flour. The highest $^{+}$a value found in sample 2. It was also found that decreasing the addition of pumpkin seed decreases the redness of the sample. The highest $^{+}$b value found in sample 2 i.e. highest degree of yellowness. Wettability percentage of the product is shown in table 4. Wettability percentage of the product is increased from 0.83 to 0.95 by decreasing the amount of pumpkin seed**.** The values are listed in Table 3 below.

Table 3: Physical parameters of biscuits

Sample	Width(mm)	Thickness(mm)	Spread factor	Weight of cookies	Wet ability Percentage (%)
Sample 1	50±0.3	15±0.55	33.5±0.2	7.74±0.04	95±0.3
Sample 2	50.3±0.3	15±0.55	33.5±0.2	9.11±0.07	86±0.35
Sample 3	51.3±0.32	15±0.55	34.2±0.15	7.35±0.04	85±0.5
Sample 4 (control)	57±0.5	11±0.50	51.81±0.15	8.55±0.04	84±0.5

Table 4

Sample	Color values		
	L	$^{+}$a	$^{+}$b
Sample 1	64.5±0.7	7.5±0.1	29.7±0.1
Sample 2	64.45±0.08	8.2±0.13	30.55±0.24
Sample 3	64.5±0.07	7.5±0.1	29.6±0.1
Sample 4 (control)	52.1±0.45	11.01±0.57	30.11±0.15

[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 biscuits made from whole pumpkin seed flour showed that protein value was highest in sample 3, that is 17.50g/100g and protein value decreases proportionally by decreasing the incorporation of pumpkin seed flour. Carbohydrate content was highest in the sample 4 (control sample). The highest fat content shown in sample 3 (9.76g/100g) and fat content decreases to 5.67 by decreasing the addition of pumpkin seed. Fibre content was highest in sample 4 (control sample) that is 8.46g/100g. Energy value of the control sample is lowest and addition of pumpkin seed increases energy value, that is highest in sample 3 (389.77kcal/100gm). Product moisture ranged between 5.32-6.2%. The values are listed in Table 4.

Table 5: Proximate values of Biscuits (g/100gm)

Sample	Protein	Carbohydrate	Fat	Fiber	Moisture	Ash	Energy (kcal)
Sample 1	13.76±0.07	68.94±0.47	5.67±0.10	7.88±0.01	5.4±0.05	1.2±0.05	364.14±0.03
Sample 2	15.64±0.10	65.36±0.03	7.69±0.01	7.30±0.15	6.2±0.05	1.24±0.02	376.86±0.02
Sample 3	17.50±0.09	61.85±0.12	9.76±0.01	6.73±0.04	5.58±0.03	1.27±0.01	389.77±0.01
Sample 4 (control)	11.87±0.02	72.53±0.78	3.55±0.05	8.46±0.05	5.32±0.05	1.00±0.15	351.41±0.06

Data is expressed in mean± S.D

Antioxidant content

Antioxidant properties of biscuits made from whole pumpkin seed flour in terms of total phenolic content showed that sample 3 has the highest value of phenolic content and this value decreases with decreasing the amount of pumpkin seed. Radical scavenging activity also decreases gradually with decreasing the amount of pumpkin seed. Carotenoid content is highest in sample 3 and 2 with a high proportion of pumpkin seed but the value decreases with decreasing the amount of pumpkin seed content in biscuits. Values are shown below in Table 5.

Table 6: Determination of total phenol assay, DPPH, Oryzanol content and Carotenoid content of biscuit

Sample	Value of Total phenol assay (mg of gallic acid equivalent/100gm)	Value of DPPH-free radical scavenging activity (%)	Carotenoid content(ppm)
Sample 1	3179.2±0.45	95.76±0.10	1.36±0.02
Sample 2	3345.43±0.02	96.88±0.11	1.41±0.01
Sample 3	3987.13±0.01	96.92±0.07	1.43±0.01
Sample 4 (control)	891.3±0.1	80.36±0.02	0.12±0.01

Data is expressed in mean± S.D

Sensory evaluation of the product

Sensory evaluation of the biscuits showed that taste of biscuits increased with the incorporation of whole pumpkin seed flour and it decreased with decreasing amount of pumpkin seed addition. So, it is shown that pumpkin seed has a positive effect on the taste of biscuits. Texture is slightly increased in biscuits by addition of pumpkin seed and there is no remarkable change in texture with decreasing the amount of seed addition. Pumpkin seed flour incorporation also improved the odor of baked samples. Sample 3 recorded the highest overall acceptability of 9.00 score. The values are listed in Table 6.

Table 7: Sensory evaluation of the biscuits

Sample	Taste	Appearance	Odor	Texture	Overall acceptability
Sample 1	7.16±0.02	8.16±0.04	8.33±0.02	7.00±0.25	8.50±0.03
Sample 2	8±0.1	7.50±0.05	8.0±0.1	8±0.1	8.66±0.01
Sample 3	8±0.1	8.16±0.04	8.0±0.1	8.50±0.03	9±0.50
Sample4 (control)	6±0.05	6.5±0.05	7.5±0.05	7.9±0.15	7±0.25

Data is expressed in mean±S.D

Previous study found that biscuits developed from wheat and pumpkin seed flour were rich in carbohydrate, fat, chlorine and calcium. This can be a healthy snack choice for undernourished children and people with calcium and chlorine deficiency (Aminuddin Syam *et al* 2019) [17] Another study shows that protein and ash contents of biscuit samples made of wheat flour and wheat flour replaced by pumpkin flour at different levels(5,10 and 15%).It describes that gradual substitution of pumpkin flour with different ratios(5, 10 and 15%) significantly increased the protein content of biscuit flour by 11.78, 16.27 and 19.14% respectively (Inas M. Yhia *et al* 2020) [18]

In our study biscuits made from whole pumpkin seed flour and oats powder also shows that increasing incorporation of pumpkin seed flour (10, 20 and 30% respectively) increases protein content of the biscuits by 13.76, 15.64 and 17.50% respectively. Biscuits are also rich in fiber content.

Conclusions

It may be concluded from the above findings that biscuits made by blending whole pumpkin seed flour and oats powder are responsible for increasing the overall nutrition of biscuits, particularly protein, fiber and beta carotene while also promoting gluten free biscuits. Since biscuits are items of mass consumption and are consumed by every age group, this can have a positive impact for alleviating protein energy malnutrition. *Cucurbita pepo* seed has the potential of being used as a nutritional supplement. When the seeds are purchased in an unorganized way, the cost may be high but if it will be purchased in bulk quantity in an organized manner, the cost is low and it has a large commercial viability as it is suitable for making healthy products.

Acknowledgments

I would like to express my sincere regards and gratitude to my supervisor and all the co-authors, School of Community Science and Technology, Indian Institute of Engineering Science and Technology, Shibpur, for their resourceful guidance, active supervision, constant encouragement, cooperation and great support during the project work and preparation of manuscript.

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