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Development and shelf-life studies of pea enriched gluten free cookies

Avanthika K.
avanthika1118@gmail.com
Sri Sathya Sai Institute of Higher Learning, Anantapur, Andhra Pradesh

Dr. Meera M.
meeramanik4@gmail.com
Sri Sathya Sai Institute of Higher Learning, Anantapur, Andhra Pradesh

ABSTRACT

Millets are gluten free and excellent for celiac disease patients. An attempt was made to develop pea incorporated millet cookies. The developed cookies were analyzed for physical parameters like weight, thickness, and diameter, spread ratio, percent spread and bulk density. The cookies were analyzed for proximate composition, mineral content, anti-nutrients and anti-oxidants. The products were packed in polyethylene pouches and stored at ambient temperature for 4months and analyzed for microbial and consumer acceptability. On storage there was increase in the moisture content of the samples from 4.65% to 6.25% and decrement in anti-nutrient and anti-oxidant content. Addition of 30% pea flour to composite flour was found acceptable by considering the overall quality of the developed cookies and the economic aspect.

Keywords— *Pea, Gluten Free, Anti- Nutrients, Millets*

1. INTRODUCTION

Bakery products play a vital role in the market of which cookies are preferred by all age groups (Masoodi *and Bashir,* 2012) Cookies are differentiated from other baked products due to lesser moisture content which facilitate the product to be free from microbial spoilage and increase in shelf stability.

Foods containing less than 20 ppm of gluten are termed as 'gluten free foods' (Rai et al, 2014). Gluten free bakery products are prepared by using different flours. Many researchers have worked on GF products by incorporating starches, prebiotics, dairy products to improve the quality of developed products (Gallagher *et al.,* 2004**).** Composite flour or fortified flour are used in cookie preparation to increase its nutritive value. Pulses have cultural importance as man's staple food (Roy *et al.,* 2010). The Canadian pulse crop industry has become the world's largest exporters of soya bean, dry peas, and lentils with a production of 4.1 ton in 2009.Pulses are rich in carbohydrates, protein, fiber, minerals and vitamins and provide health benefits (Bazzano,*et al* 2011**)**. Fortification of bakery products can be done with soy, pigeon pea, green pea, lentil to improve the nutritional quality of the products (Preedy *et al.* , 2011).Pulse flour has good moisture retention and fat binding proteins. Peas act as an alternative source in the global food industry. Milled peas such as pea flour, pea protein and pea fibers are used as replacements in cakes, cookies, soups, and sauce products (Tulbek,2016**)**.

The objective of the study is to develop pea flour incorporated cookies and to evaluate the physicochemical, sensory, anti-nutritional, antioxidant and microbial analysis of developed cookies.

2. MATERIALS AND METHODS

Foxtail millet, finger millet, pearl millet, barnyard millet, dry green peas, sugar and hydrogenated fat were purchased from Anantapur market.

2.1. Preparation of raw materials

The millets and dry green peas were purchased from local market, cleaned, dried and milled separately.

Table 1: Formulation of composite flour

	Millet /pea flour (g)				
Samples	**Pearl**	**Foxtail**	**Finger**	**Barnyard**	**Pea**
C	25	25	25	25	0
P1	22.5	22.5	22.5	22.5	10
P2	20	20	20	20	20
P3	17.5	17.5	17.5	17.5	30

Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies.

2.2. Formulation of composite flour blends

Three variations of pea flour incorporated gluten free cookies were developed. Each variation comprised of composite flour mix and pea flour in different ratio as 90:10 (variation-1), 80:20 (variation-2) and 70:30 (variation-3) with only composite flour cookies with millet flour as control (C) without any value addition (100:0).

2.3. Preparation of cookies

Different proportions of composite flour was prepared (table-1). Sugar was added to butter and creamed to which sifted flour was added, folded and kneaded to form dough. Dough was chilled for 10 minutes and the dough was portioned to 16g balls each, sheeted to a thickness of 0.6 cm and 5 cm diameter. Cookies were transferred to a lightly greased tray, baked at 150˚C for 15 minutes until golden brown color was obtained. Cookies were allowed to cool and packed in polyethylene pouches (0.298mm) and stored in dry place until further analysis.

2.4. Sensory evaluation

The cookies were assessed for their sensory characteristics by 21 panel members using hedonic scale. The results were analyzed by ANOVA.

2.5. Physical analysis

The physical parameters of cookies like weight, thickness, diameter, spread ratio, percent spread factor and water absorption capacity were analyzed by AOAC (2000).

2.6. Proximate analysis of cookies

Protein, fat, moisture, fiber and ash, were determined by AOAC (2010) and total carbohydrate by Dubois *et al*., (1956).

2.7. Mineral composition

Calcium was estimated by titrimetric method (Hawk & Scimmelson 1957), Iron and phosphorous content by Raghuramulu (2003).

2.8. Storage studies

The cookies were packed in polyethylene packaging material. The covers were sealed by using sealing machine and were stored under ambient conditions. For four months, they were assessed periodically (every month) for moisture, sensory characteristics, microbial analysis, antioxidants and anti- nutrients.

2.9. Determination of anti-nutrients

Estimation of oxalic acid by Raghuramulu, (2003), phytic acid (Davies and Reid, 1979) and tannins (Kirk and Sawyer, 1998).

2.10. Determination of anti-oxidants

Estimation of total polyphenolic content by Singleton and Rossi (1965) and total flavonoid content (Chang et al., (2002).

2.11. Microbial analysis of cookies:

The microbial analysis of cookies was performed for determining total bacterial count by standard plate count method using nutrient agar (Aneja, 2001).

2.12. Statistical Analysis

The results obtained were subjected to the student t-test (used to measure the significance level of difference observed in the case of small samples) and two-way analysis of variance (ANOVA) and the significance between the mean was calculated. Values expressed are means of three independent samples analyzed in triplicates ± standard deviation. Statistical significance was accepted at $p< 0.05$.

3. RESULTS AND DISCUSSIONS

3.1. Physical characteristics of cookies prepared from Composite flour blends

The physical parameters of cookies are depicted in table 2. The average weight of control cookies was 14.2g and pea flour incorporated gluten free cookies ranged from 13.3 to 13.7g. A reduction in weight of the value added cookies were observed compared to control cookies by partial replacement of composite flour with pea flour resulted in decrease in weight of cookies compared to control cookies due to lower bulk density of pea flour has compared to control cookies. The thickness of the developed cookies ranged from 0.68 to 0.70 and significant difference was not observed among the developed samples. The thickness of the cookies decreased due to addition of pea flour and this could be attributed to increased content of protein. Mishra & Chandra (2012) reported that width of the cookies decreased from 44cm to 36.2cm with increased level of substitution of rice bran and soya and the control cookies (100% wheat flour) had maximum width 44cm followed by T1(10% RF and SF+80%WF) had 41.35. Also thickness of the cookies increased from 9.2 to 10.6 with increased substitution level. Similar trend was observed in the current study. The spread factor, acts as an indicator to determine the quality of cookies. The effect of change in diameter and thickness are correlated with spread ratio and determined by dividing the diameter by thickness of sample (D/T), indicating the ability of the cookies to raise during baking. The percent spread factor of the control cookie and 30% pea flour incorporated cookies had the highest scores compared to other experimental cookies. The bulk density of the cookies varied from 0.57 to 0.77 g/cm^3.The water absorption capacity of the developed cookies P2 (20% incorporated pea flour) had the highest WAC score of 1.62 compared to other samples.

Table 2: Physical parameters of developed pea flour incorporated cookies.

Sample	Weight (g)	Thickness (cm)	Diameter (cm)	Spread ratio	% Spread factor	Bulk density (g/cm^3)	Water Absorption capacity
C	14.2±0.75	0.7±0	5.24±0.15	7.48±0.19	100±0	0.57±0.01	1.45±0.01
P_1	13.3±0.27*	0.68±0.04	5.04±0	7.2±0.12	95.1±1*	0.77±0.01*	1.37±0
P_2	13.3±0.25*	0.68±0.04	5.06±0.14	7.46±0.51	94.9±0.8	0.67±0	1.62±0*
P_3	13.7±0.11*	0.7±0	5.08±0	7.25±0.06	97±2.9	0.64±0	1.34±0

*Values are expressed as mean ± SD of triplicates. *P < 0.05, Significant at 5% level, NS- Not Significant*
Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies.

3.2. Effect of pea flour incorporation on proximate and chemical composition of cookies prepared from composite flour blends

The moisture content of developed cookies ranged from 4.65 to 6.25% among the developed samples. The experimental samples had highest moisture content compared to control cookies (4.65%) as given in table (3). This may be due to low water binding capacity of millet flour. However, with percent addition of pea flour, moisture content of end product increased. This may be due to increase in protein content of pea flour in cookies as protein exhibits hygroscopic nature. The low moisture content of the product, improves shelf stability and enhance consumer preference and acceptability. The ash content of developed cookies indicated a range of 0.5% to 2.02%. Nidfe *et al*., (2014) observed increased ash content from 2.15% to 2.95% in cookies prepared from soybean flour substitution. However high level of ash content indicates high mineral content in foods.

Table 3: Proximate composition of developed cookies

Sample	Moisture (%)	Ash (%)	Carbohydrate (g)	Protein (g)	Fat (g)	Fiber (g)
C	4.65±0.25	1.47±0.03	53.3±0.66	5.9±0.14	24.5±0.77	3±0
P_1	5.5±0.07	0.5±0*	53.3±0	4.99±0*	24.5±0	2.5±1.85
P_2	6±0	1.25±0.3	55.2±0.66	5.4±0.14*	24.5±0.74	2.8±1.94
P_3	6.25±0.35	2.02±0.03	57.5±0.66	6.1±0.14*	24.5±0.75	3.05±2

Values are expressed as mean ± SD of triplicates. P < 0.05, Significant at 5% level, NS- Not Significant.
Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies.

Amin et al., (2016) observed that cookies prepared with pea flour, soy flour and oat flakes showed ash content of 1.57% to 1.89% which was maximum in experimental cookies compared to control cookies. The present study also revealed that at increase substitution level ash content increased from 0.5 to 2.02%.

The total carbohydrate content of the developed cookies ranged from 53.3 to 57.5g. Omah and Okafor (2015) reported that increase in the level of carbohydrate content from 64.70-66.86% was observed in cookies developed with millet pigeon flour, cassava cortex flour and wheat flour. Among pea flour incorporated cookies, 30% level had the highest protein content of 6.1g. Hence the developed cookies could be used as nutrient rich snack food item for under nourished preschool children. The total lipid content of the cookies showed less variation compared to control and experimental cookies. The fat content of developed cookies was 24.5g. The fiber content of the developed cookies ranged from 3g to 3.05g.

Nidfe et al., (2014) reported that with increase in the substitution of soy flour with whole wheat flour at (20, 30 and 50%) an increase in the iron content of the experimental (3.19mg/100g) cookies were observed compared to control cookies (210mg/100g). Similar results were found in current study, where an increase in the substitution level of pea flour to millet flour, showed increase in the iron content from 2.5 to 3.6mg/100g which was also reported by (Nidfe et al., (2014), table (4).

Table 4: Mineral composition of developed cookies

Sample	Mineral (mg/100g)		
	Iron	**Calcium**	**Phosphorous**
C	2.5±0.25	59±1.44	169.3±2.77
P_1	3.1±0.22	54±1.41	169.3±2.78
P_2	3.4±0.28	55±1.48	182.9±0
P_3	3.6±0	61±1.42	210.3±0.55

Values are expressed as mean ± SD of triplicates. *P < 0.05, Significant at 5% level, NS- Not Significant Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies

However, calcium content of pea flour incorporated cookies ranged from 54mg/100g to 61mg/100gm. The P3 (30% level) had the highest calcium content (61mg/100g) followed by P2 (55mg/100g) and P1 (54mg/100g) respectively. Whereas phosphorous content of pea flour incorporated cookies P1 (10%) had 169.3mg/100g, P2 (20%) had 182.9mg/100g and P3 (30%) had 210.3mg/100g. Ibrahium (2015) reported that with increased level of substitution of quinoa flour an increase in calcium (Ca) and iron (Fe) content of the cookies was observed due to high mineral salt content of quinoa flour (Vilche *et al*., 2003 and Alvarez-Jubete *et al*., 2009),

compared to wheat flour. The present study indicated that with increased pea flour substitution, calcium, iron and phosphorous content of the cookies got enhanced.Similar trend was observed by Nidfe et al (2014).

3.3. Effect of pea flour incorporation in cookies on storage

The developed cookies were packed in polyethylene packaging material and stored for 4 months. The packaging material was analyzed for COBB test and water penetration test and found no water uptake by the packaging material.

3.3.1. Moisture content: Cookies are baked products and their storage depends upon moisture content, which should be low to prevent the growth of microorganisms. Good shelf life of foods is important criteria in consumer acceptability. Shelf life depends on packaging material, environmental factor and handling. The moisture content of the developed cookies incorporated with pea flour, packed in polyethylene packaging material was recorded as 4.65% to 6.25% respectively. The moisture content of control sample C increased gradually over the storage period of 4 months from 4.65% to 5.8%. Sujirtha and Mahendran (2015) reported that cookies made with 50% coconut flour and wheat flour, moisture content was found as 5.92%. On storage the moisture content increased, due to high protein content and water binding capacity of the biscuits. It was observed that in the current study, the pea flour incorporated cookies packed in polyethylene packaging material had maximum moisture gain compared to control sample. A significant difference in moisture content was found in all the experimental cookies as compared to the control cookies prepared with composite flour, at 5% level of significance during the storage period of 4 months.

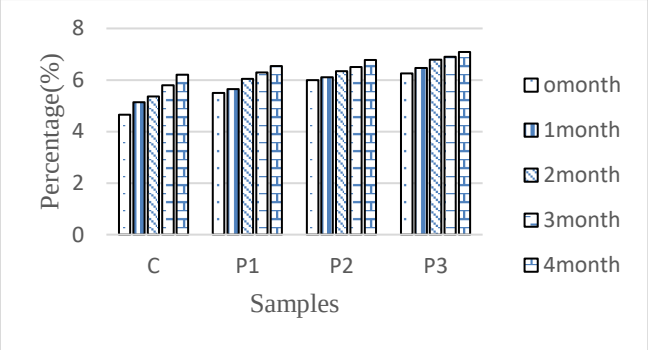

Fig. 1: Effect of storage on moisture content of developed cookies

Values are expressed as mean ± SD of triplicates. *P < 0.05, Significant at 5% level, NS- Not Significant Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies.

3.3.2. Sensory evaluation of stored cookies:

Table 5: Mean Sensory Scores of Stored Cookies

Attributes	Samples	Month				
		0	1	2	3	4
Appearance	C	7.8±0.45	7.7±0.48	7.7±0.60	7.6±0.47	7.5±0.60
	P_1	7.5±0.68	7.6±0.44	7.4±0.62	7.1±0.44	7.1±0.38
	P_2	7.6±0.57	7.5±0.58	7.4±0.67	7.2±0.40	7.2±0.45
	P_3	7.5±0.60	7.4±0.57	7.1±0.35	7.2±0.43	7.1±0.35
Color	C	7.8±0.45	7.5±0.55	7.4±0.60	7.5±0.50	7.5±0.50
	P_1	7.8±0.65	7.6±0.59	7.5±0.60	7.5±0.69	7.4±0.58
	P_2	7.9±0.71	7.7±0.78	7.5±0.55	7.5±0.40	7.4±0.25
	P_3	8±0.80	7.9±0.67	7.8±0.47	7.8±0.87	7.4±0.54
Taste	C	7.6±0.56	7.4±0.80	7.3±0.90	7.2±0.52	7.15±0.63
	P_1	7.8±0.74	7.6±0.59	7.5±0.61	7.5±0.85	7.4±0.55
	P_2	7.7±0.76	7.9±0.88	7.6±0.98	7.4±0.57	7.1±0.45
	P_3	7.9±0.85	7.7±0.77	7.6±0.44	7.5±0.80	7.3±0.41
Texture	C	7.6±0.48	7.5±0.88	7.4±0.78	7.3±0.67	7.1±0.81
	P_1	7.7±0.67	7.6±0.88	7.5±0.454	7.4±0.257	7.3±0.69
	P_2	7.9±0.52	7.85±0.85	7.7±0.66	7.5±0.62	7.4±0.38
	P_3	8.05±0.24	7.9±0.52	7.6±0.88	7.5±0.73	7.2±0.80
Flavor	C	8.02±0.67	8.04±0.90	7.5±0.55	7.6±0.45	7.4±0.53
	P_1	7.8±0.58	7.7±0.55	7.5±0.69	7.4±0.58	7.3±0.58
	P_2	7.6±0.42	7.5±0.65	7.4±0.58	7.3±0.47	7.1±0.38
	P_3	8.1±0.61	7.7±0.97	7.6±0.46	7.4±0.55	7.2±0.49
Over all acceptability	C	8±0.85	7.76±0.68	7.76±0.42	7.3±0.47	7.14±0.341
	P_1	8.04±0.56	7.7±0.423	7.6±0.58	7.5±0.40	7.3 ±0.58
	P_2	8.1±0.42	7.8±0.58	7.7±0.36	7.5±0.84	7.14±0.47
	P_3	8.2±0.58	7.8±0.54	7.8±0.37	7.5±0.85	7.4±0.55

Values are expressed as mean ± SD of triplicates. *P < 0.05, Significant at 5% level, NS- Not Significant Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies.

The developed products were subjected to sensory evaluation to know the preference level for various sensorial attributes and the scores was found to decrease over a period of four months, which was not significant. Therefore, the developed products could be stored for a period of four months without any changes in sensory parameters.

3.4. Effect of storage on anti-nutrient and anti- oxidant content of developed cookies

Effect of storage on anti-nutrient and anti-oxidant content of developed cookies was studied for a period of 0, 2 and 4 months and values are given in table (6) and (7).

Table 6: Effect of storage on anti- nutrient content of developed cookies

Sample	Month								
	Oxalates(mg/100g)			Phytates (mg/100g)			Tannins(mg/100g)		
	0	2	4	0	2	4	0	2	4
C	6±1.25	5.45±0	4.56±0	257.63±0.94	229.34±0.90	219.25±07	0.130±0	0.125±0.33	0.121±0
P1	5.42±0	4.78±0.37	3.65±0	219.27±3.88	157.35±0	131.74±1.95	0.121±1.35	0.087±0.65	0.08±0
P2	5.85±0.68	5.85±0.38	4.25±0.38	223.34±0.84	182.90±1.92	157.33±3.87	0.126±0	0.088±0.55	0.083±0.62
P3	6.37±0	6.07±0.35	4.55±0	252.99±0	186.9±0	168.45±0.09	0.132±1.35	0.089±1.35	0.081±0.65

*Values are expressed as mean ± SD of triplicates. *P < 0.05, Significant at 5% level, NS- Not Significant*
Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies.

Table 6: Effect of storage on anti- oxidant content of developed cookies

Sample	Month					
	Total polyphenol(mgGAE/100g)			Flavonoids (mgQE/100g)		
	0	2	4	0	2	4
C	170.22±4.32	154±4.09	151.81±0.07	94.45±0.55	94±0	87.23±0
P1	181.45±3.45	180.67±1.75	148.07±0.19	109.75±0.31	94.4±0.55	91±5.35
P2	183±3.097	168.0±0	153.11±2.73	116.57±1.68	109.32±1.08	102.43±0
P3	198.74±0.96	160.25±2.35	151.81±0.03	120.38±3.75	117.75±0	117.72±0

*Values are expressed as mean ± SD of triplicates. *P < 0.05, Significant at 5% level, NS- Not Significant*
Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies.

The reduction of anti-nutrient and anti-oxidant content could be due to storage conditions, type of packaging material used and baking temperature of cookies.

Sakac *et al*., (2016) observed that during storage conditions, antioxidants are subjected to many changes, resulting to lipid oxidation and the cookies prepared with rice flour and buckwheat flour, there is reduction in the total phenolic content of unpacked and packed cookies kept at different temperature (23 ± 1^0C) during storage conditions. During 9 months of storage period, there was reduction in the phenolic content of packed and unpacked cookies, reduction rate was found as 47% to 49%; where cookies were stored at 40^0C unpacked and packed cookies showed reduction rate of 48% to 45% respectively. Similar results were observed by Zielinski *et al*., (2012), in the rye ginger cake during the long storage condition, TPC content reduction rates was (2-23%) respectively. This variation could be due to antioxidant capacity which differ from rye ginger cakes and the rice flour/light buck wheat flour cookies.

3.5. Microbial analysis of developed cookies

The developed cookies were analyzed for microbial analysis by using nutrient agar as a medium. Effect of storage on microbial quality of developed cookies are given in table (7). Kukadae et al (2017) observed microbial growth of cookies prepared with guar gum incorporated composite flour on storage resulted in increase in total plate count of the cookies $0x10^3$ to $4.1x10^3$, yeast and mould count was $0x10^3$ to $2.4x10^3$ on storage of 0 to 90 days. The growth could be due to post processing contamination (Agu and Ndidimaka, 2014). In the present study, on 3 month of storage, the cookies are microbiologically stable and similar results were found in 0 month and 1 month there was no microbial growth in the samples. The microbial growth increased from 2 months to 4 months.

Table 7: Effect of storage on microbial quality of developed cookies

Sample	Total plate count($x10^3$cfu/g)				
	Month				
	0	1	2	3	4
C	1	1	2	6	16
P_1	-	-	3	6	27
P_2	1	1	5	7	29
P_3	-	-	7	8	28

*Values are expressed as mean ± SD of triplicates. *P < 0.05, Significant at 5% level, NS- Not Significant Key: C- control (100% composite flour); P1-10% pea flour incorporated gluten free cookies; P2- 20% pea flour incorporated gluten free cookies; P3- 30% pea flour incorporated gluten free cookies.*

4. CONCLUSION

The composite mix with equal proportion of millets (25:25:25:25) was partially replaced with pea flour at 10%/ 20%/ 30% and subjected to shelf life study for 4 months. Partial replacement with pea flour at 10%/ 20%/ 30% resulted in enhancement of micro and macronutrients. The shelf stability of the product revealed consumer acceptance and found safe for consumption for a period of four months. Therefore, the developed millet based pea flour incorporated cookies improved the quality and quantity of the protein content and contributes to grater scope in combating protein calorie malnutrition and micronutrient deficiencies at large for vulnerable segment of population at large especially preschoolers, school going children, pregnant and lactating mothers as supplements. The developed gluten free products could benefit the consumers for therapeutic usage especially those suffer from celiac disease

5. ACKNOWLEDGEMENT

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6. CONFICT OF INTEREST

None.

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