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Shelf Life Extension of Bread Using Anti-microbial Activity of Spices

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Abstract— **The increasing consumer awareness and their concern about food safety has drawn the attention of food scientists and technologists to the development of natural antimicrobials to preserve food instead of artificial chemical preservative. The aim of present work was to evaluate the antimicrobial activity of Cinnamon, Clove, Garlic and Ginger using their methanol and aqueous extract against food borne pathogens (*E. coli, S. aureus, B. subtilis,* and *B. cereus*) and to utilize the powdered form of spices in the shelf life extension of bread . The study was conducted to assess the effectiveness of 20%, 40%, 60%, 80% and 100% of methanol and aqueous extract of spices against test pathogens. Disc diffusion method was used to check the antimicrobial activity. Clove and Cinnamon extracts (methanol and aqueous) showed higher antimicrobial activity than Garlic and Ginger. Bread incorporated with Cinnamon powder shown more sensory acceptability than Clove bread therefore Cinnamon powder was selected for the final development of spiced bread for improvement in shelf life. Sensory point of view the Cinnamon powder incorporated at 2.5% level in bread give away better result. The shelf life study of 2.5% Cinnamon powder incorporated bread revealed improvement in shelf life by 2 days as compared to control bread.**

Keywords: **Bread, Spices, Anti-microbial activity, Shelf life, Sensory evaluation**

I. INTRODUCTION

Chemical preservatives are used to prevent the growth of food spoiling microbes in the food industry. The growing concern about food safety due to the use of artificial chemical preservative has recently led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria [1, 2]. There has been constant increase in search of alternate and efficient compounds for food preservation aimed at a partial or total replacement of chemical preservatives. Also there has been increase in awareness of consumers about foods free or with lower level of chemical preservatives because these could be toxic for human therefore plant material with antimicrobial properties have a possible application in food preservation [3, 4, 5].

Antimicrobial substances are those substances that are capable of destroying or inhibiting the growth of microorganisms [6]. Spices are one of the most commonly used natural antimicrobial agent 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 [7, 8]. Bioactive compounds present in spices that improve the shelf life and inhibit the growth of microorganisms. 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 [9, 10]. Cinnamon, clove, garlic and ginger inhibit the growth of gram positive, gram negative bacteria, yeast and molds. Major antimicrobial compounds in clove, cinnamon, garlic and ginger have been reported to be eugenol, cinnamaldehyde, allicin and gengerol respectively .

The present investigation was therefore carried out with the aim to study the antimicrobial property of various spices (cinnamon, clove, garlic, ginger) and to exploit the antimicrobial property of most effective spice for the shelf life improvement of bread.

II. MATERIALS AND METHODS

The spices (cinnamon, clove, garlic, ginger) required for the work was procured from the local market of Jalgaon (super market: D-MART) and was analysed for various parameters like moisture, ash, protein, fat by method described by S. Ranganna, 1986 [11], fiber by fiber analyser (Pelican KES 04LVA) and carbohydrates by difference. The qualitative test for the presence or absence of various phytochemicals as bioactive component in the spices like alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids was done as per the method described by G. Sibi et al, 2013 [12].

2.1 Preparation of methanol and water extract

Methanol extract of each spice was prepared by mixing 30 grams of dry spice powder in 300 ml methanol for 48 hours at room temperature. Aqueous extract of each spice was prepared by 50 grams of fine powder mixed with 250 ml of distilled water boiled for 2 hours in water bath. filtration of extract and used. The each extract was filtered and further used for the antimicrobial activity study [13, 14].

2.2 Microorganisms for antimicrobial test

Food borne pathogens including gram positive (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus*) and gram negative culture (*Escherichia coli*) were used in the study which was obtained from National Chemical Laboratory (NCL) Pune.

2.3 Preparation of culture broth

Nutrient agar broth was used as culture media to which a loop full of the culture was transferred in 50 ml and incubated at 37 ^{0}C for 24 hours and was used as inoculums..

2.4 Assessment of antimicrobial activity

Disc diffusion method was used for the evaluation of antimicrobial activity. A paper disc of 6 mm diameter containing the spice extract was aseptically placed on the bacteria containing agar Petri plates. The plates were then incubated in incubator at 37 ^{0}C for 24 hours. Ultimately the plates were examined for zone of inhibition measured by zone measuring scale

2.5 Preparation of Control Bread and Spiced Bread

Control bread was prepared by making dough by mixing maida(300 g), Sugar(15 g), baker's yeast (3 g), salt (5.6 g), oil(14.8 g) and water in a quantity to achieve suitable consistency. The dough was then subjected for various bread making operation like dough fermentation, knock back, dividing/ scaling, intermediate proof (35-43 ^{0}C), final moulding and baking (225 ^{0}C for 20 min.) [15]. The spiced bread were prepared from cinnamon and clove powder by adding to the level of 3 % . Both the spiced bread were then subjected for the sensory evaluation to find out the more acceptable spice in bread using 9 point Hedonic Scale. The various types of cinnamon bread were then prepared by incorporating the cinnamon powder to the various level (1.5 %, 2.5 %, 3.5 % and 4.5 %) in the formulation of control bread which were subjected for the sensory evaluation to find out the accepted level of cinnamon powder in bread.

2.6 Shelf Life Study of Control and Spiced (Cinnamon) Bread

Shelf life study of control bread and accepted cinnamon bread were carried out for 4 and 6 days respectively. During shelf life study the bread were evaluated microbiologically (total plate count, coliform count, yeast and mold count using standard methods) and for sensory parameters using 9 point Hedonic Scale.

III. RESULTS AND DISCUSSION

Analysis result of spices in powder form is given in Table 1 and is showing all spices are good source of mineral and clove being considerable source of clove oil.

TABLE 1
ANALYSIS RESULT OF SPICES POWDER

Parameter	Clove	Ginger	Garlic	Cinnamon
Moisture %	5.58± 0.01	9.1±0.1	3.1±0.03	2±0.03
Ash %	5.07± 0.005	6±2	3.36±0.57	5.33±0.3
Fat %	8.5±0.2	1.22±0.02	0.8±0.01	1.20±0.01
Protein %	1.18±0.02	8.29±0.11	13.10±0.04	8.2±0.04

(Values are means of 3 determination ± standard deviation)

Phytochemical analysis of the spice extract is show in the Table 2. Flavonoids, tannin, terpenoids were found to be as the major phytochemicals present in all the spice samples along with glycosides and saponins in most spices, whereas alkaloids were observed with clove and ginger only.

TABLE 2
RESULT OF PHYTOCHEMICAL ANALYSIS OF SPICE EXTRACT

Spices	Alkaloid	Flavonoid	Glycosides	Phenols	Saponins	Steroids	Tannins	Terpenoids
Cinnamon	-	+	+	+	-	+	+	+
Clove	+	+	+	+	+	+	+	+
Garlic	-	+	-	-	+	-	+	+
Ginger	+	+	+	-	+	-	+	+

(+) present, (-) absent

Table 3 is showing the comparison of various spice methanol extract for the antimicrobial activity against *E. coli, S.aureus, B. cereus* and *B. subtilis*. The methanol extract of cinnamon was observed higher antimicrobial activity at all the concentration against all bacteria as compare to other spices. 20 % is the minimum concentration to which the methanol extract from all the spices required to be diluted to show antimicrobial activity. Overall the second most effective methanol extract was from clove

followed by ginger and then garlic. Thus Cinnamon methanol extract proved to be most effective inhibitory against the tested group of bacteria as compare to other spices.

TABLE 3
RESULT OF ANTIMICROBIAL ACTIVITY OF METHANOL SPICE EXTRACT

Spices	Conc. of Extract	Zone of Inhibition (mm)			
		E. coli	*S. aureus*	*B. cereus*	*B. subtilis*
Ginger Methanol Extract	100%	16.6±1.5	22±1	11.6±0.5	12.3±0.5
	80%	12.6±0.5	14.6±0.5	10.6±0.5	11.6±0.5
	60%	10.3±0.5	9.6±1.5	9.6±0.5	10±1
	40%	8±0	6.6±0.5	6.3±0.5	5±0
	20%	1.6 ±1.57	2.6± 0.5	2.6 ±0.5	2.6 ±0.5
Garlic methanol extract	100%	14.6±3.05	12±0	12.3±0.5	10.3±0.5
	80%	11±2.6	9.6±0.5	10.3±0.5	8±1
	60%	10.6±2.08	8±0	8.6±0.5	8.3±0.5
	40%	7.6±0.5	5.6±0.5	6.6±0.57	4.3±0.57
	20%	2.6 ±0.5	2.3 ±0.5	2± 1	2.6 ±0.5
Clove Methanol Extract	100%	20.3±0.57	17.3±3.05	17.6±0.5	17.3±0.5
	80%	15±0	11.3±1.15	16±1	15±1
	60%	10.3±0.57	11.6±1.52	13±1	13.6±0.5
	40%	8.6±1.52	6±0	10.6±1.54	10.6±0.5
	20%	4.3±0.5	3.3 ±0.5	3±0	2.6 ±0.5
Cinnamon Methanol Extract	100%	24±1	22.3±0.5	17.3±0.57	20±0
	80%	14.6±0.5	22±1	15.6±0.57	14.6±0.5
	60%	13.3±0.5	14.3±0.5	15.3±0.57	13.3±0.5
	40%	11±1	12.3±2.3	14.6±2.88	11.3±0.5
	20%	4.6 ±0.5	6.3± 0.5	5.6±0.5	4±0

(All values are means of triplicate determinations ± standard deviation)

Table 4 is showing the comparison of various spice aqueous extract for the antimicrobial activity against selected bacteria (*E. coli, S.aureus, B. cereus* and *B. subtilis*). Clove and cinnamon aqueous extract shown the higher antimicrobial activity at different concentrations as compare to garlic and ginger. The Ginger aqueous extract shown no anti microbial activity at 20% as well as 40% concentrations against bacteria under test. Water extract from cinnamon, clove and garlic required to be diluted to the minimum concentration of 20% to show antimicrobial activity whereas in case of ginger it is 40%. Thus clove and cinnamon aqueous extract demonstrated to be the most effective inhibitory against tested species of bacteria. Therefore the clove and cinnamon were selected for preparing spiced bread to find out the most sensory acceptable matching spice for control bread.

TABLE 4
RESULT OF ANTIMICROBIAL ACTIVITY OF AQUEOUS SPICE EXTRACT

Spices	Conc. of Extract	Zone of Inhibition (mm)			
		E. coli	*S. aureus*	*B. cereus*	*B. subtilis*
Aq. Ginger Extract	100%	6.6±0.5	6±0	6.3±0.5	6±0
	80%	4.3±0.5	5±0	5±0	4.6±0.5
	60%	3±0	3.6±0.57	3.6±0.5	3.3±0.5
	40%	0	0	0	2±2.3
	20%	0	0	0	0
Aq. Garlic Extract	100%	8.6±0.5	6.6±0.5	6.3±1.1	6.6±0.5
	80%	7±0	5.6±0.5	6±1	5.6±0.5
	60%	3.6±0.5	3.3±0.5	4.6±0.5	4.3±0.5
	40%	3±0	2.6±0.5	3±0	3.3±0.5
	20%	1±1	1±1	0.3± 0.5	1±1

Aq. Clove Extract	100%	11.6±0.5	12.3±1.5	8.9±1.5	10.6±0.5
	80%	9.6±0.5	10	8.3±0.5	10.3±0.5
	60%	7.3±0.5	10.3±0.5	6.6±0.5	9±1
	40%	6±0	8.30.5	4.6±0.5	7.3±0.5
	20%	3 ±1	3.6 ±0.5	2.6±0.5	2.6 ±0.5
Aq. Cinnamon Extract	100%	11.3±0.5	9.3±0.5	8.3±0.5	8.3±0.5
	80%	8.6±0.5	7±0	6.6±0.5	7.6±0.5
	60%	7.3±0.5	5.6±0.5	7±0	6.3±0.5
	40%	6.3±0.5	5.3±0.5	5.6±0.5	6.3±1.15
	20%	3.3±0.5	3.3±0.5	4.3±0.5	3.6±0.5

(All values are means of triplicate determinations ± standard deviation)

The sensory evaluation result of control, clove incorporated and cinnamon incorporated bread is presented in Table 5. The result reveled Cinnamon powder incorporated bread to be more acceptable than Clove powder incorporated bread. Also sensory point of view the Cinnamon powder incorporated bread not differ much than control. Therefore the Cinnamon powder was selected for the final development of spiced bread.

TABLE 5
SENSORY EVALUATION RESULT OF CONTROL AND SPICED (CLOVE/ CINNAMON) BREAD

Parameter	Control Bread	Cinnamon Bread	Clove Bread
Appearance	7.6 ± 0.3	6.8 ± 0.4	5.9 ± 0.5
Color	7.7 ± 0.4	6.8 ± 0.2	5.8 ± 0.5
Texture	7.6 ± 0.5	6.7 ± 0.3	5.7 ± 0.3
Flavor	7.5 ± 0.5	6.9 ± 0.3	5.9 ± 0.4
Overall acceptability	7.6 ± 0.4	6.8 ± 0.3	5.8 ± 0.4

(All values are means of scores given by 10 panelist ± standard deviation)

Sensory evaluation result of bread incorporated with different level of cinnamon is given in Table 6. Out of all the bread prepared with Cinnamon , the sensory score of bread incorporated with 2.5 % level Cinnamon scored highest score in all sensory parameter. The final spiced bread was therefore developed by incorporating 2.5 % level Cinnamon powder.

TABLE 6
SENSORY EVALUATION RESULT OF BREAD INCORPORATED WITH DIFFERENT LEVEL OF CINNAMON

Parameter	Control Bread	Cinnamon Powder Levels in Bread			
		1.5 %	2.5 %	3.5 %	4.5 %
Appearance	7.8 ± 0.5	7.3 ± 0.6	7.6 ± 0.5	6.8 ± 0.6	5.7 ± 0.4
Color	7.6 ± 0.6	7.1 ± 0.4	7.5± 0.4	6.8 ± 0.6	5.8 ± 0.6
Texture	7.7 ± 0.6	7.2 ± 0.6	7.5 ± 0.5	6.6 ± 0.5	5.8 ± 0.4
Flavor	7.6 ± 0.4	7.5 ± 0.5	7.7± 0.6	6.6 ± 0.5	5.6 ± 0.5
Overall acceptability	7.6 ± 0.5	7.2 ± 0.6	7.5 ± 0.5	6.7± 0.6	5.7 ± 0.5

(All values are means of scores given by 10 panelist ± standard deviation)

Result of chemical analysis of bread is shown in Table 7. The result reveled no significant change in the chemical composition of control bread due to the incorporation of Cinnamon powder to the level of 2.5 %. Only slight increase in ash and crude fiber was observed.

TABLE 7
RESULT OF CHEMICAL ANALYSIS OF BREAD

Parameter	Control Bread	2.5 % Cinnamon Bread
Moisture %	33.2 ± 0.44	31.9 ± 0.05
Protein %	7.41 ± 0.02	7.47 ± 0.02
Ash %	0.71 ± 0.01	0.82 ± 0.01
Fat %	1.30 ± 0.01	1.27 ± 0.0
Crude fiber %	0.33 ± 0.02	0.61± 0.01
Carbohydrates (by diff.) %	56.94 ± 0.43	42.1 ± 0.04

(All values are means of triplicate determinations ± standard deviation)

Result of microbial analysis (bread associated bacteria, yeast and mold) and sensory evaluation of control and 2.5 % cinnamon powder incorporated bread for shelf life study is given in Table 8 and Table 9 respectively. As per the microbial and sensory result the shelf life of control bread was found to be 3 days as in day 4 the microbial load increased than the acceptable limits and also the sign of spoilage observed in sensory evaluation. The shelf life of 2.5 % cinnamon powder incorporated bread was found to be 5 days as in day 6 the spoilage started as reveled in sensory evaluation and which was also confirmed from the microbial analysis as microbial load increased than the acceptable limits. Thus it was concluded that incorporation of cinnamon powder to the level of 2.5 % increased the shelf life of bread by 2 days.

TABLE 8
RESULT OF MICROBIAL ANALYSIS OF CONTROL AND CINNAMON BREAD FOR SHELF LIFE STUDY

Parameter	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	C. B	Cinn.B	C. B	Cinn.B	C. B	Cinn.B	C. B	Cinn.B	C. B	Cinn.B	C. B	Cinn.B
TPC	$1X10^2$	NIL	$2X10^3$	$1X10^2$	$8X10^3$	$1X10^3$	$12X10^3$	$3X10^3$	-	$9X10^3$	-	$1.2X10^4$
Coliform	NIL	NIL	$3X10^1$	NIL	$8X10^1$	$1X10^1$	$11X10^1$	$4X10^1$	-	$9X10^1$	-	$14X10^1$
Y & M	NIL	NIL	$2X10^1$	NIL	$4X10^1$	$1X10^1$	$12X10^1$	$3X10^1$	-	$9X10^1$	-	$16X10^1$

(C.B- Control Bread, Cinn. B -Cinnamon Bread)

TABLE 9
RESULT OF SENSORY EVALUATION OF CONTROL AND CINNAMON BREAD FOR SHELF LIFE STUDY

Parameter	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
	C.B	Cinn.B	C. B	Cinn.B	C. B	Cinn.B	C. B	Cinn.B	C. B	Cinn.B	C. B	Cinn.B
Appearance	7.5 ±0.6	7.6±0.5	6.2± 0.5	7.4± 0.6	6.5± 0.4	6.8± 0.5	5± 0.4	6.1± 0.5	-	6.1± 0.4	-	5.8± 0.5
Color	7.4± 0.8	7.5± 0.6	6.5± 0.5	7.5± 0.7	6.6± 0.5	6.5± 0.4	5.2± 0.4	6.2± 0.2	-	6± 0.3	-	5.6± 0.3
Texture	7.5± 0.6	7.7± 0.5	6.5± 0.7	7.4± 0.4	6.8± 0.3	6.7± 0.3	5.4± 0.6	6.3± 0.5	-	6.1± 0.4	-	5.4± 0.4
Flavor	7.6± 0.6	7.5± 0.7	6.2± 0.5	7.3± 0.5	6.3± 0.3	6.5± 0.4	5.2± 0.5	6.2± 0.4	-	5.8± 0.5	-	5.1± 0.5
Overall accept.	7.5± 0.5	7.5± 0.5	6.3± 0.6	7.4± 0.5	6.6± 0.4	6.6± 0.4	5.6± 0.5	6.2± 0.3	-	6± 0.4	-	5.5± 0.4

C.B- Control Bread, Cinn. B -Cinnamon Bread
(All values are means of scores given by 10 panelist ± standard deviation)

IV. CONCLUSION

Following conclusions are drawn from the study:

1)All the spices (cinnamon, clove, garlic, ginger) used in the study shown antimicrobial activity against selected micro-organisms.

2)20% is the minimum concentration to which the methanol extract from all the spices required to be diluted to show antimicrobial activity.

3)Water extract from cinnamon, clove and garlic required to be diluted to the minimum concentration of 20% to show antimicrobial activity whereas in case of ginger it is 60%.

4)Out of all the spices incorporated in the bread, cinnamon shown high acceptability on the basis of sensory evaluation and the optimum level of cinnamon powder to be incorporated to produce bread of acceptable quality was found to be 2.5%.

5)The shelf life study of bread incorporated with optimum level of cinnamon powder shown increased shelf life by 2 days as compared to control bread.

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