

ANTIOXIDANT SYNERGY EFFECT OF ROSEMARY AQUEOUS EXTRACT AND GREEN TEA FLAVANOL-RICH CONCENTRATE FOR SUPERIOR PROTECTION OF BUFFALO MEATLOAVES

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Abstract

Phenol-containing extracts of green tea and rosemary have potential for use as food antioxidants. Due that rosemary and green tea extracts were considered as "natural" ingredient, it had more acceptances by the consumer than synthetic antioxidants. The objective of this work was to study the antioxidant effects and the synergistic interactions between the water-soluble extract obtained from rosemary (WSR), green tea water extract (TWE) or its flavanol-rich concentrate (GTF). Total phenolics in each extract were determined according to the Folin-Ciocalteu procedure. WSR, TWE and GTF alone or in combinations were examined first on buffalo meat emulsion homogenates (30% fat) as single or combined ingredients and their antioxidative activities were determined based on β -carotene changes in buffalo meat lipids after 10, 20, 40, and 60 min of heating at 50°C. Results so obtained were compared with those of samples containing α -tocopherol and BHA/BHT as commonly used food antioxidants. The most effective inhibitors of β -carotene decomposition were the mixture of WSR/GTF, followed by BHA/BHT, GTF, WSR, WSR/TWE, TWE then α -tocopherol in descending order. Besides, in subsequent experiments buffalo meatloaves were prepared containing 500 ppm WSR; 400 ppm WSR + 100 ppm GTF; 300 ppm WSR + 200 ppm GTF or 500 ppm GTF. The loaves were baked to an internal temperature of ~ 74 °C then stored at 4°C. Samples were analyzed for lipid oxidation (TBA values) and total psychrotrophic bacterial counts (PCs) throughout 6 days of refrigerated storage to assess non-microbial and microbial shelf-life properties. Results suggest that WSR and GTF may be used as meat antioxidants that are safe and health promoting. Super antioxidative effect was achieved by utilizing the mixture of WSR₃₀₀/GTF₂₀₀ that exhibited also some antimicrobial activity.

Key words: Buffalo meat; natural antioxidants; rosemary extract; green tea extract; meatloaf

INTRODUCTION

Apart from microbial spoilage, oxidative reactions are the major cause of deterioration in muscle foods (1). Oxidative reactions lead to degradation of lipid and proteins, resulting in deterioration of many quality characteristics such as flavor, color, texture, nutritive value and safety of the food (2). Oxidation of unsaturated fatty acids in cooked meat triglycerides prior to serving and product reheating causes the development of stale or rancid flavor commonly known as "warmed-over flavors" (3, 4, and 5). In Egyptian food, increased consumption of precooked meatloaves has raised the concern of oxidative rancidity or formation of warmed-over flavor since it may keep warm for a variable time prior to serving.

One approach to minimize lipid oxidation and warmed-over flavors development is to incorporate an antioxidant in the meat product formulation (6 and 7). As consumers are increasingly concerned about the long-term safety of synthetic antioxidants, interests in the development and use of natural alternatives have increased markedly (8 and 9). Natural antioxidants are required at higher levels than artificial products, so the importance of identifying active components and optimizing usage is emphasized.

The use of some natural extracts from rosemary (*Rosmarinus officinalis*) as food antioxidants is well-established (10, 11 and 12). The antioxidant properties of rosemary were found to be mainly due to rosmarinic acid, carnolic acid and γ -lactone diterpenes

like carnosol (13 and 14). The antioxidants effects of rosemary extract and its major component had been observed in ground pork products (15); several food systems (16); bulk oils and oil-in-water emulsions (17 and 18). Antioxidant synergism in food systems speculated that the components of rosemary extract might be used as a substitute for ascorbic acid to enhance the antioxidative activity of α -tocopherol (19).

Green tea flavanols (GTFs) are polyphenolic compounds that have recently received much attention as functional ingredients to a range of foods and beverages owing to their various biological activities (20). Their beneficial properties are thought to include antiatherosclerotic, anticarcinogenic, antihypertensive, antioxidative and hypolipidemic effects (21 and 22). Catechins and other flavonoids had recognized as efficient antioxidants for scavenging oxygen radicals and chelating metal ions (23). Tea catechins was reported to function as potent antioxidants in lipid model systems (24); canola oil (25); fish oil (23); food emulsions (26), fish meat (27), pork (28) and minced poultry muscles (29). The antioxidative activity of green tea catechins (GTC) extract was compared with that of rosemary extract in canola oil, pork lard and chicken fat (30). They found that the GTC extract was much more effective than the rosemary extract against lipid oxidation. The present study aimed to analyze the effectiveness of water-soluble extracts of rosemary (WSR), green tea (TWE) and green tea flavanol-rich concentrate (GTF) and their possible synergistic effects in lowering the lipid oxidation of cooked buffalo meatloaves in comparison to such properties of some commonly used food antioxidants, such as α -tocopherol and BHA/BHT.

MATERIAL AND METHOD

Meat

About 6 kg of meat (mainly biceps femoris, quadriceps, semimembranosus and semitendinosus muscles) from round portion of male buffalo carcass of good finish were obtained from a local meat market within 5 h of slaughter. The meat was chilled at $4 \pm 1^\circ\text{C}$ for 20 h post mortem. After removal of intramuscular fat and connective tissues, the lean meat was minced coarsely and then

more finely by passing through 8 mm and 4 mm plates respectively. Buffalo kidney fat from the same carcass were ground and utilized for each trial of the experiments.

Additives and chemicals

Rosemary (*Rosmarinus officinalis* L.), Chinese green tea (*Camellia sinensis*) were purchased from a local herb-shop (Harraz, Cairo, Egypt). Gallic acid, α -Tocopherol, β -carotene, Tween 20 caffeine and catechin were purchased from Sigma Chemical, Co. Ltd. (St. Louis, MO, USA). Tenox 4 [a 50/50 blend of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)] was obtained from Eastman Co. The Folin-Ciocalteu reagent was purchased from Merck Ltd. (Lutterworth, U.K.). Good-Quality table salt (Bono) was procured from a local market. All other chemicals were reagent grade "AnalaR".

Extraction preparation mode:

I- Extraction of water-soluble rosemary extracts (WSR)

Samples of ground dried rosemary leaves, weighing 12.5 g were soaked in 250 ml of distilled water and mixed using an electric mixer at room temperature for 1 h. Subsequently the extracts were filtered through Whatman No. 1 filter paper. The extraction was repeated with 250 ml distilled water and the combined extracts were concentrated under vacuum at 40°C using Buchi Rotavapor-R evaporator and dried on a Virtis Model 6201 freeze dryer at -60°C .

II- Preparation of green tea water extracts (TWE)

TWE were prepared by adding 20 g of ground green tea leaves to 400 ml of freshly prepared hot water (80°C) for 1 h. The mixture was then cooled to room temperature and filtered through a Whatman No. 1 filter paper. The infusion was concentrated under vacuum at 40°C using Buchi Rotavapor-R evaporator and dried on a Virtis Model 6201 freeze dryer at -60°C .

III- Preparation of green tea flavanol-rich extracts (GTF)

GTF were prepared according to the procedure described elsewhere (Wanasundara and Shahidi, 31). The ground tea leaves (20 g) were firstly soaked in 400 ml of hot water (80°C) for 1 h. The mixture was then cooled to room temperature and filtered through a

Whatman No 1 filter paper. The collected filtrate was mixed with an equal volume of chloroform and allowed to separate to remove caffeine and pigments. The upper aqueous layer was extracted two times with an equal volume of ethyl acetate to extract the total GTFs from the remaining aqueous phase. The ethyl acetate extracts were combined and evaporated to dryness under vacuum at 40 °C, using a rotary evaporator.

All the dried extracts were weighing to determine the extraction yield and storing at -18°C until use. For estimation their total antioxidative effects according "beta-carotene assay" (as shown below), approximately 25 mg of the dried extracts was dissolved in distilled water to give a concentration of 1 mg of extract/ml of water.

Determination of total phenolic compounds.

Total phenolics in each extract were determined using Folin-Ciocalteu reagent (32) and the results were expressed as gallic acid equivalents (GAE).

Catechins and caffeine determination.

TWE and GTF were analyzed for catechins and caffeine contents (33). Catechins and caffeine were quantified using a Shimadzu LC-10AD HPLC.

Experimental design

Two separate experiments were conducted: The first one was for determining the antioxidant effects of water-soluble extract from rosemary (WSR), green tea water extract (TWE) and green tea flavanol-rich concentrate (GTF) on buffalo meat homogenates (30% fat), as model system, based on β -carotene changes during heating at 50°C. In the second part of the study, Buffalo meat is reformed into meatloaves. The feature of buffalo meatloaves containing 500 ppm WSR, or 500 ppm GTF and their combinations (400 ppm WSR + 100 ppm GTF or 300 ppm WSR + 200 ppm GTF) were evaluated by monitoring lipid oxidative stability, microbiological quality and sensory properties after baking and in refrigeration storage at 4°C to characterize the most satisfactory formulation for meatloaf manufacture.

Experiment A: Antioxidant effects based on β -carotene changes during heating at 50°C (β -carotene bleaching test).

Preparation of buffalo meat homogenates

Model system meat homogenates (batter)

containing 30% fat were prepared (34). Representative samples from lean buffalo meat and kidney fat were initially analyzed for fat content. Finely minced buffalo lean meat (28 g kg⁻¹) and buffalo kidney fat (900 g kg⁻¹) were used to formulate lean meat in high fat level (30%). Calculation was performed using the Person's square method. The bulk was thoroughly homogenized in a Horbat type mixer for 3 min until a viscous meat emulsion was formed, then it was subdivided into two equal amounts. One-half was used for fresh evaluation, whereas the other part was stored under conditions of frozen storage (-20°C) for 90 days.

β -carotene assay for antioxidant activity

Antioxidant activity was estimated based on β -carotene changes (co-oxidation in the presence of lipid) during heating at 50°C using the method of Al-Saikhan et al. (35) that modified by Szczepanik and Stodolnik (36).

Experiment B: Performance of antioxidant-treated cooked buffalo meatloaves

Preparation of buffalo meatloaves:

Ground buffalo lean meat was blended in appropriate proportion with minced buffalo kidney fat to formulate lean meat had 15% fat level using the Pearson' square as described above. Meatloaf bulk was prepared by manually mixing buffalo lean meat, kidney fat, salt (20 g kg⁻¹) and onion paste (70 g kg⁻¹) following the meatloaf recipe as depicted by Rhee and Myers (37) with some alterations to stimulate the Egyptian preference. Freshly prepared buffalo meatloaf blend was divided into portions to which one of the following 6-antioxidant treatments was applied: (1) control (no antioxidant), (2) 500 ppm WSR; (3) 400 ppm WSR + 100 ppm GTF; (4) 300 ppm WSR + 200 ppm GTF; (5) 500 ppm GTF and (6) 200 ppm BHA/BHT.

WSR and GTF dry extracts as well as their mixtures were dispersed in about 2 mL of distilled water before adding to the meatloaf formulation. No other spices or herbs were further appended in the meatloaf recipe.

Freshly baked wheat loaves, weighing approximately 100 g, were used and plugged with 150 g buffalo meatloaf mixture. A total of 30 meatloaves were formed, 5 for each treatment. The loaves were vertically cut into halves and each half loaf was covered

individually with butter foil coded by the treatment number. The loaves were baked in a preheated conventional oven at 176.7°C to an internal temperature of ~ 74°C as monitored using a thermocouple (Omega, Model 199, Engineering Stanford, CT USA), (38). Freshly cooked meatloaves for each treatment were placed onto tray covered with aluminum foil for refrigerated storage (4°C). The loaves were kept at 4°C for 6 days.

Analytical methods

Chemical analysis and cook yield.

Moisture, protein, fat (ether-extractable) and ash contents were determined according to methods 934.01, 978.02, 920.39 and 938.08 of the AOAC (39). Cooked weight was expressed as percent of raw weight for cook yield determination.

Determination of pH. The pH was measured at room temperature using a pH meter (model 420 A, Orion Laboratory Products Division Inc., Boston, MA).

Determination of iron content. The total iron content was determined (40) and expressed as $\mu\text{g iron g}^{-1}$ muscle. Heme iron was determined using the modified method of Clark et al. (41).

Fatty acid analysis. Total lipid was extracted according to the procedure of Folch et al. (42). The fatty acids methyl esters were prepared using benzene: methanol: concentrated sulfuric acid (10:86:4) and methylation was carried out for one hour at 80-90°C (43). The composition of fatty acids were achieved by Gas liquid chromatography analysis using PYE Unicam model PV 4550 capillary Gas chromatography fitted with flame ionization detector, the column (1.5 m \times 4 mm) packed with diatomite C (100-120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8 °C/min from 70°C to 190°C then isothermally at this temperature for 20 min and nitrogen flow rate was 30ml/min. Detector and injector temperatures, hydrogen and air flow rates and chart speed were 300°C, 250°C, 33 ml/min, 330 ml/min and 2 cm/min respectively. The presented fatty acids were identified according to an authentic sample of fatty acids chromatographed under the same conditions.

Measurement of lipid oxidation. The extent of lipid oxidation was determined by the 2-thiobarbituric acid reactive substances

(TBARS) (44).

Sensory evaluation. Eight panelists from faculty staff and graduated students of the Food Science Department, Minia University were used to evaluate sensory properties of freshly cooked meatloaves samples. 10 mm thick from the loaves were served to the panelists. Color, flavor, texture and overall acceptability were evaluated on 8-point descriptive scales (45).

Microbial sampling and analysis.

Total psychrotrophic bacteria test was utilized to detect microbial loads of precooked meatloaves that was not reheated after refrigerated storage on the day of test as described in ICMSF (46). Following incubation at $4 \pm 1^\circ\text{C}$ for 10 days plates showing 30-300 colonies were counted and expressed as \log_{10} (Colony-forming units (CFU)) g^{-1} sample.

Statistical analysis.

Data were analyzed with the GLM (General Linear Model) program using statistical analysis system (47). Mean values were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSIONS

Extract yields and total phenolics

The extract yields and total phenolics content data for the water-soluble extracts are shown in Table 1. The extract yield of green tea water extract (TWE) was higher than that of rosemary extract (WSR) (18.66 and 13.72, respectively). The yield of TWE was closely related to that previously reported (48). Actually, the extract of GTF was compromised by a decreased yield (9.48%) than TWE. The total phenolics content ranged from 69.54 mg GAE/g for WSR to 104.96 mg GAE/g for GTF. No correlation was found between the amount of extractable components (extract yields) and total phenolic contents. Results coincided with those reported (49) and (50).

Catechin and caffeine in green tea extracts

The percentages of total catechins and caffeine in TWE and GTF were illustrated in Fig. 1. A comparison of total phenols and catechins analyzed in this study showed most of the phenolics determined by Folin-Ciocalteu reagent to be catechins. The HPLC analysis showed that GTF had more total catechins (95.9%) compared to those of TWE

(62.2%). It is also interesting to remark that caffeine was presented in negligible amount in GTF (1.4%) contrary to TWE (23.6%).

Therefore, it could designate GTF as a decaffeinated extract (51).

Table 1: Extraction yield and total phenolics content⁽¹⁾ of WSR; TWE and GTF.

Parameter	Type of extract		
	WSR	TWE	GTF
Yield (g/100g dry leaves)	13.72 ± 1.61	18.66 ± 2.13	9.48 ± 0.90
Total phenolics [mg gallic acid equivalent (GAE)/g extract]	69.54 ± 2.50	87.40 ± 4.25	104.96 ± 1.77

(1) Results are expressed as means ± SD of duplicate determinations.

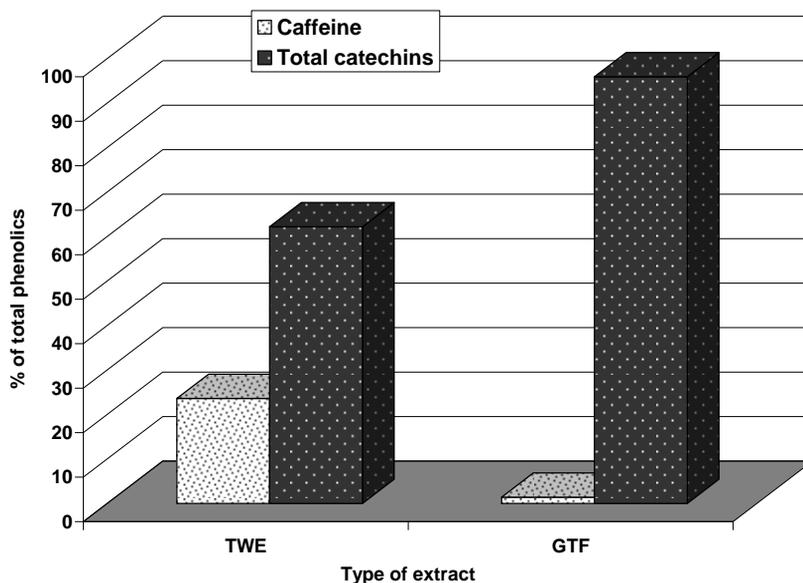


Fig. 1: Percentage contribution of total catechins and caffeine in green tea water-extract (TWE) and flavanols enrichment green tea concentrate (GTF)(1).

(1) Results are expressed as means of duplicate HPLC determinations.

Experiment A: *β-carotene assay*

The lipid analysis of buffalo meat homogenates model used for the present study constituted of 50.64% unsaturated fatty acids from which 40.43% were monounsaturated and 10.21% were polyunsaturated (Table 2).

Table 2: Percentage composition of fatty acids of buffalo meat homogenates.

Fatty acid (% total fatty acids)	Buffalo homogenates lipid
Myristic (14:0)	3.83
Palmitic (16:0)	25.48
Palmitoleic (16:1)	4.20
Stearic (18:0)	17.95
Oleic (18:1 ω -9)	36.23
Linoleic (18:2 ω -6)	8.87
Linolenic (18:3 ω -3)	1.34
Σ SFA ⁽¹⁾	47.26
Σ MUFA ⁽²⁾	40.43
Σ PUFA ⁽³⁾	10.21
Σ PUFA / Σ MUFA	0.25
Other fatty acids	2.10

⁽¹⁾SFA: saturated fatty acids

⁽²⁾MUFA: monounsaturated fatty acids

⁽³⁾PUFA: polyunsaturated fatty acids

Results in Table 3 demonstrated that the level of β -carotene degradation (discoloration) changed the most in control samples (without antioxidant additives). Furthermore, the prolongation of heating from 10 to 60 minutes inflicted increased losses of β -carotene. Addition of aqueous extract of rosemary (WSR) markedly stabilized buffalo homogenates lipid. A similar pattern was observed after supplementation of aqueous tea extract (TWE) which in general had lower antioxidative potential than WSR. Results are in accordance with other findings (52 and

53). Therefore, it could be suggested that the antioxidative activity was not actually related to the total phenols contents of the extracts as it mainly depended on the actual phenols present in each extract.

Moreover, results in Table (3) clearly showed that the antioxidant activity of GTE exceeded that of TWE and WSR, which could be related to its higher level of polyphenolic catechins (95.9%). Tea extract showed dual effects in the meat homogenates model system that was dependent on the ability of both reducing iron and scavenging oxy-radicals (54).

Table 3: Effect of antioxidant supplementation on β -carotene degradation level⁽¹⁾ [%] in lipid of buffalo meat homogenates during heating.

Additives	Heating time (min.)			
	10	20	40	60
Control	20.3 ± 0.9	33.6 ± 0.5	51.3 ± 1.1	60.8 ± 2.7
WSR	10.4 ± 0.1	13.9 ± 0.3	16.8 ± 0.2	19.6 ± 0.1
TWE	13.0 ± 0.4	16.3 ± 0.2	18.7 ± 0.5	23.9 ± 0.2
GTF	8.8 ± 0.1	11.6 ± 0.3	15.9 ± 0.1	18.3 ± 0.4
WSR+TWE [1:1 w/w]	7.6 ± 0.5	13.5 ± 0.2	17.3 ± 0.4	22.1 ± 0.6
WSR+GTF [1:1 w/w]	1.5 ± 0.3	2.9 ± 0.1	4.6 ± 0.8	9.8 ± 0.1
Tenox 4 [BHA/BHT]	6.2 ± 0.2	9.7 ± 1.1	10.9 ± 0.3	14.1 ± 0.2
α -tocopherol	18.7 ± 0.6	26.4 ± 0.3	30.7 ± 1.5	36.2 ± 2.9

⁽¹⁾ Results are means of duplicate determinations ± SD.

The use of BHA/BHT distinctly inhibited oxidative changes of β -carotene though it was more effective than WSR, TWE, and GTF. BHA/BHT inflicted a 14.1 percentage point decline after an incubation of 60 min at 50°C, while WSR, TWE and GTF used alones, showed a decline of 19.6, 23.9 and 18.3 percentage points, respectively (Table 3). Accordingly, addition of single natural additives to lipid of buffalo meat homogenates appeared to be less efficient than the synthetic antioxidants in inhibition of β -carotene degradation. On the other hand, α -tocopherol inhibited the lowest values of β -carotene stability and the lowest effectiveness of the additives, resulted in increased losses of β -carotene.

Interestingly, among all lipid additives used, the most effective inhibition of β -carotene changes was observed for the mixture of WSR + GTF that was more protective than BHA/BHT. With use of the latter additive, β -carotene level declined by

only 9.8% percentage point after an incubation of 60 min at 50 °C illuminating that there was a positive synergism observed between WSR with GTF. However, β -carotene stability was less markedly affected by interactions between WSR and TWE. In general, compounds tested by this method gave the following order of efficiency:

WSR+GTF>BHA+BHT>GTF>WSR>WSR+TWE>TWE> α -tochopherol > control.

Moreover, three month storage of meat homogenates at -20°C reduced more β -carotene stability in lipids then did fresh homogenates after incubation of 60 min at 50°C (Fig. 2). The above suggests that frozen storage and heating is associated with complex processes of lipid transformation. The synergistic activity of WSR and GTF, yet again demonstrated the strongest protective properties in relation to the percentages of the available β -carotene in the lipids of buffalo homogenates sampled after 3 months of frozen storage.

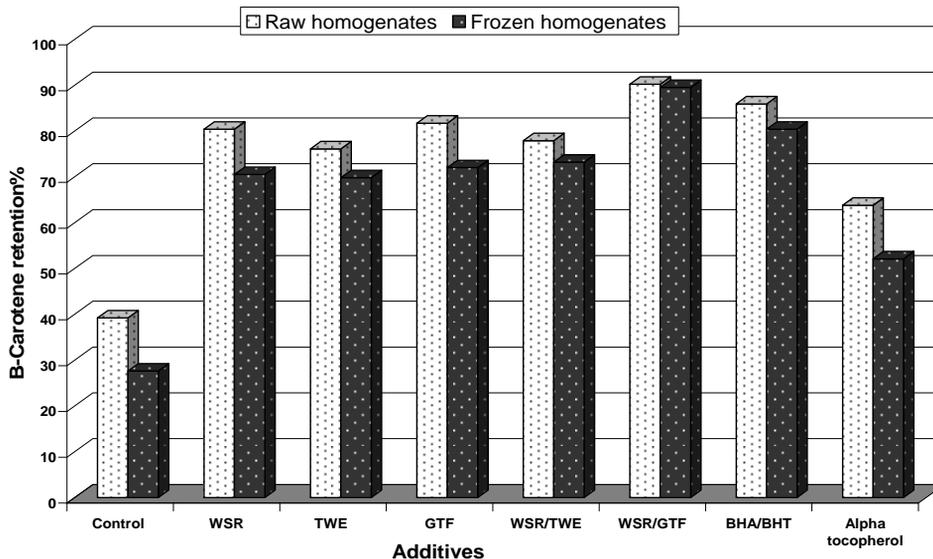


Fig. 2: Effect of freezing storage⁽¹⁾ of buffalo meat homogenates on β -carotene retention (%) in the lipids after 60 min heating at 50°C⁽²⁾.

⁽¹⁾ Freezing storage at -20°C for 90 days.

⁽²⁾ Results are means of duplicate determinations.

Experiment B: Evaluation of antioxidant-treated meatloaves

Physicochemical evaluation of buffalo meatloaf raw preparation

Table 4 shows the physicochemical characteristics of raw meat mixture used for meatloaf preparation. The pH value of the meatloaf raw mixture was 5.72. In addition, it had high moisture content (64.87%) and decreased fat and protein contents (13.59 and 17.65% respectively). Moreover, the results clearly indicate that buffalo meat mixture had high concentrations of heme, non-heme and total iron (26.45, 8.84 and 35.29 $\mu\text{g/g}$ respectively). Ionic iron released from meat pigments during heating is a potent catalyst for lipid oxidation and warmed over flavor (WOF) in precooked meats (55 and 56).

Sensory properties of antioxidant-treated meatloaves

Table 4: Physico-chemical properties of raw mixture for buffalo meatloaf preparation

Parameters ⁽¹⁾	Buffalo meat mixture
pH	5.75 \pm 0.03
Moisture (%)	64.87 \pm 0.40
Fat (%)	13.59 \pm 1.26
Protein (%)	17.65 \pm 0.91
Ash (%)	2.92 \pm 0.15
Iron value ($\mu\text{g/g}$):	
Total iron	35.29 \pm 0.33
Heme iron	26.45 \pm 0.17
Non Heme iron ⁽²⁾	8.84 \pm 0.15
Cooking yield (%)	76.41 \pm 1.58

(1) Values are expressed as means of three determinations \pm SD

(2) Nonheme iron = Total iron content – Heme iron content

The sensory scores of cooked meatloaves are summarized in Table 5. It was clear that meatloaves made with 500 ppm WSR was the most liked as they scored topmost in flavor and had significantly ($P < 0.05$) the highest sensory scores for overall acceptance than those contained other antioxidant additives. On the other hand, meatloaves contained of 500 ppm GTF alone scored lowest in Flavor, probably due to odor of the native green tea extract. Results coincided

with those previously obtained (57). However, insignificant differences ($P>0.05$) were observed between color and texture of meatloaves among the tested samples.

Table 5: Sensory properties of freshly cooked buffalo meatloave supplemented with different antioxidant additives

Additives	Sensory scores ⁽¹⁾			
	Color	Flavor	Texture	Acceptability
Control	6.82 ^{ad}	6.13 ^c	6.27 ^a	6.16 ^c
WSR ₅₀₀ ⁽²⁾	7.07 ^a	7.56 ^a	6.52 ^a	7.30 ^a
GTF ₅₀₀ ⁽³⁾	6.91 ^a	5.15 ^d	6.39 ^a	5.82 ^d
WSR ₄₀₀ +GTF ₁₀₀ ⁽⁴⁾	6.85 ^a	7.09 ^b	6.47 ^a	6.87 ^b
WSR ₃₀₀ +GTF ₂₀₀ ⁽⁵⁾	6.79 ^a	6.92 ^b	6.60 ^a	6.64 ^b
[BHA/BHT] ₂₀₀ ⁽⁶⁾	6.88 ^a	6.26 ^c	6.41 ^a	6.19 ^c

^{a-d} Means within a column with different superscript letters are significantly different at $P<0.05$.

(1) 8- point descriptive scales (8, high desirable; 1, very undesirable).

(2) WSR500: addition 500 mg water soluble rosemary.

(3) GTF500: addition 500 mg green tea flavanol enrichment concentrate.

(4) WSR400+GTF100: mixture of 400 mg WSR and 100 mg GTF.

(5) WSR300+GTF200: mixture of 300 mg WSR and 200 mg GTF.

(6) [BHA/BHT]200 :addition of 200 mg Tenox 4 (BHA+BHT).

Lipid oxidative stability of cooked meatloaves during refrigerated storage

The effect of different antioxidant extracts on TBARS values of precooked buffalo meatloaves over 6 days of refrigerated storage and reheated is shown in Table 6. TBARS values at the start of storage were significantly ($P<0.05$) higher in control samples (no antioxidant) indicating that onset of lipid oxidation occurs during meatloaf processing. The increased in TBARS value in the control sample was due to that during cooking of meat, an extensive disruption or destruction of cellular structure may occur, allowing mixing of various meat constituents, including unsaturated fatty acids and pro-oxidants (58). In addition, salt has been shown to have an accelerating effect on lipid oxidation (59). The pro-oxidative activity of NaCl is due to its ability to release iron from heme pigments and other heme binding molecules (60). It is obvious that TBARS values were affected by storage time and antioxidant treatments (Table 6). TBARS content increased rapidly and steadily with storage time for the control (about 578% increases after 6 days). WSR and GTF used individually demonstrated inhibiting influence on rate of lipid oxidation. However, GTF showed higher protection of lipid oxidation at all storage times than WSR. similar observation was also reported (30).

Data in Table (6) gave a more clear-cut result that there was a significant ($P<0.05$) synergistic interaction between rosemary aqueous extracts (WSR) and green tea

polyphenols (GTF). Combination of WSR₄₀₀ and GTF₁₀₀ exerted inhibitory effect on TBARS formation equivalent to 200 ppm BHA/BHT, and significantly ($P<0.05$) greater than 500 ppm GTF alone. Apparently, the mixture of WSR₃₀₀ + GTF₂₀₀ provided the most effective antioxidative activity in terms of lowest TBARS values (until the latter stages of storage. Overall TBARS values tended to decrease in the order:

$$\text{WSR}_{300} + \text{GTF}_{200} > \text{WSR}_{400} + \text{GTF}_{100} = (\text{BHA/BHT})_{200} > \text{GTF}_{500} > \text{WSR}_{500}$$

The enhanced effect of added tea catechins to rosemary extract could be attributed to its higher number of hydroxyl groups that are able to scavenge more radicals (61). In addition, tea catechins are more effectual to inhibit iron release from heme during cooking (62).

Microbial quality of cooked meatloaves during refrigerated storage

The microbial feature of meat depends on many factors, including initial microbial quality, storage conditions, processing condition and microbial quality of additives (63). Microbiological evaluation of the loaves during storage gave significant increases in the counts (\log_{10} cfu/g) of psychrotrops (Table 7). The total psychrothrophic count increased but was well below the incipient spoilage level of 10^7 CFU g^{-1} at the end of storage (64) indicating minimum post cooking contamination. Varied observations were noted on the microbial quality of meatloaves containing the antioxidant additives. Control samples and those with 500 ppm WSR

showed no significant differences ($P>0.05$) between them. Apparently, water miscible natural extract of rosemary (WSR) had no added benefit of reducing microbial growth under the condition used. On the other hand, meatloaves with added 500 ppm GTF showed the lowest microbial growth at all tested storage times and caused a reduction of 0.96

\log_{10} (CFU/g) after 6 days of refrigerated storage compared to the control. Moreover, meatloaves included WSR and GTF mixtures exhibited significantly ($P>0.05$) some antibacterial activities compared to the control. The mode of antimicrobial action of GTF requires further investigation.

Table 6: TBARS (\pm SD)⁽¹⁾ of meatloaves treated with different antioxidant additives during refrigerated storage and reheating

Additives ⁽²⁾	TBARS (mg malonaldehyde/kg sample)		
	Storage period (days)		
	0	3	6
Control	^a 2.03 _y ± 0.08	^a 5.89 _x ± 0.28	^a 11.73 _w ± 0.19
WSR ₅₀₀	^b 1.31 _v ± 0.02	^b 1.92 _x ± 0.04	^b 3.66 _w ± 0.10
GTF ₅₀₀	^c 1.18 _v ± 0.05	^c 1.35 _x ± 0.09	^c 3.07 _w ± 0.12
WSR ₄₀₀ +GTF ₁₀₀	^d 0.91 _x ± 0.03	^e 1.03 _x ± 0.02	^d 1.78 _w ± 0.15
WSR ₃₀₀ +GTF ₂₀₀	^e 0.76 _x ± 0.01	^f 0.72 _x ± 0.05	^e 0.94 _w ± 0.07
[BHA/BHT] ₂₀₀	^d 0.88 _y ± 0.04	^d 1.15 _x ± 0.02	^d 1.92 _w ± 0.21

^{a-y} Means within a column with different superscript letters are significantly different at $P<0.05$.

^{w-y} Means within a row with different superscript letters are significantly different at $P<0.05$.

⁽¹⁾ SD= standard division, n=4.

⁽²⁾ See table 5 for abbreviations.

Table 7: Evaluation of psychrotrophic bacteria in meatloaves treated with different antioxidants during refrigerated storage (4 ± 1°C)

Additives ⁽¹⁾	Psychrotrophs \log_{10} CFU/g		
	Storage period (days)		
	1	3	6
Control	3.97 ^a ± 0.08	4.20 ^a ± 0.12	5.09 ^a ± 0.10
WSR ₅₀₀	3.81 ^{ab} ± 0.14	4.05 ^{ab} ± 0.10	4.96 ^{ab} ± 0.05
GTF ₅₀₀	2.62 ^e ± 0.04	3.06 ^e ± 0.03	4.13 ^e ± 0.16
WSR ₄₀₀ +GTF ₁₀₀	3.35 ^c ± 0.15	3.77 ^c ± 0.17	4.88 ^{bc} ± 0.09
WSR ₃₀₀ +GTF ₂₀₀	2.94 ^d ± 0.07	3.32 ^d ± 0.02	4.75 ^{cd} ± 0.04
[BHA/BHT] ₂₀₀	3.50 ^{bc} ± 0.23	3.87 ^{bc} ± 0.11	4.61 ^d ± 0.06

^{a-b} Means within a column with different superscript letters are significantly different at $P<0.05$.

⁽¹⁾ See table 5 for abbreviations.

CONCLUSIONS

The results showed that aqueous extracts derived from rosemary leaves or green tea polyphenols had the potential to reduce the oxidation of buffalo meatloaves and extend their shelf life. The antioxidant combination composed of 300 ppm WSR + 200 ppm GTF (by weight of the meat) provided the most effective antioxidative activity in terms of lowest TBARS values until the latter stages of storage. GTF probably exerts its antioxidant effect by chelating soluble iron. The combined extracts showed in addition some antibacterial activity that led also to a significant extension of meatloaf shelf life. The availability of these natural antioxidants and their possible co-antioxidant or synergistic effects suggests an interesting way of improving meat stability and preventing degenerative diseases caused by fat.

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