

## Herbal Fortification of Bread with Fennel Seeds

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### Summary

Fennel seeds are a source of many nutrients, *i.e.* sugars, minerals, essential fatty acids, vital vitamins, protein and fibre. They are also rich in essential oil and many flavonoids. The sweet-flavoured fennel seeds are very popular worldwide and are extensively used in culinary and therapeutic fields. Bread fortified with fennel seed powder is thus likely to have higher consumer acceptability than white or other fortified bread. In this study, bread samples were prepared by supplementing white flour with fennel seed powder at the levels of 3.0, 5.0, 7.0, 10.0 and 15.0 % and their physical, sensory, antioxidant and staling characteristics were determined. Crumb moisture increased and reached the maximum at 7.0 % level of supplementation with gradual increase in crumb firmness. An attempt has been made to understand the role of fennel seed powder in the bread samples during storage. Antioxidant activity (*i.e.* total phenolic content, ferric reducing antioxidant power and 2,2-diphenyl-1-picrylhydrazyl radical scavenging) showed an increase up to 7.0 % level of supplementation. Bread with fennel seed content between 5.0 and 7.0 % showed the highest acceptability among the fortified bread samples.

*Key words:* fennel seeds, sensory evaluation, crumb moisture, crumb firmness, starch retrogradation, antioxidants

### Introduction

Herbal fortification of white bread is a new trend to improve its nutritional value. Herbs are rich in minerals, vitamins, flavouring agents and natural antioxidants. Roots, stems, leaves or seeds of herbal plants have long been used in cooking and in naturopathy all over the world. In a recent communication, we have reported the fortification of white bread with coriander leaf powder. This supplementation imparted a spicy flavour, greatly improved taste and sensory properties, and enhanced the level of natural antioxidants (1). Another commonly used herb is fennel, which deserves special consideration as a potential candidate for fortification of white bread.

Fennel (*Foeniculum vulgare* var. *dulce*) is an edible herb commonly used for savoury formulations, sauces, liqueurs, confectionary, *etc.* The herb has immense applications in therapeutic and culinary fields worldwide.

Traditionally, fennel extracts have been used as antispasmodic, diuretic, carminative, analgesic, antipyretic, and anti-inflammatory agent. The herb can be used to treat skin disorders, conjunctivitis and blepharitis (2–6). It is also highly recommended for the treatment of diabetes, bronchitis, chronic coughs, kidney stones and has galactogenic properties (7–9). The fennel essential oil consists of anethole, estragole and fenchone as the major constituents (3,4). Fennel is also rich in sugars (10), minerals (11), essential fatty acids (7,12), proteins and fibres (13). Essential oil and extracts of fennel seeds have strong antioxidant and antimicrobial activities (2,14). Oktay *et al.* (14) suggested that it can be used against oxidative deterioration, and thus can be used as a food supplement or in pharmaceutical industry. Fennel seeds contain numerous flavonoid antioxidants like kaempferol and quercetin. These compounds function as powerful antioxidants protecting human body from cancer, infection, ageing and degenerative neurological diseases.

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Fennel seeds are a concentrated source of metals like copper, iron, calcium, potassium, manganese, selenium, zinc and magnesium. The seeds indeed are a storehouse for many vital vitamins, *i.e.* vitamins A, E, C as well as many of the B-complex components like thiamin, pyridoxine, riboflavin and niacin.

Unlike herb leaves that wither and lose most of their nutritional value during storage at room temperature, the herbal seeds like fennel have additional advantage of being stored for more than a year without any significant loss of their beneficial properties.

Against the background of this information, the present investigation was undertaken with clear objectives of: (i) evaluating the effects of fennel seed powder on the sensory, textural and baking characteristics of the fortified bread samples, (ii) examining its staling properties (crumb firmness and crumb moisture content) over a four-day storage period at ambient temperature, and (iii) assessing the extent of augmentation of its antioxidant content.

## Materials and Methods

### Bread ingredients

Commercial bread-making wheat flour with (in %): moisture 13.2, ash 0.5, protein 11.15 and gluten 10.62 (15) was used in bread preparation. Sugar, shortening (refined oil), and salt were purchased from the local stores of Jadavpur, Kolkata, India. Compressed baker's yeast (Saf Yeast Company Pvt. Ltd., Mumbai, India) was used as the leavening agent. Glycerol monostearate (Loba Chemie Pvt. Ltd., Mumbai, India) was used in the bread formulation.

### Preparation of fennel powder

Packaged fennel seeds were bought from the local grocery shop of Jadavpur. They were then heated to  $(41\pm 2)$  °C for 1 h in a hot air oven and then ground to powder in a commercial kitchen grinder (Prestige Stylo Mixer Grinder, Prestige, Bangalore, India). The obtained powder was then sifted to obtain the fennel powder of particle size less than 150  $\mu\text{m}$  (BS 100).

### Preparation of composite flour

To obtain the flour blends for bread preparation, 100 g of wheat flour was mixed with 3.0, 5.0, 7.0, 10.0 and 15.0 % of fennel powder.

### Chemicals

The 2,4,6-tripyridyl-*s*-triazine (TPTZ; HiMedia Laboratories Pvt. Ltd., Mumbai, India), anhydrous ferric chloride (Rankem, New Delhi, India), ferrous sulphate heptahydrate, acetone, methanol, ethanol, Folin-Ciocalteu reagent, sodium carbonate (Merck Specialities Pvt. Ltd., Mumbai, India), gallic acid (sd fine-chem Ltd., Mumbai, India) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) were used in the investigation.

### Bread preparation

The bread ingredients included (in %): compressed yeast 2.5, sugar 5.0, salt 2.0, shortening 5.0, glycerol monostearate 1.5 and water 60.0 per 100 g of wheat flour. Flour blends were prepared by supplementing 3.0, 5.0, 7.0, 10.0 or 15.0 % of the fennel seed powder to 100 g of flour. The dry ingredients, shortening and activated yeast were mixed with the required amount of water and then kneaded for about 10 min until the dough was elastic and of desired consistency. The compressed yeast was activated with 1 g (approx.) of each flour and sugar in 10 mL of water at 36 °C for 15 min. The dough was then rounded and kept in a bowl for the first proofing at room temperature for about 40 min. A wet cloth was covered over the bowl to maintain a relative humidity of 80–90 %. After the first proofing, the dough was punched and worked up lightly so that the excess CO<sub>2</sub> could escape and the gas cells were redistributed in size and space. The dough was then shaped to fit lightly in greased bread moulds, and kept for final proofing for about 1 h at  $(40\pm 1)$  °C. Finally, after second proofing, the loaves of bread in moulds were baked in a rotary oven (CM HS108, Chanmag Bakery Machine Co. Ltd., New Taipei City, Taiwan) at  $(220\pm 2)$  °C for 20 min. The prepared bread samples were cooled for about 1 h at room temperature and subsequently analyzed for their relevant physical and chemical properties. Fresh bread samples were stored at room temperature  $(29\pm 1)$  °C in Zip-foil<sup>®</sup> metallised food bags (UFELX LIMITED, Noida, India) for four days, and crumb firmness and crumb moisture content were determined on subsequent days (1,16).

### Bread analyses

Bread samples were weighed and the volume was determined by the seed displacement method (17). Specific volume (in mL/g) was calculated by dividing loaf volume by loaf mass. Crumb and crust colour of the fresh bread loaves were measured with Hunter Lab colour measurement system, ColorFlex 45/0, D65, 10° observer (Hunter Associates Laboratory Inc., Reston, VA, USA). Colour readings were expressed in CIELAB system of colour measurement as  $L^*$  (whiteness/darkness),  $a^*$  (redness/greenness) and  $b^*$  (yellowness/blueness). Crumb moisture content of the samples was determined by AA-CC approved oven method 44–19 (15). Crumb firmness of the loaves was determined by Instron Universal Testing Machine, Table Model 4301 (Instron Ltd., High Wycombe, Bucks, UK) in the compression mode fitted with a 100-N load cell (18,19). The bread samples were sliced and the middle slices, having a height of 25 mm, were placed horizontally under a flat plate probe. Firmness of the bread crumb was measured using a 40-mm diameter cylindrical probe at 20 mm/s compression speed to 50 % crumb thickness compression.

### Sensory evaluation

Tests on sensorial properties of the fresh loaves of bread were carried out by a panel of 10 members consisting of students and the staff of the Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, India. A nine-point hedonic scale ranging from like extremely (9) to dislike extremely

(1) was used to evaluate the colour, flavour, taste, texture and overall acceptability of the bread formulations. The panellists were seated in individual sensory booths. The bread samples were sliced and placed on white plates. Samples were assigned random codes. Each panellist was served a control bread sample along with the test samples, and water was provided for rinsing mouth in between. The evaluation process was repeated three times. Appearance was judged on the basis of size, shape and uniformity of the bread samples. Colour was evaluated as the increase in the intensity of the light brown colour of the crumb and the brown colour of the crust of bread samples. Aroma was judged according to the sweet, pleasant smell of the fennel seeds. Taste was assessed on the basis of the mouthfeel of the samples. Texture was evaluated according to the softness and springiness of the bread samples.

### Antioxidant activity

#### Total phenolic content

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method (20,21). Samples (2 g) were homogenized in 80 % aqueous ethanol at room temperature (approx. 25 °C) and centrifuged in cold at 10 000×g for 15 min, and then the supernatant was extracted. The obtained residue was re-extracted twice and the supernatants were collected, poured into Petri dishes and evaporated to dryness at room temperature. The residue was dissolved in 5 mL of distilled water. A volume of 100 µL of these extracts was diluted to 3 mL with water and 0.5 mL of Folin-Ciocalteu reagent was added. After 3 min, 2 mL of 20 % sodium carbonate were added and the contents were mixed thoroughly. A colour developed and the absorbance was measured at 750 nm in a UV-Vis spectrophotometer (U 2800, Hitachi, Tokyo, Japan) using gallic acid as a standard. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh material.

#### Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay was based on the reduction of Fe<sup>3+</sup>-TPTZ to a blue coloured Fe<sup>2+</sup>-TPTZ (22), according to the method adapted by Wanyo *et al.* (23). A mass of 2 g of each sample was extracted with 1:1 aqueous acetone solution (10 mL per g) in an ultrasonic bath for 1 h. The solution was then centrifuged at 12 000×g for 15 min and the supernatant liquid was collected for further analysis. The FRAP reagent was freshly prepared by mixing acetate buffer (pH= 3.6), TPTZ solution (10 mM TPTZ in 40 mM HCl) and 20 mM FeCl<sub>3</sub> solution at a ratio of 10:1:1. Sample volumes of 100 µL were taken and 3 mL of FRAP reagent were added. The absorbance was measured after 0 and 30 min (following incubation at 37 °C) at 593 nm. The antioxidant potential of the extracts was determined from a standard curve plotted for different concentration of FeSO<sub>4</sub>·7H<sub>2</sub>O solution. The results were expressed as mg of FeSO<sub>4</sub> per 100 g of sample.

#### DPPH radical scavenging assay

The DPPH assay was based on the methods of Brand-Williams *et al.* (24) and Michalska *et al.* (25). To prepare the extract, bread samples were sliced (3 cm width and 1

cm thickness) and dried in an oven at 40 °C for 24 h. The dried material was ground in a grinder to obtain powdered bread, which was then extracted with 80 % aqueous methanol (10 mL per g) for 2 h at 37 °C. Samples were then centrifuged at 12 000×g for 15 min. The collected supernatant was used in the assay. The DPPH radical solution was prepared by dissolving 10 mg of DPPH in 25 mL of 80 % methanol. Bread extracts (100 µL) were then mixed with 250 µL of DPPH solution and 2.0 mL of 80 % methanol, shaken vigorously and allowed to stand at room temperature for 20 min. The decrease in the absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The percentage of inhibition or the percentage of discolouration was calculated as follows:

$$\text{Inhibition}/\% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad /1/$$

### Statistical analysis

All the studies were repeated three times and the mean values were calculated. All the experimental data were analyzed with analysis of variance (ANOVA) using STATISTICA v. 8 (StatSoft Inc., Tulsa, OK, USA). The mean values were compared and grouped by Fisher's least significant difference test at a significance level of  $p \leq 0.05$  using STATISTICA v. 8.

## Results and Discussion

### Bread loaf size

The volume of the bread samples (control and treated) showed a significant decrease ( $p \leq 0.05$ ) when increasing the level of fennel seed addition, although the loaf mass did not show any significant difference up to 7.0 % level of supplementation (Table 1). Gluten network impairment due to wheat flour substitution and interaction between gluten and fibre (26) led to lowering of the CO<sub>2</sub> retention capacity of the dough, thus decreasing the loaf volume. Sivam *et al.* (27) also stated that two main reasons for reduced loaf volume in fibre-supplemented bread samples are the dilution of gluten and the interactions among fibre components, water and gluten. When 7.0 % of wheat flour was substituted, the fibrous fennel seed powder absorbed and retained water during dough making and baking processes respectively, thus increas-

Table 1. Physical properties of bread samples supplemented with fennel seed powder at different levels

<i>w</i> (fennel seed)	<i>m</i> (loaf)	<i>V</i> (loaf)	<i>v</i> (loaf)
%	g	mL	mL/g
0.0	(152.6±1.62) <sup>b</sup>	(490.6±2.14) <sup>a</sup>	(3.21±0.025) <sup>a</sup>
3.0	(151.3±2.04) <sup>b</sup>	(474.8±2.92) <sup>b</sup>	(3.14±0.026) <sup>b</sup>
5.0	(152.6±2.01) <sup>b</sup>	(464.7±1.98) <sup>c</sup>	(3.05±0.030) <sup>c</sup>
7.0	(152.4±2.88) <sup>b</sup>	(448.6±3.39) <sup>d</sup>	(2.94±0.035) <sup>d</sup>
10.0	(156.3±0.86) <sup>a</sup>	(413.8±1.74) <sup>e</sup>	(2.65±0.006) <sup>e</sup>
15.0	(159.1±1.30) <sup>a</sup>	(391.0±1.26) <sup>f</sup>	(2.46±0.021) <sup>f</sup>

Data represent mean value of three samples ( $N=3$ )±standard deviation (S.D.)

Mean values with different superscripts (a–f) within the same column are significantly different ( $p \leq 0.05$ )

ing the loaf mass. Specific loaf volume of the bread samples showed a decreasing trend when increasing the fennel seed powder supplement.

### Bread loaf colour

The crust of the control and treated bread samples showed more or less decreasing variation of  $L^*$ ,  $a^*$  and  $b^*$  values with increasing fennel seed powder supplementation, with some anomalies at 10.0 and 15.0 % (Table 2). The crust colour is mainly influenced by the Maillard browning reaction rather than the colour of the herb. The crumb  $L^*$  value of the bread samples showed almost significant decrease ( $p \leq 0.05$ ) with increasing herb addition. On the other hand,  $a^*$  and  $b^*$  values of the bread crumbs increased almost significantly ( $p \leq 0.05$ ) with the increase in fennel seed powder supplementation. This is due to the light brown colouration of the fennel seed powder, which has a greater impact on the lightness ( $L^*$ ) and redness ( $a^*$ ) of the treated than on the untreated bread samples.

### Sensory properties

Sensorial properties, *i.e.* appearance, aroma, colour, taste, texture and overall acceptability of the bread samples were evaluated and the results are shown in Table 3. The appearance of the bread samples was not significantly different among the bread samples supplemented with 0, 3.0, 5.0 and 7.0 % fennel seed powder. The shape of loaves with 10.0 and 15.0 % fennel seed powder was different and not uniform.

White bread has a typical yeasty aroma. The loaves of bread supplemented with 7.0 and 10.0 % fennel seed powder scored significantly higher than the white bread, suggesting that the panel favoured the sweet aroma of fennel over the yeasty aroma of white bread. Loaves of bread with 3.0 and 5.0 % fennel had faint aroma of fennel, which was not sufficient to mask the yeasty aroma. On the other hand, bread with 15.0 % powder had a strong aroma of fennel, which was not preferred by the panel.

With respect to colour, the light brown colour of the fennel seed powder was preferred by the panellists. Loaves of bread with 5.0 and 7.0 % were most preferred by the panellists. With 3.0 % powder, the amount of fennel was not sufficient to bring in any significant change in the colouration whereas with 10.0 and 15.0 % fennel powder, the colour of the crumbs was dark and not appreciated by the panel.

Supplementation of fennel seed powder up to 10.0 % was found to enhance the taste of bread samples. The addition of 7.0 and 10.0 % fennel was found to be optimal. Loaves supplemented with 3.0 and 5.0 % fennel had lower scores owing to the lower amount of fennel. In the case of 15.0 % supplementation, the taste was impaired due to bitterness.

In general, supplementation with fennel seed powder had a negative effect on the texture of the bread. There was a progressive degeneration in the texture with increasing levels of fennel seed powder, which was further corroborated by statistical analysis.

Table 2. Colour parameters of the bread samples supplemented with fennel seed powder at different levels

$w(\text{fennel seed})$ %	Crust			Crumb		
	$L^*$	$a^*$	$b^*$	$L^*$	$a^*$	$b^*$
0.0	(45.8±0.46) <sup>a</sup>	(17.6±0.13) <sup>a</sup>	(31.2±0.24) <sup>a</sup>	(78.0±0.13) <sup>a</sup>	(0.6±0.09) <sup>e</sup>	(21.6±0.49) <sup>e</sup>
3.0	(38.2±0.14) <sup>b</sup>	(15.9±0.11) <sup>b</sup>	(23.6±0.19) <sup>c</sup>	(65.6±0.15) <sup>b</sup>	(0.7±0.04) <sup>e</sup>	(26.9±0.32) <sup>d</sup>
5.0	(37.3±0.38) <sup>cd</sup>	(14.8±0.18) <sup>d</sup>	(23.1±0.26) <sup>d</sup>	(60.0±0.40) <sup>c</sup>	(1.1±0.02) <sup>d</sup>	(27.7±0.23) <sup>c</sup>
7.0	(37.1±0.45) <sup>d</sup>	(13.5±0.11) <sup>e</sup>	(23.9±0.61) <sup>c</sup>	(59.6±0.35) <sup>c</sup>	(1.9±0.13) <sup>c</sup>	(29.5±0.37) <sup>b</sup>
10.0	(38.0±0.34) <sup>b</sup>	(15.4±0.20) <sup>c</sup>	(24.7±0.30) <sup>b</sup>	(55.8±0.11) <sup>d</sup>	(2.7±0.08) <sup>b</sup>	(30.6±0.02) <sup>a</sup>
15.0	(37.8±0.08) <sup>bc</sup>	(14.6±0.05) <sup>d</sup>	(23.8±0.08) <sup>c</sup>	(53.1±0.37) <sup>e</sup>	(3.5±0.15) <sup>a</sup>	(29.5±0.33) <sup>b</sup>

Data represent mean value of three samples ( $N=3$ )±S.D.

Mean values with different superscripts (a–e) within the same column are significantly different ( $p \leq 0.05$ )

Table 3. Sensorial properties of the bread samples supplemented with fennel seed powder at different levels

$w(\text{fennel seed})$ %	Appearance	Aroma	Colour	Taste	Texture	Overall acceptability
0.0	(8.6±0.49) <sup>a</sup>	(5.6±0.50) <sup>e</sup>	(5.6±0.57) <sup>e</sup>	(6.3±0.48) <sup>e</sup>	(8.6±0.50) <sup>a</sup>	(7.2±0.61) <sup>e</sup>
3.0	(8.5±0.51) <sup>a</sup>	(6.3±0.45) <sup>d</sup>	(6.5±0.51) <sup>c</sup>	(7.0±0.62) <sup>d</sup>	(8.2±0.61) <sup>b</sup>	(7.5±0.51) <sup>d</sup>
5.0	(7.7±0.48) <sup>b</sup>	(6.9±0.40) <sup>c</sup>	(7.6±0.50) <sup>b</sup>	(7.5±0.51) <sup>c</sup>	(8.0±0.53) <sup>bc</sup>	(8.3±0.47) <sup>b</sup>
7.0	(7.9±0.57) <sup>b</sup>	(7.4±0.50) <sup>b</sup>	(8.5±0.51) <sup>a</sup>	(8.5±0.51) <sup>a</sup>	(7.8±0.41) <sup>c</sup>	(8.6±0.50) <sup>a</sup>
10.0	(6.8±0.55) <sup>c</sup>	(8.0±0.53) <sup>a</sup>	(6.0±0.64) <sup>d</sup>	(8.0±0.59) <sup>b</sup>	(6.7±0.47) <sup>d</sup>	(7.9±0.61) <sup>c</sup>
15.0	(5.9±0.58) <sup>d</sup>	(5.0±0.46) <sup>f</sup>	(5.1±0.55) <sup>f</sup>	(5.4±0.56) <sup>f</sup>	(5.4±0.49) <sup>e</sup>	(5.7±0.58) <sup>f</sup>

Data represent mean value of three samples ( $N=3$ )±S.D.

Mean values with different superscripts (a–f) within the same column are significantly different ( $p \leq 0.05$ )



In terms of overall acceptability, with the addition of 5.0 and 7.0 % of fennel the most superior bread samples were obtained. This may be attributed to higher scores in aroma, taste and colour with respect to the white bread. The loaves with 3.0 and 15.0 % fennel powder were either found to have less or more than optimal amount of fennel necessary for overall acceptability.

#### Crumb moisture content

The data in Fig. 1 show that throughout the 4 days of storage, the moisture content steadily increased to a maximum value and then decreased with the increase in supplementation level. The maxima occurred somewhere around 7.0 % fennel addition on all the 4 days of storage, with the exception of bread sample with the addition of 10.0 % fennel at 3 days of storage.

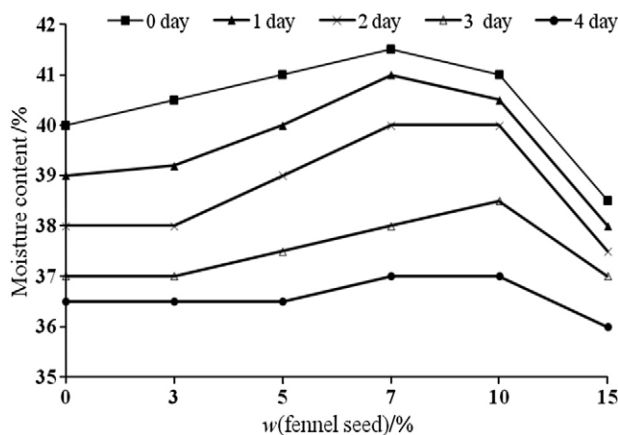


Fig 1. Crumb moisture of the bread samples with fennel seed powder supplementation at different storage days

The commonly accepted level of moisture content in bread is known to be within a range of 35–40 % (28). In this case, bread samples during 4 days of storage were found to have a minimum moisture content of 36 % and in some cases above 40 %. This may be reasoned by the fact that the introduction of fennel powder in bread lead to a higher retention of crumb moisture. The highest moisture content was seen in bread with 7.0 % fennel at day zero.

Fig. 1 also shows that, at any particular substitution level, crumb moisture content has a decreasing trend with longer storage. This was consistent with the time-dependent staling process.

#### Crumb firmness

The variation of crumb firmness with the addition of different levels of fennel during different storage periods is shown in Fig. 2. At 0, 1 and 2 days of storage, crumb firmness continued to increase steadily with the addition from 5.0 up to 15.0 %. The trend was somewhat different after 3 and 4 days of storage, when a steady increase in firmness of bread samples with 3.0 % fennel was observed.

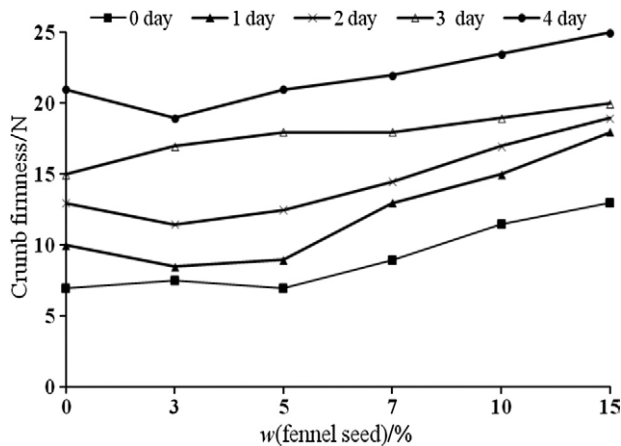


Fig 2. Crumb firmness of the bread samples with fennel seed powder supplementation at different storage days

#### Role of fennel seed powder

It is generally accepted that the moisture in bread is almost exclusively retained by the gluten network that constitutes the dough matrix. In a filled system, however, the filler (fennel seed powder) itself can absorb water quite readily and with increasing proportion of water, the dough suffers decrease in viscosity and, hence, in firmness. On the contrary, fennel powder absorbs water rather slowly, has significant water saturation limit, and yet maintains its solid consistency to act as reinforcing filler to the dough matrix. There are at least four important but complicating factors that determine both the moisture content and firmness of the finished dough and, in turn, of the finished bread: (i) when the volume of water per 100 g of dry mix of wheat flour and fennel powder is kept constant at 60 mL, the firmness of the dough matrix decreases with increasing proportion of fennel powder because the same volume of water is mostly absorbed by a smaller amount of wheat flour compared to control dough; (ii) a higher proportion of fennel powder, which in fact reinforces the matrix, increases the viscosity or the firmness of the finished dough/bread; (iii) there is an equilibration of moisture between the matrix and the dispersed phase all along the processes of mixing, subsequent proofing and baking, and then retrogradation of starch during storage of bread, and (iv) the cellulosic fibre content of fennel may offer surface-induced nucleation and crystallization of amylopectin chains leading to an enhanced rate of staling and to increased crumb firmness. Crystalline amylose has a reported melting point at around 150 °C, but amylopectin shows melting point over a range of 60–80 °C, and can absorb water to the extent of about 37 % of its mass (29). The released water of crystallization is largely retained by the filler cellulosic fibre. The net effect is the stiffening of the gluten matrix without any significant decrease in total moisture content. Rationalization and quantification of these factors in predicting the crumb moisture and firmness need further experiments, which are beyond the scope of the present investigation.

In loaves of bread without the addition of fennel, crumb firmness increased with the decrease in moisture content. During storage, the stretched amylopectin chains reorganize themselves to a more compact *para*-crystalline network and release water molecules bound within interchain spaces, but this interrelation cannot be extended to filled bread systems like the one of fennel-supplemented bread. Throughout the entire process of bread-making, from preparation to consumption, a continuous redistribution of moisture takes place between the dough (as the matrix) and the fennel powder (as the filler). Thus, the water released during retrogradation of starch may be in part or completely absorbed by fennel powder if its moisture content is below its saturation level. The net effect is that the total moisture content of the bread suffers only a little or no change, but with an enhanced firmness. It seems that the fennel seed powder serves as a buffer to prevent moisture loss due to starch retrogradation.

To summarize these results, the conventional interpretation of increasing crumb firmness as being exclusively due to decreasing crumb moisture needs a critical introspection into the basic nature and the level of supplementation of the agents used for fortification of white breads.

#### Antioxidant activity

The graphs in Fig. 3 show the relationship between total phenolic content, DPPH radical scavenging and FRAP assay. Total phenolic content is an important parameter to study as it determines the polyphenolic content of the sample which is interrelated with the antioxidant activity of the sample (21).

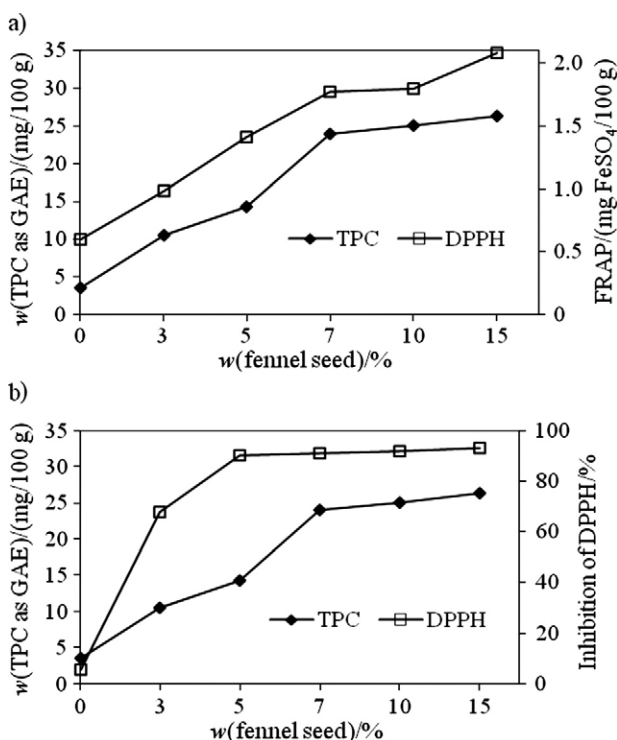


Fig 3. Relationship between total phenolic content (TPC) and: a) FRAP and b) DPPH assays of the bread samples

Graphically, Fig. 3a shows a parallel evolution of both TPC and FRAP with increasing level of fennel seed powder. However, this does not hold in the case of TPC and DPPH radical scavenging, where the seemingly parallel trend of DPPH scavenging breaks at 5.0 % level of supplementation (Fig. 3b). Except for DPPH, in either case of TPC or FRAP, there was a steady increase of TPC with increasing fennel seed powder content up to the supplementation of 7.0 % fennel. Above this level, the rate of increase slowed down and the graph gradually flattened out. In the case of DPPH, above the supplementation of 5.0 %, the same behaviour was observed. One important reason is that upon a certain level, the polyphenolic compounds may interact with the wheat starch or protein molecules during dough preparation/baking, forming large complexes and degrading some of the phenolic compounds, thus reducing antioxidant activity (30–32). Thus, at the supplementation level of 7.0 % fennel, the phenolic antioxidants coexist naturally with other components of wheat. Therefore, from this study, we can infer that the bread samples with 7.0 % fennel seed powder had an optimized antioxidant activity.

#### Conclusions

Fennel seeds are a sweet spice used worldwide in various cuisines. Bread fortified with fennel seed powder showed high moisture content in the crumbs, rich antioxidant content and good consumer acceptability when up to 7.0 % of fennel seed powder was added. Crumb firmness followed an increasing trend with the increase in the fennel seed powder content. It can thus be inferred that the optimum supplementation level of fennel seed powder ranged between 5.0 and 7.0 % per 100 g of wheat flour.

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