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Study on the impact of pumpkin seed flour on whole wheat bread characteristics

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Abstract
Whole wheat bread is increasingly consumed around the globe in the recent decades. Although it remains to be healthy alternative than the bread made with all-purpose flour, consumers have to compromise the bread characteristics. Pumpkin seeds rich in protein content can be used to improve the protein of wheat bread and modify the bread properties. Very few researches have been done on the incorporation of pumpkin seed flour in whole wheat bread. Hence, the present study focuses on the effect of the pumpkin seeds on whole wheat bread characteristics. Physical and sensory properties revealed that replacement of pumpkin seed flour at 5%, 10% and 15% to the whole wheat flour improved the bread properties. The study showed high values of loaf volume (485 ml), specific volume (1.9 ml/g) and less baking loss (7.42%) at 15% pumpkin seed flour addition. Proximate composition of bread samples showed high crude protein (12.22%) at 15% addition of pumpkin seed flour to the wheat bread. However, whole wheat bread made of 10% pumpkin seed flour had higher acceptability than the control and other bread formulations.

Keywords: Whole wheat flour, pumpkin seed flour, bread characteristics, sensory evaluation

Introduction
Plant seeds provide nutrition to the people in many parts of the world. Pumpkins belonging to the *Cucurbitaceae* family are grouped into *Cucurbia pepo, Cucurbita moschata, Cucurbita maxima amd Cucurbita mixta* based on their stem morphology. Except the former species, the other three species have been cultivated globally with high production rates. Pumpkin contributes both as a vegetable and medicine in many countries including China, Yugoslavia, Argentina, India, Mexico, Brazil and America Research in pumpkin has gained more attention in the recent decades because of its medicinal and biological value. Only a very few research has been carried out in pumpkin seeds and its value addition especially in bread. While pumpkin pomace is being processed to obtain various products like juice, pomace, pickles, jam and other dried products**.**

Pumpkin contains succulent flexible stem with trifoliate leaves, two cotyledons growing annually at the growth rate of 0.6 m by 5.0 m. Pumpkin seeds are rich in essential amino acids like lysine, tyrosine, tryptophan, methionine and essential fatty acid (linolenic acid) (H Glew *et al*., 2006) [7], Cr, Na, K, P and good amount of Mg, Zn, Cu, Mo, Se (Imaeda *et al*., 1999) [9]. Pumpkin seeds contain high levels of Iron and so it is recommended for children and adolescents prone to anaemia caused due to iron deficiency Pumpkin seeds are also rich in phytosterols, polyunsaturated fatty acids, antioxidant vitamins such as carotenoids and tocopherol.

Studies have shown that pumpkin seed reduces the risk of hypercholesterolemia (Gossell-Williams *et al*., 2011) [6], bladder stone, diabetes, hypertension (E.M.K. El-Mosallamy, A Sleem, Abdel-Salam, Shaffie, & Kenawy, 2011) [2], tumour growth (especially gastric, lung, breast and colorectal cancer (Craig, 1997; Huang *et al*., 2004) [1, 8]. It also acts against inflammation, acidity, bacteria, intestinal parasites and worms (M Ptitchkina, V Novokreschonova, V Piskunova, & Morris, 1998) [10].

Nutritional profile of pumpkin alters with species and cultivator and is found to be high. Around the globe, protein content of pumpkin seeds ranged from 24.5 % to 36.0 %. For this reason, fluted pumpkin seed flours have been used as protein supplements in various foods. Nutty taste present in the pumpkin seed flour makes it feasible to create new food products of a high nutritional value. Another engrossing benefit of pumpkin seed flour is that it is gluten-free, therefore it can be recommended to the patients suffering from gluten intolerance or

celiac disease (Patel 2013). Pumpkin seed flour was also used in promoting nutritional status of undernourished 3 children in Kenya as an adequate source of dietary energy, protein and fat (Ward and Ainsworth 1998). Tryptophan, an essential amino acid and 5-hydroxytryptophan (5-HTP), intermediate metabolite of tryptophan in the formation of neurotransmitter serotonin present in pumpkin seed are promoted as treatment of depression (Eagles 1990). Though pumpkin seeds are loaded with functional nutritional compounds, anti-nutritional factors limit their application and consumption. Anti-nutrients are shown to be predominantly high in mature older seeds than younger seeds. Therefore, younger seeds are preferred for consumption. Researchers have concluded that anti-nutritional properties of pumpkin seeds decreased with treatments like germination and fermentation (Giami, 2004) [4-5].

Earlier studies have shown that the addition of pumpkin seed in wheat bread production has resulted in increased loaf volume, sensory properties and good baking performance when added in certain levels beyond which the bread was unacceptable (Giami & Barber, 2004) [4-5]. Addition of pumpkin seed proteins enhanced the in vitro protein digestibility of bread (Mansour, Dworschák, Gergely, Hóvári, & Pollhamer, 1999) [11].

Recent attention on agro waste utilisation reduces food wastage and retains the available resources. Pumpkin seed, by-product of pumpkin processing, available in large quantity is rich in oil and protein. Hence, utilisation of pumpkin seeds in popular global food, bread, maximises its protein profile and modifies the bread characteristics.

In the present study, effect of the pumpkin seed flour incorporation in the whole wheat bread was evaluated.

Materials and Methods

Ingredients

Wheat flour was produced by milling wheat grains (Sambha variety) using miller. Local pumpkin seeds processed in the month of May, 2019 was used for the study. Seeds were grounded using blender and stored at 4^0C for future use. Honey (Lion Honey), salt, yeast, oil, was purchased from Consumer Co-operative Warehouse, Thanjavur, Tamil Nadu.

Bread Dough Formation

Ingredients such as whole wheat flour, pumpkin seed flour, salt, honey, oil, yeast, improver, and water were weighed according to the formulation as mentioned in Table 1 and kneaded using Spiral Mixer of 10kg capacity. All the ingredients were mixed by straight-dough mixing method by mixing at low speed for 2 minutes followed by medium speed for 3 minutes and high speed for 5 minutes. After dough mixing, the dough was portioned, rounded, transferred to bread pans and fermented at $\pm35^0$C for 120 minutes. This fermentation time was calculated using the dough cylinder test. Fermented samples were baked at 165^0C for 20 minutes and cooled to room temperature. Cooled bread samples were packed in polyethylene bags and stored at room temperature of $\pm35^0$C for further analysis.

Table 1: Recipe used in Bread dough formulation

Ingredient	Control	T1	T2	T3
Whole Wheat Flour	100	95	90	85
Pumpkin seed flour	-	5	10	15
Water	70	70	70	70
Salt	1.0	1.0	1.0	1.0
Yeast	1.5	1.5	1.5	1.5
Honey	15	15	15	15
Fat/ Oil	7.0	7.0	7.0	7.0
Improver	0.3	0.3	0.3	0.3

* % values are based on the total flour weight (100 g)

Determination of Physical Properties of Bread

Loaf volume, weight and specific volume

The weight of bread loaves was measured using a digital weighing scale and the volume of the breads was measured by rapeseed displacement method (AACC, 2010). The specific volume of the bread loaf was calculated using the formula below.

$$\text{Specific Volume (cm}^3\text{/g)} = \frac{\text{Bread loaf volume}}{\text{Bread loaf weight}}$$

Baking loss

The weight of the dough and bread (after 2 hours of drying in room temperature) was noted (Kim *et al*. (2003) and the percent weight loss of the bread samples was calculated as:

% weight loss = (A- B /A) 100

Where, A = weight of dough; B = weight of baked bread.

Crumb and crust Color

Hunter Lab next generation Color flexEZ spectrophotometer (Hunter associates laboratory, Reston, VA, USA) was used to determine the L, a and b color values of the bread crust and crumb. The instrument was calibrated using a standard white and black plate. The total color difference was calculated using the following equation.

$$\Delta E=\sqrt{L^{*2} + a^{*2} + b^{*2}}$$

Texture analysis

Bread hardness was analyzed using the Stable Micro Systems Texture Analyzer (TA-XT plus). Different ingredients in bread formulation contribute to its texture. Cylindrical probe of 36 mm diameter was used for analyzing the bread. 30 kg load cell at a pretest and posttest speed of 0.5 mm/s and 10.0 mm/s respectively was used for the analysis. (74-10A).

Sensory Analysis

9-point hedonic scale was adopted to analyze the sensory attributes of the bread. 20 semi-trained panel members carried out sensory analysis to determine the sensory attributes such as color, texture, appearance, taste and overall acceptability. Samples with specific alpha-numeric codes were given to the panelists and the hedonic scale indicated the following terms.

9=Extremely like, 8=Like very much, 7=Like moderately, 6=Like slightly, 5=Neither like nor dislike, 4=Dislike slightly,

3=Dislike moderately, 2=Dislike very much, 1=Extremely dislike.

Proximate Analysis

AOAC Method (2000) was followed for all the proximate analysis. Percent moisture is calculated by finding the weight lost when the sample is subjected to 130^0C for 1 hr. Crude oil was determined using the Soxhlet apparatus with hexane as the solvent. Crude protein was calculated from the nitrogen content determined by the Kjeldahl method using the formula below.

$$\% \text{ Nitrogen} = \frac{\text{Vol. of 0.1N H2SO 4 used } 0.0014 \times 100}{\text{weight of the sample (g)}} \times 100$$

Percent crude protein = Percent N x 6.25

Combusting the sample at 550^0C for 6 hours in a muffle furnace gave the ash (mineral) content. Difference in crude protein, lipid, moisture and ash from 100 resulted in the amount of total carbohydrate (dry weight basis).

Data Analysis

The data were interpreted using analysis of variance (ANOVA test) in SPSS software. The significant level was established at $p<0.05$. Mean was found by Least Square Difference (LSD)

Results and Discussion

Baking Characteristics of bread

Pumpkin seeds contain high levels of linolenic and oleic acid. Oilseed flours usually decreases the specific volume of the bread. However, this phenomenon is influenced by its quantity. Contradictory results were also obtained during addition of oil seeds like flaxseeds. Loaf volume is an important attribute which determines the acceptability of the bread. The results in Table 2 show that T3 has high loaf volume than other bread formulations. (El-Soukkary, 2001) [3] reported that the loaf volume of the bread made of all-purpose wheat flour decreased with increase in the addition of raw pumpkin seed meal. In contrast, bread made of whole wheat flour with addition of pumpkin seed flours has positive effect in loaf volume with increase in pumpkin seed flour proportion.

Table 2: Effect of pumpkin seed flour on physical properties of the whole wheat bread

Bread	Loaf Volume	Specific Volume	Baking Loss
(ml)	(ml/g)	(%)	
Control	449±2.82^d	1.536±0.01^d	9.18±0.03^a
T1	465±2.82^c	1.674±0.02^c	7.98±0.04^b
T2	473±0.70^b	1.809±0.00^b	7.79±0.06^c
T3	485±4.24^a	1.901±0.01^a	7.42±0.09^d

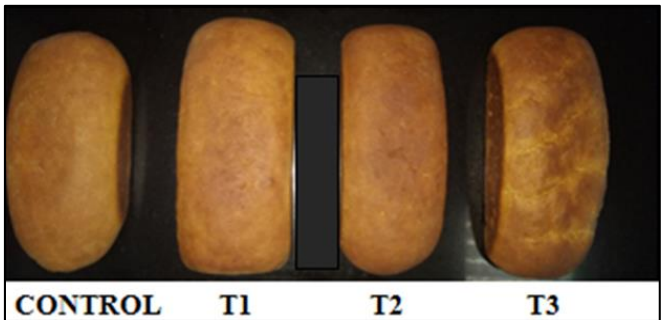

Fig 1: Bread loaf made of 100% whole (Control), 95% wheat flour & 5% pumpkin seed flour (T1), 90% wheat flour & 10% pumpkin seed flour (T2) and 85% wheat flour & 15% pumpkin seed flour (T3)

Specific volume is the ratio of loaf volume and its weight. Specific volume of the bread increased with increase in pumpkin seed flour with significant difference ($p<0.05$) between the bread samples. This result can be attributed to the observation of (Mentes, Bakkalbaşı, & Ercan, 2008) who noticed positive effect of bread volume till 10% and negative effects began after 20% addition of flax seed flour. Baking loss is the quantity of moisture removed from the dough during baking. With the addition of pumpkin seed flour, baking loss decreased compared to the control bread with high baking loss (9.18%). All the samples showed significant difference at 5% level of significance.

Bread Colour

Colour values of bread crumb and crust mentioned in Table 3 and Table 4 indicates that the lightness (L) value increased with increase in pumpkin seed flour. The total colour difference (ΔE) of bread crumb and crust increased with increasing seed flour addition from 5 to 15%. However, the bread crumb of T2 (58.035) had no significant difference with ΔE value (58.25) that of the control. Significant difference existed only between T1 and T3 at 5% level of significance. Whereas, the crust colour value (ΔE) of the control and treated (T1, T2 and T3) whole wheat bread was found to be significantly indifferent. This result may be due to addition of lesser amount of pumpkin seed flour to whole wheat bread (Pareyt, Finnie, Putseys, & Delcour, 2011).

Table 3: Effect of pumpkin seed flour on crumb colour of whole wheat bread

Bread	L	a	b	ΔE
Control	53.004±1.19	7.546±0.36	22.946±0.75	58.25±1.36
T1	51.454±1.81^a	7.63±0.39	23.23±0.51	56.936±1.56^a
T2	52.692±1.27	7.416±0.2	23.166±0.52	58.035±1.36
T3	54.047±0.47^a	7.554±0.12	23.18±1.23	59.4099±0.51^a

Table 4: Effect of pumpkin seed flour on crust colour of whole wheat bread

Bread	L	a	b	ΔE
Control	31.218±1.61	12.446±1.35	20.096±1.72	39.224±1.85
T1	28.944±1.87	12.667±0.89	18.646±1.30	36.205±1.08
T2	29.086±1.85	12.740±1.15	18.740±0.19	36.877±1.62
T3	29.170±1.45	11.796±1.86	17.678±1.87	37.301±0.59

Bread Hardness

The effect of the addition of pumpkin seed flour on whole wheat bread hardness is mentioned in Table 5. T1 and control was found to have similar hardness indicating that addition of 5% pumpkin seed flour has no influence on bread texture. Bread hardness was observed to be increasing with the addition of pumpkin seed flour from 5 to 15%. However, there was no significant difference between the hardness of the whole wheat bread and the pumpkin seed flour (5-15%)

enriched bread samples. This result is in agreement with who proposed that increasing the addition of chia seed flour (oilseed flour) in wheat bread increased the hardness (Pizarro *et al*., 2014; Steffolani, Martinez, León, & Gómez, 2015).

Table 5: Effect of pumpkin seed flour on whole wheat bread hardness

Bread	Bread Hardness (g)
Control	0.646±0.57
T1	0.652±0.50
T2	0.947±0.58
T3	1.284±0.22

Sensory Evaluation

Sensory evaluation results (Table 6) revealed that the overall acceptability of T2 (6.75) was highest among the other bread formulation. This may be due to the better taste than other samples. There was no significant difference found between the control and treated bread samples. This is in agreement with (El-Soukkary, 2001) [3] who concluded that no significant difference for colour, texture and overall acceptability between control and pumpkin seed flour enriched bread. Taste and flavour of the bread is attributed to the maillard reaction where high production of aroma compounds occurs with high protein levels in bread due to high protein in pumpkin seed. From the sensory evaluation results, taste of the bread highly influenced the overall acceptability of the product.

Table 6: Effect of pumpkin seed flour on mean sensory scores of the whole wheat bread

			Sensory Attributes		
Bread	**Colour**	**Appearance**	**Texture**	**Taste**	**Overall Acceptance**
Control	6.5±1.64	6.16±1.47	6.0±1.67	6.0±1.47	5.66±1.63
T1	6.83±0.75	6.83±0.98	6.16±0.75	6.41±0.49	6.5±0.54
T2	6.83±0.98	6.91±0.91	6.08±1.68	7.0±0.63	6.75±0.75
T3	7.0±0.63	7.0±0.63	5.19±1.62	6.16±1.16	6.33±1.03

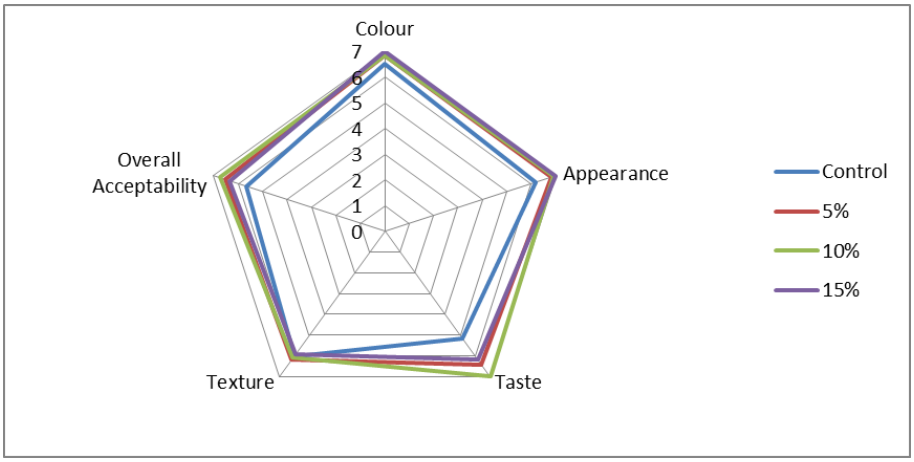

Fig 2: Effect of pumpkin seed flour on sensory attributes of whole wheat bread

Proximate composition

Whole wheat bread and bread enriched with 5-15% pumpkin seed flour evaluated for its proximate composition is mentioned in Table 7. Crude protein, crude fat and ash content increased with increase in addition of pumpkin seed flour. However, significant difference (p<0.05) existed only in crude fat and protein levels of the whole wheat bread and treated samples. Moisture content decreased with increasing pumpkin seed flour addition from 5 -15%. This can be correlated with the increasing hardness of the pumpkin seed flour enriched wheat bread. Crude fat and protein increased with increasing addition of pumpkin seed flour from 5 to 15%. Adding higher levels of seed flour may lead to rancidity. From the results, we can conclude that adding pumpkin seed flour increase the protein and fat content of the whole wheat bread.

Table 7: Proximate composition of whole wheat bread with enriched pumpkin seed flour in 5%, 10% and 15%

Bread	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)	Carbohydrates (%)
Control	20.245±0.06^{c}	10.003±0.05^{d}	1.520±0.02^{d}	0.755±0.02	67.476±0.11^{a}
T1	20.365±0.02^{a}	10.605±0.02^{c}	1.810±0.02^{c}	0.675±0.20	66.544±0.27^{b}
T2	20.272±0.02^{b}	10.930±0.05^{b}	2.585±0.03^{b}	0.960±0.01	65.270±0.05^{c}
T3	19.107±0.03^{d}	12.220±0.04^{a}	4.555±0.02^{a}	0.975±0.07	63.139±0.06^{d}

Conclusion

Whole wheat bread made with the addition of pumpkin seed flour enriches the protein content in the bread. It is evident from the results that increased addition of pumpkin seed flour enhances the bread characteristics. However, the sensory score was limiting since the taste of the pumpkin seed flour was not highly appealing. Also, it can be concluded that inclusion of pumpkin seed flour below 15% did not much have difference in the sensory scores. Hence, pumpkin seed flour can be added to the whole wheat bread to improve the nutritional value and physical properties of the bread.

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