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OPTIMIZATION OF SUSTAINABLE PROTEIN EXTRACTION METHODS OF A PROMISING PROTEIN SOURCE FOR THE DEVELOPMENT OF PROTEIN ENRICHED COOKIES

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Abstract

One of the serious public health concerns all over the world today is malnutrition, especially in children below the age of five years, which accounts for an estimated 45% of child deaths in low- and middle-income countries. This paper examines the role of semi waste materials such as the seed and peels of pumpkins to combat malnutrition. This study also seeks to uncover the use of waste products such as pumpkin seeds and peels in combating malnutrition. The edible portion of pumpkin seeds consists of an unsaturated fatty acid, protein, minerals, and phenolic compounds; therefore, it adds nutritional value into food designed for development of children. The research also describes how protein isolates can be prepared from pumpkin seeds and peels by using enzymatic extraction, micellization and isoelectric precipitation methods. These isolates were evaluated by gel electrophoresis (SDS-PAGE), proximate analysis of protein cookies. Results obtained showed that enzymatic extraction had the highest protein content (91.96±0.17%) than the other approaches. Proximate composition analysis shows it is promising to use pumpkin seed protein isolate in the production of fortified foods.

1. INTRODUCTION

Malnutrition, especially among children below the age of five years, is still regarded as one of the major public health problems worldwide. According to the World Health Organization (WHO) 2020, 45 million children were found to be underweight, 38.9 million were overweight and 149 million were stunted in growth. Approximately 45% of mortality under five years of age is due to malnutrition, especially in countries with low and middle income Katoch, 2022). Malnutrition is defined as deficiency, excess, or imbalanced consumption of essential nutrition which contributes to poor mental and sensory development of children, thus limiting their productivity in future. People have improper prenatal care, families display low literacy, disadvantaged social situations, environmental contamination, nutritionally poor foods, disease attack, lack of resources and poverty (Adedokun and Yaya, 2021).

There are a number of causes of malnutrition including insufficient breastfeeding, unhygine condition, relative poverty and poor nutrition that results in a low consumption

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of both macronutrients and micronutrients. Hence, it is important for the community to participate in the malnutrition screening of children below five years. Mid-Upper Arm Circumference (MUAC) and Weight-for-Height Z-score (WHZ) are the most common parameters that are used in the diagnosis of acute malnutrition (Reyes et al., 2015). In children aged six months up to five years suffering from Severe Acute Malnutrition (SAM) registered a MUAC of 11.5cm or less or a WHZ of more than -3SD. For instance, Moderate Acute Malnutrition (MAM), which is a MUAC of less than 12.5 cm or WHZ of between -2 to -3 standard of (Million et al., (2016), WHZ is recognized as the best indicator of malnutrition while MUAC is also acceptable especially in the field settings. MUAC is associated with the screening of both acute malnutrition and severe acute malnutrition (SAM) for cut- off observation (Bhasin et al., 2016). The pattern of relationship between these two measures, MUAC and WHZ, among different age groups of Pakistani children can help further in undertaking proper identification of SAM and MAM. It has been argued that there need to be cut-off points for specific ages concerning the MUAC to reduce the effects of malnutrition on health (Dani et al., 2018).

The industries of fruit production and processing invariably produce wastage that is detrimental to the environment and incurs enormous losses. The fruit and vegetable by products can comprise 25 to 60% of the weight of the fruit. Amongst the Cucurbitaceae family, it bears Cucurbita pepo, Cucurbita maxima and Cucurbita mixta. Normally the seeds are disposed off after consumption, however these days they are industrially processed and sold as a salty snack (Mariod et al., 2017). This study found that pumpkin seeds contain 24.46% protein, 5.26% moisture, 3.26% ash, 38.53% oil and 14.77% cellulose (Milovanovic et al., 2014; Nyam et al., 2013). Besides, they are rich in tocopherol, polyunsaturated fatty acids, carotenoids, phytosterols, iron and magnesium (Al-Farga et al., 2016; Bialek et al., 2015). The seeds were especially rich in the essential amino acids, methionine, tryptophan, tyrosine and lysine. Due to their impressive iron content, scholars endorse the use of pumpkin seeds in children who suffer from iron-deficiency anemia (Patel, 2013).

Pumpkin seeds contain significant levels of magnesium, potassium, calcium, phosphorus, manganes, and very less amount of sodium. They also possess some polyunsaturated trace elements such as zinc, copper, and iron which act as antioxidants and enhance the function of antioxidant enzymes. The low amount of sodium and high quantity of potassium in pumpkin seeds is advantageous for maintaining a healthy heart (Dotto and Chacha, 2020). Zinc is essential for male health, cell protection, and the formation of protein structures. The role of zinc in the organization of protein structures, cell protections, and male well-being is very crucial. Since the mineral contents are adequate and most needed in pumpkin seeds, they can be used to improve the quality of fortified foods especially in baked products (Aghaei et al., 2014). Pumpkin seeds are very high in minerals such that they contain magnesium, potassium, calcium, phosphorus, manganese but have very low sodium. They also possess traces of other minerals like zinc, copper and iron which act as antioxidants and support the functioning of various antioxidants-based enzymes. The sodium levels in pumpkin seeds are lower and the potassium level is higher. Therefore, pumpkin seeds are good for the cardiovascular

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system (Dotto et al., 2019). Zinc is well known to be very useful in the structural formation of proteins, cell protection as well as male health. Considering the mineral content of dried pumpkin seeds, these components are appropriate for improving the nutritional quality of especially the fortifiable food products including baked goods (Aghaei et al., 2014).

Plants are the primary natural source of bioactive components and the most important sources of functional foods. Pumpkin seed has been shown to be abundant in phenolic compounds such as coumarin, unsaturated fatty acid, flavonoid, pyrazine, phytosterol, pigment, squalene, triterpenes, and vitamin E (Abou-Zeid et al., 2018). Pumpkin seeds powder contained the highest concentration of total phenolics (224.61±1.60 mg GAE/100g) than peel and also total flavonoids (139.37±1.07 mg CE/100g powder) than the peel extract. The pulp extract had less total carotenoids and β-carotene than the peel and seeds extracts (Zeb et al., 2021). Similar to vitamins and amino acids, Acorda et al. (2019) observed that zinc, iron, manganese, calcium, sodium, copper, magnesium, potassium and phosphorus were contained in pumpkin seeds as well.

Pumpkin peel flour contains water, ash, proteins, fat, and carbohydrates of 7.58g, 5.56g, 17.99g, 7.02g, and 61.85g respectively (Staichok et al., 2016). The pumpkin peel has better content of dietary fiber, protein, calcium and magnesium than the pulp with lower content of potassium, carbohydrates and fat. Furthermore, pumpkin peel is also a good source of carotenoid that has their own health benefits (Song et al., 2017). Moreover, Cuco *et al.* (2019) demonstrated that pumpkin peel is an essential resource of physiologically active chemicals. The addition of pumpkin peel flour to beef burgers has improved their physical, sensory and cooking characteristics due to its high mineral and dietary fiber content (Hartmann et al., 2020). Zeb et al. (2021) found higher sodium content 17.87±0.22mg/100g powder and potassium 1592±20.3mg/100g powder and iron 41.50±0.45mg/100g powder in the flesh of pumpkin than in its peels and seeds. An additional surface content of 15.21±0.07 mg Z /100g was recorded in the pumpkin seed harvested in Pakistan (Hussain et al. 2021)

2. METHODOLOGY

The present study was conducted at the Food Technology Lab, Food Processing and Preservation Lab and Food Analytical Lab Department of Food Science and Technology, Govt College Women University, Faisalabad

2.1. Raw Material Procurement

The whole raw material will purchase from the market. All the standards will be purchased from Sigma Aldrich (Sigma- Aldrich Tokyo, Japan Merck (KGaA Merck Dermstadt, Germany).

2.2. Proximate Analysis

Proximate analysis of cookies was carried out after the preparation of product according to the method of AOAC (2019).

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2.3. Preparation of Protein Isolates

2.3.1. Isoelectric Precipitation

The pumpkin seed and peel protein isolates were produced according to the method of Osemwota *et al.* (2021).

2.3.2. Micellization

The protein isolates were prepared according to the method of Khalid *et al.* (2016).

2.3.3. Enzymatic Extraction

The pumpkin seed and peel protein isolates were produced according to the method of described in Prommaban *et al.* (2021).

2.4. Gel Electrophoresis

The SDS-PAGE analysis was carried out according to the Procedure of Osemwota *et al.,* (2021).

2.5. Statistical Analysis

The data will be analyzed statistically by using software (Statistix 8.1) (Montgomery *et al.,* 2013).

3. RESULT AND DISCUSSION

3.1. Proximate Analysis

The proximate analysis of pumpkin seed and peel mean values are summarized in Table 1. Moisture content of pumpkin seed and peel flour were 4.96±0.45 and 7.10±0.65% respectively A similar effect of moisture content was found in a study of pumpkin flour sample that published by Marcel et al. the pumpkin peel result was similar and same as George and Shamaail (2020). The moisture of defatted meal analyzed after oil extraction was 2.96±0.05 and 0.96±0.06% for pumpkin seed meal (PSM) and peel meal (PM), respectively at the end of oil extraction the (%) of protein content for pumpkin seed flour and for seed meal were 35.67 ± 1.49 and 49.01 ± 0.02, respectively. The maximum protein content was in pumpkin seed meal. The achieved outcomes coincide with the ones reported by Sert et al. (2022). The values for the protein of pumpkin peel flour and peel meal were 12.54±0.49 and 26.55±0.5%, respectively. This result is in line with the interpretation of Indrianingsih et al. (2019). Fat content of pumpkin seed and pumpkin meal were 32.21±1.08 and 17.01±1.11%, respectively. These results were align with the findings of Ike *et al.* (2020), who revealed that pumpkin seed flour was rich in fat 33.12%. The fiber content of pumpkin seed flour, peel flour, seed meal and peel meal were 3.72±0.64, 12.69±0.36, 16.59±0.4 and 20.7±0.27% whereas ash content were 3.77±0.39, 3.77±0.39, 9.37±0.24 and 10.1±0.18%, respectively. The highest value was recorded for maximum NFE of 62.44±0.31% for pumpkin peel flour Amin et al. (2019) also reached similar findings in their studies.

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3.2. Mineral Content

The mineral content of pumpkin seed flour and pumpkin peel flour are described in Table 2. The result depicted that pumpkin seed flour contained phosphorus, potassium, zinc, calcium and iron in amount of 981.36±6.67, 804.5±8.32, 13.58±0.81, 31.17±0.99 and 18.12±1.23mg/100g, respectively. A similar significant result of phosphorus content was seen in a study of pumpkin flour sample that was reported by Marcel *et al.* (2020). Pumpkin peel flour exhibited phosphorus, potassium, zinc, calcium and iron which were 1.36±0.06, 1194.65±48.75, 18.05±0.73, 18.05±0.73, and 1.68±0.31 and 15.24±0.62 mg/100 g, respectively. Likewise, VIDHYA *et al.* (2022) explicated the higher amount of potassium and iron in pumpkin peel was 1243.74 and 15.87 mg/100g.

Table 1: Proximate Analysis of Pumpkin Seed Flour, Pumpkin Peel Flour, Pumpkin Seed Meal, and Pumpkin Peel Meal

Proximate Analysis (%)	Pumpkin Seed Flour	Pumpkin Peel Flour	Pumpkin Seed Meal	Pumpkin Peel Meal
Moisture	4.96±0.45	7.10±0.65	2.96±0.05	0.96±0.06
Protein	35.67±1.49	12.54±0.49	49.01±0.02	15.55±0.25
Fat	32.21±1.08	2.22±0.39	17.01±1.11	1.02±0.05
Fiber	3.72±0.64	12.69±0.36	16.59±0.4	20.7±0.27
Ash	3.77±0.39	2.77±0.29	9.37±0.24	10.1±0.18
NFE	19.67±1.44	62.68±0.51	17.97±0.9	51.67±0.82

Table 2: Mineral Content of Pumpkin Seeds Flour and Pumpkin Peel Flour

Minerals	Pumpkin Seed Flour (mg/100g)	Pumpkin Peel Flour (mg/100)
Phosphorus	981.36±10.54	1.36±0.06
Potassium	804.5±8.32	1146.28±12.74
Zinc	13.58±0.81	18.05±0.73
Calcium	31.17±0.99	1.68±0.31
Iron	18.12±1.23	15.24±0.62

3.3. Protein Isolates

The moisture, ash and protein content of synthesis of pumpkin seed protein isolates are presented in Table 3. The Protein recovery of 91.96±0.17% by enzymatic extraction of protein from pumpkin seed are higher than those obtained by micellization and isoelectric precipitation. Such as, Bucko et al. (2016) also noted the protein content of the protein isolates obtained by using enzymatic digestion approach was 92.13%. The protein isolates obtained through Isoelectric precipitation showed the protein content of 86.64±0.44% and this findings in agreement with the study done by Coşkun and Gülseren, (2020). The slight variation in results of protein isolates was due to the different varieties and operating conditions. Protein isolate by micellization process contained protein 83.07±0.45, Ash 1.71±0.27 and Moisture 5.33±0.34. On the other hand, processing by enzymatic extraction exhibited 2.52±0.34 ash and moisture content of 5.76±0.22%. In addition, isoelectric precipitation isolates moisture content within 10.76±0.33% and 3.97±0.09% ash content.

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3.4. Gel Electrophoresis- SDS-PAGE

SDS-PAGE method is used to separate proteins according to their molecular weight. Figure 1 showed the SDS-PAGE results of protein isolates from pumpkin seed meal, identified as EPI, IPI and MPI compared to a standard marker. The meal isolates varied in size from 6.5 to 117kDa with different low molecular weight fraction observed in the electropherogram. The EPI electrophoretic analysis displayed several polypeptide bands ranging in size from 14.5 to 117 kDa with distinct bands observed at approximately 25 and 117 kDa. On the other hand, IPI showed bands at 25 and 45kDa. MPI had bands at 25 and 38kDa, although lower in number than the other isolates. Slight variations in band mobility were noticed between EPI, IPI and MPI. The difference is due to changes in protein structure, content and interaction with salts in the isolates. According to the findings of Bucko *et al.* (2016), the PSPI analysis revealed that all three bands that were found remained visible however, the strength of the bands at about 24kDa and the low molecular weight band 14-20 kDa was enhanced.

Table 3: Protein Isolates of Pumpkin Seed Prepared by Isoelectric Precipitation, Micellization and Enzymatic Extraction

Protein isolates methods	Protein %	Ash Content %	Moisture Content %
Micellization	83.07±0.45	1.71±0.27	5.33±0.34
Isoelectric precipitation	86.64±0.44	3.97±0.09	10.76±0.33
Enzymatic extraction	91.96±0.17	2.52±0.34	5.76±0.22

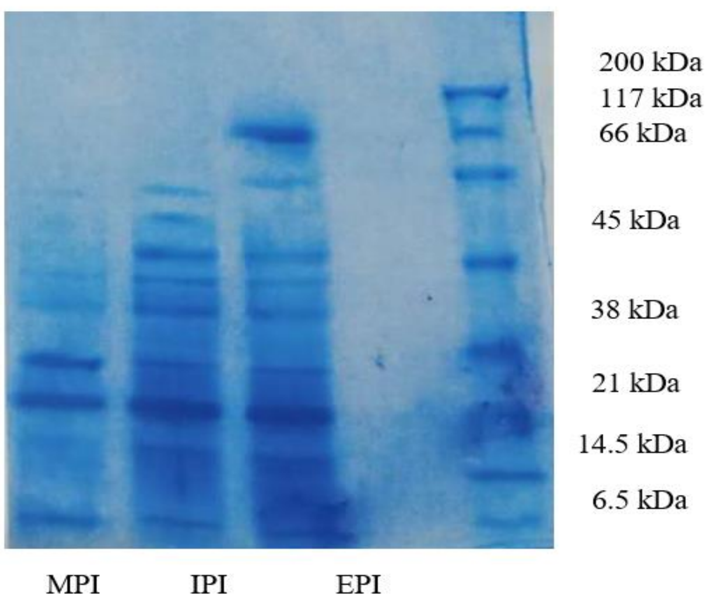

Figure 1: Electropherogram of pumpkin seed meal protein isolates

EPI= Enzymatic protein isolates

IPI= Isoelectric protein isolates

MPI= Mizallied protein isolates

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3.5. Proximate Composition of Cookies

The proximate analysis carried out for the protein isolate cookies indicated that moisture, crude protein, ash, crude fat, crude fiber, and nitrogen free extract (NFE) all had significant differences between treatments. The proximate composition of cookies are described in Table 4. Moisture content however raised significantly with the values on T0 5.15% and T6 7.65% meaning that the composite flour had a higher moisture absorption. The content of crude protein also increased from 8.79% (T0) to 25.76% (T6) in line with the findings of Dhiman et al. (2018) regarding the protein level of the flour used in the composite preparation. Likewise, ash content was recorded to increase from 1.47% (T0) to 3.49% (T6), which is an increase in mineral content observed by Racheal et al (2016). The crude fat content of the flour samples ranged from 7.38 % (T0) to 15.14 % (T6), while the crude fiber content of the flour sample rose from 1.17 % (T0) to 3.33 % (T6), showing the increase dietary fiber level with composite flour, as noted by saima et al. (2015). Increasing NFE level from T0 (81.18%) to T6 (52.26%) however correlated with the increase in protein content and fiber. This was similar to the studies done by Oyet and Chibor, (2020) on biscuits made from composite flour. The cookies nutritional value especially the thickness was enhanced with the addition of composite flour.

Table 4: Mean Values for Proximate Composition of Protein Isolate Cookies

Treatments	Moisture %	Crude Protein %	Ash %	Crude Fat %	Crude Fiber %	NFE %
T_0	5.15±0.10^G	8.79±0.08^G	1.47±0.1^G	7.38±0.05^G	1.17±0.1^G	81.18±0.03^A
T_1	5.54±0.04^F	11.37±0.15^F	1.89±0.1^F	8.87±0.05^F	1.42±0.07^F	76.44±0.11^B
T_2	6.04±0.04^E	12.36±0.05^E	2.68±0.15^D	9.18±0.1^E	1.77±0.1^E	74.52±0.25^C
T_3	6.31±0.03^D	14.03±0.06^D	2.70±0.1^D	11.14±0.06^D	2.17±0.11^D	63.65±0.08^D
T_4	6.51±0.03^C	18.69±0.25^C	2.89±0.09^C	13.17±0.15^C	2.50±0.05^C	62.72±0.32^E
T_5	6.97±0.04^B	23.22±0.10^B	3.12±0.1^B	14.31±0.26^B	2.80±0.06^B	56.54±0.46^F
T_6	7.65±0.09^A	25.76±0.11^A	3.49±0.15^A	15.14±0.06^A	3.33±0.29^A	52.26±0.50^G

T_0 =100% (control)

T_1 =95% straight grade flour, 5% Blends

T_2 =90% straight grade flour, 10% Blends

T_3 =85% straight grade flour, 15% Blends

T_4 =80% straight grade flour, 20% Blends

T_5 =75% straight grade flour, 25% Blends

3.6. Physical Characteristic of Cookies

The analysis of variance (ANOVA) performed on the physical parameters of cookies with protein isolate indicated that treatments differed significantly in their thickness, width and spread factor ($p < 0.01$). The mean values of physical parameters of cookies are presented in Table 5. The inclusion of blends, thickness rose to 14.04mm (T6) from 11.04mm (T0). The dough volume appeared denser and higher due to use of composite flour which is higher in protein and fiber content noticed by Dhiman et al 2018. The width

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of the cookies varied, the highest values were noted in T0 and T6, while T1 had the lowest width of 50.14 mm, which means that the expansion and binding formation of protein had an impact on stiffness and spreading of the dough after baking. The spread factor, which is the ratio of width to thickness, was significantly reduced with increasing composite flour from 51.77mm (T0) to 34.10mm (T6) which show more spreading without increasing the thickness of cookies when less composite flour was used. This agreed with Alshehry (2023) who reported similar behaviours in cookies that were made with partial replacement of wheat flour with pumpkin seeds powder. The effects of composite flour incorporation on the texture and appearance of the cookies were due to the changes in their physical properties.

Table 5: Effect of Treatments for Physical Parameters of Protein Isolate Cookies

Treatments	Mean Values		
	Thickness (mm)	**Width (mm)**	**Spread Factor**
T_0	11.04 ± 0.06^G	$56.96A \pm 0.16^A$	$51.77^A \pm 0.11^A$
T_1	11.39 ± 0.06^F	50.14 ± 0.10^F	$51.23A \pm 0.06^A$
T_2	11.86 ± 0.07^E	51.11 ± 0.59^E	$49.93A \pm 0.76^B$
T_3	12.21 ± 0.02^D	51.65 ± 0.07^D	$45.88B \pm 1.93^C$
T_4	13.18 ± 0.05^C	52.19 ± 0.31^C	$41.59C \pm 2.57^D$
T_5	13.85 ± 0.06^B	53.88 ± 0.22^B	$39.14D \pm 0.17^E$
T_6	14.04 ± 0.04^A	55.96 ± 0.10^A	$34.10E \pm 0.09^F$

T_0 =100% (control)

T_1 =95% straight grade flour, 5% Blends

T_2 =90% straight grade flour, 10% Blends

T_3 =85% straight grade flour, 15% Blends

T_4 =80% straight grade flour, 20% Blends

T_5 =75% straight grade flour, 25% Blends

T_6 =70% straight grade flour, 30% Blends

CONCLUSION

Malnutrition, which results from inadequate nutritional intake and poverty, affects more than 1.5 billion people in world. Low-cost, nutrient-dense foods like pumpkin seeds can improve diet quality and lower the risk of malnutritionn. Promoting nutrient-dense foods, like pumpkin seeds, which are high in protein, fiber, minerals, and antioxidants, is another way to address this issue.it contains good levels of zinc, magnesium, iron and a good source of essential amino acids that will help with cell protection and heart health and may be helpful in iron-deficiency prevention in children. Research indicates that by-products of pumpkin, especially peel, contain nutritional values and can enhance the nutritional quality of fortified food products (e.g. baked goods). This study focuses on improving food quality and providing low-cost nutrition for malnourished communities through the use of pumpkin-based products.

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