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Quality and Antioxidant Properties of Pan Bread Enriched with Watermelon Rind Powder

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ABSTRACT

The aim of the study was to investigate the effect of replacement levels different (0, 3, 6, 9 and 12 %) of watermelon rind powder (WMRP) as partially substitute for wheat flour in producing pan bread for improving the antioxidant potential of pan bread and increasing shelf life by increasing the phenolic compounds content of pan bread without compromising its sensory quality. The results showed that the pan bread containing (6, 9 and 12 % watermelon rind powder) had the highest phenolic content 18.85, 25.64 and 33.04 mg /100g on dry weight, respectively. The content of total flavonoids in pan bread with different levels of WMRP was higher 3:9 fold when compared to pan bread control. The addition of WMRP at different levels to pan bread, was effective in enhancing antioxidant activity, as evaluated by means of DPPH, which increased to 3.10, 3.40, 3.65 and 3.90 mmol Trolox/kg in pan bread containing different levels WMRP compared with pan bread control (2.95 mmolTrolox/kg).The improving of the antioxidant potential of pan bread led to increasing shelf life (10 days) compared with pan bread control (6 days) by increasing its phenolic compounds content in pan bread. Analysis of sensory results of pan bread showed that addition of WMRP to the dough improved the sensory properties of pan bread and increased acceptable quality attributes such as taste, color and odor. It could be WMRP at level of 6-12% suggest that addition of WMRP into the formula of pan bread can increase antioxidant potential and increasing shelf life by increasing the phenolic compounds content as well as improve the sensory properties of pan bread. Also, pan bread fortified with WMRP, may be placed on the market as a functional food.

Key words: Watermelon rindpowder, pan Bread,enriched,antioxidant properties, quality; substitution

Introduction

Nowadays, the food and agricultural product processing industries generate substantial quantities of phenolic-rich by-products, which could be valuable natural sources of antioxidants to be employed as ingredients. Phytochemicals, including phenolic compounds, are suggested to be the major bioactive compounds contributing to the health benefits of fruits, vegetables and grains (Mateo Anson *et al.,* 2010 and Roldan *et al.,* 2008). Flavonoids are a group of phenolic compounds with antioxidant activity that have been connected to reducing the risk of major chronic diseases (Boots *et al.,* 2011; Gawlik-Dziki *et al.,* 2011).
Phenolic compounds are widely distributed in foods, such as fruits, vegetables and cereals (Dykes and Rooney, 2007; Liyana-Pathirana and Shahidi, 2006). As naturally occurring antioxidants, phenolic compounds have been reported to possess diverse beneficial bioactivities, including anti-allergic, antiviral, anti-inflammatory and anti-mutagenic properties (Yao *et al.,* 2004). Meanwhile, a large number of in vitro and animal studies have also suggested that phenolic compounds may be effective in protecting against cancer, and cardiovascular diseases. The protective effects might be mediated through their action as antioxidants to prevent oxidative damage induced by reactive oxygen species to some important biomolecules (like DNA, lipids and proteins) under pathological conditions (Hollman, 2001; Yao *et al.,* 2004).

Recently, phenolic antioxidants have been viewed as an important class of food ingredients either as food additives or as novel ingredients to introduce extra health benefits to various food products. Considering the fact that heat treatment is a widespread processing method in the food industry, a salient question is whether these thermal processes would lead to significant alterations in the antioxidant capacities of phenolic additives. It has been reported that total antioxidant activities of tomatoes and carrots were enhanced with thermal processing (Dewanto *et al.,*2002; Patras *et al.,*2009) while antioxidant capacities of soybeans were lowered with similar processing (Xu and Chang, 2008).

Watermelon is an important crop grown in the warmer regions of the world. Half of a watermelon fruit is edible while the other half, consisting of about more 35% rind and 15% peel goes to waste (US Department of Agriculture, 2004). Watermelon is utilized for the production of juices, nectars and fruit cocktails, etc.

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(Ahmed,1996 and Bawa and Bains, 1977) whereas major by-product "the rind" is utilized for the products such as pickle, preserve, pectin and other products, etc.

El-Badry *et al.,* (2014) investigated that the addition effect of watermelon rind powder at different levels on some important quality properties such as rheological, physical, chemical properties, and sensory attributes of pan bread produced. The present results showed that more than 38 % from the total weight of the watermelon fruit as the waste may be used as a good source of the dietary fiber, ash, indispensable amino acids, phenolic and flavonoid compounds, also the rheological study was appeared increasing in stability and better product conservation by addition of watermelon rind powder.

Watermelon contains moderate but significant quantities of phenolics (Perkins-Veazie *et al.,* 2002 and 2007; and Brat *et al.,* 2006). Among phenolics, flavonoids reduce low density lipoprotein (LDL) oxidation and quench reactive oxygen radicals, decreasing thereby the risk of cardiovascular diseases and cancers (Pietta, 2000 and Lila, 2004). Although the biological effect of flavonoids is, in general, attributed to their antioxidant activity, recent investigations indicate that they might affect signaling pathways in animal cells (Williams *et al.,* 2004).

The therapeutic effect of watermelon has been reported and has been ascribed to antioxidant compounds (Leong and Shui, 2002; Lewinsohn *et al.,* 2005). The citrulline in watermelon rinds gives it antioxidant effects that protect you from free-radical damage. Additionally, citrulline converts to arginine, an amino acid vital to the heart, circulatory system and immune system. These researchers speculate that watermelon rind might relax blood vessels as cancer and cardiovascular diseases (Rimando and Perkins-Veazie, 2005).The rind is usually discarded; they are edible, and sometimes used as a vegetable.

In developing functional bakery products (such as bread), it is important to develop a product with physiological effectiveness and consumer’s acceptance in terms of appearance, taste and texture (Siró *et al.,* 2008). Bread is an important staple food made of wheat flour, salt and yeast, and consumed worldwide (Fan *et al.,* 2006). Nowadays consumers prefer to eat healthier foods in order to prevent non-communicable diseases. For this reason industry and researchers are involved in optimizing bread making technology to improve the variety, quality, taste and availability of food products such as bread (Hathorn *et al.,* 2008). Among the ingredients that could be included in bread formulation there are herbs and spices, which are important part of the human diet. They have been used for thousands of years to enhance the flavor, color and aroma of food and also for their preservative, anti-oxidative, antimicrobial and other medicinal values.

This study investigate the impact of replacement of wheat flour by watermelon rind powder at different levels (0, 3, 6, 9 and 12 %) in producing pan bread for improving the antioxidant potential of pan bread and increasing shelf life by increasing its phenolic compounds content without compromising its sensory quality, and improve the most important quality characteristics of pan bread.

Material and Methods

Materials:

Watermelon Fruits (*Citrullus lanatus*) used in this investigation were obtained from local market in Cairo, Egypt. Waste materials used were namely watermelon rinds (WMR). Soft wheat flour (72% extraction) was obtained from The Five Stars Co. Adabia, Suez, Egypt. Instant active dry yeast, shortening, sugar (sucrose) and salt (sodium chloride) were purchased from the local market, Cairo, Egypt.

All chemicals used in this study for chemical analysis were analytical grade and purchased from El-Gamhouria Trading Chemicals and Drugs Company, Egypt.

Methods:

Preparation of watermelon rind powders:

The watermelon rind was separated from the washed fresh watermelon fruits and cut into small pieces by a sharp knife for water removing by spread in trays of air dryer, and dried at 50±5°C till its moisture content reached to 11%, and then the dehydrated pieces were ground in a laboratory disc mill (Braun AG Frankfurt Type: KM 32, Germany) to fine powder.

Pan bread processing:

The straight dough process was performed in pan bread preparation according to the method described by Curie *et al.* (2002). The ingredients were: 100 g wheat flour, 1.5 g instant active dry yeast, 2.0 g salt, 2.0 g sugar, 3.0 g shortening and water (according to farinograph test). Treatment (1-4) containing watermelon rind powder as partially substitute for wheat flour at different levels (0, 3, 6, 9 and 12 %) were placed in a mixing bowl at 28 ±2.0°C and mixing for 6 min, after mixing, the formulated dough was rounded manually by folding for 20 times, then the bulk dough was leaved to rest for 10 min. The prepared dough (120 g) was placed in lightly greased a baking pan (5 · 9 · 8). The dough were proved for 80 min in a cabinet at 30± 0.5 °C and 85% relative humidity then baked for 20 min at 250 °C in an electrical oven. Before measurements, the baked breads were cooled at room temperature (25± 2.0 °C) for 60 min and then packed in polyethylene bags and stored for 12 days at room temperature (25± 2.0 °C).

Chemical analysis:

Moisture, protein (N×5.7), ether extracts, ash and fiber were determined in tested samples according to A.O.A.C., (2005).Total carbohydrates were calculated by difference.

Determination of the total phenolics (TP) content:

The total phenolics (TP) content was conducted according to the modified Folin–Ciocalteu colorimetric method Singleton *et al.* (1999) at 760 nm using an UV/Visible spectrophotometer (Spekol 11 No. 849101, Carl Zeiss JENA). Gallic acid was used as a standard, and then the results were expressed as Gallic acid equivalents (GAE) per 100 g dry matter.

Identification and quantification of Phenolic compounds by HPLC analysis:

Extraction and measurement of phenolic compounds in watermelon rind powder was analyzed by HPLC according to the method of Goupy *et al.,* (1999) as follows:

Weighed sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2 µm Millipore membrane filter then 1-3 ml was collected in a vial for injection into HPLC Hewlett-Packard (series 1050) equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set as 280 nm and quaternary HP pump (series 1100). The column temperature was maintained at 35 0c. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1 ml/min. Phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of Phenolic compounds concentration. The Phenolics compounds were determined in Ministry of Agricultural and Land Reclamation, Agricultural Research Center, Food Technology Research Institute.

Determination of the total flavonoid content:

Total flavonoid was analyzed according to the method described by Bahorun *et al*., (2004). The absorbance of extracted flavonoids was measured at 510 nm on a spectrophotometer (Spekol 11 No. 849101, Carl Zeiss JENA) against the blank (distilled water) and the total flavonoids content was expressed as mg rutin equivalents/100 g (dry weight basis).

Determination of the total antioxidant activities (TAA) by determination of DPPH radical scavenging activity.

DPPH (1,1- diphenyl-2-Picrylhydrazyl) radical scavenging activity was measured according to the method of Yen and Chen (1995) with modification (Pasko *et al.,* 2009). For measurement of sample scavenging activity 0.4 mL of methanolic acetate buffer was added to the cuvettes containing the increasing volumes of sample (e.g. 0, 0.1, 0.2, 0.3, 0.45, 0.6 mL) with adequate volumes of methanol to make total volume of 1 mL. Acetate buffer was made from 0.2 mol/L solutions of sodium acetate and acetic acid in methanol mixed at the volume ratio 7.9:2.1. The pH of the buffer was 5.2. 1 mL of DPPH stock solution (12 mg DPPH was dissolved in 100 mL of methanol; absorbance 1.3) was added to each cuvette, then absorbance was measured after 24 h. The absorbance of the resultant solution was determined using JascoUV-530 spectrometer (Japan) at 514 nm. The total antioxidant activities (TAA) were estimated as 6-hydroxy-2,5, 7, 8-tetramethyl-2-carboxylic acid (Trolox)equivalents (TEAA) by interpolation to 50% inhibition (TEAA50).

Microbiological aspects

The microbiological analysis were carried out including the determination of total bacterial count (TBC) using nutrient agar medium (Oxoid 1982, UK), yeast & mold count using potato dextrose agar (PDA) Oxoid 1982, UK.

Sensory evaluation of pan bread:

Sensory properties of pan bread were evaluated after cooling by 10 members from the Food Science and Technology Department, Faculty of Agriculture, Al-Azhar University, and semi training staff members of the Egyptian Baking Technology Center, Egypt comprise panelists group for Shape, Odor, Taste, Crust color, Crumb color, Crumb texture and Overall acceptability according to the method described by Pyler (1973).

Statistical analysis:

All data were statistically analyzed by using SPSS (version 16.0 software Inc. Chicago, USA) of completely randomized design as described by Gomez and Gomez (1984). Treatment means were compared using the least significant differences at 0.05 levels of probability and Standard Error.

Results and Discussion:

Quality characteristics of pan bread as affected by partial substitution of wheat flour with watermelon rind powder (WMRP):

The effect of replacement of wheat flour by watermelon rind powder as partially substitute in producing pan bread at different levels (0, 3, 6, 9 and 12 %) on the improving the antioxidant potential of pan bread for increasing shelf life by increasing its phenolic compounds content without compromising its sensory quality.

Total phenolic, flavonoid compounds and DPPH of watermelon rind powder, and its content in pan bread before kneading and after bread-making.

Phenolics compounds have showed wide range of cumulative biological affects including anti-inflammatory, antibacterial, vasodilator actions, anticarcinogenic, antiviral, antithrombotic, antiallergic, and hepatoprotective affects (Haggag *et al.* 2011). The biochemical, chemical, epidemiological and clinical evidences support the chemo protective effects of phenolic substances against oxidative stress facilitated disorders (Turner *et al.*, 2005; Del Bano *et al.,* 2006 and Jayaram and Dharmesh, 2011). Recent awareness regarding their anti-cancer potential built up a pressure on food and pharmaceutical industries to explore more and more phenolic resource and innovates efficient to utilization from the natural sources of Phenolics compounds especially agro wastes by adding extracts or whole dried waste which was rich with Phenolic compounds to food as natural sources. Watermelon and watermelon rind contains substantial level of phenolic antioxidants (Al-Sayed and Ahmed, 2013 and Tillie*et al.,* 2011). In recent decades, flavonoids have been the focus of much research, due to their potential as health promoting phytochemicals. Flavonoids exhibit antioxidant and antimicrobial properties and have been investigated extensively regarding their ability to lower the risk of cardiovascular diseases Volden *et al.,* (2009).

The health promoting phytochemicals including total phenolic, total flavonoids contents as antioxidant compounds and the total antioxidant activities (TAA) by determination of DPPH radical scavenging activity in pan bread formula containing watermelon rind powder before kneading and after bread-making were determined and the obtained results are shown in Table (1 and 2).From the obtained data (Table 1 and 2), it could be noticed that the flavonoids compounds content was the major antioxidants compounds found in watermelon rind powder, it was represented about 380.95 mg /100g. The total phenolic in watermelon rind powder was lower than the total flavonoids which was 229.85 mg /100g as shown in Table (1 and 2).

From the same Table (1 and 2), it could be also observed that the total phenolic content in pan bread formula before kneading and after pan bread-making at different level of partially substituted of wheat flour with watermelon rind powder was decreased from 11.65 in pan bread before kneading to 8.70 mg /100g in pan bread after bread- making at level 3% of WMRP, and from 33.04 to 26.35 mg /100g at level 12% of WMRP, as compared with the control sample which decreased from 4.96 to 3.62 mg /100g; respectively.

Also, the total flavonoids was decreased from 12.60 to 8.80 mg /100g at substitution level (3%), and from 30.25 to 26.94 mg /100g at substitution level (12%) as compared with control samples which was decreased from 6.87 to 3.11 mg/100g pan bread formula before kneading and after pan bread-making; respectively.

On the other hand, the total antioxidant activities (TAA) determined by DPPH of Wheat flour, watermelon rind powder and pan breads before and after bread-making were expressed as mmol of Trolox per 1 kg dry weight. DPPH before bread-making was highest in WMRP 8.90 mmol of Trolox per 1 kg d.w compared with wheat flour (3.19mmol of Trolox per 1 kg d.w) led to increasing the DPPH by increasing replacement levels (3, 6, 9 and 12%) of WMRP in pan bread (3.10, 3.4, 3.65 and 3.9 mmol of Trolox per 1 kg d.w, respectively). Also, DPPH after bread-making was increasing by increasing replacement levels of WMRP in pan bread from 1.1mmol of Trolox per 1 kg d.w for pan bread control to 2.85mmol of Trolox per 1 kg d.w for pan bread 12% WMRP. Their results were relatively comparable with the present data by Dewanto *et al.,*2002; Patras *et al.,*2009 and Xuand Chang, 2008.

From the former discussion, it could be mentioned that all substitution level of wheat flour by watermelon rind powder led to supplementation of produced bread by considered amounts of natural antioxidants especially in samples substituted at level 9 and 12 %watermelon rind powder.

Table 1: Total phenolic, flavonoid compounds and DPPH of watermelon rind powder and its content in pan breads before kneading.(M± SE)

Properties	Wheat flour	WMRP*	Substitution levels				
			Control	3% WMRP	6% WMRP	9% WMRP	12% WMRP
			Pan Breads formula before Kneading				
Total Phenolics (mg /100g)	60.56±2.38	229.85±1.95	4.96±1.60 a	11.65±1.45 b	18.85±1.50 c	25.64±1.45 d	33.04±1.55 e
Total Flavonoids (mg /100g)	7.89±2.38	380.95±1.50	6.87±2.55 a	12.60±1.18 b	18.33±1.15 c	23.90±1.20 d	30.25±1.25 e
DPPH [mmol Trolox/kg]	3.19 ±0.11	8.90 ± 0.52	2.95±0.23 a	3.10±0.19 b	3.40±0.20 c	3.65±0.28 d	3.90±0.31 e

WMRP watermelon rind. M± SE: Means± standard error for total phenolic, flavonoid compounds and DPPH; the means within the same row having different superscript are significantly varied (P ≤ 0.05).*
DPPH: 1,1-diphenyl-2-picrylhydrazyl. Trolox: 6-hydroxy-2,5,7,8-tetramethyl-2- carboxylic acid.

Table 2: Total phenolic, flavonoid compounds and DPPH of watermelon rind powder and its content in pan breads after bread-making.(M± SE)

properties	Wheat flour	WMRP*	Substitution levels				
			Control	3% WMRP	6% WMRP	9% WMRP	12% WMRP
			Pan Breads after bread-making				
Total Phenolics (mg /100g)	60.56±2.38	229.85±1.95	3.62±1.05 a	8.70±1.09 b	15.05±1.08 c	21.85±1.55 d	26.35±1.55 e
Total Flavonoids (mg /100g)	7.89±2.38	380.95±1.50	3.11±1.50 a	8.80±1.55 b	14.90±1.45 c	20.89±1.60 d	26.94±1.30 e
DPPH [mmolTrolox/kg]	3.19 ±0.11	8.90 ± 0.52	1.10±0.13 a	1.85±0.11 b	2.10±0.16 c	2.50±0.13 d	2.85±0.17 e

WMRP watermelon rind. M± SE: Means± standard error for total phenolic, flavonoid compounds and DPPH; the means within the same row having different superscript are significantly varied (P ≤ 0.05).*
DPPH: 1,1-diphenyl-2-picrylhydrazyl. Trolox: 6-hydroxy-2,5,7,8-tetramethyl-2- carboxylic acid

Identification of phenolic compounds in watermelon rind

Phytochemicals compounds such as phenolic compounds that have much more valuable materials that known to be have healthy effect for their bioavailability in human body where its acts as antioxidants, anticarcinogenic and also considered as chemo preventive for inhibition of pathogenic bacteria. It was found by many workers that phenolic compounds in diets could play this role in our bodies (Hodges *et al.,* 2006; Stojceska *et al.,* 2008; Słupski *et al.,* 2009; Picchi *et al.,* 2012 and Gowers, 2010). Therefore, it was established as healthy materials, as reported by other workers (Dixon, 2007; Clout, 2009; Gil-Izquierdo *et al.* 2002 and Scalbert *et al.* 2005).

Phenolic compounds could protect against degenerative diseases involving oxidative damage due to their antioxidant action and their potential as health promoting phytochemicals, also exhibited antioxidant and antimicrobial properties and have been investigated extensively regarding their ability to lower the risk of cardiovascular diseases (antioxidant properties, disease prevention and activity against toxins Kondratyuk, and Pezzuto, (2004) and volden *et al.,* (2009).

Recently the role of phenolic compounds from foods and beverages in the prevention of free radical-mediated diseases has become more important due to healthy benefits; the discovery of the link between per oxidation of low-density lipoproteins (LDL) and arteriosclerosis. The emphasis placed by the European Commission on enhancing the nutrient content of food crops through traditional plant breeding as well as food-processing technologies confirms the importance of phenolic compounds in terms of health benefits to the international community Prakash and Gupta 2009.

To make pan bread have this effects, in present work, watermelon rind powder (WMRP) was partially substituted from wheat flour in produced pan bread at different levels (0, 3, 6, 9 and 12 %) for the improving the antioxidant potential of pan bread by increasing its phenolic compounds content without compromising its sensory quality.

The health promoting phytochemicals such as phenolic contents as antioxidants which are naturally occurred in watermelon rind were determined in powder, as shown in Table (3).

The obtained results by HPLC analysis (Table 3), indicated that the Syringic acid was the major of phenolic compounds in watermelon rind powder it was represented about 3662.21 ppm followed by Pyrogallol (2628.01 ppm), Chlorogenic (358.95 ppm), Gallic acid (135.72 ppm), E-Vanillic (82.18 ppm) and Epicatechen (59.96 ppm). Other phenolic compounds was Identified in extracts of watermelon rind powder, but their amount were ranged between 1.11-28.20ppm. The mentioned data are in accordance with those reported by Al-Sayed, and Ahmed, (2013).

According to Gil-Izquierdo *et al.* (2002), Scalbert *et al.* (2005) and Kondratyuk and Pezzuto, (2004), the daily intake of phenolics may be about 600-1000 mg per day without any side effects, which is about 10 times higher than that of daily intake of vitamin C and 100 times higher than those of daily intake of vitamin E and carotenoids.

Generally, it could be concluded that watermelon rind powder contained considerable amount from phenolic compounds which characterized as antioxidant properties, disease prevention and activity against toxins.

Table 3: Identification of phenolic compounds extracted from watermelon rind powder.

Watermelon rind powder			
Phenolic compounds	(ppm)	Phenolic compounds	(ppm)
Syringic	3662.21	P-Coumaric	13.52
Pyrogallol	2628.01	Vanillic	8.83
Chlorogenic	358.95	4-Aminobenzoic	8.71
Gallic acid	135.72	Iso-Ferulic	8.66
E-Vanillic	82.18	Ferulic	8.00
Epicatechen	59.96	Salicylic	6.75
Catechol	28.20	Caffeine	5.24
Catechin	22.46	P.oH.benzoic	5.20
Ellagic	20.98	2,4,5 MethoxyCinnamic	5.18
Benzoic	20.22	Cinnamic	2.76
Protocatechuic	18.34	α-Coumaric	1.24
Caffeic	18.00	Coumarin	1.11

Chemical Composition of the pan bread produced as affected by different replacement levels of watermelon rind powder.

The chemical composition of the pan bread produced as affected by different replacement levels (3, 6, 9 and 12 %) of watermelon rind powder comparing to control sample (without addition watermelon rind powder) was listed in table (4).

As shown in the obtained results (table 4), it could be noticed that the protein and carbohydrates content were slight decreased as the substitution level increased from 3 to 12% of watermelon rind powder in pan bread produced, which were ranged from 11.75-11.18 and 84.21-83.28 % as compared with the control sample which were represented 11.90 and 84.58, respectively. Contrariwise, fat was gradually increased as the substitution level increased from 3 to 12% of watermelon rind powder as compared with the control sample. The same behavior was also observed for both ash and fiber contents which were increased gradually by increasing the percent of watermelon rind powder from 3 to 12%, but this increase was more obvious in case of fiber content, whereas it was increased from 1.80 in the control sample to 4.80 % in pan bread contained 12% WMRP. Also, the ash content was increased from 1.37 in the control sample to 2.93 % in pan bread contained 12% WMRP. Their results were relatively comparable with the present data by El-Badry *et al.,* 2014.

Finally, it could be seen that as the levels of the watermelon rind powder increased in pan bread formula, the ash and fiber were increased. These results may be due to a gradual reduction of carbohydrates as the results of replacement of watermelon rind powder and also increasing the fiber contents.

Table 4: Proximate Chemical Composition of pan bread partially substituted of wheat flour with watermelon rind powder (WMRP)M± SE.

Chemical		Replacement levels				
Composition (%)	WMRP	Control	3%	6%	9%	12%
Moisture	12.05±0.38	36.80±0.30a	37.90±0.29a	39.95±0.22b	42.75±0.30c	43.90±0.31c
Protein	8.95±0.28	11.90±0.28a	11.75±0.32a	11.58±0.29a	11.35±0.30a	11.18±0.27a
Fat	2.33±0.16	2.15±0.11 a	2.22±0.15 a	2.40±0.17 b	2.54±0.10c	2.61±0.14cd
Ash%	11.90±0.32	1.37±0.28a	1.81±0.27b	2.13±0.23c	2.52±0.27d	2.93±0.25e
Fiber%	15.85±0.20	1.80±0.22a	2.70±0.22b	3.35±0.22c	4.05±0.22d	4.80±0.22e
Carbohydrates	76.82±0.49	84.58±0.46 a	83.89±0.42 a	84.21±0.49 a	83.59±0.52 a	83.28±0.55 a

WMRP watermelon rind .M± SE: Means± standard error for Chemical Composition; the means within the same row having different superscript are significantly varied (P ≤ 0.05).*

Sensory evaluation of pan bread

The organoleptic quality criteria (Shape, Odor, Taste, Crust color, Crumb color, Crumb texture and overall acceptability) of pan bread partially substituted of wheat flour with watermelon rind powder levels (3, 6, 9 and 12 %) were evaluated. The means sensory scores of pan bread partially substituted of wheat flour with watermelon rind powder samples are presented in Table (5).

From the obtained data (Table 5), it could be seen that there was no significant variation between samples containing of watermelon rind powder up to 6% for all tested organoleptic properties (Shape, Odor, Taste, Crust color, Crumb color, Crumb texture and overall acceptability) and also described as excellent properties when compared to the control sample. pan bread produced by partially replacement of their wheat flour with watermelon rind powder at level 3 and 6 % characterized with a good sensory properties which having Overall acceptability 8.90 and 8.51 respectively, followed with a level of 9 and 12 % watermelon rind powder were recorded Overall acceptability 7.73 and 7.17 when compared with control sample 8.92. As well as pan bread contained 3 and 6 % watermelon rind powder was recorded high score described as high quality. The mentioned data are in accordance with those reported by (El-Badry *et al.,* 2014).

In general, it could be showed that pan bread produced by partially replacement of their wheat flour with watermelon rind powder at level 3 and 6 % characterized with a good sensory properties followed with a level of 9 and 12 % characterized with a good sensory properties and acceptability when compared with control sample.

Table 5: Sensory evaluation of pan bread partially substituted of wheat flour with watermelon rind powder (WMRP).M± SE:

Sensory properties	Pan Breads				
	Control	3% WMRP	6% WMRP	9% WMRP	12% WMRP
Shape	8.98±0.89a	8.74±0.72a	8.35±0.78a	7.24±1.20b	7.23±1.30b
Odor	8.80±1.77a	8.83±1.56a	8.21±1.86ab	7.53±2.78cb	7.02±3.42c
Taste	8.58±1.76a	8.70±1.19a	8.21±1.50ab	7.78±1.88b	7.07±2.53c
Crust color	8.79±1.70a	8.89±1.32a	8.49±1.21a	7.68±1.66b	6.67±2.18c
Crumb color	8.76±1.70a	8.87±1.32a	8.50±1.21a	7.69±1.66b	6.68±2.18c
Crumb texture	8.99±0.89a	8.78±0.72a	8.41±0.78a	7.28±1.20b	7.29±1.30b
Overall acceptability	8.92±7.40a	8.90±5.50a	8.51±6.18a	7.73±8.02b	7.17±10.71c

WMRP watermelon rind. M± SE: Means± standard error for Sensory evaluation; the means within the same column having different superscript are significantly varied (P ≤ 0.05).*

Microbiological status of pan bread samples during storage at room temperatures for 12 days

In view of safety evaluation of any processed foods, either after preparation or after storing, to be ready for human consumption, the microbiological quality is mainly undertaken. In present work, the microbiological values in forms of total count bacteria (TC) and total counts for mold and yeasts (M&Y) were estimated in both pan bread partially substituted of wheat flour with watermelon rind powder levels (3, 6, 9 and 12 %) and they compared with pan bread (control). Also, this microbial quality was done in present work to evaluate the effect of watermelon rind powder additions on the rate of microbial growth during storage. Along the storage periods (12 days) the (TC) and (M&Y) were examined at the beginning of storage (zero time),and after every two days during the storage period. The obtained results are listed in Table (6 and 7) for total aerobic count bacteria and total mold and yeast count (log cfu/g) of pan bread samples during storage at room temperatures for 12 days.

Directly after processing and cooling at room temperature of pan bread samples packaged in polyethylene-packages at zero time and periodically every two days were microbiological analyzed. It could be primarily observed from table (6) that the pan bread samples have no detected of growth bacteria at zero time but detected of total count bacteria in the second day in pan bread control (2.3 log cfu /g) and increasing of TC in pan bread control during storage even arrived the maximum in the sixth day (5.3 log cfu /g), While in pan bread substituted of wheat flour with 3% WMRP total count bacteria in the second day was (1.8 log cfu /g) and increasing of TC during storage even arrived at the maximum in the eightieth day (5.5 log cfu /g), comparing with pan bread substituted of wheat flour with (6, 9 and 12 %) WMRP having of total count bacteria in the second day (1.6, 1.5 and 1.3 log cfu /g, respectively) and increasing of TC during storage tin arrived the maximum in the tenth day (5.3, 4.9 and 4.5 log cfu /g, respectively) of storage.

Table 6: Total Aerobic Count of Bacteria (log cfu/g) of pan bread samples during storage at room temperatures for 12 days.

Storage period (days)	Pan bread samples				
	Control	3% WMRP	6%WMRP	9%WMRP	12%WMRP
0	0.00	0.00	0.00	0.00	0.00
2	2.3	1.8	1.6	1.5	1.3
4	3.4	2.4	2.3	2.2	1.9
6	5.3	3.1	2.7	2.5	2.1
8	*	5.5	4.1	3.8	3.4
10	*	*	5.3	4.9	4.5
12	*	*	*	*	*

WMRP watermelon rind. M± SE: Means± standard error for Total Aerobic Count Bacteria; the means within the same column having different superscript are significantly varied (P ≤ 0.05).*
**: spoiled*

For M&Y counts, through storage period, the presented data in table of (7) reflected varied results than that observed with TC values. It could be noted that pan bread control contained higher counts of M&Y (2.1 , 3.2 and 5.2 log cfu /g) after 2, 4 and 6 days of the storage period, respectively than pan bread substituted of wheat flour with watermelon rind powder levels 3, 6, 9 and 12 % (1.7, 2.3, 2.8 and 5.2 log cfu /g) after 2, 4, 6 and 8 days of storage, respectively for pan bread 3% WMRP, (1.5, 2.2, 2.7, 3.9 and 5.1 log cfu /g) after 2, 4, 6,8 and 10 days of storage, respectively for pan bread 6% WMRP, (1.4, 2.1, 2.5, 3.3 and 4.6 log cfu /g) after 2, 4, 6,8 and 10 days of storage, respectively for pan bread 9% WMRP and (1.1, 1.7, 2.1, 3.1 and 4.4 log cfu /g) after 6,8 and 10 days of storage, respectively for pan bread 12% WMRP.

The reduction of TC and M&Y counts may be attributed for substitution with watermelon rind powder at deferent levels containing phenolic compound which inhibit microbial growth and subsequently reduced microorganisms growth or the death of some organisms (Frazier, 1978 and Egorova, 1982). Also, during storage there is a slow increase in microbial numbers more rapid at first stage and slower thereafter. The effect of poly phenols as microbial inhibitors is clearly shown in pan bread fortified with WMRP than pan bread control. This may be because pan bread fortified with WMRP has high level of poly phenols than pan bread control.

In general, it could be concluded that these variations of TC and M&Y counts between pan bread control and pan bread containing watermelon rind powder (levels 3, 6, 9 and 12 %) may due to the presence of phenolic compounds in pan bread which reduction of microbial growth led to increasing the shelf life.

Table 7: Mold and Yeast Count (log cfu/g)of pan bread samples during storage at room temperatures for 12 days.

Storage period (days)	Pan bread samples				
	Control	3% WMRP	6% WMRP	9%WMRP	12%WMRP
0	0.00	0.00	0.00	0.00	0.00
2	2.1	1.7	1.5	1.4	1.1
4	3.2	2.3	2.2	2.1	1.7
6	5.2	2.8	2.7	2.5	2.1
8	*	5.2	3.9	3.3	3.1
10	*	*	5.1	4.6	4.4
12	*	*	*	*	*

WMRP: watermelon rind. M± SE: Means± standard error for Mold and Yeast Count Bacteria; the means within the same column having different superscript are significantly varied (P ≤ 0.05).
**: spoiled*

Conclusion

The replacement of wheat flour with watermelon rind powder in producing pan bread with different levels (0, 3, 6, 9 and 12 %) led to the improving of the antioxidant potential of pan bread and increasing the shelf life of stored pan bread at room temperature due to its phenolic compounds content without side affection its sensory quality. These results indicate that consumers may be accepting pan bread containing WMRP.

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