

Evaluation of the Nutraceutical Properties of Enriched Bread

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ABSTRACT

Nowadays, people are more concerned with their health and more focused on purchasing products that boost their bodies' defenses. Therefore, there has been an increase in interest in the development of new goods as well as the use of frequently wasted food byproducts. This study explores the formulated enriched bread with different seeds (melon, pumpkin, sesame). In the first phase, the fortified bread was made by using different ingredients in four different compositions of seeds (6%, 12%, 18%, and 24%). In the second phase, the proximate composition (moisture, ash, crude fiber, fat, protein, carbohydrate {CHO}, iron, calcium), antioxidant analysis (vitamin C, DPPH, total phenols), phytochemical screening (alkaloids, flavonoids, glycosides, phenolic compounds, saponins, tannins, phytosterols), and shelf-life study (pH, temperature) were done. In the third phase, sensory evaluation was done by a 9-point hedonic scale. In the fourth phase, the statistical analysis was done by using mean, standard deviation, and One-Way Analysis of Variance (ANOVA). The results showed that proximate composition and antinutrient analysis of variant D was higher. Also, they had a significant difference ($p < 0.05$), except for vitamin C and DPPH. In phytochemical screening, all phytochemicals are present in all variants. The shelf life is maximum up to 5-6 days. And according to the sensory evaluation, variant A was more acceptable. In conclusion, fortifying bread with seeds represents a promising strategy to enhance both the nutritional and sensory aspects of this staple food, promoting a healthier and more enjoyable dietary option for consumers.

HIGHLIGHTS

- ① Developing novel and inventive products that have never been produced before is the main topic of this paper.
- ① This product is made from seeds, which contributes to its nutritious balance.
- ① The product was prepared in four different compositions among which variant A was most acceptable.

Keywords: Fortified bread, nutrient analysis, seeds, shelf-life, sensory evaluation

The contemporary era's increase in life quality has a direct impact on the pace of population expansion in the world, causing overuse of human resources and increasing waste output. "Waste" refers to things that are no longer in use or materials that are useless. As a result, both environmental damage and ineffective waste management are caused by increased trash output (van Ewijk and Stegemann 2020; Singh *et al.* 2022). Consumers today place a greater emphasis on buying goods that strengthen their body's defenses because they have grown concerned with their well-being. In order to preserve their health and prevent many different ailments including weight gain, diabetes, and heart

disease, consumers today prefer foods with high fiber and protein fiber contents. As a result, there has been a growing interest in the creation of new products and the utilization of food by-products that are often discarded (Bhatt and Gupta 2015; Singh *et al.* 2020).

Bread which is a baked product made using of wheat flour constitutes one of the ancient and most

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widely consumed foods and is enjoyed by people of all ages worldwide (Bhatt and Gupta 2015). In terms of weight, form, crust hardness, softness, crumb cell structure, and color, there are several types of bread. Every population acknowledges the value of bread as a highly practical food source (Ijah *et al.* 2014). Bread has a high caloric content and is high in fat, but it is deficient in other elements including protein, vitamins, and minerals. Numerous research has looked into the possibility of replacing wheat flour with other types of flour in order to improve the nutritional content of bread (Bolarinwa *et al.* 2019). Given the widespread use of bread, fortifying it is a smart approach to avoid vitamin shortages and raise the intake of antioxidants and fiber. A growing number of people are requesting the use of wholegrain cereals as well as the enrichment of bread with different edible seeds, which are a valuable source of antioxidants and nutritional fiber (Benítez *et al.* 2018).

A member of the Cucurbitaceae family, melon or cantaloupe (*Cucumis melo* L.) is one of the most widely grown cucurbits every year in regions of India, Africa, Asia, and Europe. It is an annual vine that has some hair on it. Despite preferring a hot temperature, it thrives in all tropical and subtropical regions of the planet. The melon's center is filled with a lot of seeds as shown in Fig. 1 (a). The seeds are thrown away and the fruit is eaten whole. The output of melon seeds is estimated at 782,205 tonnes, and its use for melon seed farming is 893,855 hectares, according to the Food and Agriculture Organisation. It is a common fruit because of its great flavor and use in traditional medicine as a therapeutic herb. The primary conditions for which this is advised include gout, anemia, atherosclerosis, rheumatism, cardiovascular, renal, and liver illnesses. Melon seeds have medicinal properties such as analgesic, anti-ulcer, anti-cancer, anti-diabetic, anti-inflammatory, antioxidant, anti-microbial, anthelmintic, and free radical scavenging activity actions (Dhami 2021; Kumar *et al.* 2022). Due to its delicious flavor and diverse chemical structure, melon is a great source of naturally active chemicals for people. Melon seeds include 4.5% moisture, 2.4% ash, 25.0% crude protein, 23.3% crude fiber, 25.0% crude fat, and 19.8% CHO. Melon contains glucose, fructose, vitamins A, D, C, E, and K, as well as certain B-group vitamins. Melon contains

4.6 to 18% sugar and up to 4.5% pectin by weight. Melon also contains minerals including sodium, magnesium, calcium, phosphorus, potassium, and selenium. Different aromatic compounds are also found in melon along with all of the aforementioned makeup (Khalid *et al.* 2021).

A member of the Cucurbitaceae family, pumpkin (*Cucurbita pepo* L.) is a medium plant sized grown for its fruit and edible seeds. Pumpkin seeds are large, plentiful, and palatable as shown in Fig. 1 (b). However, the majority of these seeds are wasted as agro-industrial waste. Due to the nutritious and health-promoting qualities of the seeds, pumpkin has attracted a lot of attention lately. Toasted pumpkin seeds are loved in several areas of the world. In Eritrea, Africa, pumpkin seeds are used to cure tapeworms. The treatment of problems of the bladder, prostate, and kidneys is another traditional use (Patel and Rauf 2017). The seed contains medicinal benefits including hyperlipidemia, hypertension, anti-ulcerative, anti-cancer, anti-diabetic, antifungal, antibacterial, antioxidant, and anti-inflammation actions in addition to being a great source of protein (Syed *et al.* 2019; Batool *et al.* 2022). Due to their abundance of antioxidants such as carotenoids and tocopherols, pumpkin seeds provide a unique chance to include this ingredient in specialty loaves of bread. Although the notion of adding nutrients to meals is not new, the kinds of foods chosen and the number of nutrients added will depend on the individual's specific nutritional demands. Oil from pumpkin seeds is extracted and utilized in cooking or as emollients (Jasper *et al.* 2020). They are rich in protein, pyrazine, pigments, squalene, coumarins, phytosterols, saponins, triterpenoids, flavonoids, phenolic compounds and their derivatives, polyunsaturated and monosaturated fatty acids, carotenoids, provitamins, and vitamin E (tocopherols). Pumpkin seeds are also an excellent source of minor minerals including zinc, iron, manganese, calcium, copper, and sodium. They are also a good source of magnesium, potassium, and phosphorus (Dotto and Chacha 2020).

A member of the Pedaliaceae family, sesame (*Sesamum indicum* L.) is one of the oldest oil crops. The output of sesame seeds has grown dramatically over the past three decades, rising from 6.3 million hectares (Mha) and 2.8 million tonnes (Mt) in 2002

to 11 Mha and 6.2 Mt in 2016. The top sesame-producing nations are India, Tanzania, and Sudan, producing 1.1 Mt, 0.8 Mt, and 0.7 Mt, respectively. It is mostly grown in Asia and Africa (FAOSTAT 2017 and FAOSTAT 2018). As a result of its mellow flavor and aromatic odor, sesame is becoming more and more popular around the globe and also increased the number of qualities and nutritious goods that use them. Sesame seeds have long been used in food preparation and are used in a variety of ways in people's lives. Sesame may be divided into three categories based on the color of the germplasm: white sesame, black sesame, and yellow sesame as shown in Fig. 1 (c). Black and white sesame are among the most prevalent and extensively farmed dominating species. Protein, dietary fiber, fat, minerals (especially calcium and iron), and vitamins are all abundant in sesame seeds. Due to the significant levels of essential and non-essential amino acids present in comparison with different seed proteins, it is regarded as a significant protein source in terms of nutrition. Sesame seeds have a higher protein content (17-40%) than both meat (18-25%) and grains (7-13%) (Abbas *et al.* 2022). Sesame seeds are known as the "crown of eight grains" and are considered an "all-purpose nutrient bank" due to their high nutritional content (Li and Lu 2015). Sesame seeds are extremely beneficial to human health and have a wide range of pharmacological effects, including anti-cancer, antioxidant, anti-inflammatory, anti-melanogenic, auditory protection, anti-cholesterol, and anti-aging properties. They also have a protective effect on the heart, liver, and kidneys (Wei *et al.* 2022).

The objective of the current study seeks (i) to develop

a useful or nutritious bread by incorporating different seeds like melon seeds, pumpkin seeds, and sesame seeds at different ratios as 6%, 12%, 18%, and 24% respectively; (ii) to analyze the proximate composition, antinutrients analysis, phytochemical screening, and shelf-life study of formulated bread with readymade bread (Control); (iii) to conduct the acceptability evaluation; and (iv) statistical analysis were done.

MATERIALS AND METHODS

Collection of Raw materials

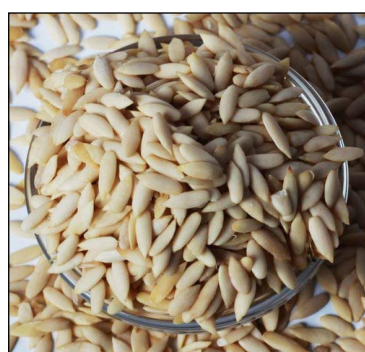
The raw materials like muskmelon seeds, pumpkin seeds, sesame seeds, multigrain flour, and yeast of the food product were collected from the market of Banasthali Vidyapith, Tonk, Rajasthan.

Food Product Development

The fortified bread was made by using different ingredients in four different variants of seeds. The fortified bread was prepared in the Cooking Laboratory of Banasthali Vidyapith, Rajasthan.

Proximate Composition

The proximate composition of formulated bread was analyzed by moisture, ash, crude fiber by acid and alkali treatment method, fat by soxhlet method, and protein by micro-kjeldahl method. The mineral composition includes iron by wong's method, and calcium by titrimetric method. The amount of CHO was calculated by deducting the sum of the contents of the moisture, ash, protein, fat, and fiber from 100 (Raghuramulu 2003 and Sharma 2007).



(a)



(b)



(c)

Fig. 1: (a) Melon seeds; (b) Pumpkin seeds; and (c) Sesame seeds

Antioxidant Analysis

The antioxidant analysis was done by vitamin C by the titrimetric method (Raghuramulu 2003).

The scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical was used to assess the antioxidant activity of the alcoholic extract. 4 ml of a methanolic solution of DPPH (0.004% w/v) were combined with 0.1 ml of different strengths of the extracts in methanol. After 30 minutes, the preparations' absorbance was measured at 517 nm using a UV spectrophotometer, and the corresponding percentage inhibition of standard concentrations (10-100 µg/ml) was compared. By graphing the inhibition percentage vs extract concentration, the extract concentration that provides 50% inhibition (IC₅₀) was determined. Positive controls such as ascorbic acid were employed throughout all assays (Chand *et al.* 2012).

The Folin-Ciocalteu technique was used to measure the total phenolic content (TPC), and the standard procedure was then used. The sample was treated with sodium carbonate and the Folin-Ciocalteu reagent before being submerged for 30 minutes at 40 °C in water. At a wavelength of 720 nm, spectrophotometric analysis was evaluated. Gallic acid equivalent (GAE)/100 g of the sample was used to indicate the TPC (Singleton *et al.* 1999).

Phytochemical Screening

Before the analysis extract was prepared. The qualitative analysis was done by using alkaloids (Mayer's Test), flavonoids (Shinoda Test), glycosides (Legal's Test), phenolic compounds, saponins (Froth Test), tannins, and phytosterols (Liebermann Buchard's Test) (Harborne 1973; Raghuramulu 2003).

Shelf-life Study

The shelf-life of bread was determined by pH (potential of hydrogen) and temperature. The pH meter was used for pH determination (Sharma 2007).

Determination of temperature, before use, ensure the thermometer is clean and dry. Place the thermometer into the food and wait until the temperature reading has stabilized before reading. Clean and sanitize the thermometer after measuring the temperature of the food (Sharma 2007).

Sensory Evaluation

The sensory evaluation consists of judging the quality of food by a panel of judges. The evaluation deals with measuring, analyzing, and interpreting the qualities of food as they are perceived by the sense of smell, taste, touch, and hearing. Acceptance of the food depends upon the 9-point hedonic scale performa. It was done formally by the laboratory and by semi-trained panel members with the help of a triangle test (Chaudhary *et al.* 2023).

Statistical Analysis of Data

The mean, standard deviation, and One-Way ANOVA Test by GraphPad Prism 8.0.2 (263) software were utilized as the statistical approach for the analysis of the data for the current study. The means of the three sample analyses' values were determined. At a 5% probability level, a significant difference was found ($p < 0.05$) (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Food Product Development

The development of fortified bread with four different variants (A, B, C, and D) – low, moderate, high, and very high composition of seeds as shown in Table 1. The control variant was white bread which was obtained from the market.

Table 1: Composition of Fortified Bread

Ingredients (100 g)	Fortified Bread			
	Variant A	Variant B	Variant C	Variant D
Melon seeds	2	4	6	8
Pumpkin seeds	2	4	6	8
Sesame seeds	2	4	6	8
Multigrain flour	64	58	52	46
Tutti fruity	10	10	10	10
Salt	5	5	5	5
Sugar	5	5	5	5
Yeast	10	10	10	10

Variant A contains 6% seeds compositions (low) like 2% melon, 2% pumpkin, and 2% sesame seeds respectively; Variant B contains 12% seeds compositions (moderate) like 4% melon, 4% pumpkin, and 4% sesame seeds respectively; Variant C contains 18% seeds composition (high) like 6% melon, 6% pumpkin, and 6% sesame seeds respectively; Variant contains 24% seeds composition (very high) like 8% melon, 8% pumpkin, and 8% sesame seeds respectively.

Proximate Composition

Table 2 shows the proximate composition of different variants of fortified bread with the Control variant. The results indicated that moisture, ash, crude fiber, fat, protein, iron, and calcium were higher in variant D as compared to variants A, B, C, and Control. So, variant D was highly nutritive as compared to other variants because it contains a higher composition of seeds which increased the nutritive value of fortified bread. Only the CHO content was higher in the Control variant and in fortified bread variant A was higher. And all variants of fortified bread with Control had a significant difference ($p < 0.05$) in proximate composition as shown in Fig. 2. According to the study (Elinge *et al.* 2012; Ugwuona and Nwamaka 2016), the results were supported.

Antioxidant Analysis

Table 3 shows the antioxidant analysis of different variants of fortified bread with the Control variant. The results indicated that vitamin C is higher in variant D. It contains a higher amount of seeds composition as compared to other variants A, B, C, and Control. And all variants of fortified bread with Control had no significant (ns) difference ($p < 0.05$) in vitamin C as shown in Fig. 3.

In DPPH, variant D had strong antioxidant activity at a concentration of 0.02 $\mu\text{g/ml}$ while the standard is ascorbic acid. According to the study Yamin *et al.* (2021), free radical scavenging activities of the seeds flour were found to increase as increasing its concentration. The IC_{50} value of variant D has a low value as compared to other variants. The lower the IC_{50} value higher the antioxidant property

in variants. So, variant D has higher antioxidant property as compared to other variants. But the all variants of fortified bread with Control had no significant (ns) difference ($p < 0.05$) in DPPH as shown in Fig. 3.

Total Phenolic Content (TPC) is higher in variant D as compared to other variants. This is because variant D contains 24% composition of seeds that was higher. And all variants of fortified bread with Control had a significant difference ($p < 0.05$) in TPC as shown in Fig. 3.

Phytochemical Screening

Phytochemical intake offers several health benefits (Agarwal and Chauhan 2022). Table 4 shows the phytochemical screening of different variants of fortified bread with the Control variant. The results indicated that all phytochemical constituents like alkaloids, flavonoids, glycosides, phenolic compounds, saponins, tannins, and phytosterols were present in all the variants such as A, B, C, and D. In the Control variant, alkaloids, flavonoids, and phytosterols were absent. The results were supported by the study (Bhatt and Gupta 2015).

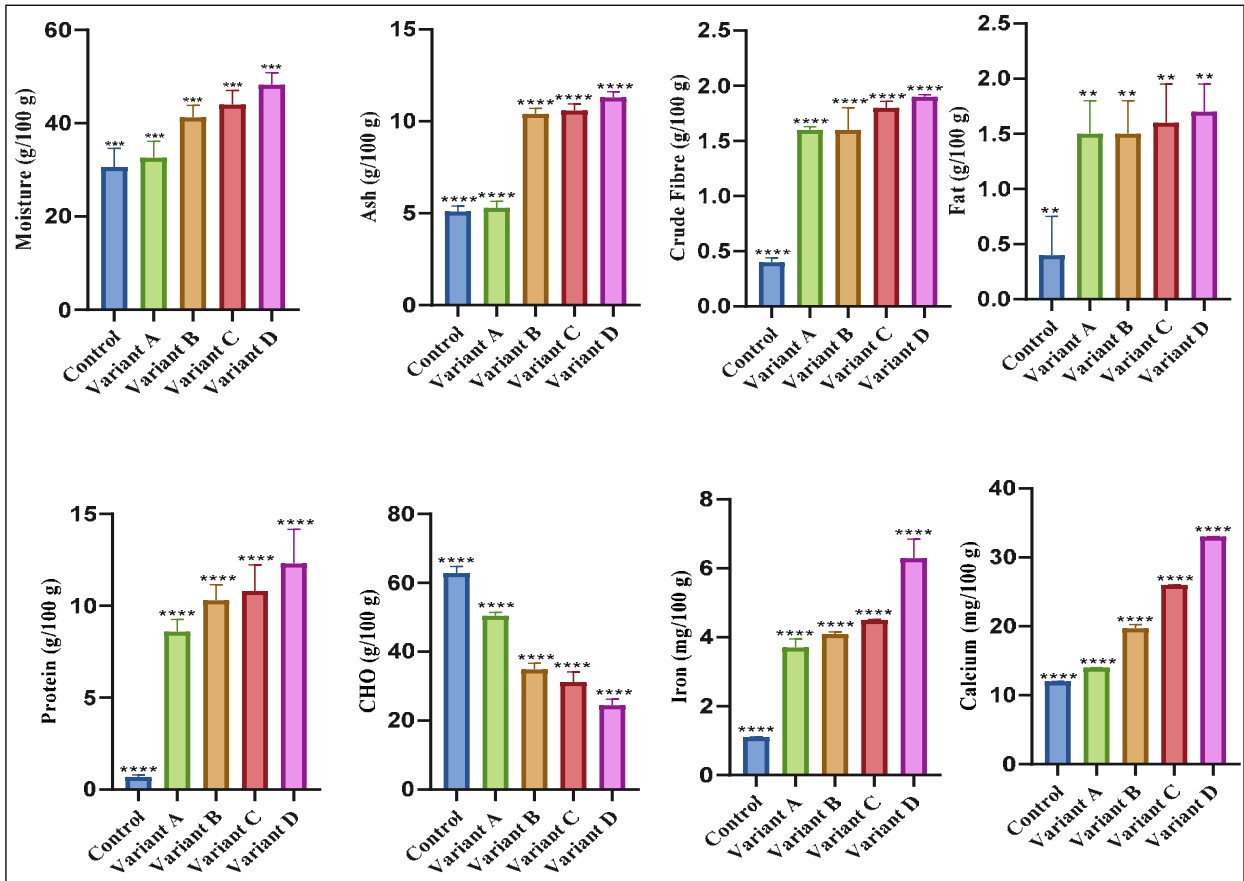
Shelf-life Study

The produced bread was subjected to a shelf-life examination during the first week. pH and temperature studies were conducted mostly over three days in alternate. In the food business, pH is a crucial quality indicator that affects factors like shelf life and quality. pH, which is an acronym for "potential of hydrogen", describes the degree (intensity) of an acidic or alkaline substance. On the other hand, food temperature allows us to

Table 2: Proximate Composition of Fortified Bread with Control Bread

Proximate Composition (100 g)	Control	Variant A	Variant B	Variant C	Variant D
Moisture (g)	30.6 \pm 4.04**	32.6 \pm 3.51**	41.3 \pm 2.51**	44.0 \pm 3.00**	48.3 \pm 2.51**
Ash (g)	5.1 \pm 0.30****	5.3 \pm 0.35****	10.4 \pm 0.30****	10.6 \pm 0.35****	11.3 \pm 0.30****
Crude Fibre (g)	0.4 \pm 0.04****	1.6 \pm 0.03****	1.6 \pm 0.20****	1.8 \pm 0.06****	1.9 \pm 0.02****
Fat (g)	0.4 \pm 0.35**	1.5 \pm 0.30**	1.5 \pm 0.30**	1.6 \pm 0.35**	1.7 \pm 0.25**
Protein (g)	0.7 \pm 0.10****	8.6 \pm 0.66****	10.3 \pm 0.84****	10.8 \pm 1.43****	12.3 \pm 1.85****
CHO (g)	62.8 \pm 1.97****	50.4 \pm 1.05****	34.9 \pm 1.72****	31.2 \pm 2.98****	24.5 \pm 1.73****
Iron (mg)	1.1 \pm 0.01****	3.7 \pm 0.25****	4.1 \pm 0.06****	4.5 \pm 0.02****	6.3 \pm 0.55****
Calcium (mg)	12.0 \pm 0.02****	14.0 \pm 0.02****	19.7 \pm 0.56****	26.0 \pm 0.03****	33.0 \pm 0.03****

Values are expressed as Mean \pm SD. Values followed by **, ***, **** were significant difference ($p < 0.05$).



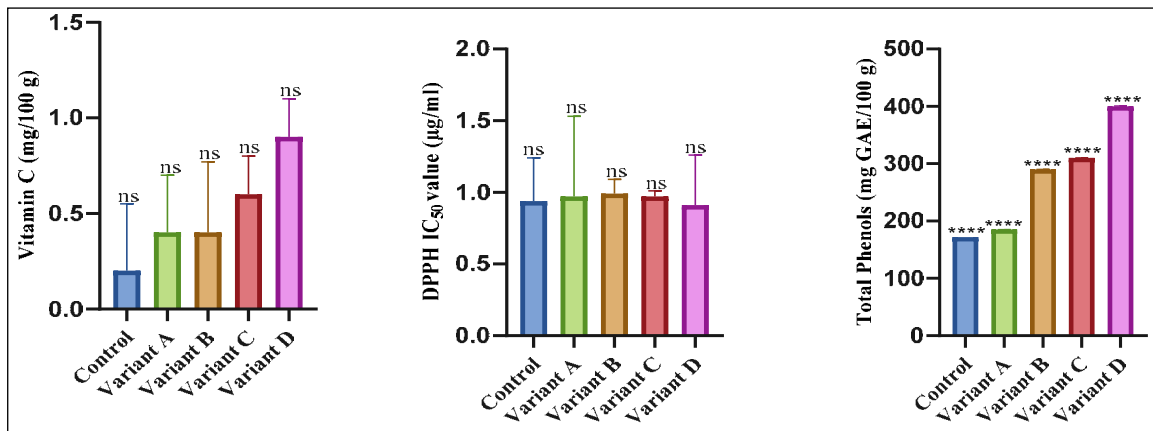
Values followed by **, ***, **** were significant difference ($p < 0.05$)

Fig. 2: Proximate Composition of Fortified Bread with Control Bread

Table 3: Antioxidant Analysis of Fortified Bread with Control Bread

Antioxidant analysis	Control	Variant A	Variant B	Variant C	Variant D
Vitamin C (mg/100 g)	0.2±0.35 ^{ns}	0.4±0.30 ^{ns}	0.4±0.37 ^{ns}	0.6±0.20 ^{ns}	0.9±0.20 ^{ns}
DPPH IC ₅₀ value (µg/ml)	0.94±0.30 ^{ns}	0.97±0.56 ^{ns}	0.99±0.10 ^{ns}	0.97±0.04 ^{ns}	0.91±0.35 ^{ns}
Total phenols (mg GAE/100 g)	172±0.01****	185±0.20****	290±0.35****	310±0.21****	400±0.43****

Values are expressed as Mean±SD. Values followed by **** were significant difference ($p < 0.05$) and ^{ns} were no significant difference ($p < 0.05$).



Values followed by **** were significant difference ($p < 0.05$) and ^{ns} were no significant difference ($p < 0.05$).

Fig. 3: Antioxidant Analysis of Fortified Bread with Control Bread

Table 4: Phytochemical Analysis of Fortified Bread with Control Bread

Phytochemical Analysis	Control	Variant A	Variant B	Variant C	Variant D
Alkaloids	-	+	+	+	+
Flavonoids	-	+	+	+	+
Glycosides	+	+	+	+	+
Phenolic Compounds	+	+	+	+	+
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Phytosterols	-	+	+	+	+

+ is present; - is absent.

Table 5 Shelf-life Study of Fortified Bread with Control Bread

Parameters	Control	Variant A	Variant B	Variant C	Variant D
pH	5.4±0.10	5.5±0.25	5.6±0.15	5.5±0.10	5.5±0.40
Temperature (°C)	30.6±2.08	31.3±4.16	30.3±6.50	31.6±5.68	32.6±5.50

Values are expressed as Mean±SD.

Table 6: Sensory Evaluation of Fortified Bread with Control Bread

Attributes	Control	Variant A	Variant B	Variant C	Variant D
Appearance	8.6±0.59	8.3±0.73	7.8±0.73	7.4±0.88	7.4±1.18
Taste	8.4±0.99	8.0±1.19	7.9±1.05	7.2±1.10	6.6±1.51
Texture	8.3±0.98	8.0±0.91	7.7±0.91	7.3±0.91	7.3±1.34
Flavor	8.6±0.59	8.2±0.83	7.9±0.85	7.5±0.94	7.1±1.16
Color	8.7±0.47	8.3±0.58	8.1±0.87	7.8±0.87	7.5±1.05
Overall Acceptability	8.6±0.88	8.5±0.99	8.2±0.83	7.3±1.03	7.1±1.25

Values are expressed as Mean±SD.

determine how long it will last on the shelf in the environment.

Table 5 shows the shelf-life study of different variants of fortified bread with the Control variant. The results indicated that baked bread cannot be stored for a long time. Its shelf life is maximum up to 5-6 days. It's very important to store baked bread in a refrigerator because refrigerator cooling air increases bread's shelf-life. The results were supported by the study (Bhatt and Gupta 2015; Fatima *et al.* 2023).

Sensory Evaluation

Table 6 indicates the sensory evaluation of fortified bread with control bread. As of according to the 9-point hedonic scale perform the attributes like appearance, taste, texture, flavor, color, and overall acceptability (8.6±0.88) were higher in Control as compared to other variants of fortified bread like variant A (6% seeds composition),

variant B (12% seeds composition), variant C (18% seeds composition), and variant D (24% seeds composition) respectively.

Except Control, the overall acceptability was higher in variant A (8.5±0.99) because of low seeds composition in fortified bread, than in variants B, C, and D. As other attributes like appearance, taste, texture, flavor, and color were also higher in variant A as compared to other variants like B, C, and D. The least overall acceptability was in variant D (7.1±1.25) due to incorporated very high composition of seeds in fortified bread.

However, variants A and B were found to be the most preferred fortified bread. Variants C and D were the least acceptable but their nutritive value was higher than other variants of fortified bread. Results showed that the increase in the composition of seeds in fortified bread reduces the acceptability of sensory parameters, this data supports in the study of (Ugwuona and Nwamaka 2016). But it also



shows that incorporating seeds was suitable for the bread's development.

So, our investigation comes to an end that increasing the composition of seeds in the fortification of bread enhances the nutritive value but the taste was not good. And also, there is no study found that all the seeds that we used in our study like melon seeds, pumpkin seeds, and sesame seeds were incorporated in the multigrain flour to make bread.

CONCLUSION

The bread which is a baked product made using wheat flour has a high caloric content and is high in fat, but it is deficient in other elements including protein, vitamins, and minerals. Increased vitamin intake and increased consumption of fiber and antioxidants can both be achieved by fortifying bread. Wholegrain cereals are becoming more and more popular, and bread is being enriched with a variety of edible seeds, which are an excellent source of nutrients. The study concluded that melon, pumpkin, and sesame seeds are very good sources of nutrients along with antioxidant properties, as well as good therapeutic properties. From the highlights of the present study, variant D (24% seeds composition) was highly nutritive as well as all phytochemicals were present. In sensory evaluation, variants A, B, and C were more acceptable as compared to variant D.

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