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Research Article

Lipid Oxidation, Color Changes, and Microbiological Quality of Frozen Beef Burgers Incorporated with Shirazi Thyme, Cinnamon, and Rosemary Extracts

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In this study, the oxidative stability of beef burgers incorporated with Shirazi thyme, cinnamon, and rosemary extracts was compared with that of BHT-incorporated and antioxidant-free samples. The chemical composition, TBARS, metmyoglobin, pH, color, and microbial and sensory characteristics were evaluated during storage at −18°C for 2 months. The results indicated that Shirazi thyme and cinnamon extracts did not change the colorimetric properties significantly ($P < 0.05$). Incorporating natural antioxidants led to a significant ($P < 0.05$) reduction in TBARS (36.58–46.34%) and metmyoglobin (16.25–18.47%) as compared to control. Except for the control sample, total microbial counts of burgers were lower than the maximum allowed limit. Burgers formulated with Shirazi thyme revealed the lowest amount of total count. Regarding the sensory characteristics, the overall acceptability of different samples decreased in the order of cinnamon > BHT > Shirazi thyme > rosemary > control. Finally, the results showed that these plant extracts can be utilized as an alternative to synthetic antioxidants in formulation of burgers.

1. Introduction

Meat and meat products are among the most important protein sources in the daily diet of people living in developed countries. Beef burger is almost the most popular meat product consumed by millions of people from all over the world. The common processes (such as mincing, cooking, and salt addition) applied in the production of burgers enhance the formation of reactive oxygen species; therefore, the resultant product is highly vulnerable to oxidation [1]. Lipid and protein oxidations have been reported as the principal reason for the decreased quality of burgers during storage resulting in decreasing the shelf life [2–4].

Application of antioxidants is the best strategy to prevent oxidation reactions [5]. Synthetic antioxidants such as BHT, TBHQ, and BHA have various adverse human health effects including allergy, headache, asthma, and dermatitis. Therefore, the application of natural antioxidants (such as herbal essential oils and extracts) is of interest and can be observed in a growing number of research works [6–8] (Aliakbarlu et al. 2016). Moreover, consumers are increasingly demanding for green-labeled food products such as those containing natural antioxidant. Natural compounds can be obtained from natural sources such as plants, fruits, vegetables, oil seeds, spices, team, honey, bee pollen, and cereals [9]. A lot of extracts have been approved as GRAS (Generally Recognized as Safe); that is, the addition of these chemicals or substances into food is considered safe by experts, and their application is exempted from the usual Federal Food, Drug, and Cosmetic Act (FFDCA) [10].

The potential of natural antioxidants in preventing the lipid oxidation in different food products has been evaluated by many researchers around the world [11]. It has been reported that the extracts of kinnow rind, pomegranate rind,

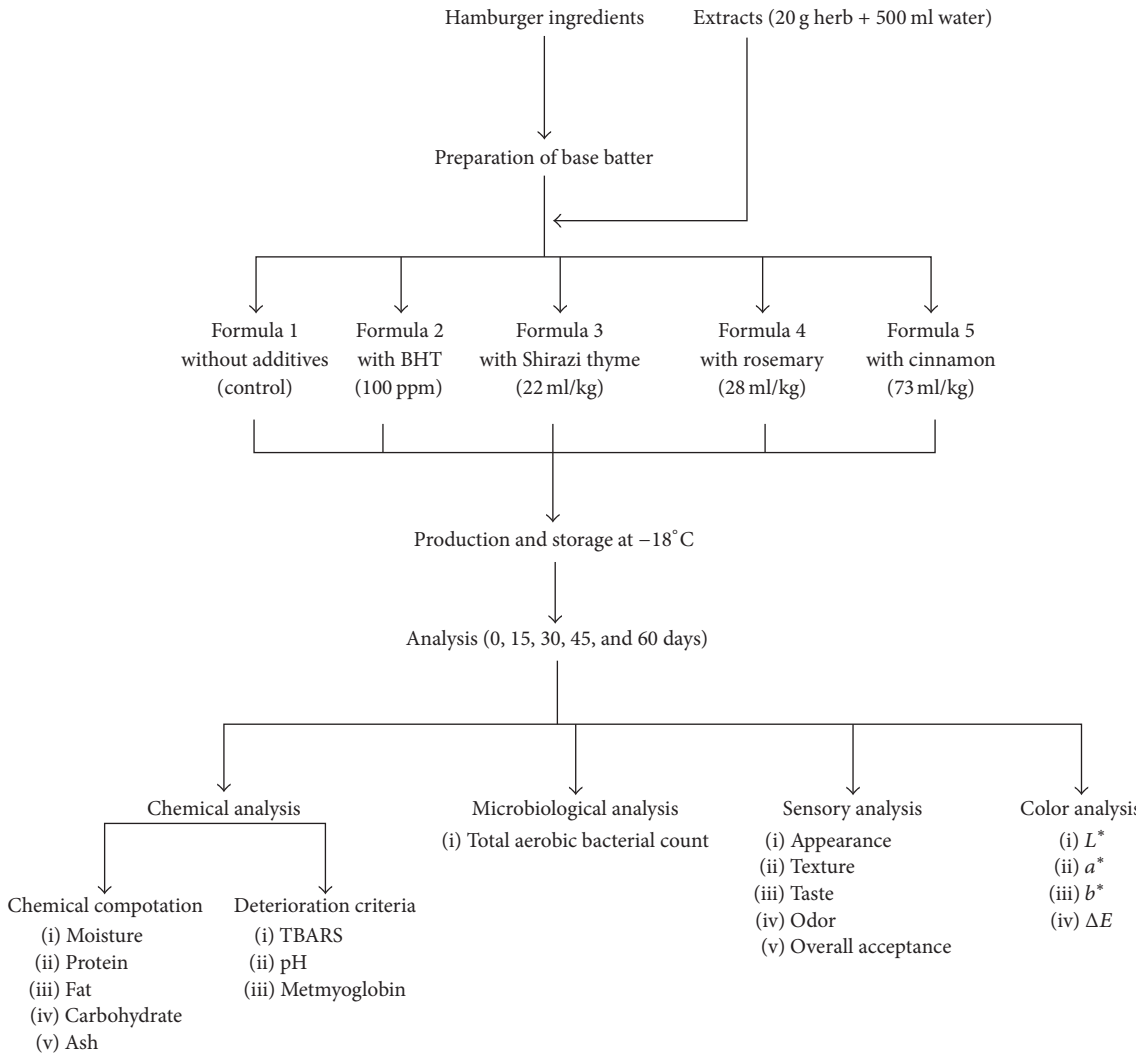

Figure 1: Schematic diagram of experimental design.

and seed powders were significantly able to decrease the lipid oxidation in goat meat patties [12]. The effects of natural (sage and rosemary) and synthetic (BHT) antioxidants on protein oxidation, discoloration, and texture of refrigerated patties produced from pig liver have been studied by Estévez et al. [13]. Authors reported that the antioxidant properties of natural and synthetic antioxidants were similar. In treated samples, the increase in the carbonyl content as a result of oxidation was significantly ($P < 0.05$) lower than control samples. Moreover, antioxidants could successfully protect heme molecules from degradation. Among different natural antioxidants, it has been reported that the extracts of Shirazi thyme, cinnamon, and rosemary reveal substantial antioxidant capacity [14].

The main objective of the current study was to compare the effects of Shirazi thyme, cinnamon, and rosemary extracts (denoted as natural antioxidants) with those of BHT on protein and lipid oxidations and physicochemical, microbial, and sensory characteristics of frozen beef burgers during storage. The results of this study may have potential implications in substituting the synthetic antioxidants with nature-made ones.

2. Materials and Methods

A schematic representation of beef burger production is shown in Figure 1.

2.1. Materials. Beef flanks (18% fat) from freshly slaughtered animals (24 hours post slaughter) were purchased from a local market (Shiraz, Iran) and transported to the laboratory in insulated polystyrene ice-boxes. The meat was ground (Meat Grinder, Philips, HR2743, Amsterdam, Netherlands) through a perforated plate with a hole diameter of 5 mm. Thiobarbituric acid (Sigma-Aldrich, St. Louis, MO, USA), 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, St. Louis, MO, USA) and diphenyl-2-picrylhydrazyl (DPPH$^\bullet$) (Sigma-Aldrich, St. Louis, MO, USA), soy protein isolate (SPI, 90% protein, Sonic Co., India), onions, salt, spices, and rusk were also used in this study.

2.2. Plant Extracts. Shirazi thyme *(Zataria multiflora)*, cinnamon *(Cinnamomum zeylanicum),* and rosemary *(Rosmarinus officinalis)* were purchased from a local grocery. The genus and species were certified by experts from the Herbarium of Biology Department (Shiraz University, Shiraz, Iran). Dried plants were powdered with a mill and then kept in polyethylene bags at room temperature before extraction.

2.2.1. Extract Preparation. Plant powder (20 g) was thoroughly mixed with boiling distilled water (500 mL) for 5 min followed by filtration through Whatman grade No. 1 filter papers and concentration in rotary evaporator (Buchi Rotavapor R, Switzerland) at 50°C [15].

2.2.2. DPPH Radical Scavenging Activity. Antioxidant activity of natural extracts and BHT was calculated using DPPH free radical method described by Çam et al. [16]. The amounts of extracts, required to replace BHT, were calculated based on the IC_{50} of BHT. The required amounts of natural extracts should have equal antioxidant activity to BHT.

2.3. Beef Burger

2.3.1. Burger Preparation and Storage. The formulation of beef burger used in this study was composed of beef (70%), onions (13.4%), rusk (4%), water (8%), SPI (3%), salt (1%), black (0.3%), and red (0.2%) peppers. Ground meat, salt, water, and minced onion were mixed together. After that, SPI was added and then mixed for 15 min to obtain a homogenous mixture. Rusk and spices were added in the next step. Finally, natural extracts and/or BHT were incorporated into the homogenate and then mixing process was continued. The concentration of BHT was 100 ppm. A burger maker (9 cm internal diameter) was used to shape the mixture into patties of approximately 90 g and 5 mm thickness. Burgers (15 patties per treatment) were then packed in light-resistant polyethylene containers and frozen at −18°C. Analyses were performed during 15-day intervals for two months. Before analysis, the samples were thawed at +4°C and then hand-mixed for 30 s.

2.3.2. Chemical Analysis. Moisture, protein, fat, and ash contents of burgers were determined according to the AOAC methods [17]. Carbohydrate content was estimated by subtracting the total amounts of moisture, protein, fat, and ash from 100.

2.3.3. pH Measurement. Meat sample (10 g) was homogenized with 50 mL deionized water for 1 min. pH was measured at room temperature using a digital pH meter (Suntex TS-1, Taiwan) equipped with a probe-type combined electrode (Ingold) through direct immersion of electrode into the mixture [17].

2.3.4. Instrumental Color Evaluation. Color attributes of beef burgers were measured by $L^*a^*b^*$ method described by Yam and Papadakis [18]. The values of L^* (brightness), a^* (redness-greenness), and b^* (yellowness-blueness) were measured on the whole outer surfaces of beef burger. A wooden box ($50 \times 50 \times 60\,cm^3$) equipped with a natural daylight source (6500 K) and a digital camera (Canon Powershot A540 of six megapixels resolution) in a vertical position and a distance of 25 cm from the samples was used for taking photos. Adobe Photoshop® CS6 was applied to determine the average surface color.

2.3.5. Determination of Lipid Oxidation. Lipid oxidation was monitored by measuring thiobarbituric acid reactive substances (TBARS). Meat sample (4 g) was homogenized with 20 mL trichloroacetic acid solution (20% w/v) and then centrifuged at $3000g$ for 10 min. The supernatant (2 mL) was mixed with 2 mL thiobarbituric acid solution (0.1% w/v in double distillated water) followed by heating in a water bath at 100°C for 30 min and then cooling to room temperature. Therefore, TBARS were extracted in chilled atmosphere. The absorbance of each extract was measured at 520 nm in a spectrophotometer (spec 1650PC, Shimadzu, Japan). 1,1,3,3-tetraethoxypropane was used to develop the standard curve for TBARS assay. TBARS values were reported as mg of malonaldehyde per kg of beef burger [7].

2.3.6. Metmyoglobin Measurement. A modified method of An et al. [19] was used to measure the metmyoglobin content in raw beef burgers. The sample (4 g) was mixed in a homogenizer with 20 mL of phosphate buffer (0.04 M, pH 6.8) and then stored at 1°C for 1 h. The mixture was then centrifuged at $3500g$ and 4°C for 30 min. Finally, the supernatant was filtered through Whatman grade No. 1 filter paper. The absorbance of the solution was measured at 525, 572, and 700 nm. Metmyoglobin (%) was calculated according to the following equation:

$$\text{Metmyoglobin (\%)} = \left[1.395 - \left[\frac{(A_{572} - A_{700})}{(A_{525} - A_{700})}\right]\right] \times 100. \quad (1)$$

Total Aerobic Bacterial Count. Total aerobic bacteria were quantified every 15 days using the method described by Aliakbarlu et al. (2016). Beef burgers (10 g) were aseptically transferred into individual stomaching bags containing 90 mL of sterile saline (0.9%) and homogenized for 2 min. Serial 10-fold dilutions were prepared in saline and 100 μL from appropriate dilutions was spread on the surface of plate count agar for total aerobic count (TMC) and then incubated at 35°C for 24 h.

2.3.7. Sensory Evaluation. Sensory analyses have been performed by 20 trained panelists from the Department of Food Science and Technology (Shiraz University). Panelists were both male and female in the age range of 23–28 years old. Panelists were selected based on their previous experiences in consuming traditional beef burgers. Moreover, they receive a preparatory session before the sensory test to train them how to describe all evaluated factors completely. Burgers were fried at 150°C in a forced draft oven to a core temperature of 72°C and kept warm in the oven for 3–8 min until the sensory testing [20]. Rectangular pieces of approximately

2 cm were cut from the center of each burger and then served at room temperature. Each panelist randomly evaluated three pieces of all formulations and asked to give a numerical value between 1 and 9 for the following attributes: taste, 1 (imperceptible) to 9 (extremely intense); texture attributes and juiciness 1 (extremely dry) to 9 (extremely moist); appearance, 1 (extremely soft) to 9 (extremely tough); and odor 1 (imperceptible) to 9 (extremely intense). Tap water was provided for panelists to rinse their mouth between different samples. At the end of evaluation, each panelist was asked to give an overall score from 1 (dislike very much) to 9 (like very much) for the overall acceptability of different formulations.

2.4. Statistical Analysis. A completely randomized block design with five treatments (including antioxidant-free (control) treatment and those incorporated with BHT, cinnamon, rosemary, and Shirazi thyme extracts) was used in this study. Experiments were performed during a 60-day storage period at 15-day intervals (0, 15, 30, 45, and 60). Independent blocks (developed from three different batches) were replicated three times at each sampling point. A two-way analysis of variance (ANOVA) and Duncan's multiple range tests (SAS 8.0 software, SAS Institute, Inc., Cary, NC, USA) were performed to analyze the effect of treatments, storage period, and their interaction on the physicochemical, microbial, and sensory characteristics of beef burgers at a confidence level of 0.05. In the analysis models, the treatments, storage times, and their interaction were assigned as fixed effects and the replications as random effects.

The given scores of different sensory attributes were compared between the treatments using general linear model (GLM). Duncan's multiple range tests were used for comparison of means at a confidence level of 0.05. Treatments and assessors were considered as main effects and the replications as random effects. Data was reported as mean ± standard error (SE).

3. Results and Discussion

3.1. Antioxidant Activity of Extracts. The IC50 values (defined as the concentration of an antioxidant required to reduce the initial DPPH concentration by 50% using DPPH free radical method described by Çam et al. [16]) of natural extracts (including Shirazi thyme, cinnamon, and rosemary) and synthetic BHT were 0.022, 0.373, 0.062, and 0.107 mg/mL, respectively. Therefore, the amounts of Shirazi thyme, cinnamon, and rosemary concentrated extracts required to replace 100 ppm BHT in formulation were 22, 73, and 28 mL per kg of beef burger, respectively. Cinnamon is a source of bioactive compounds such as cinnamaldehyde, eugenol, and coumarin [21]. The effect of cinnamon extract as direct scavengers of free radicals has been studied by Roussel et al. [22]. Carnosic acid and carnosol are the main bioactive compounds present in the rosemary extract [23]. According to Sharififar et al. [24], the major chemical compounds present in Shirazi thyme were carvacrol (33.65%), thymol (37.59), *p*-cymene (7.72%), γ-terpinene (3.88%), and β-caryophyllene (2.06%).

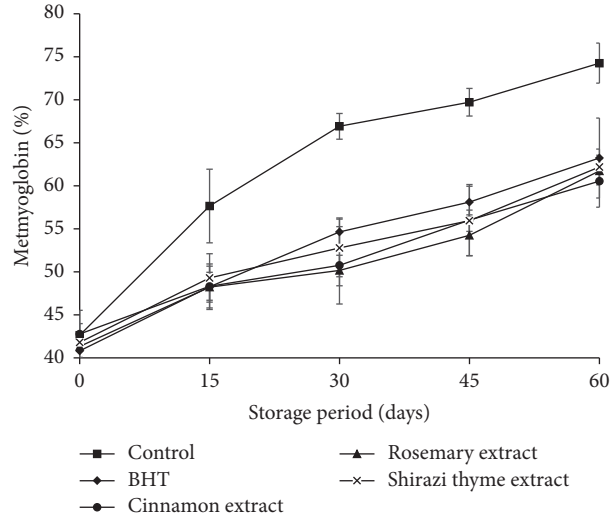

Figure 2: Effect of incorporating Shirazi thyme, cinnamon, and rosemary extracts on the metmyoglobin (%) developed in raw beef burgers during frozen storage at −18°C.

3.2. Physicochemical Properties of Beef Burger. The results indicated that the samples contained 59.13% moisture, 19% protein, 12% fat, 7.72% carbohydrate, and 2% ash.

3.3. Color Stability. When assessing meat, a consumer pays a great attention to its color, which, as a visual impression, is induced mainly by the presence of pigments but it also depends on tissue composition and meat structure. Hence, the color of fresh meat is an important quality parameter that determines a consumer's response and decision to buy or not to buy that product at retail. Changes in L^*, a^*, and b^* values of beef burgers during frozen storage are shown in Table 1. No significant difference ($P > 0.05$) was observed between the samples incorporated with natural antioxidants and those incorporated with BHT at production time (day 0), resulting in low ΔE values at early stages of storage. All of the formulations showed significant decrease in a^* during frozen storage. Formulation incorporated with rosemary extract showed lower amounts of a^* at the end of storage time. The significant decrease in a^* values indicated the red color reduction of products during storage; this is despite the fact that BHT- and extract-incorporated treatment significantly reduced the Met-mb development ratio in comparison to control one (Figure 2). During the first 30 days of storage, L^* values increased significantly ($P < 0.05$) then remained constant. During frozen storage, changes in the amounts of L^* in cinnamon- (6.42%) or Shirazi thyme-incorporated samples (5.97%) were less than those in other formulations. Samples incorporated with rosemary extract showed the least redness (4.33).

A significant decrease in a^* values of raw pork patties containing grape seed extract and bearberry has been reported over a 12-day storage by Carpenter et al. [25]; however, b^* values (particularly of control samples) increased significantly ($P < 0.05$). Similarly, Nuñez de Gonzalez et al. [26] reported that a^* value of beefs stored under refrigeration

Table 1: Effect of addition of Shirazi thyme, cinnamon, and rosemary extracts and BHT on L^*, a^*, b^*,and ΔE values of raw beef burgers during frozen storage at −18°C.

Parameter	Sample	Storage period (days)				
		0	15	30	45	60
L^*	Control	60.33 ± 0.88 g**	58.00 ± 0.58 hi	71.33 ± 0.34 abc	71.67 ± 0.34 ab	72.67 ± 0.67 a
	BHT	58.67 ± 0.88 ghi	57.33 ± 0.34 i	68.33 ± 0.34 de	69.33 ± 0.34 cd	70.67 ± 0.67 abc
	Cinnamon extract	60.33 ± 0.67 g	54.33 ± 0.34 j	59.67 ± 0.67 gh	65.33 ± 0.67 f	68.00 ± 0.58 de
	Rosemary extract	57.33 ± 0.34 i	54.33 ± 0.82 i	69.67 ± 0.88 bcd	71.33 ± 0.88 abc	71.67 ± 0.34 ab
	Shirazi thyme extract	57.33 ± 1.46 i	56.67 ± 0.67 i	57.00 ± 0.58 i	67.00 ± 0.58 ef	68.33 ± 0.67 de
a^*	Control	11.33 ± 0.34 a	8.33 ± 0.34 de	8.33 ± 0.34 de	7.67 ± 0.34 ef	6.67 ± 0.34 fgh
	BHT	11.00 ± 0.58 ab	6.67 ± 0.34 fgh	7.67 ± 0.67 ef	7.00 ± 0.58 fg	6.67 ± 0.34 fgh
	Cinnamon extract	9.33 ± 0.34 cd	7.33 ± 0.34 efg	6.67 ± 0.34 fgh	5.67 ± 0.34 hij	5.33 ± 0.34 ijk
	Rosemary extract	11.67 ± 0.34 a	5.33 ± 0.34 ijk	5.33 ± 0.34 ijk	4.67 ± 0.34 jk	4.33 ± 0.34 k
	Shirazi thyme extract	10.00 ± 0.58	6.67 ± 0.34 fgh	6.33 ± 0.34 ghi	5.67 ± 0.34 hij	5.33 ± 0.34 ijk
b^*	Control	23.33 ± 0.34 kl	25.00 ± 0.58 ij	32.33 ± 0.34 bc	32.67 ± 0.67 b	34.00 ± 0.58 a
	BHT	22.33 ± 0.34 lmn	23.33 ± 0.34 kl	31.00 ± 0.58 cd	31.33 ± 0.67 bcd	31.67 ± 0.67 bcd
	Cinnamon extract	21.67 ± 0.34 mno	22.67 ± 0.34 lm	25.33 ± 0.34 ij	26.00 ± 0.58 i	27.67 ± 0.34 h
	Rosemary extract	20.33 ± 0.340	21.00 ± 0.58 no	29.00 ± 0.58 fgh	30.67 ± 0.34 de	31.33 ± 0.67 bcd
	Shirazi thyme extract	23.00 ± 0.00 klm	24.33 ± 0.34 jk	28.33 ± 0.34 gh	29.33 ± 0.34 efg	30.33 ± 0.34 def
ΔE***	Control	0.00 ± 0.00 f				17.00 ± 0.25 a
	BHT	2.72 ± 0.69 e				14.09 ± 0.18 bc
	Cinnamon extract	2.82 ± 0.72 e				10.78 ± 0.95 d
	Rosemary extract	4.42 ± 1.04 e				15.59 ± 1.02 ab
	Shirazi thyme extract	3.54 ± 0.30 e				12.29 ± 0.80 cd

** Data represent averages of three independent repeats ± standard errors.
** Means with different letters are significantly different ($P \leq 0.05$).
*** For each storage time, the color difference (ΔE) was calculated by comparing the color attributes of different formulations with those of control sample.

for 10 weeks decreased significantly ($P < 0.05$). The results of the current study showed that high antioxidant activity of natural extracts can be potentially used to develop natural color stabilizers, particularly for controlling L^* and b^* values in frozen beef burger.

As mentioned already, samples incorporated with BHT and natural extracts did not show any significant difference in ΔE value at the beginning of the storage time. However, at the end of storage, the lowest and highest changes in ΔE were observed in cinnamon-incorporated and control samples. Red meat color is an important property of visual appearance and could influence meat purchasing decisions more than any other quality parameters. The color of meat products and meat is influenced by metmyoglobin percentage in muscle. Initially, the myoglobin was changed into oxymyoglobin (light pink color), which could result in brighter red meat, and then oxymyoglobin was oxidized into metmyoglobin during storage [7].

3.4. Metmyoglobin Content Assay. The amount of metmyoglobin has been positively linked to the extent of protein oxidation in meat products. The heme complex of heme (in) proteins consists of iron in the ferrous state (Fe^{+2}) which turns into the ferric state (Fe^{+3}) via a process called autoxidation [27]. The effects of natural extracts on the changes of metmyoglobin content are shown in Figure 2. The maximum amount of metmyoglobin (74.26%) was measured in antioxidant-free samples. The presence of antioxidants resulted in a significant decrease (16.25–18.47%) in the amounts of metmyoglobin developed during storage which was attributed to the strong antioxidant activity. An et al. [19] evaluated the percent metmyoglobin of the pork jerky samples incorporated with different kimchi powder concentrations. It has been reported that the metmyoglobin contents (%) of the samples prepared with various kimchi powder levels (ranged from 81 to 83%) were significantly ($P < 0.05$) lower than that of control sample (85%) [19]. There was no significant difference between the amounts of metmyoglobin under the influence of natural extracts or BHT indicating that these natural extracts can be utilized as a substitute for BHT to prevent discoloration.

The myoglobin reactions with peroxides result in the formation of lipid oxidation promoting compounds [28]. The lowest amount of metmyoglobin was measured in the samples formulated with cinnamon extract indicating that it was more effective than the other antioxidants (either natural or synthetic) in preventing metmyoglobin formation. A reverse relationship between the metmyoglobin level and a^* value was observed in this study. A decrease in a^* values corresponding to the decreased redness of lamb meat as a result of myoglobin oxidation (metmyoglobin formation) has been reported previously [29, 30].

Metmyoglobin content increased during storage. The lowest increasing rate was observed in the samples incorporated with rosemary extract. The highest amount of metmyoglobin was observed in the control sample at the end of storage. Changes in the metmyoglobin content of control sample became significant after 15 days.

Table 2: Thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde per kg) of beef burger during frozen storage at −18°C.

Sample	Storage period (days)				
	0	15	30	45	60
Control	0.12 ± 0.01*	0.15 ± 0.02	0.20 ± 0.02	0.23 ± 0.01	0.41 ± 0.04
BHT	0.10 ± 0.01	0.13 ± 0.01	0.15 ± 0.02	0.18 ± 0.01	0.23 ± 0.02
Cinnamon extract	0.11 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	0.17 ± 0.02	0.24 ± 0.02
Rosemary extract	0.11 ± 0.01	0.13 ± 0.02	0.17 ± 0.02	0.19 ± 0.02	0.22 ± 0.02
Shirazi thyme extract	0.12 ± 0.01	0.14 ± 0.02	0.17 ± 0.02	0.18 ± 0.01	0.26 ± 0.02

*Data represent averages of three independent repeats ± standard errors.

3.5. Oxidative Stability. Initiation is the step in which a fatty acid radical is produced. Two notable initiators in oxidative stability are $OH^{\bullet}$ and $HOO^{\bullet}$, which combines with a hydrogen atom to make water and a fatty acid radical. The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide, or a cyclic peroxide if it had reacted with itself. This cycle continues, as the new fatty acid radical reacts in the same way. The radical reaction stops when two radicals react and produce a nonradical species. The lipid oxidation leads to produce of some components causing off-flavors and reduced nutritional quality such as malondialdehyde and 4-hydroxynonenal.

Thiobarbituric acid test can be used to determine the secondary products of lipid oxidation in different foods such as meat and its products [31]. Thiobarbituric acid reactive substances (TBARS) values of different formulations (as a function of storage time) are shown in Table 2. Except for control sample, the variations in TBARS values of the other formulations revealed a similar pattern. Due to the absence of antioxidant in formulation, the observed changes in TBARS values of control were significantly ($P < 0.05$) higher than others (86.36% higher than rosemary). Natural extracts were as effective as BHT for preventing lipid oxidation in beef burgers. This observation indicated that natural herbal extracts are suitable replacers for synthetic antioxidant. It has been reported that rosemary oleoresin (77.04%) and a mixture of BHT and BHA (78.69%) was equally efficient in inhibiting the lipid oxidation of breakfast sausage containing 25% turkey meat [32]. These findings are also in good agreement with those of Mielnik et al. [33], who reported lower TBARS values after cooking of mechanically deboned turkey meat already stored in dark cold and treated with rosemary (88.34%) and grape seed extracts (83.83%). Sánchez-Escalante et al. [34] reported that incorporating rosemary essential oil into beef patties led to a significant ($P < 0.05$) decrease (64.00%) in TBARS values during cold storage. The essential oil of rosemary was also effective in preventing (72.43%) the rancidity of heat-treated turkey meat products [35]. It has also been reported that rosemary extract addition into deboned poultry meat can protect (98.20%) the product from cooking-induced oxidation [36].

Regardless of sample type examined, TBARS values increased significantly during the frozen storage and reached to a maximum amount of 0.41 mg malonaldehyde per kg in antioxidant-free (control) sample after 60 days. At TBARS values of 2.0, off-flavors are definitely detectable, and the meat is considered to be unacceptable [37].

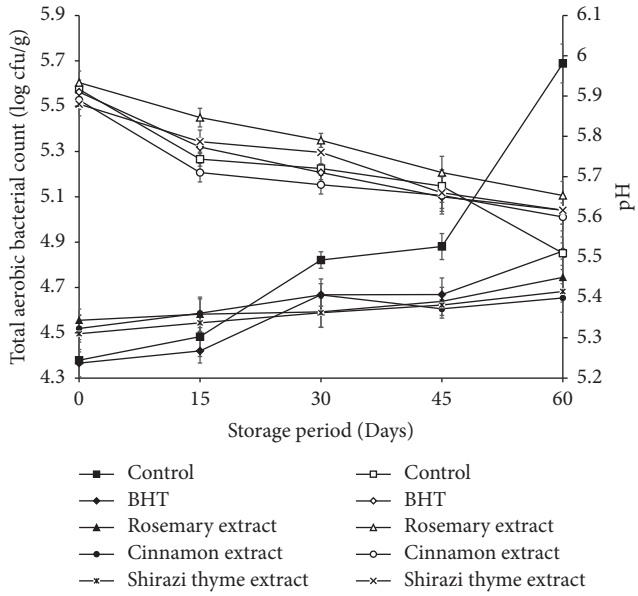

Figure 3: Total aerobic bacterial count (log cfu/g) (solid symbols) and pH value (open symbols) of different beef burger formulations as a function of storage time.

3.6. Changes in pH Values. Changes in pH values of beef burgers during frozen storage are shown in Figure 3. All formulations revealed relatively similar pH values just after production ranging from 5.88 to 5.93, coinciding with the ISIRI 2008. Mohamed and Mansour [38] similarly reported that no significant differences ($P > 0.05$) were observed in the pH values of beef patties after incorporating natural herbal extracts.

A downward pattern of different rates was observed in the pH values of all formulations during frozen storage. As no microbial growth was expected during storage, the decrease in the pH values could be attributed to the microbial growth during thawing (by consuming sugar and producing organic acids). Control sample had the lowest pH (5.51) at the end of storage. Emiroğlu et al. [39] reported that the pH values of

all fresh ground beef patty samples treated with thyme and oregano generally decreased after sixth day of storage ($P > 0.05$).

Also the result showed that, to decrease pH, amount of lipid oxidation and metmyoglobin significantly increased. Lapidot et al. (2005) evaluated lipid peroxidation of grilled red turkey muscle (Donor Kabab) as affected by pH. In this study, they show indeed that lipid peroxidation and myoglobin of a real fast food (turkey Doner Kebab, shawarma) is significantly more rapidly oxidized at pH 3.0 than at pH 5.0.

3.7. Microbial Growth. As shown in Figure 3, total aerobic bacterial counts of antioxidants-incorporated formulations were not significantly ($P < 0.05$) different. The highest amount of total count was observed in control sample (Figure 3). Shirazi thyme-incorporated beef burgers had the lowest microbial count which could be attributed to the high antimicrobial properties resulting from thymol and carvacrol. The antibacterial mechanism of thymol and carvacrol is the disruption of the cytoplasmic membrane, which raises its permeability and depolarizes its potential. Total count of frozen raw beef burger should be lower than 6 log cfu/g [40]. Except for the control sample, the microbial counts of beef burgers were lower than the maximum allowed count. A relatively good correlation was observed between the pH values and total aerobic counts. For example, control sample had the lowest pH and the highest microbial count. Rosemary extract (1000 ppm) inhibitory effect on the microbial growth of surface-applied beef steaks has been reported by Djenane et al. [41]. However, Sánchez-Escalante et al. [34] reported that rosemary did not affect the microbial counts of beef patties during storage (3 $\log_{CFU/g}$).

An increase in the microbial counts was observed during storage, but not beyond the standard range. As a rule of thumb in good manufacturing and hygienic practices during preparation of beef burger and its related products, rapid and proper storage is important for decreasing the microbial growth and enhancing the shelf life.

Vieira et al. [42] studied the effect of frozen storage conditions (temperature and length of storage) on microbiological and sensory quality of rustic crossbred beef at different states of ageing. The result showed that psychrotrophic bacteria increased (1.38 $\log_{CFU/g}$) significantly during 90-day storage.

3.8. Sensory Properties. Color, flavor, and texture are the most important sensory attributes which influence the acceptability of meat products by consumers [43]. Sensory evaluation results of cooked beef burgers are shown in Figure 4. Generally, incorporating Shirazi thyme, cinnamon, and rosemary extracts into beef burger formulations had no significant influence on the sensory properties. Although samples formulated with Shirazi thyme and cinnamon extracts with 69.44 and 71.67% acceptability obtained the highest taste scores by panelists ($P < 0.05$). The antioxidant properties of natural extracts were the main reason for the increased sensory scores of natural extract-incorporated formulations through preventing the formation of oxidation-mediated off-flavors and off-odors (short-chain aldehydes and ketones) during storage. Moreover, the presence of pleasant volatile constituents in natural extracts (particularly in cinnamon and Shirazi thyme) led to an increased sensory score in comparison to the BHT-incorporated formulation.

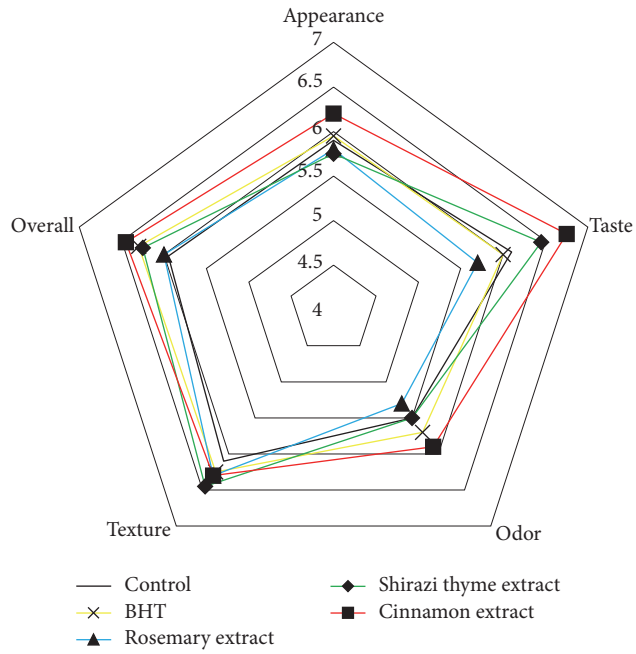

Figure 4: Sensory evaluation of different beef burger formulations; a 9-point descriptive scale (1 = extremely poor and 9 = highly acceptable) was used during evaluation.

A significant increase in the flavor scores of beef patties incorporated with antioxidant mechanically deboned poultry meat during frozen storage was similarly reported by Mohamed and Mansour [38].

4. Conclusion

This work aimed to study the possible replacement of synthetic antioxidant BHT with natural extracts (including Shirazi thyme, cinnamon, and rosemary extracts) in beef burger formulation. Different samples were prepared; then different characteristics such as protein and lipid oxidation and physicochemical, microbial, and sensory properties were evaluated during frozen storage. Herbal extracts could inhibit the lipid oxidation in formulated burgers. Moreover, it was demonstrated that natural herbal extracts were generally better than BHT in preventing the lipid and protein oxidations as well as improving the sensory attributes of beef burgers. Therefore, it can be concluded that natural extracts of Shirazi thyme (0.022 mg/mL), cinnamon (0.373 mg/mL), and rosemary (0.062 mg/mL) can be used as natural substitutes for BHT (0.107 mg/mL). This substitution may have potential implication for developing green-labeled meat product. In future studies, it would be informative to study the effect of mixed extracts and their possible synergistic or antagonistic effects in extending the shelf life of meat products.

Additional Points

Practical Applications. Beef burger containing natural antioxidant is one of the most important functional food for human consumption attributable to its nutritional properties and health effects. However, the nutraceutical properties of this food have improved due to alternative BHT with extracts. The present investigation demonstrated the effects of adding various natural antioxidants to the beef burger, so as to monitor the changes that occur to the physicochemical, antimicrobial, and sensorial properties of the beef burger.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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