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Shelf life study on the quality of traditional Punjab sweet products supplemented with partially defatted peanut cake flour and dehydrated spinach leaves powder

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Abstract
Indian sweets like *Pinni* and *Panjir*i are used in different meal programmes at child care centers in Northern parts of India to eradicate malnutrition. *Pinni* and *Panjiri* were developed using 15 per cent partially defatted peanut cake flour (DPF) and 2 per cent dehydrated spinach leaves powder (SPF) to improve the nutritive content especially protein content. The products were stored in three different packaging materials like glass jars, aluminium zip pouch + glass jar and plastic zip pouch + glass jar for a period of 90 days at ambient temperature and were analysed for their nutritional and microbial parameters before and after 90 days of storage every fortnightly. Shelf life studies revealed that the nutritional parameters of all the products reduced but it was better retained in glass jars and aluminium zip pouch + glass jar. The microbial quality of the products reduced after 60 days of storage. However, aflatoxin as well as *E.coli* and *Staphylococcus spp.* was not detected.

Keywords: Partially defatted peanut cake flour, Dehydrated spinach leaves powder, Nutritional parameters, Microbial quality, Aflatoxin

Introduction
India is the major producer of peanut production after China. A high protein peanut press cake which is usually used as animal feed is now considered as a highly nutritional underutilized by product derived after the oil extraction process of peanuts. Peanut press cake, however, can be used for human food if it is processed from food-grade peanuts under hygienic environments. Peanut flour has a relatively high protein content, bland flavor, and light tan color which allow it to be incorporated into a wide range of foods. Peanut flour is made from crushed, partly defatted peanuts and is very low in saturated fat and cholesterol. It is also a good source of dietary fiber, thiamin, folate, potassium and zinc, and a very good source of protein, niacin, magnesium, phosphorus, copper and manganese (Fekria *et al.*, 2012) [6]. Utilization of defatted groundnut meal with mild processing treatment is becoming increasingly popular in other countries.

Proper storage of partially defatted peanut cake flour (DPF) is very important as the product contains some amount of oil to prevent rancidity and microbial contamination. Appropriate food packaging materials should be used as it can retard product deterioration, retain the beneficial effects of processing, extend shelf-life, and maintain or increase the quality and safety of food. Plastic packaging offers a large range of barrier properties but is generally more permeable than glass or metal. Thus, Package design and construction play a significant role in determining the shelf life of a food product. Materials that have traditionally been used in food packaging include glass, metals (aluminum, foils and laminates, tinplate, and tin-free steel), paper and paperboards, and plastics (Kenneth and Betty, 2007) [8]. In the recent years, food packaging involves combining several materials to effectively utilise each material's functional or aesthetic properties. Hence, the purpose of this study was to compare the shelf life on the nutritional and microbial quality of value added products prepared using partially defatted peanut cake flour.

Materials and Methods
Procurement of raw materials
Ingredients required for the development of *Pinni* and *Panjiri* like Wheat flour, Chick pea

flour, Raw peanuts, spinach leaves as well as other ingredients like ghee and sugars etc. were procured from the local market of Ludhiana in a single lot.

Processing of defatted peanut cake flour (DPF) and dehydrated spinach leaves powder (SPF)

Peanuts were purchased and checked for any infestation or damage. They were then roasted, de-skinned. Oil was extracted by using oil extraction machine. The residual cake was collected and dried in the oven at 65 ºC for half an hour. Dried cake was ground to fine powder.

Spinach leaves were procured, washed and dried in a tray drier at 60°C for 5-6 hours (constant weight was achieved in this time). The dried spinach leaves were ground to fine powder stored in separate airtight containers.

Formulation and development of the products

Two traditional Indian sweets *Pinni* and *Panjiri* were developed keeping the basic ingredient used for all the products as cereal-pulse mix flour. Pulse flour was taken in one-third amount of cereal flour. This mix was replaced for the main ingredient listed in the standard procedure. Wheat flour was used as a cereal source and chickpea flour was used as a pulse source. The standard procedures used for product development were as follows:

Pinni

Ingredients

Wheat flour	60.5g
Chick pea flour	22.5g
Partially defatted peanut flour	15g
Spinach leaves powder	2g
Powdered sugar	70 g
Saturated fat	60 g

Procedure

Add saturated fat in a thick pan, add wheat and chick pea flour and cook on slow fire till light brown colour. Add peanut flour in it, mix thoroughly and cook for few minutes. Remove from fire, add powder sugar and mix well. Grease a tray and spread the mixture over it. Roll out small balls called *pinni* of desired sizes when warm.

Panjiri

Ingredients

Wheat flour 60.5g
Chick pea flour 22.5g
Partially defatted peanut flour 15g
Spinach leaves powder 2g
Powdered sugar 70g
Saturated fat 50g

Procedure

Heat fat in thick pan and roast wheat flour and chickpea flour on slow fire. Add peanut flour flour mix well and roast for 2 minutes. Add powder sugar and mix it, cook for 2-3 minutes.

Storage of the products

The products were stored separately in three packaging materials namely glass jars, aluminium zip lock pouch + glass jar and plastic zip lock pouch + glass jar to compare the shelf life quality of the products at (30 ± 2^0C) for a periods of 90 days.

Nutritional Analysis of the value added products

The developed value added products were then subjected to nutritional analysis for proximate and mineral using standard procedures. *Invitro* protein digestibility was also analysed. The analysis was conducted before and after storage of the products in different packaging materials

1. **Proximate Composition** - Proximate composites like Moisture, Crude Protein, Crude Fat, Crude Fiber, Crude Ash, Carbohydrates and Energy were analysed using standard procedures suggested by AOAC (2000) [2].
2. **Minerals** - Elements namely iron and calcium were estimated using atomic absorption spectrophotometer (AAS, Varian model) after wet digestion (Piper, 1950) [14].
3. ***In vitro* protein digestibility -** It was carried out by the by macro kjeldahl method (Akeson and Stachman 1964) [1].
4. **Aflatoxins** - The samples were extracted by using the method of Barabalok *et al*. (1974) [3] and further detected using the Pressure Mini Column methods (PCM) Sashidhar *et al*. (1989) [19].

Microbial estimation of the value added products

The products from different packaging materials were estimated for their microbial quality every fortnightly using standard procedures (David and Frankhausar, 2010) [4]. The parameters analysed and respective medias used are as follows:

T**able 1:** Microbial parameters analaysed and specific media used

Test performed for	Media Used
Aerobic Plate Count /Total Plate count	Nutrient Agar
Yeast count	Glucose Yeast Extract Agar
Mold count	Potato Dextrose Agar
Total coliforms	Urinary Tract Infections Media
Staphylococcus aureus	Urinary Tract Infections Media
Escherichia coli	Urinary Tract Infections Media

Statistical Analysis

The nutritional attributes were analysed using paired t-test for fresh and stored samples. While to compare the quality of the products between the packaging materials, one-way anova was. applied. The values are expressed as Mean±SE (Standard error)

Results and Discussions

The effect of storage on the developed products developed incorporating partially defatted peanut cake flour and dehydrated spinach leaves powder packed in different packaging materials namely glass jars, aluminium zip lock pouch + glass jar and plastic zip lock pouch + glass jar was studied and a reduction in the proximate and mineral content was observed for all three packaging materials. Significant reduction was observed for all the nutrient composition for products stored in plastic zip lock pouch + glass jar.

Effect of storage on the proximate composition of the value added products

The results of the proximate composition of pinni and panjiri are presented in Table 2 and Table 3. The moisture content increased significantly ($p < 0.05$) for *pinni* stored in glass container + plastic zip lock pouch from 4.91 to 5.56 per cent as shown in Table 2.

Significant reduction in the crude fat content was observed in *pinni* stored in glass container + aluminium zip pouch and in glass container + plastic zip pouch and the reduction was 18.06 to 17.85 per cent and 18.06 to 17.56 per cent respectively. Srivastava *et al.* (2011) [22] studied the effect of storage on VCM (Virgin coconut meal) based *laddoo*s packed in polypropylene (PP, 75 µ) and metallised polyester (MP, 75 µ) which were heat sealed. The samples packed in PP lost moisture and became hard and brittle, while those packed in MP remained soft. Peroxide and thiobarbituric acid values were higher in PP packed samples as compared to those packed in MP. Multigrain *laddoos* were evaluated before and after storage in polypropylene packages for their proximate composition (Naidu *et al.*, 2013) [11]. A decrease in the proximate composition of *laddoos* were observed after 15 days of storage.

Table 2: Proximate composition of *Pinni* after storage in different packaging material (dry weight basis)

Proximate composition (g / 100 g)	Packaging Material		
	Glass container	Glass container + Aluminium zip lock pouch	Glass container + Plastic zip lock pouch
Moisture (BS)	4.91±0.01	4.91±0.01	4.91±0.01
(AS)	5.11^{b}±0.08	5.05^{b}±0.04	5.56^{a}±0.11
Paired t-value	3.76*	3.12*	17.90**
Crude protein (BS)	20.18±0.03	20.18±0.03	20.18±0.03
(AS)	19.98^{a}±0.02	19.92^{b}±0.01	19.28^{c}±0.02
Paired t-value	0.99^{NS}	2.38^{NS}	4.55*
Crude Fat (BS)	18.06±0.03	18.06±0.03	18.06±0.03
(AS)	17.93^{a}±0.01	17.85^{b}±0.15	17.56^{c}±0.07
Paired t-value	3.45^{NS}	7.39*	4.55*
Crude Fiber (BS)	3.84±0.01	3.84±0.01	3.84±0.01
(AS)	3.77^{a}±0.01	3.79^{a}±0.02	3.71^{a}±0.04
Paired t-value	6.24^{NS}	2.96^{NS}	2.11^{NS}
Total ash (BS)	3.20±0.23	3.20±0.23	3.20±0.23
(AS)	3.22^{a}±0.01	3.19^{a}±0.35	3.16^{a}±0.32
Paired t-value	0.09^{NS}	1.96^{NS}	3.27^{NS}
Carbohydrates (BS)	41.92±0.21	41.92±0.21	41.92±0.21
(by differences) (AS)	49.99±0.03	50.20±0.18	50.73±0.05

Values are Mean±SE with different superscripts are significantly different *(p<0.05) **(p<0.01)
BS- Before storage AS- After storage NS- Not significant

Significant increase ($p < 0.05$) in the moisture content of *panjiri* stored in glass container and glass container + aluminium zip lock packet was observed while the increase was even more significant ($p < 0.01$) for *panjiri* stored in glass container + plastic zip lock packet as depicted in Table 3. Reduction in the crude protein and fat content of *panjiri* was observed when stored in the different packaging materials but the reduction was not significant when stored in glass container as well as glass container + aluminium zip lock packet. Significant reduction in the crude fat was also observed. Shelf life of *panjiri* stored in polyethylene and laminated pouches for a period of three months were studied by Salve *et al.* (2011) [18]. They observed that both the materials retained fair nutrient composition of *panjiri* during storage. Higher fatty acid value as well as moisture was observed. Significant decrease in ash, protein, fat, and ascorbic acid content of supplementary foods was observed with increasing storage period packed in HDPE and LDPE and were stored at ambient temperature (Mishra and Mishra 2014) [10].

Table 3: Proximate composition of *Panjiri* after storage in different packaging material (dry weight basis)

Proximate composition (g / 100 g)	Packaging Material		
	Glass container	Glass container + Aluminium zip lock pouch	Glass container + Plastic zip lock pouch
Moisture (BS)	4.53±0.08	4.53±0.08	4.53±0.08
(AS)	4.77^{c}±0.02	4.80^{b}±0.12	5.04^{a}±0.28
Paired t-value	3.18*	4.07*	11.54**
Crude protein (BS)	18.96±0.06	18.96±0.06	18.96±0.06
(AS)	18.71^{a}±0.08	18.78^{a}±0.15	18.51^{b}±0.19
Paired t-value	2.81^{NS}	4.28^{NS}	12.11**
Crude Fat (BS)	26.96±0.02	26.96±0.02	26.96±0.02
(AS)	26.49^{a}±0.01	26.54^{b}±0.01	26.13^{c}±0.02
Paired t-value	1.92^{NS}	1.96^{NS}	5.50*
Crude Fiber (BS)	3.86±0.04	3.86±0.04	3.86±0.04
(AS)	3.81^{a}±0.02	3.85^{a}±0.05	3.80^{b}±0.13
Paired t-value	2.24^{NS}	0.180^{NS}	3.28^{NS}
Total ash (BS)	2.89±0.02	2.89±0.02	2.89±0.02
(AS)	2.83^{a}±0.01	2.80^{a}±0.12	2.75^{b}±0.09
Paired t-value	5.50*	7.39*	5.94*
Carbohydrates (BS)	42.32±0.08	42.32±0.08	42.32±0.08
(by differences) (AS)	43.39±0.03	43.23±0.01	43.77±0.06

Values are Mean±SE with different superscripts are significantly different *(p<0.05) **(p<0.01)
BS- Before storage AS- After storage NS- Not significant

Effect of storage on the mineral composition of the value added products

The products were analysed for minerals like calcium and iron. Significant decrease in the mineral content of the developed products after three months of storage is shown in Table 4. Kavitha and Parimalavalli (2014) [7] reported a non significant decrease in iron content of weaning food porridge. Elhardallou *et al.* (2015) [5] reported significant decrease ($p < 0.05$) in calcium content and increase in moisture content of supplementary foods formulated from legumes and dates after storage of five months.

Table 4: Mineral composition of the developed products after storage in different packaging material (dry weight basis)

Products in different packaging materials	Calcium			Iron		
	BS	AS	t-value	BS	AS	t-value
Pinni Glass Jar	50.23±0.01	49.16^{a}±0.04	8.59*	4.86±0.01	4.56^{b}±0.02	8.56*
Al zip pouch+glass jar	50.23±0.01	49.32^{a}±0.05	6.35*	4.86±0.01	4.61^{a}±0.12	7.45*
Plastic zip pouch+glass jar	50.23±0.01	49.09^{b}±0.01	9.12*	4.86±0.01	3.90^{c}±0.00	10**
Panjiri Glass Jar	48.68±0.02	47.93^{a}±0.04	3.13*	4.89±0.02	3.98^{a}±0.01	3.91^{NS}
Al zip pouch+glass jar	48.68±0.02	47.91^{b}±0.05	4.23*	4.89±0.02	4.18^{a}±0.02	2.61^{NS}
Plastic zip pouch+glass jar	48.68±0.02	47.56^{c}±0.02	5.66*	4.89±0.02	3.72^{b}±0.03	5.23*

Figures with different superscripts are significantly different* ($p < 0.05$), **($p < 0.01$)
BS- Before storage AS- After storage NS- Not significant

Effect of storage on the *in-vitro* protein digestibility of the value added products

The *in -vitro* protein digestibility decreased in both the developed products after storage period of three months as shown in Table 5. The highest *in-vitro* protein digestibility was observed in *pinni* followed by *panjiri*. Nicole and co workers (2010) [12] also reported a decrease in the *in-vitro* protein digestibility Ready-to-Eat composite porridge flours made by Soy-Maize-Sorghum-Wheat when stored in plastic pouches for 6 months. Preethi and Chimmad (2010) [15] showed decrease in *in-vitro* protein digestibility of supplementary foods packed in ordinary zip lock packet after storage period of 45 days. *Chikki* was prepared from peanut was enriched by incorporating soy protein isolate, calcium carbonate, ferrous fumerate, vitamin A and folic acid to meet the growing demand for health foods by Pallavi and co workers (2014) [13]. The authors reported a Protein digestibility corrected amino acid score of nutra *chikki* was 0.78 whereas that of control *chikki* was 0.73 An increased *in vitro* protein digestibility with a high amino acid profile in supplementary foods prepared using rice flour soybean flour, black sesame seed, and rice bran oil were reported by Satusap *et al.* (2014) [20].

Table 5*: In-vitro* protein digestibility of the developed products after storage in different packaging material (dry weight basis)

Products in different packaging materials	*In-vitro* digestibility		
	BS	AS	t-value
Pinni Glass Jar	80.13±0.30	78.49^{a}±0.31	5.78*
Al zip pouch+glass jar	80.13±0.30	78.52^{a}±0.23	6.28*
Plastic zip pouch+glass jar	80.13±0.30	77.90^{b}±0.21	8.51**
Panjiri Glass Jar	75.28±0.32	74.78^{a}±0.01	6.95*
Al zip pouch+glass jar	75.28±0.32	74.65^{a}±0.22	6.98*
Plastic zip pouch+glass jar	75.28±0.32	74.20^{b}±0.08	7.38**

Figures with different superscripts are significantly different *($p < 0.05$), **($p < 0.01$)
BS- Before storage AS- After storage NS- Not significant

Aflatoxin in the developed products

Aflatoxins were not detected in any of the samples before and after the storage period. Purohit and Rajyalakshmi (2011) [16] observed that laddoo and other snacks prepared with peanut flour were not detected with aflatoxin B1. Soher *et al.* (2013) [21] studied the contamination of peanut samples by aflatoxins as well as in chicken burgers incorporated with deoiled peanut flour. They noticed that aflatoxin G1 (AFG1) was absent in all peanut samples; whereas, trace amounts of aflatoxin G2 (AFG2) was detected and recorded 0.170, 0.905 and 0.760 µg/kg, respectively for three samples out of five of the burger which was contributed by the growth in chicken meat.

Microbial quality of the value added products

An increase in the total plate count and fungal count was storage. *Pinni* and *Panjiri* showed an increase (2-3x10^{-4}cfu/g) after the 75-90 days of storage. The fungal count increased which varied from 4-8x10^{-5}cfu/g in *pinni* and 5-7x10^{-5}cfu/g in *panjiri* after 90 days of storage. The highest increase in the TPC as well as fungal counts was found for the products stored in glass container + plastic zip pouch. The total plate count of freshly prepared virgin coconut meal incorporated instant halwa mix ranged 150 - 180 colonies/g after 12 months of storage in polypropylene (PP, 75 µ) and laminates of metallised polyester (MP, 90 µ). Yeast and mold counts in fresh and stored samples were below 25 cfu/g (Khan *et al.*, 2012) [9]. Naidu and co workers (2013) [11] also observed that the microbial count in multigrain *laddoos* were in safe limits upto two months of storage in polypropylene packages. Raja *et al.* (2014) [17] observed increasing trend in the total plate count (log cfu/g) and yeast and mould count (log cfu/g) for the control as well as treatment samples of fish curls incorporated with corn flour, black gram flour and peanut flour on storage in LDPE pouches.

Harmful pathogenic organisms like *E.coli* and *Staphylococcus spp*. were not detected in any of the *pinni and panjiri samples*. *Escherichia coli* and *Staphylococcus aureus* counts were detected in chicken burger and chicken burger supplemented with 20 per cent roasted defatted peanut flour (R-DPF) as 5.00 and 6.20 log10 CFU/g respectively) by Soher *et al.* (2013) [21].

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