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Effect of fermentation on functional properties of pumpkin seed flour

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Abstract
Present investigation evaluates the effect of processing on functional properties of pumpkin seed powder. Pumpkin seed were made into powder and subjected to natural and controlled single culture (*L. acidophilus* and *L. rhamnosus*; 37 °C, 36hr.) fermentations. A sharp decline was observed in pH of all three fermentations accompanied by increased titratable acidity with increasing time. Fermentations significantly ($P \leq 0.05$) increased the water absorption capacity while bringing a significant ($P \leq 0.05$) reduction in oil absorption capacity and swelling power.

Keywords: Pumpkin seed powder fermentation, functional properties, *L. acidophilus*, *L. rhamnosus*

Introduction
Fruit and vegetable processing industries generate enormous amount of byproduct wastes in form of skin, seeds, stones, unused flesh etc., some of which have high nutritional value. One such domestic as well as industrial waste is pumpkin seed. Pumpkin seeds have come to be known as nutritional powerhouse, as these seeds are excellent source of not only protein and fibre but also micronutrients mainly zinc, phosphorous, magnesium, potassium and selenium.
Fermentation is a known process for improving the nutritional value of products. At the same time it also affects the functional properties of food. Functional properties are the essential physicochemical properties of foods that are a result of complex interactions between the food components as a result of their molecular structures and conformations. Functional characteristics help us understand how new proteins, fat, carbohydrates (starch and sugars), and fibre may behave in specific food systems (Kinsella, 1982; Kaur and Singh, 2006; Chen et al., 2016) [8, 10, 5]. Controlled fermentation with probiotics can further add to therapeutic values of developed food products (Sindhu and Khetarpaul, 2003; Sindhu et al., 2013) [18, 16].
Considering the nutritional potential of pumpkin seeds, the present study was designed to study the effect of fermentation on functional properties of pumpkin seed flour.

Materials and Methods
Pumpkins (*Cucurbita sp*.) were procured in a bulk from the local market and seeds were separated from the pulp.

Processing of pumpkin seeds
Fermentation: Pumpkin seeds with seed coat were grinded in electrical grinder and subjected to three type of fermentations:
- Natural fermentation (37 °C, 36hr.)
- Controlled fermentation with *L. acidophilus* ATCC4356 (37 °C, 36hr.)
- Controlled fermentation with *L. rhamnosus* ATCC7469 (37 °C, 36hr.)

All processed pumpkin seeds were dried in hot air oven (60 °C) for 6-8 hours and made into flour using electric grinder.

pH and Titratable acidity
Titratable acidity in freshly fermented sample was determined as lactic acid per ml AOAC (2012) [3].

Functional properties

All processed pumpkin seed flours were studied for Water absorption capacity (Wang and Kinsella, 1976) [20], oil absorption capacity (Singh and Singh,1991) [19] and Swelling power (Robertson *et al*., 2000) [14].

Statistical analysis

The data obtained were subjected to statistical analysis for analysis of variance in a complete randomized design. Critical difference was used to check the significance of differences between treatments. Level of significance was set at $P \leq 0.05$.

Results and Discussion

pH and titratable acidity

The pumpkin seed flours were subjected to natural as well as controlled fermentations (*L. acidophilus* and *L. rhamnosus*) for varying time periods (Table 1). A sharp decline was observed in pH of all three fermentations accompanied by increased titratable acidity with increasing time. During fermentation, developing microorganisms convert glucose to lactic acid which is responsible for the decline in pH of the food product. A rapid drop in pH with corresponding increase in titratable acidity during fermentation has been reported in earlier studies (Sindhu and Khetarpaul, 2005; Khanna and Dhaliwal, 2013) [17, 9].

Functional properties

Table 2 presents the effect of different fermentations on functional properties of pumpkin seed flours. The unprocessed pumpkin flour had Water absorption capacity 178.00 ml/100g, Oil absorption capacity 82.90 ml/100g and Swelling power 2.79 ml/g. All the processing methods resulted in significantly ($P \leq 0.05$) higher water absorption capacity as compared to unprocessed

pumpkin seed flour. Highest per cent increase (66.35) was obtained after *L. acidophilus* fermentation while Natural and *L. rhamnosus* fermentation resulted in 59.74 and 59.37 per cent increase, respectively. The higher water absorption capacity of all processed samples may be due to protein denaturation. Severe denaturation has been reported to destroy hydrophobicity; (Hutton and Cambell, 1981; Fagbemi *et al*., 2006) [7, 6]. The proteolytic activity during fermentation breaks the peptide bonds of protein causing an increase in polar groups and thereby increasing hydrophilicity of the proteins (Beuchat, 1976) [4].

Oil absorption capacity of unprocessed, naturally fermented, *L. acidophilus* fermented and *L. rhamnosus* fermented pumpkin seed flours was 82.90, 77.60, 79.33 and 78.17 ml/100g, respectively. The process of fermentation brought significant ($P \leq 0.05$) decrease as compared to unprocessed flour. The lower capacity of fermented pumpkin seed flours to bind and retain oil than unprocessed flour, suggests that conformational changes of starch and protein molecules could have occurred during fermentation that exposed less hydrophobic than hydrophilic groups. The results of this study are in line with studies who reported increased WAC and decreased OAC of fermented African oil bean seed flour (Akubor and Chukwu,1999) [2] and pumpkin seed flour (Akintade *et al*., 2019) [1]. Contrary to these, Oloyede et al. (2016) [11] reported a significant ($P < 0.05$) increase in the oil absorption capacity of moringa flour samples with increase in fermentation time.

All fermentations significantly ($P \leq 0.05$) reduced the swelling power as compared to unprocessed flour. The Swelling power is the measure of the ability of starch to assimilate water. It reflects the extent of associative forces within the granules. It is related with starch as well as protein content of the flour (Ramírez- Wong *et al*., 2007) [13]. A reduced swelling power therefore reflects the effect of fermentation on starch as well as protein. Fermentation has been known to modify macromolecules and affect functionality (Oloyede et al., 2016; Oyeyinka et al., 2020) [11, 12].

Functional properties are the fundamental physico-chemical properties that can affect product development specially in case of bakery products. High water absorption helps to reduce moisture loss in packaged bakery goods; moreover water-holding is indispensably required to maintain freshness and a moist mouth feel of baked foods. Oil absorption and swelling power also affect the retention of flavor and texture of baked foods. Effect of fermentation on functional properties of food ingredients has been attracting interest of scientists. Simwaka *et al.* (2017) [15] observed that Water Solubility Index increased while water absorption index and oil absorption capacity decreased after fermentation in complementary foods formulated from pumpkin seeds, amaranth, finger millet and sorghum grains.

Table 1: pH and titratable acidity (% lactic acid) of fermented pumpkin seed flours

Fermentation		6 h	12 h	18 h	24 h	30 h	36 h	CD ($P \leq 0.05$)
Natural	pH	6.21±0.06	4.62±0.03	4.51±0.02	4.25±0.03	4.14±0.03	4.03±0.02	0.17
	Titratable acidity	0.04±0.02	0.08±0.03	0.11±0.03	0.16±0.06	0.17±0.05	0.18±0.11	0.03
L.acidophilus	pH	6.04±0.03	4.61±0.02	4.11±0.01	3.78±0.03	3.67±0.01	3.51±0.02	0.15
	Titratable acidity	0.11±0.02	0.13±0.03	0.19±0.06	0.21±0.07	0.23±0.02	0.27±0.03	0.05
L. rhamnosus	pH	6.11±0.04	4.72±0.03	4.34±0.02	4.16±0.03	3.96±0.06	3.88±0.02	0.22
	Titratable acidity	0.05±0.02	0.09±0.06	0.12±0.08	0.16±0.11	0.18±0.01	0.21±0.13	0.02
Values are mean ± SE of six independent determinations.								

Table 2: Functional properties of processed pumpkin seed flours

Treatment	Water absorption capacity (ml/100g)	Oil absorption capacity (ml/100g)	Swelling power (ml/g)
Unprocessed	178.00±0.06	82.90±0.06	2.79±0.18
Natural fermentation	284.33±6.89 (+59.74)	77.60±0.12 (-6.39)	2.39±0.04 (-14.34)
L. acidophilus fermentation	296.10±8.50 (+66.35)	79.33±0.23 (-4.31)	2.47±0.02 (-11.47)
L. rhamnosus fermentation	283.67±9.33 (+59.37)	78.17±0.12 (-5.71)	2.05±0.05 (-26.52)
CD ($P \leq 0.05$)	0.89	0.67	0.21
Values are mean ± SE of six independent determinations. Values in parenthesis indicate per cent change over control.			

Conclusion

It may be concluded that pumpkin seeds which are otherwise discarded as vegetable waste can successfully be used for value addition using simple processing methods such as fermentation. The fermented powder developed from pumpkin seeds have functional properties making them suitable for product development. Such developed products will have a good protein and mineral profile and therefore can be used to combat malnutrition.

Conflict of interest: None

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