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Utilization of some herbs extracts for improving the quality of pan bread

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ABSTRACT

The present study aimed to evaluate the benefits of some herbs such as green tea, rosemary and ginger extracts at three levels (5, 10, and 15 %) for making pan bread. Fractionation of phenols, flavonoids and acrylamide were determined. Sensory evaluation, color index and staling of pan bread were also measured. The results showed that such extracted materials have a high content of polyphenol compounds such as pyrogallol, catechin, catechol, epicatechein, caffeine, chlorogenic and salicylic acid in addition to flavonoides compounds. The results showed that 10% green tea extract have the best results in total score in pan bread than rosemary and ginger extracts. Meanwhile, the highest reducing ratio of acrylamide was showed of using 10% extracts of green tea in pan bread comparing with the same ratio of rosemary and ginger. After three days of storage, total bacterial counts were increased in control, meanwhile pan bread made using extracts showed a slight appears in bacterial counts. Staling of pan bread used in investigation were arrangement as green tea, ginger and rosemary extracts at 10%, respectively. It can be concluded that the green tea extract at 10% can be used as functional additives on bread quality.

Keywords: green tea, rosemary, ginger, phenols, flavonoids, pan bread, sensory evaluation, acrylamide, color, spoilage, microbial and staling.

Introduction

Functional foods are classified as fortified, enriched or enhanced foods that provide health benefits with essential nutrients such as vitamins, minerals and antioxidants. Phytochemicals and phenolic antioxidants in plant including fruits, vegetables, herbs and spices are recognized as active ingredient which is responsible for human health (Ibrahim *et al.,* 2015).

General attitudes concerning functional foods have an important role in human nutrition, which is a modern global trend in the food industry. Researchers are involved in optimizing bakery products, making technology to improve the quality, taste, functionality and bioavailability of food products (Annunziata and Vecchio 2011).

Tea is the most widely consumed beverage worldwide and is rich in polyphenolic compounds known as the tea flavonoids. Green tea is prepared from fresh tea leaves and contains mainly catechins. Extract of green tea (EGT) is demonstrated as an excellent antioxidant applied in many food matrixes (Gramza *et al*., 2006). It is well known that four kinds of flavanols are the main ingredients in EGT: epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) (Zhang *et al*. 2006). Tea flavonoids (such as catechins, theaflavins and thearubigins) possess strong antioxidant properties, i.e. they may protect the body from damage caused by free radical-induced oxidative stress. (Langley-Evans, 2000).

Ginger contains natural organic materials beneficial to health and enhances resistance to infectious diseases by increasing non-specific and specific immune mechanisms. The rhizome of ginger has shown to be effective in the control of bacterial, viral, fungal and parasitic diseases in humans and aquacultures (Shakya, 2015).

Rosemary (*Rosmarinus officinalis L., Lamiaceae*) is widely cultivated all over the world as an ornamental and aromatic plant, and has been commonly used for flavouring food, and for different medicinal purposes. Recently, essential oil isolated from rosemary and monoterpenes as its main active compounds have been of great interest due to their various health benefits and therapeutic effects (Al-Sereiti *et al.,*1999 and Ngo *et al.,* 2011).

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Concerning bakery products, Hedegaard *et al.* (2008) reported that addition of aqueous rosemary extract, rosemary oil, dried rosemary leaves to wheat dough reduced the content of acrylamide in wheat buns by 62%, 67% and 57%, respectively. Significant acrylamide reduction was reported by Zhang and Zhang (2007) in fried bread sticks upon addition of antioxidants from extract green tea (EGT).

The present study aimed to utilize some herbs extracts (green tea, rosemary and ginger) for improving the nutritional, functional properties and the quality of pan bread.

Materials and Methods

Materials:

Wheat flour (72% extraction) was obtained from 6th October for Milling and Marketing Co., 6th October City, Egypt. Instant active dry yeast, crystal white sugar, sodium chloride, corn oil, green tea, rosemary powder and ginger powder were obtained from the local market, Cairo, Egypt.

Methods of Analyses:

Phenol and flavonoid compounds of the different methanolic extracts (1 gram sample: 100 ml methanol 80%) of Green tea, rosemary and ginger powder were determined according to Goupy *et al*. (1999) and Mattila *et al.* (2000). The supernatant was collected in vials for injection into a HPLC instrument (Hewlett packecd, series 1050) composed of a C_{18} hypersil BDS column with a particles size of 5 µm. Separation was carried out with methanol and actonitrile as the mobile phase, using a flow rate of 1 ml/min. Quantification of the flavonoid compounds was carried out using a standard flavonoid calibration curve.

Antioxidant activity of sample extracts was studied through the evaluation of the free radical-scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The results were expressed as percentage of inhibition of the DPPH radical and was calculated according to Alothman *et al.* (2009).

Total phenolic concentration in the extract was determined spectrophotometrically according to the Folin-Ciocalteu method of Singleton *et al.* (1999). The total phenolic content was calculated from the calibration curve using gallic acid as a standard and the results were expressed as milligram of gallic acid equivalents per gram dry weight of extract (mg GAE/g d.w.) for the dried extract.

The amount of total flavonoids in the extracts was measured spectrophotometrically as reported by Lin and Tang (2007). Briefly, 500 µL of each extract was mixed with 1.50 mL of 95% ethanol, 0.10 mL of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.10 mL of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1 M) and 2.80 mL of distilled water. After incubation for 40 min, absorbance was measured at 415 nm using a spectrophotometer. To calculate the concentration of flavonoids, we prepared a calibration curve using quercetin as standard. The flavonoid concentration is expressed as quercetin equivalents in mg per gram of extract. All assays were carried out in triplicate.

Fractionation of phenolic compounds after hydrolysis were conducted using high performance liquid chromatography (HPLC). Which equipped with a variable wave length detector (Agilant, Germany) 1100, auto sampler, Quaternary pump degasser and column compartment. Analyses were performed on a C_{18} reverse phase (BDS 5µm, Labio, Czech Republic) packed stainless-steel column (4×250 mm, i.d.). To determine phenolic compounds after hydrolysis, samples were prepared according to the method described by Jakopič *et al.* (2009). The chromatographic conditions (mobile phase, gradient program, temperature of column) were similar to those described by Schieber *et al.* (2001). All chromatograms were plotted at 280 nm to estimated phenolic acids.

Acrylamide was determined according to the method described by Mastovska and Lehotay (2006).

Pan bread Preparation:

The straight dough method for pan bread production was carried out according to the method described by AACC. (2002). Exactly 5, 10 and 15 g of green tea , rosemary and ginger /100 ml boiling distilled water (w/v) for 10 min. The extracts were filtered using filter paper (4 to 7 m pore size, Whatman International Ltd., Kent England) and leave to cool at room temperature 25± 2ºC. Exactly 60 ml of each extract was used for Kneading and preparing the different dough of pan bread

according to Farinograph water absorption determination. Bread dough was prepared by mixing wheat flour (100 g), instant active dry yeast (1.5 g), crystal white sugar (5 g), sodium chloride (1.5 g), corn oil (3 g) and kneading water (60 ml of extract) in the mixer bowl for 2 min, fermented dough was left to rest for 20 min at 28- 30^{o} C (first proofing) then the dough was divided into 150 g per piece. The pieces were hand moulded and placed into pans for final proofing at 32- 35^{o} C and 80- 85 % relative humidity in fermentation cabinet for 60 min. Dough was baked in electrically oven at 210- 220^{o} C for 15- 20 min. After baking, loaves were separated from the metal pan and allowed to cool at room temperature before sealed in polyethylene bags to prevent moisture loss then stored at room temperature (25± 2^{o} C).

Sensory evaluation of pan bread:

Pan bread was evaluated for its characteristics by ten panelists from the staff of the Cereal Technology Research Section, Agric. Res. Center Egypt. The scoring scheme was established according to the method described by AACC (2002). The characteristic of evaluation was total score (100) which divided in different items such as general appearance (15), crust color (15), crumb color (15), distribution of crumb (15), taste (20) and flavor (20).

Measurement of color of pan bread:

Pan bread color was measured by Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and light trap supplied by the manufacturer. Color was expressed using the CIE *L**, *a**, and *b** color system. A total of three spectral readings were taken for each sample. Lightness (*L**) (dark (0) to light (100)), the redness (*a**) values [(+) reddish to (-) greenish], and the yellowness (*b**) [(+) yellowish to bluish (-)] values were estimated.

Microbiological examination:

Microbialogical tests including total bacterial counts, yeasts and molds, were determined using the standard procedures outlined in the American Public Health Association **(**APHA, 1992).

Staling of pan bread:

Staling rate of bread during storage for 3 days was determined using penetrometer apparatus according to the method described by Maleki and Seibel (1972).

Statistical analysis

Data were expressed as the means ± SD. Statistical analysis was carried out using one – way analyses of variance, ANOVA (Rao, and Blane, 1985).

Results and Discussion

Phenolic compounds profiles of green tea, rosemary and ginger extracts:

The results of phenolic compounds content of green tea, rosemary and ginger extracts were recorded in Table (1). The data of fractionation showed that catechein (27.74mg/100g), epicatechein (67.5mg/100g), Caffeine (190.43mg/100g), vanillic acid (190.1 mg/100g) and e-Vanillic acid (71.18 mg/100g) were major phenolic compounds in green tea extract. Langley-Evans (2000) reported that the tea flavonoids (such as catechins, theaflavins and thearubigins) possess strong antioxidant properties, i.e. they may protect the body from damage caused by free radical-induced oxidative stress. The major phenolic compounds of rosemary extract were e-Vanillic acid (303.34 mg/100g), benzoic acid (106.99 mg/100g) and ellagic acid (88.31 mg/100g). Xie *et al*. (2017) found that the rosemary extract is a rich source of phenolic compounds that showed high antioxidant performance in both *in vitro* chemical assay studies and real food applications. Water soluble compounds from rosemary extract can be used for different types of food applications. This makes rosemary extract a highly versatile and functional antioxidant for the food industry and markets.

Data cleared that the major phenol compounds of ginger extract were pyrogallol acid (27.3 mg/100g) and e-Vanillic acid (14.43 mg/100g).

Table 1: Phenolic compounds profiles of green tea, rosemary and ginger extract (mg/100g).

Phenolic compounds	Green tea	Rosemary	Ginger
Gallic Acid	23.46	0.56	0.48
Pyrogallol Acid	5.73	22.81	27.30
4-Amino-benzoic Acid	2.53	0.45	0.52
Protocatchuic Acid	18.97	8.61	0.74
Catechein	27.74	13.17	2.02
Chlorogenic acid	9.73	36.52	0.46
Catechol	3.80	25.98	0.26
Epicatechein	67.50	12.01	0.74
Caffeine	190.43	5.93	0.25
P-OH-benzoic acid	9.16	10.65	0.85
Caffeic acid	0.55	5.45	0.41
Vanillic acid	190.1	14.33	0.65
p-Coumaric acid	3.23	15.24	1.24
Ferulic acid	1.49	18.11	0.51
Iso-Ferulic acid	2.44	24.72	0.76
Rrversetol	1.07	57.10	0.77
Ellagic acid	7.11	88.31	1.96
e-Vanillic acid	71.18	303.34	14.43
Alpha-Coumaric acid	2.70	20.19	0.17
Benzoic acid	33.23	106.99	2.23
3,4,5-Methoxy-Cinnamic	1.03	5.55	0.72
Coumaric acid	0.47	39.35	1.62
Salicylic acid	1.51	15.95	2.52
Cinnamic acid	0.40	2.60	0.51

Wang *et al*. (2004) stated that green tea extract contain polyphenols which are natural antioxidants. Also, green tea is utilized as active ingredient in wide range of applications especially in food and nutraceutical. Ginger has been shown to exhibit antioxidant effects (Fuhrman *et al.* 2000).

Fractionation of flavonoid compounds of green tea, rosemary and ginger extracts (mg/100g) were shown in Table 2. The major flavonoid compound in green tea and ginger was Hisperdin, meanwhile, the major flavonoid compounds in rosemary were Hisperdin, narengin and rosmarinic.

Table 2: Flavonoid compounds profiles of green tea, rosemary and ginger extract (mg/100g).

Flavonoid	Green tea	Rosemary	Ginger
Luteolin	13.01	53.90	1.28
Narengin	4.93	320.82	1.31
Rutin	1.34	33.64	0.22
Hisperdin	117.98	492.1	8.81
Rosmarinic	3.91	305.44	2.99
Quercetrin	4.36	12.76	0.31
Quercetin	1.23	64.1	0.86
Narngenin	5.89	41.93	1.40
Hispertin	3.42	40.81	0.52
Kampferol	2.44	16.81	0.51
Apegnin	0.21	19.98	0.25

Rosemary contained polyphenolic compounds and flavonoids (e.g., homoplantaginin, cirsimaritin, genkwanin, gallocatechin, nepetrin, hesperidin, and luteolin derivatives) (Baser and Buchbauer, 2010).

Table 3 showed that the highest total phenolic, flavonoid contents and antioxidant activity was found in green tea extract than that found in the rosemary and ginger. Shannon *et al.* (2018) mentioned that green tea has a good source of dietary phenolic antioxidants. Mošovská *et al.* (2015) who reported that total flavonoid content were 14.15 mg quercetin /g ginger extract.

Table 3: Total antioxidant activity of green tea, rosemary and ginger extracts.

Parameters	Green tea	Rosemary	Ginger
DPPH (%)	63	39	28
Total phenolic contents (mg /g GAE*)	123	103	70
Total flvonoids as quercetin mg/g)	40	33	19

*GAE: Gallic acid equivalent.

Sensory evaluation of the resultant pan bread:

Sensory characteristics of the resultant pan bread (crust color, crumb distribution, crumb color, taste, flavor, general appearance and total score) are presented in Table (4). The results showed that there is no significant difference in crust color, Crump distribution, crumb color, taste, flavour, general appearance and total score at 5 and 10 % for green tea and rosemary extracts relative control. Mean while 15 % green tea and rosemary extracts showed that a significant difference comparing with control. Also, the results recorded that 15% of rosemary, green tea and ginger extracts have lower and significantly difference than other samples (61.8 rosemary, 44.5 green tea and 72.3 ginger) comparing with control (95.3). From the obtained data it could be concluded that 10% green tea extract have the best results in crust color, crumb distribution, crumb color, taste, flavour, general appearance and total score in pan bread than Rosemary and ginger extracts. These results are in agreement with those reported by Wang *et al.* (2007) who stated that the green tea extract has good effect and can be used as functional ingredient on bread quality. Moreover, the green tea extract was significantly contributed in the taste of the bread, besides that, it also involved in hardness, sweetness, stickiness and astringency of the bread. Since the green tea contains excellent source of antioxidant, fortification with bread will provide functional food product with additional health benefits.

Table 4: Sensory evaluation scores (Mean ±SD) of pan bread made using different extracts of rosemary, green tea and ginger.

Samples			Crust color (15)	Crumb distribution (15)	Crumb color (15)	Taste (20)	Flavor (20)	General appearance (15)	Total score (100)
Control			$14.5^{a} \pm 0.57$	$14.0^{a} \pm 0.82$	$14.5^{a} \pm 0.57$	$19.0^{a} \pm 0.82$	$19.3^{a} \pm 0.96$	$14.0^{a} \pm 0.82$	$95.3^{a} \pm 0.95$
Different extracts (g sample / 100 ml water)	Green tea	5%	$13.5^{ab} \pm 0.57$	$13.5^{a} \pm 0.57$	$13.8^{ab} \pm 0.95$	$18.8^{a} \pm 0.50$	$17.3^{a} \pm 0.95$	$13.0^{ab} \pm 0.82$	$89.8^{a} \pm 1.70$
		10%	$13.3^{ab} \pm 0.95$	$14.0^{a} \pm 0.82$	$14.0^{ab} \pm 0.82$	$19.3^{a} \pm 0.95$	$18.5^{a} \pm 0.58$	$13.8^{a} \pm 0.50$	$92.8^{a} \pm 2.21$
		15%	$4.5^{e} \pm 1.29$	$13.0^{a} \pm 0.81$	$4.50^{d} \pm 1.29$	$10.0^{d} \pm 5.25$	$8.3^{c} \pm 1.71$	$6.5^{d} \pm 2.38$	$44.5^{d} \pm 8.39$
	Rosemary	5%	$13.3^{ab} \pm 0.95$	$13.5^{a} \pm 0.57$	$14.5^{a} \pm 0.58$	$18.5^{a} \pm 1.29$	$17.5^{a} \pm 1.29$	$14.0^{a} \pm 0.82$	$91.3^{a} \pm 3.10$
		10%	$13.0^{abc} \pm 0.82$	$13.3^{a} \pm 0.95$	$13.5^{ab} \pm 0.57$	$18.5^{a} \pm 1.29$	$18.3^{a} \pm 1.25$	$13.0^{ab} \pm 0.82$	$89.5^{a} \pm 4.51$
		15%	$9.8^{d} \pm 0.95$	$13.3^{a} \pm 0.95$	$6.5^{c} \pm 1.29$	$8.5^{b} \pm 1.29$	$12.3^{b} \pm 2.21$	$11.5^{bc} \pm 1.29$	$61.8^{c} \pm 1.25$
	Ginger	5%	$13.0^{abc} \pm 1.41$	$14.0^{a} \pm 0.82$	$14.0^{ab} \pm 0.82$	$18.8^{a} \pm 0.95$	$18.5^{a} \pm 0.57$	$12.5^{abc} \pm 1.29$	$90.8^{a} \pm 3.68$
		10%	$11.5^{c} \pm 1.29$	$14.0^{a} \pm 0.82$	$14.0^{ab} \pm 0.82$	$19.0^{a} \pm 0.82$	$17.3^{a} \pm 0.96$	$11.5^{bc} \pm 1.29$	$89.5^{a} \pm 5.97$
		15%	$12.3^{bc} \pm 0.95$	$10.8^{b} \pm 1.70$	$12.5^{b} \pm 2.08$	$7.5^{b} \pm 1.29$	$18.5^{a} \pm 0.57$	$10.8^{c} \pm 1.70$	$72.3^{b} \pm 3.86$

Means in the same column with different letter are significantly different *($P \leq 0.05$).*

Effect of different extracts on acrylamide level of the resultant pan bread:

Acrylamide level of the resultant pan bread made using different herbs extracts were recorded in Table 5. The data showed decreasing in acrylamide level in all pan bread samples comparing with control. More decreasing in acrylamide level pan bread was observed for green tea extract 10% which showed the highest decrease in acrylamide level. It contained 1.15 ppm followed by the bread produced using extract of rosemary and ginger 2.54 and 3.55 ppm, respectively, this result agree with Hedegaard *et al.* (2008) who reported that adding aqueous rosemary extract, rosemary oil and dried rosemary leaves to a bread model, reduced the content of acrylamide by 62, 67 and 57%, respectively, compared with bread without rosemary.

Hedegaard, *et al.* (2008) evaluated the effect of antioxidants on the content of acrylamide in wheat bread as a heated food product often spiced with herbs such as rosemary, which are known to have antioxidative properties and are used in many types of food. They found that rosemary decreased the acrylamide formation when added to dough prior to baking. Zhang *et al.* (2008) demonstrated that two kinds of natural antioxidants "Antioxidant of Bamboo Leaves (AOB)" and "Extract of Green Tea (EGT)", of which the main functional ingredients were flavonoids, lactones and phenolic acids, could be significantly reduced the acrylamide level.

Table 5: Effects of green tea, rosemary and ginger extracts on acrylamide content (ppm) in pan bread.

Samples	**Acrylamide content (ppm)**		
Control	7.94		
Extracts of	Green tea	Rosemary	Ginger
5g /100 ml	1.50	4.17	6.07
10g /100 ml	1.15	2.54	3.55

Miyoshi *et al*. (2015) reported that green tea which contain catechins, epigallocatechin, gallate and caffeine are strong antioxidants which bind with proteins, lipids and nucleic acids led to decrease the acrylamide content in pan bread contenting green tea extract.

Color coordinators of the samples:

The color value of pan bread made using herbs extracts were recorded in Table 6. Chroma measurement showed a consistent trend in bread color with the Table 4. For bread made using green tea extract, the L* value reduced had higher values from 65.95 to 54.91 while increased in bread made using rosemary and ginger extract 59.36 and 62.66, respectively. On the other hand the (a*) value increased in the three samples compared with control sample. This means that the brightness decreased and red color increased. It is to note that the b* value i.e. yellow color showed an increased trend compared to the control sample. Nevertheless, the L*, a* and b* values were all significantly different between the control and the bread sample contenting herbs extractes. This results are in agreement with Surdyk *et al*. (2004) who reported that the Maillard reaction is also responsible for color formation during food processing. This fact gave rise to the question of whether a correlation exists between acrylamide content and color intensity. Studies on the relationship between color and acrylamide content in bread crusts with different acrylamide content showed that the color varied from almost white to dark brown. A highly significant correlation ($P<0.001$) was found between color and acrylamide content in these crusts when the breads were baked with the same recipe.

Table 6: The CIE color values of the resultant pan bread made using extracts ginger, rosemary and green tea

CIE color	**Control**	**Green tea extract**	**Rosemary extract**	**Ginger extract**
L*	65.95 ^{a} ±1.01	54.91 ^{c} ±0.93	59.3 6 ^{bc} ±1.06	62.6 6 ^{ab} ±5.34
a*	7.60 ^{c} ±0.42	8.82 ^{bc} ±1.00	10.97 ^{a} ±0.69	9.36 ^{b} ±0.94
b*	29.68 ^{b} ±1.11	29.80 ^{b} ±0.35	32.10 ^{a} ±1.13	30.02 ^{b} ±0.25

Means in the same row with different letter are significantly different at ($P \leq 0.05$).

Total bacterial, Molds and yeast counts of pan bread made using extracts containing 10% green tea, rosemary and ginger:

Total bacterial, molds and yeast counts of pan bread made using extracts of green tea , rosemary and ginger were recorded in Table 7. Total bacterial, molds and yeast counts of pan bread showed no appears at zero time. After 24 hours total bacterial counts showed a low count growth (6×10^{1} - 4×10^{2}) in control and slightly appears (2×10^{1} - 1×10^{2}) green tea, rosemary (3×10^{1}-2×10^{2}) and ginger (4×10^{1}-2×10^{2}) but molds and yeast not detected. Total counts increased in control after 48 h (9×10^{1}-7×10^{2}) and 72 h (16×10^{1}-14×10^{2}) mean while molds and yeast was not detected at 48 h and

appears at 72 h (8×10^1-6×10^2), also green tea and rosemary extracts showed a slightly appears in total counts (4×10^1 - 2×10^2) - (6×10^1 - 4×10^2) and (6×10^1 - 3×10^2) - (8×10^1 - 4×10^2) respectively , but molds and yeast were not detected on pan bread made using such extracts. Meanwhile ginger extracts appeared a slightly increase in total counts (7×10^1 - 4×10^2) - (11×10^1 - 7×10^2) than green tea and rosemary extracts but, molds and yeast also were not detected. The data means that pan bread made using extracts was decreased growth of microbes (total counts, molds and yeast) and may be refernd to the green tea, rosemary and ginger extracts contained antimicrobial substances. Friedman (2007) studied an antibacterial, antitoxin, antiviral, and antifungal activities of teas and tea flavonoids and he reported that these metabolites include phenolic compounds, the so-called six catechins, and the methyl-xanthine alkaloids, caffeine, theobromine, and theophylline. These phenolics of tea are also known for their antibacterial activity. Campo *et al*. (2000) studied the antioxidant activity of rosemary extracts and they found that the antimicrobial effects were reported to be strongly dependent on the composition of the medium.

Table 7: Total bacterial, Molds and yeast counts of the resultant pan bread during storage for 3 days at room temperature.

Pan bread samples	Time (hr)	Total bacterial counts		Yeast and molds counts	
		$\times 10^1$ CFU/g	**$\times 10^2$ CFU/g**	**$\times 10^1$ CFU/g**	**$\times 10^2$ CFU/g**
Control	zero	N.D	N.D	N.D	N.D
	24	6	4	N.D	N.D
	48	9	7	N.D	N.D
	72	16	14	8	6
Green Tea extracts	zero	N.D	N.D	N.D	N.D
	24	2	1	N.D	N.D
	48	4	2	N.D	N.D
	72	6	4	N.D	N.D
Rosemary extracts	zero	N.D	N.D	N.D	N.D
	24	3	2	N.D	N.D
	48	6	3	N.D	N.D
	72	8	4	N.D	N.D
Ginger extracts	zero	N.D	N.D	N.D	N.D
	24	4	2	N.D	N.D
	48	7	4	N.D	N.D
	72	11	7	N.D	N.D

N.D: Not detected growth of microorganism

Saeed *et al.* (2013) revealed that there is a great potential of using spices extracts as natural antimicrobials for controlling food spoilage and pathogenic bacteria, signify the fact that natural products like spices can be seen as alternatives to chemical preservatives used in various food industries so as to minimize their side effects and simultaneously improving the shelf life of the food products.

Staling determination of the resultant pan bread:

The effects on shelf life of pan bread made using 10% extracts were shown in Fig 1. The green tea extracts has a good indication and delayed staling of pan bread comparing with rosemary and ginger extracts after storage for 3 days. Staling of pan bread used in investigation was arrangement as green tea, rosemary and ginger extracts respectively.

Kim and Park (2002) reported that characteristics for stalling indicated that quality of pan bread was significantly extended by addition of green tea extract. Senanayake (2013) reported that green tea extract, contained a natural source of antioxidant, include catechins (epicatechin and epigallocatechin) which has been successfully used to enhance the shelf-life of bakery products such as pizza toppings, cereals and snack foods and others.

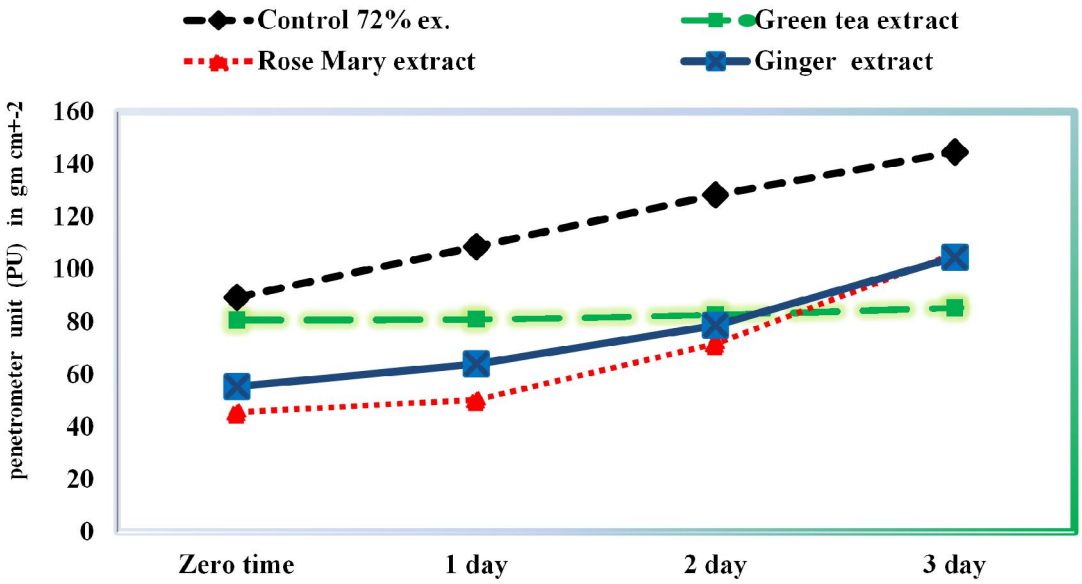

Fig. 1: Staling of pan bread made using extracts

Conclusion:

It could be concluded that green tea, rosemary and ginger extracts can be used in baking products to improve the nutritional value and to the its amounts of antioxidant, antimicrobial activity, reduce acrylamide, delayed staling and prolonging the shelf life of pan bread.

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