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Studies on the Effects of Spice Extracts on the Quality Characteristics and Storage Life of Bread

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Abstract—*Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavor. The objective of this study was to prepare antimicrobial bread using different natural antimicrobials (aqueous extracts of clove, ginger and cinnamon in different concentrations) to obtain an acceptable product. The breads prepared were then analyzed for their physical, sensory, textural and microbiological properties. In physical parameters of bread baking loss, loaf volume, loaf weight, specific volume of bread was analyzed. Baking loss of bread varied from 1.6 to 2.2. The highest loaf weight 333.23g was observed in bread with clove extract (4%) and lowest in bread with ginger extract (3%). Loaf volume of control bread with different extracts varied from 129.97cm^3 to 340cm^3 being the maximum in bread with cinnamon extract (2%) and minimum in bread with ginger extract (2%).Textural characteristics of bread was determined as hardness, cohesiveness, springiness, gumminess, chewiness and. Maximum hardness was observed in bread with ginger extract (2%) whereas minimum found in cinnamon extract (4%). Bread containing 2-4% clove extract, ginger extract and cinnamon extract had a mold free shelf life for more than 6 days whereas control bread spoiled within 4 days.*

1. INTRODUCTION

The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria. Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavor. Spices and herbs inhibit the growth of both Gram–positive and Gram– negative food borne pathogens or spoilage bacteria, yeast, and molds [1]. The active ingredients of plants against microorganisms are mostly some of the secondary metabolites (i.e. alkaloids, glycosides etc.) that are present in abundance in herbs and spices commonly used in Indian food preparations [2].

Bread is one of the most popular foods and it is prepared from a yeast, flour and water, usually by baking. Generally, bread's shelf life is confined by several destructive processes including fungi growth, moisture loss and staling, Sixty percent of bread spoilage is attributed to the mold's activity (*Penicillium species and Aspergillus niger*). In addition to their detectable growth, the fungi are responsible for the development of off flavor, producing mycotoxins and allergenic compounds. Wheat (*Triticum aestivum*) flour of both hard and soft wheat classes has been the major ingredient of leavened bread for many years because of its functional proteins.

Ginger (*Zingiber officinale*) belongs to Zingiberaceae family. The part of the plant used is rhizome. In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3- one is the most abundant constituent in the gingerol series. The active constituents of ginger inhibit multiplication of colon bacteria. These bacteria ferment undigested carbohydrates causing flatulence, this can be counteracted with ginger. It inhibits the growth of *Escherichia coli, Proteus sp, Staphylococci, Streptococci and Salmonella*. Ginger has strong antibacterial activity and to some extent antifungal properties. Ginger inhibits *Aspergillus,* a fungus known for production of aflatoxin, a carcinogen [3]. Cinnamon (*Cinnamomum)* belongs to family Lauraceae. It is valued for its dried inner bark of the tree *Cinnamomum verum*. Cinnamon has been reported to have remarkable pharmacological effects in the treatment of type II diabetes. Apart from health benefits of being a natural antioxidant, cinnamon possess strong antimicrobial properties to act as natural preservatives in bread. Essential oil such as trans-cinnamaldehyde, caryophyllene oxide, L-borneol, L-bornyl acetate, eugenol, bcaryophyllene, E-nerolidol, and cinnamyl acetate was reported by Tung *et al*. [4]. Some other constituents are Terpinolene, α-Terpineol, α-Cubebene, and α-Thujene. Matan *et al*. [5] reported antimicrobial activity of Cinnamon bark. Cinnamon is known for its high total phenol content and antioxidant activity [6], which accelerates its use in bread. Clove (*Syzygium aromaticum*) belongs to family Myrtaceae. It has been reported to have antioxidant, antiseptic, local anesthetic, anti-inflammatory, rubefacient (warming and soothing), carminative and anti-flatulent properties. The spice contains health benefiting essential oils such as eugenol**.** The objective of this study was to prepare antimicrobial bread using different natural antimicrobials (aqueous extracts of clove, ginger and cinnamon in different concentrations) to obtain an acceptable product. The breads prepared were then analyzed for their physical, sensory, textural and microbiological properties.

2. MATERIALS AND METHODS

2.1 Procurement of raw materials

Clove, ginger, cinnamon, sugar, vanaspati ghee and refined wheat flour were purchased from local market in Rohtak. All the chemicals and reagent used for the analysis were of analytical grade.

2.2 Preparation of clove, cinnamon and ginger extract

The aqueous extracts of all the three spices were prepared by using the method of Saeed & Nadeem [7]. The dark colored extracts were stored in the refrigerator (4^0C) for further use.

2.3 Preparation of bread

Sugar and yeast were dissolved in a part of slightly warm water; a small amount of flour was added into it and allowed to stand for 5-10 minutes. Flour, active yeast, salt, milk powder, water and fat were put into the dough mixer fitted with blade and blended together with on and off quick turns. The 2 ml of 10 ppm solution of ascorbic acid and 1 ml of 10 ppm solution of potassium bromate were added. Water was added (poured) until a ball of proper consistency of dough was formed. The kneaded Dough was allowed for fermentation (90 min, 30^0C), covered with sterilized wet muslin cloth and allowed to rise in bulk. The dough was punched many times and placed in greased tin, it was covered and placed in a warm place for proofing (50 min.). Then it was baked in a preheated oven at 225^0C for 20-25 min. The baked bread was removed from the oven and allowed to cool before its further study.

2.4 Physico-chemical and functional properties of flours

Refined wheat flour was evaluated for their proximate composition i.e. moisture, protein, ash, fat and fiber using standard methods [8]. Water and oil absorption capacities were determined using method of Beuchat [9]. Water or oil absorption capacity was determined as water or oil absorbed by the flour sample and the results were expressed as g/g of sample. The bulk density was determined according to the method as described by Okaka and Potter [10]. The bulk density was calculated as weight per unit volume of sample. The least gelation concentration was determined using method of Coffman and Garcia [11] with some modifications. The flour dispersions with concentrations of 20 to 40% with an interval of 2% were prepared and the least gelation concentration was determined as that concentration when the sample from inverted tube did not slip. The method of Okaka and Potter [10] with some modifications was used for determining the swelling capacity. The swelling power was expressed as g/g of dry flour. The method described by Narayana and Narasinga [12] was used for the determination of foam capacity (FC) and foam stability (FS). The whole wheat meal flour of each wheat variety was tested for Pelshenke value according to AACC (2000) [8] method.

2.5 Quality evaluation

2.5.1 Texture profile analysis (TPA) of bread

Bread texture (hardness, springiness, cohesiveness, chewiness, gumminess and resilience) was determined by using TA.XT plus texture analyzer. Bread slices (2 cm thick) were placed in between the two load cell (5kg) plates of machines, and the load cell was slowly brought to a lower level, so that it touches the sample. Parameters like diameter, speed (5mm/sec), the percent compression (10mm), and no. of cycles (two) were inputted before starting compression of the sample. Then, the load cell started slowly moving downwards compressing the sample in 5 sec. wait between first and second compression cycles.

2.5.2 Crumb firmness of bread

Slices of each loaf (2 cm) were taken for analysis of the crumb firmness, using TA.XT plus texture analyzer. A modified AACC standard method was used with a cylindrical probe (diameter 15 mm). The probe compressed the slices 40% at a test speed of 1.7 mm / sec. The young modules reading between 3 and 20% were taken as a measure of bread crumb firmness. The measurements were carried out on 36 mm thickness slice taken from the center of the bread loaf. The measurements were carried out on four loaves from each batch.

2.5.3 Loaf weight, specific volume and loaf volume of bread

The Loaf weight (W) of bread was measured after cooling for 1 h with using digital scale. Specific volume of bread was calculated using standard method by dividing volume (cc) by weight (gm). Loaf volume of bread was measured by using rapeseed replacement method according to the procedure AACC (2000) [8]. The loaf was put in a metallic container with known volume (V_C). The container was topped up with rapeseed, the loaf was removed and the volume of rapeseed was noted (V_R), loaf volume (V_L) was taken calculated according to the following formula:

$$V_L\ (cm^3) = V_C - V_R$$

2.6 Microbiological analysis

The plate count agar (PCA) and potato dextrose agar (PDA) were used for the enumeration of bacteria, yeast and mold in the bread sample. Total bacterial count was carried out using the pour plate method described by Harrigan [13]. 1 ml homogenized sample was aseptically poured in the sterilized petriplates followed by pouring of about 10-15 ml of PCA in the petriplates. Allow to cool the media and place the inverted petriplates in the incubator at 37^0C temperature. Observed and count the formed colonies (cfu/ml) after 24 hours. Yeasts and moulds were enumerated by pour plate method described by Harrigan [13]. 1 ml of homogenized sample was aseptically poured in the sterilized petriplates and about 10-15 ml of PDA were poured in the petriplates. Allow to settle the media and place the inverted petriplates in the incubator at 25-27^0C for 7 days. Observe and count the colonies each day.

2.7 Sensory analysis of bread

Bread sample was analyzed for sensory characteristics. Sensory quality characteristics were evaluated by a panel of 10 semi trained members using a 9-point hedonic scale [14]. The bread was evaluated for their color, appearance, flavor, texture and overall acceptability.

2.8 Statistical analysis

The data were analyzed using ‘OPSTAT’ statistical software. ANOVA was applied in a completely randomized model. The values were represented as mean $\pm$ SD. The mean were compared at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Proximate composition of flour

The chemical composition of refined wheat flour is given in Table 1. The moisture content of refined wheat flour is 12.2%. The similar observations were recorded by Yadav *et al.* [15]. The moisture content of refined wheat flour is influenced by the milling techniques as well as storage condition [16]. The fat content of flour is 0.87%. The ash content of flour is 0.39%. The protein content of flour is 10.08% and the crude fiber content of refined wheat flour is 0.49%.

3.2 Functional properties of refined wheat flour

Table 1 shows the functional properties of refined wheat flour. The bulk density of refined wheat flour was 0.70 g/cm^3.Akubor and Obiegbuna [17] reported that bulk density of a sample could be used in determining its packaging requirements as this relates to the load the sample can carry if allowed to rest directly on one another. WAC of refined wheat flour was 1.05%. OAC of flours improve mouth feel and flavor retention (Kinsella, 1976). The OAC content of refined wheat flour was 2.39%. The fat absorption can also be influenced by the lipophilicity of protein [18]. The high OAC could suggest the presence of a large proportion of hydrophobic groups as compared with the hydrophilic groups on the surface of protein molecules [19]. Swelling power determines the extent to which a flour sample increases in volume when soaked in water in relation to its initial volume. The SP content of flour was 6.96%. Moorthy and Ramanujam [20] reported that the swelling power of flour granules was an indication of the extent of associative force within the granules. The water solubility value of refined wheat flour was 5.90 g/g. The value of foam capacity of flour was 12.3%. The foam stability value was 7.33%. Pelshenke value of refined wheat flour was 183 minutes. The Pelshenke test is one of the most important tests used by the wheat scientists to evaluate their breeding material for the assessment of gluten strength.

Table 1: Chemical composition and functional properties of refined wheat flour

Sample	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Crude fiber (%)	BD (g/cm3)	WAC (%)	OAC (%)	SP (g/g)	FC (%)	FS (%)	LGC (%)	Pelshenke value(min)
Refined Wheat Flour	12.20 ±0.01	0.87 ±0.02	0.39 ±0.01	10.08 ±0.01	0.49 ±0.02	0.70 ±0.01	1.05 ±0.02	2.39 ±0.02	6.96 ±0.13	12.30 ±1.20	7.33 ±0.30	20	183

The values are expressed as the mean ± SD of three independent determinations. The values are the means compared at significance level of 5%.

Where, BD= bulk density, WAC= water absorption capacity, OAC= oil absorption capacity, SP= swelling power, FC= foaming capacity, FS= foaming stability, LGC= least gelation concentration

Table 2: Texture profile analysis (TPA) of breads

Samples	A	B	C	D	E	F	G	H	I	J
Hardness (g)	588.86	378.70	532.37	1219.66	494.50	636.01	300.42	1446.52	713.85	427.26
Cohesiveness	0.86	0.85	0.87	0.91	0.88	0.86	0.90	0.90	0.89	0.88
Gumminess	508.01	324.64	467.38	1100.91	438.73	547.79	273.06	1302.05	637.73	379.65
Springiness	0.96	1.00	1.31	0.99	1.06	1.13	2.63	0.99	3.23	0.99
Chewiness	489.15	324.64	614.30	1098.17	468.05	619.66	720.35	1295.61	2064.74	378.71
Resilience	0.53	0.52	0.54	0.61	0.58	0.56	0.56	0.66	0.60	0.60
Adhesiveness (g/sec.)	-0.10	-	-	-0.19	-	-	-0.12	-0.21	-	-

Where, A= control bread, B= clove 2%, C= clove 3%, D= clove 4%, E= cinnamon 2%, F= cinnamon 3%, G= cinnamon 4%, H= ginger 2%, I= ginger 3%, J= ginger 4%

The results regarding Pelshenke value obtained in the present study were in close agreement with the earlier found of Randhawa *et al.* [21] who found Pelshenke values varying from 108- 185 minutes among different Pakistani wheat varieties.

3.3 Texture properties of breads

The hardness of control bread and breads prepared using extracts in different concentration varied from 300.42 to 1446.52g (Table 2). The sample H (bread with 2% ginger extract) showed highest hardness whereas sample G (bread with cinnamon 4%) showed lowest value. The highest value of springiness was found in sample I (3.23) where as lowest value was found in sample A (0.96) and resilience (which express the ability or speed of material to return to its original shape after a stress) showed lowest value for sample B (0.52) where as highest value was found in sample H (0.66). Gumminess & chewiness are secondary parameters. Chewiness is the most indicative characteristics of bread. The highest value of gumminess was found in sample D (1100.91) & lowest in sample G (273.06). The highest value of chewiness was found in sample H (1295.61) & lowest in sample B (324.64). The highest value of cohesiveness was found in sample D (0.91) where as lowest value was found in B (0.85).

3.4 Physical characteristics of breads

The breads prepared using extracts of clove, cinnamon and ginger were analyzed for their physical properties such as baking loss, loaf volume, loaf weight and specific volume (Table 3). Baking loss of the breads varied from 1.6 to 2.2%. The maximum baking loss occurred in control bread (A) whereas bread with clove extract 3% (C) had minimum (1.6%) baking loss. The highest loaf weight (333.23g) was observed in bread with clove extract 4% and lowest (298.13g) in bread with ginger extract 3%. Loaf volume of control bread and bread with different extracts varied from 129.97 cm^3 to 340 cm^3 being the maximum in bread with cinnamon extract 2% and minimum in bread with ginger extract 2%. The highest Specific volume of bread was in bread with cinnamon extract 4% and lowest in bread with cinnamon extract 2%.

Table 3: Physical characteristics of breads

Sample	Baking Loss (%)	Loaf volume (cm3)	Loaf weight (g)	Specific volume (cm3//g)
A	2.20±0.15	141.72±0.01	322.15±0.06	2.20±0.03
B	1.86±0.01	276.17±0.03	311.99±0.08	1.28±0.03
C	1.60±0.03	274.53±0.07	320.15±0.06	1.67±0.02
D	2.03±0.03	160.35±0.01	333.23±0.15	2.08±0.02
E	1.73±0.03	340.00±0.01	312.17±0.09	1.10±0.02
F	2.00±0.01	159.06±0.04	318.50±0.06	2.00±0.01
G	2.10±0.06	113.14±0.03	324.62±0.01	3.00±0.01
H	1.80±0.03	129.97±0.01	308.55±0.06	2.38±0.03
I	1.76±0.03	177.33±0.34	298.13±0.09	1.68±0.03
J	1.90±0.01	333.54±0.01	304.61±0.03	1.11±0.01

Where, A= control bread, B= clove 2%, C= clove 3%, D= clove 4%, E= cinnamon 2%,

F= cinnamon 3%, G= cinnamon 4%, H= ginger 2%, I= ginger 3%, J= ginger 4%

The values are the mean ± SD of three independent determinations.

The values are the means compared at significance level of 5%.

3.5 Sensory characteristics of breads

The highest overall acceptability score 8.25 was obtained for control bread, bread with ginger extract 2%, and bread with ginger extract 3%. The color of all bread samples was acceptable by all panelists (Table 4). The control bread (8.75) was most preferred in terms of color, the color of sample B (6.75) was the least preferred as compared to other bread samples. The taste of the bread refers to the sweet sensation caused in the mouth by contact with the bread due to the sweetening agent. The score for taste of control bread and sample J (8.25) bread with ginger extract (4%) was preferred by maximum number of panelists where as the taste of sample B (7) bread with clove 2% extract, H (7) bread with ginger 2% extract and D (7) bread with clove 4% were least preferred by the panelists. Aroma of sample G (8.5) was preferred by all the panelists where

Table 4: Sensory characteristics of breads

Samples	A	B	C	D	E	F	G	H	I	J
Aroma	8.00±0.02	6.75±0.25	7.25±0.47	6.75±0.25	6.75±0.25	8.00±0.01	8.50±0.28	7.00±0.40	8.00±0.01	7.75±0.25
Taste	8.25±0.25	7.00±0.48	7.75±0.25	7.00±0.40	7.25±0.40	7.25±0.25	7.25±0.25	7.00±0.01	7.75±0.25	8.25±0.47
Color	8.75±0.25	6.75±0.25	7.50±0.50	8.00±0.06	7.00±0.09	8.00±0.04	8.50±0.28	7.50±0.28	8.25±0.25	8.00±0.00
Appearance	7.75±0.25	6.25±0.25	7.00±0.02	7.75±0.25	7.25±0.25	7.75±0.25	9.00±0.01	8.00±0.01	8.50±0.28	7.75±0.25
Texture	8.00±0.01	6.00±0.01	7.25±0.25	7.25±0.25	7.25±0.25	7.50±0.28	7.75±0.25	7.50±0.28	8.75±0.25	7.50±0.28
Overall Acceptability	8.25±0.25	6.75±0.25	7.50±0.28	7.75±0.25	7.50±0.25	8.00±0.01	7.75±0.25	8.25±0.25	8.25±0.25	8.00±0.00

Where, A= control bread, B= clove 2%, C= clove 3%, D= clove 4%, E= cinnamon 2%, F= cinnamon 3%, G= cinnamon 4%, H= ginger 2%, I= ginger 3%, J= ginger 4%

as aroma of sample B, D and E (6.75) were less preferred by panelists. Texture is the quality of bread that can be decided by touch the degree to which it is rough or smooth, hard or soft. The maximum number of panelists preferred the sample I (8.75) bread with ginger 3% extract and sample B (6) bread with clove 2% extract was the less preferred by all the panelists.

3.6 Microbial analysis of different breads

The shelf life of bread is limited by several deterioration processes including fungal growth, loss of moisture and staling [22]. It is said that about 60% of bread spoilage was caused by molds while yeasts only accounts for 15%. Fungi are responsible for off flavor development, mycotoxins production and allergenic compounds [23]. Mold spores are generally killed by the baking process in fresh bread and other baked products. Therefore, for bread to become moldy, it must be contaminated either from the air, bakery surfaces, equipment, food handlers or raw ingredients after baking during the cooling, slicing or wrapping operations. This means that all spoilage problems caused by molds must occur after baking [24].

Table 5: Bacterial count of different breads (cfu/g)

Samples	A	B	C	D	E	F	G	H	I	J
0-Day	1x103	-	-	-	-	-	-	-	-	-
2-Day	3 x103	1 x103	2 x103	-	2 x103	-	-	1 x103	-	-
4-Day	3 x104	2 x103	2 x103	-	3 x103	1 x103	-	2 x103	-	-
6-Day	3 x104	3 x103	2 x103	-	4 x103	2 x103	-	3 x103	1 x103	-
8-Day	7 x104	5 x104	3 x104	1 x104	5 x104	4 x104	2 x104	4 x104	2 x104	1 x104
10-Day	9 x104	6 x104	4 x104	2 x104	7 x104	5 x104	3 x104	5 x104	3 x104	2 x104
12-Day	12 x104	8 x104	6 x104	3 x104	8 x104	6 x104	4 x104	7 x104	4 x104	3 x104
14-Day	14 x104	9 x104	8 x104	4 x104	9 x104	7 x104	5 x104	9 x104	6 x104	4 x104

Where, A= control bread, B= clove 2%, C= clove 3%, D= clove 4%, E= cinnamon 2%, F= cinnamon 3%, G= cinnamon 4%, H= ginger 2%, I= ginger 3%, J= ginger 4%

Table 6: Fungal/ mold count for different breads (cfu/g)

Samples	A	B	C	D	E	F	G	H	I	J
0-Day	-	-	-	-	-	-	-	-	-	-
2-Day	-	-	-	-	-	-	-	-	-	-
4-Day	74	52	-	-	55	-	-	48	-	-
6-Day	91	83	66	61	81	68	70	73	64	62
8-Day	120	111	109	112	115	108	107	113	115	109
10-Day	135	122	119	126	124	128	120	124	126	123
12-Day	149	138	130	138	139	140	132	138	132	130
14-Day	157	149	141	140	152	151	144	149	144	141

Where, A= control bread, B= clove 2%, C= clove 3%, D= clove 4%, E= cinnamon 2%, F= cinnamon 3%, G= cinnamon 4%, H= ginger 2%, I= ginger 3%, J= ginger 4%

The results reveals that all the breads using extracts of ginger, clove and ginger possessed activity against all the food associated bacteria. The aqueous extracts were strongly active against bacteria, fungi and mold growth. The Table 5 and 6 shows the effect of extracts of cinnamon, clove and ginger in bread during the storage period of 14 days. Fungal growth appeared after 48 h of storage. The maximum no. of viable fungal colonies was recorded in the control sample. Control bread which contained no preservative spoiled within 4 days. There was no yeast, mold and total viable count in all breads during the storage of 0-4 days. In bread the total viable count of bacteria ranged from 1×10^4 to 14×10^4 and yeast and mold growth 48 to 157cfu/g during the storage period of 14 days. Bread containing 2-4% clove extract, ginger extract and cinnamon extract had a mold free shelf life for more than 6 days as compared to control bread. These results agreed with those of Branen *et al.* [25] and inhibitory effect of cinnamaldehyde and eugenol on mold growth was also reported in previous study [26-27].

4. CONCLUSION

The study concluded that bread of acceptable quality can be prepared with the use of natural antimicrobials as it increases the shelf life of bread and enhances the flavor also. Bread containing cinnamon extract, ginger extract and clove extract in concentration up to 2-4 % had a mold free shelf life for 6 days.

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