© 2017 IJSRST | Volume 3 | Issue 4 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Effects of Rosemary and Thyme Extracts on Acrylamide Formation in Fried Beef

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ABSTRACT

This study examined the effects of rosemary and thyme extracts on acrylamide formation in fried beef. Six samples including T0 (the control sample), T1 (1% thyme and 1% rosemary), T2 (1% thyme), T3 (2% thyme), T4 (1% rosemary), and T5 (2% rosemary) were prepared. The samples were deep fried at 200 °C for 10 minutes. Physicochemical properties (pH and moisture content), oxidative indexes (peroxide value, anisidine value, and total oxidation index), acrylamide level, and sensory properties (flavor, texture, color, aroma, and overall acceptance) of the samples were investigated after frying. A completely randomized factorial design was adopted and all analyses were conducted using SPSS 16.0. According to the results, the addition of rosemary and thyme extracts to beef samples decreased acryl amide formation. The lowest levels of acrylamide were observed in T0 and T1 samples, respectively. The extracts had no significant effects on pH and anisidine values. Increasing the levels of extracts in the samples significantly reduced the peroxide value and total oxidation index, but increased the moisture content. The sensory results indicated that addition of rosemary and thyme extracts to the beef samples did not lead to any significant effects on flavor, texture, color, and aroma. Moreover, T1 and T5 samples had the highest and lowest scores, respectively. In conclusion, both rosemary and thyme extracts could significantly decrease the acrylamide level generated in fried beef. Moreover, 2% rosemary and a combination of 1% thyme and 1% rosemary had the strongest effects on reducing acrylamide and oxidative indexes. However, the samples containing 2% rosemary did not provide acceptable sensory indices. Therefore, T1 can be introduced as the best sample.

Keywords: Acrylamide, Fried Beef, Oxidative Indexes, Rosemary, Thyme

I. INTRODUCTION

Acrylamide (C3H5NO;2-propenamide), is a colorless, non-volatile, water-soluble crystalline solid with a molecular weight of 71.08 kDa [1]. Following the confirmation of its carcinogenicity in rodents, acrylamide was introduced as a potential carcinogen to humans (Group 2A) by the International Agency for Research on Cancer (IARC) in 1994. Later in 2002, the World Health Organization (WHO) Consultation endorsed this classification. The presence of acrylamide monomer in food was recently reported. This monomer is potentially toxic to the nervous system. It is a carcinogen in rodents and possibly humans which can lead to gene mutations and DNA damage [2,3]. People's diet exposes them to various levels of acrylamide. High levels of acrylamide were initially found in foods treated at high temperatures [4,5]. Further investigations revealed a variety of factors, e.g. food composition, high temperature (more than 120°C), high carbohydrate and free asparagine contents, reduced sugars, pH, water content, ammonium bicarbonate, along with high concentration of competing amino acids to affect acrylamide levels in foods [6-8]. Under normal circumstances, a person on average consumes about 0.85 µg per kg body weight of acrylamide per day [9]. Lipid oxidation has been proposed as a minor pathway, with acrylic acid as a direct precursor formed by way of acrolein by oxidative degradation of lipids [10].

Polyphenols are natural compounds present in various fruits, vegetables, herbs, spices, and grains. Over 8000 phenolic structures have been identified to date. Plant polyphenols contain an aromatic ring bearing one or several hydroxyl substituents. Based on the structure, they could be divided into several groups including

flavonoids, phenolic acids, hydroxycinnamic acids, and flavolans. Some polyphenol-rich foods are consumed by a large population in many countries. Some plant extracts containing a large amount of polyphenols have been recently recommended as food additives. Since plant polyphenols are safer and more consumer-friendly than synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), they serve as a major constituent of people's diets [11].

The beneficial effects of rosemary (Rosmarinus officinalis) and its constituents, particularly caffeic acid derivatives including rosmarinic acid, in the prevention of bronchial asthma, spasmogenic conditions, peptic ulcer, inflammatory diseases, hepatotoxicity, ischemic heart disease, cataract, cancer, and poor sperm motility have been previously reported [12]. The plant has also been found to show antimicrobial, antioxidant, anti-carcinogenic, and cognition-improving activity [13]. Carnosic acid and carnosol are believed to be responsible for the antioxidant effects of rosemary extract [14,15].

Thyme, belonging to the Labiatae family, is widely used as an aromatic agent in the European cuisine. Antioxidative effects of thyme are caused by its polyphenolic compounds such as flavonoids (Luteolin) [16,17]. The essential oil of this plant is also known as a rich source of thymol and carvacrol which both have high antioxidant activity [18].

In an attempt to improve public health, this research used rosemary and thyme extracts, as natural antioxidants, to decrease the acrylamide formation rate during the frying process of beef.

II. METHODS AND MATERIAL

A. Chemicals

A number of chemicals including ethanol, acetic acid glacial, chloroform, potassium iodide, sodium tiosolphate, methanol, isooctane, para anisidine, formic acid, sodium hydroxide, potassium hexacyanoferrate trihydrate, zinc sulfate, and acetonitrile were used in this study. All chemical were purchased from Merck & Co., Inc. (USA).

B. Rosemary and Thyme Extract Preparation

In order to prepare the extracts, 50 g of plant powders (either rosemary or thyme) were mixed with 1 L of ethanol for 24 h at room temperature using an overhead stirrer (Heidolph MR Hei-standard, Germany). Vacuum filtering was then performed (Platinum, JB industriles INC, USA) and the obtained extract was transferred to a water bath set at 45 ºC and concentrated under reduced pressure using a rotary evaporator (Heidolph, Laborata 4000-efficient, Germany). The final product was kept at 4 ºC until the experiments [19].

C. Beef Treatments

Meat samples were prepared in Pak Telise Factory (food industry 202). Fresh beef was first obtained. The muscles were then separated from the bone, cut into cubes of approximately 7.5 × 5.0 × 2.5 cm3 (sample weight: 100-120 g), marinated in a prepared sauce, and stored at 4 °C overnight. The marinade sauce contained oil, salt, red and black pepper, ginger, garlic powder, and citrate [20]. Antioxidant solutions containing either rosemary or thyme extract (at 1% and 2% concentrations) along with a solution containing a combination of 1% rosemary and 1% thyme extract were injected to meat pieces and the samples were rolled by a tumbler machine for about 45 minutes. The samples were then fried at 200 ºC for 10 minutes [21] and allowed to cool down in the room temperature (Table I).

TABLE I
THE LIST OF TREATMENTS EXAMINED IN THIS STUDY

Treatments	Description
T_0	Control (without additive)
T_1	Sample containing 1% rosemary extract and 1% thyme extract
T_2	Sample containing 1% thyme extract
T_3	Sample containing 2% thyme extract

T_4	Sample containing 1% rosemary extract
T_5	Sample containing 2% rosemary extract

D. **Analytical Procedure**

1) *Determination of Acrylamide*: The method described by Gökmen et al. was used to determine the acrylamide content of meat samples [22]. In the first stage of the procedure, 1 g of ground sample was extracted with 10 mM formic acid in three steps (10, 5, and 5 mL totaling 20 mL). In order to separate fat from the samples, cold centrifugation at 0 °C was performed at 5000 rpm (2370 g) for 10 min (Hettich Universal 320, Germany). Carrez clarification was then applied to precipitate the co-extracted colloids and the obtained extract was cleaned up by Oasis MCX cartridge. The remaining extract after discarding the first eight drops (to prevent any dilution) was transferred to an autosampler vial. Carrez I and II solutions were prepared by dissolving respectively 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 mL of water. A Hewlett-Packard UPLC system coupled to a TQ detector operating in the electrospray ionization (ESI) mode was used to analyze the samples. Chromatographic separations were performed on an Acquity UPLC HSS T3 column equilibrated at 40 °C (100, 2.1 mm i.d., 1.8 μm). The mobile phase included 10 mM formic acid with 0.5% methanol. The isocratic flow rate was set at 0.3 mL/min and the Waters ACQUITY FTN autosampler was held at 10 °C during the analysis. The capillary, cone, and extractor voltages of the electrospray source were set at 0.75 kV, 21 V, and 4 V, respectively. Its source and desolvation temperatures were set at 120 and 450 °C, respectively. The desolation gas (nitrogen) and collision gas (argon) flow rates were also set at 900 L/h and 0.25 mL/min, respectively. Multiple reactions monitoring (MRM) of two channels was conducted to identify acrylamide. The precursor ion 72 was fragmented and the product ions 55 and 44 (collision energies of 9 and 12 V, respectively) were monitored. During the whole process of MRM, the dwell time was 0.2 s. An external calibration curve was created in the range between 1 and 100 ng/mL (1, 2, 5, 10, 20 and 100 ng/mL, r2 = 0.99) used to determine the acrylamide concentration. The calculated values were expressed as parts per billion (ppb; ng/g coating).

2) *PH Measurement*: Based on the method described by Dzudie et al. [23], a digital pH meter (3510 pH Meter, Jenway, England) was used to measure the pH of a homogenized solution of the sample (5 g sample in 20 ml distilled water).

3) *Moisture Content Measurement*: As indicated by the Iranian national standard No. 745 [24], the moisture content of 0.5 g beef samples was measured after oven-drying at 105 °C and the obtained values were reported as percentage of wet weight.

4) *Determination of Peroxide Value (POV):* At first, lipids of the meat samples were extracted by the method described by Bligh and Dyer [25]. The samples were minced twice and then homogenized with chloroform: methanol: water (1:1:2) for 8 min at 4 °C using a Polytron homogenizer. Distilled water was then added and the solution was homogenized again for 2 min. The homogenate was vacuum filtered with Buchner funnel and the filter was washed with chloroform. A separating funnel was then applied to extract the organic layer. After drying the organic layer in a rotary evaporator, the obtained oil was weighted, solubilized in a predetermined volume of chloroform, and kept at -40 °C.

The method recommended by the AOCS International was used to determine the POV [26]. For this purpose, 3 g of the samples were transferred to 250 ml glass stopper Erlenmeyer flasks and placed in a 60 °C water bath for 3 min to allow the fat to melt. Afterward, the fat was dissolved in 30 ml of an acetic acid-chloroform solution (3:2 v/v) by agitating for 3 min. Meat particles were then removed by passing the samples through Whatman filters under vacuum (Platinum, JB industrials INC, USA). The filtrate was then mixed with a saturated potassium iodide solution (0.5 ml), agitated for 1 min, allowed to rest in the dark for 1 min, and added with 30 ml distilled water. In the next stage, 0.5 ml 1% starch indicator was added and the color was changed to dark blue. The titration was performed against a standard sodium thiosulfate solution (0.01 N) until the blue color disappeared and the light color appeared. The POV was then calculated based on the following equation and reported as milliequivalent (meq) peroxide per kg of the sample:

$$POV = S \times N \times 1000/W$$

where POV, S, N, and W are the peroxide value (meq/kg), the titration volume (ml), the normality of the sodium thiosulfate solution (N = 0.01), and the sample weight (kg), respectively.

5) *Anisidine Value (AnV) Calculation*: The AnV is defined as 100 times the optical density measured in a 1 cm cell of a solution containing 1 g of the substance to be examined in 100 ml of a mixture of solvents and reagents. In order to determine the AnVs, 0.5-4 g of oil were accurately weighed, transferred into a 25 ml volumetric flask, dissolved, made up to volume with iso-octane, and mixed. The absorbance of the fat solution against pure iso-octane at 350 nm was then measured in a glass cell. Then, 5 ml of the fat solution and 5 ml iso-octane were pipetted into test tubes A and B, respectively. In the next step, 1 ml anisidine reagent was added to the test tubes and the tubes were stoppered, shook vigorously, and left in a dark place for 10 min. The absorbance of the content of tube A against tube B was measured at 350 nm in a 1 cm glass cell [27]. The AnV was finally calculated as:

$$\frac{25 \times (1.2A_1 - A_2)}{m}$$

where A1 and A2 are respectively the absorbance of test solutions B and A at 350 nm and m is the mass (g) of the substance to be examined in test solution (a) in grams.

6) *Total Oxidation (TOTOX) Index Calculation*: As indicated by the method recommended by the AOCS International [26], the following equation was used to calculate the TOTOX value based on the potentiometric readings of POV and AnV:

$$TOTOX = 2POV + AnV$$

7) *Sensory Evaluation*: Sensory evaluation of the fried beef samples was conducted by faculty members and graduate students in the Food Engineering Department who were experienced in the sensory analysis of different meat products. These experienced panelists examined the samples in terms flavor, texture, color, and overall acceptability. They were asked to rate how much they liked each treatment on a seven-point hedonic scale (7 = excellent; 6 = very good; 5 = good; 4 = moderate; 3 = slightly bad; 2 = bad; 1 = very bad) [20].

E. Statistical Analysis

A factorial randomized design was employed using SPSS software (SPSS 16.0). All measurements were carried out in triplicate and the results were compared through one-way analysis of variance (ANOVA). Differences between means were determined via Duncan's multiple range test. P values less than 0.05 were considered as significant.

III. RESULTS AND DISCUSSION

A. Acrylamide Formation

The acrylamide concentrations in the fried samples are shown in Fig. 1. Statistical analyses showed that rosemary and thyme extracts had significant effects on the acrylamide contents of fried beef, i.e. the highest amount of acrylamide (61.5 µg/kg) was found in the control sample and higher levels of rosemary and thyme extracts in meat samples significantly decreased acrylamide concentrations. Sample containing 2% rosemary extract () and the combined sample (41/3 µg/kg) had the lowest amounts of acrylamide (37.2 and 41.3 µg/kg, respectively).

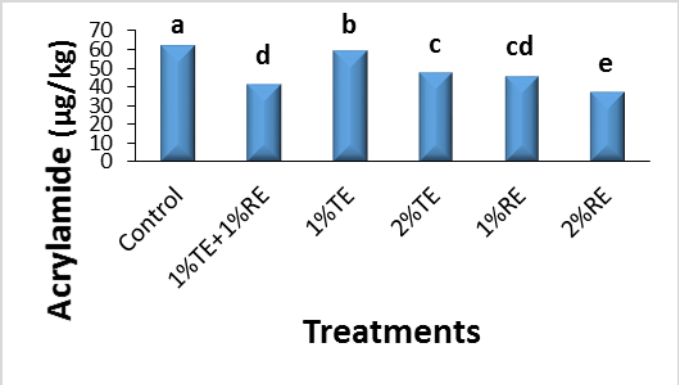

Figure 1: Effects of thyme and rosemary extracts on acrylamide formation in fried beef both on screen and on a black-and-white hardcopy

Different mechanisms can justify the effectiveness of rosemary and thyme extracts and other antioxidants in decreasing acrylamide formation during the frying process. In a possible mechanism, the radical scavenging activity of these antioxidants is believed to prevent the formation of hydroperoxide and oxidation products by stabilizing radicals during the induction and propagation steps of lipid oxidation [28]. In agreement with our findings, Demirok and Kolsarici confirmed the effects of

antioxidants on decreasing acrylamide formation during the production of fried chicken drumsticks and chicken wings [20]. Napolitano et al. studied the production of French fries and reported similar findings [29].

B. PH

Fig. 2 shows the effects of rosemary and thyme extracts on the pH of fried beef. None of these extracts significantly changed the pH of fried beef and the pH remained between 6.41 and 6.62. Likewise, Behnam and Aliakbarlou [30] and Hozhabri et al. [31] indicated that the addition of thyme and oregano essential oils to chicken meat and thyme essential oil to Mahabadi goat kid meat did not have significant effects on pH values.

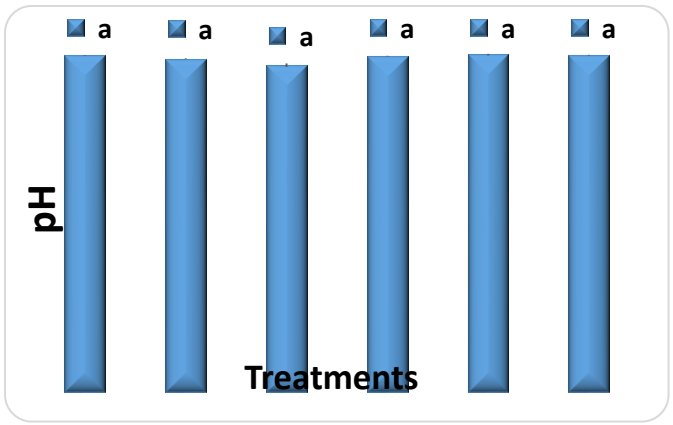

Figure 2: Effects of thyme and rosemary extracts on pH of fried beef

C. Moisture Content

The moisture content of fried meat samples is shown in Fig. 3. As seen, rosemary and thyme extracts had significant effects on moisture content of fried meat. In fact, while the control sample had the lowest level of moisture (64.17%), adding rosemary and thyme extracts to meat samples enhanced the maintenance of moisture content. This could be caused by the effect of natural antioxidants on water holding capacity (WHC) of meat samples. The highest level of moisture (71.95%) was detected in the sample containing 2% rosemary extract. Similarly, Demirok and Kolcarici found that the addition of green tea extract to chicken wings significantly increased their moisture content [20].

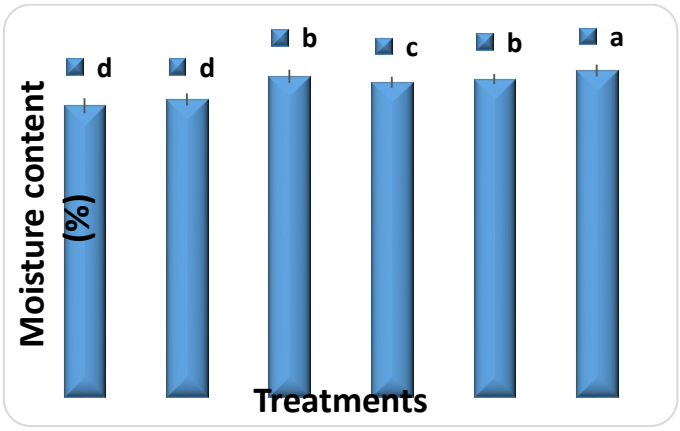

Figure 3: Effects of thyme and rosemary extracts on moisture content of fried beef

D. POV

Although peroxides are one of the primary products of oxidation, they are rapidly transformed to aldehydes or combined with proteins. Therefore, they can serve as oxidation indicators only during an early stage of rancidification [32].

Fig. 4 presents the POVs for all examined samples after the frying process. The obtained results showed that the usage of different treatments had significant effects on the POV of beef samples. The highest POV (0.192 meq/kg) belonged to the control sample andadding rosemary and thyme extracts to meat samples significantly decreased the POV. The sample containing 2% rosemary extract and that containing 1% of both extracts had the lowest amount of POV (0.100 meq/kg). The presence of copper and some enzymes might have been responsible for the higher POV of the control compared to other samples. Researchers have attributed the antioxidant activity of rosemary extracts to the presence of carnosic acid and its derivative carnosol [33,34]. In a study on chicken sausage, Sallam et al. found higher POV in the control sample than in the sample containing garlic [35]. Likewise, Pirouti et al. evaluated sausage production and reported the highest and lowest POVs in the control sample and the samples containing 1%-3% thyme extract [36].

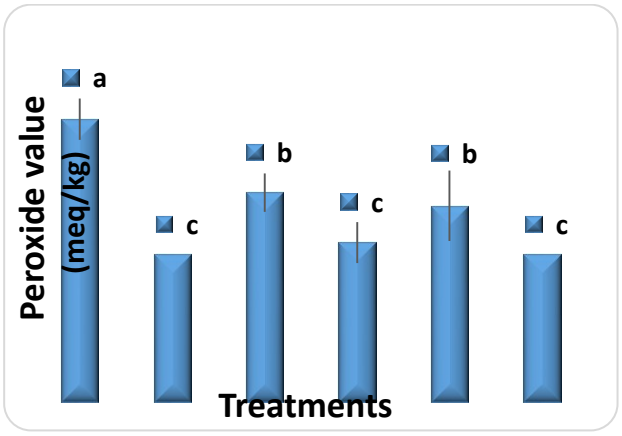

Figure 4: Effects of thyme and rosemary extracts on the peroxide value (POV) of fried beef

E. **AnV**

The AnV, an indicator of 2-alkenals and 2,4-dienals in both animal fat and vegetable oil, was investigated as a measure of secondary products of lipid oxidation. Considering the transitory nature of peroxides formed in an oxidizing fat/oil, evaluating a combination of POV and AnV facilitated the measurement of peroxide breakdown products present in the oil [27].

The AnV of fried meat samples are summarized in Fig. 5. Apparently, rosemary and thyme extracts did not have any significant effect on the AnV of fried meat. Meanwhile, due to the absence of antioxidants in the control sample, this sample had the highest AnV (0.437). Addition of herbal extracts decreased the AnV and the samples containing 2% rosemary and a combination of 1% rosemary and 1% thyme extracts had the lowest AnV (0.284 and 0.354, respectively). Ghafari also showed that red cabbage extract could decrease the AnV and delay lipid oxidation rate in beef burger [37].

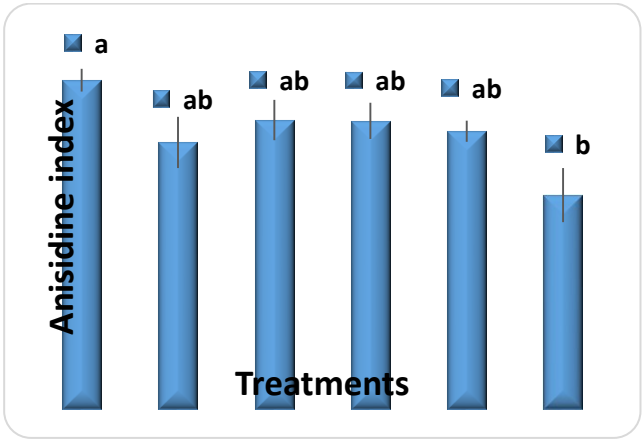

Figure 5: Effects of thyme and rosemary extracts on the anisidine value (AnV) of fried beef

F. **TOTOX Value**

The oxidation process in oils can be thoroughly investigated by simultaneous assessment of POV and AnV. TOTOX, calculated based on the POV and AnV, is a mathematical predictor of oxidative stability and the extent of oil deterioration [38].

Fig. 6 demonstrates the TOTOX values of the control sample and the experimental samples containing different concentrations of rosemary and thyme extracts. As seen, adding rosemary and thyme extracts to the samples significantly decreased the TOTOX value. Increased levels of these extracts elevated the concentrations of phenolic components and thus decreased the TOTOX value. As a result, the control sample had the highest TOTOX value (0.820) and samples containing 2% rosemary and a combination of 1% thyme and 1% rosemary extracts had the highest TOTOX values (0.484 and 0.554, respectively). Rosemary contains several compounds, e.g. rosmarinic acid, carnosic acid, and carnosol, which are responsible for its antioxidant effect. These phenolic components delayed lipid oxidation through hydrogen donation to free radicals [39]. Formanek et al. controlled by citrus preparations. Meanwhile, both oil and water soluble rosemary extracts resulted in complete elimination of rancidity [40].

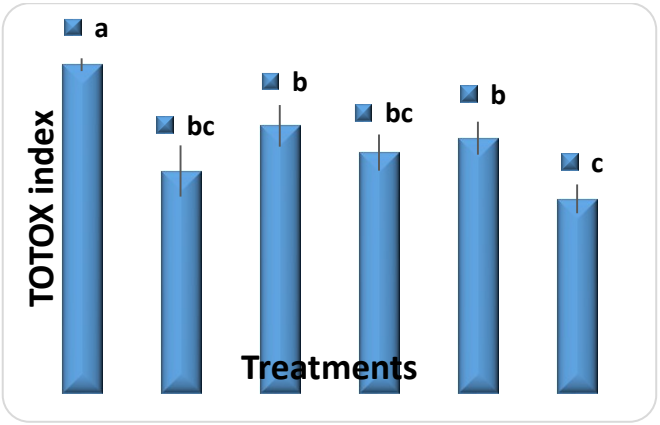

Figure 6: Effects of thyme and rosemary extracts on total oxidation (TOTOX) value of fried beef

G. **Sensory Evaluation**

Sensory evaluation is essential in determining the overall acceptability of a food product [41]. Table II summarizes the results of the sensory evaluation of fried beef samples by consumer panelists. According to the

results of statistical analyses, adding rosemary and thyme extracts did not have significant effects on flavor, texture, color, and smell of beef. The highest and lowest levels of overall acceptability were detected in cases of the combined sample and the sample containing 2% rosemary extract (5.9 and 4.0, respectively).

TABLE III
THE SENSORY PROPERTIES OF FRIED BEEF SAMPLES CONTAINING ROSEMARY AND THYME EXTRACTS

Treatments/ Scores	**Flavor**	**Texture**	**Color**	**Odor**	**Overall acceptance**
Control	5.0 ± 0.14 ^{ab}	5.7 ± 0.16 ^{a}	5.1 ± 0.19 ^{a}	5.2 ± 0.29 ^{a}	4.7 ± 0.25 ^{bc}
Combined sample	5.5 ± 0.27 ^{a}	5.7 ± 0.23 ^{a}	5.7 ± 0.22 ^{a}	5.7 ± 0.23 ^{a}	5.9 ± 0.36 ^{a}
Sample with 1% TE	5.2 ± 0.19 ^{ab}	5.2 ± 0.42 ^{ab}	5.4 ± 0.39 ^{a}	4.9 ± 0.41 ^{a}	5.0 ± 0.34 ^{abc}
Sample with 2% TE	5.2 ± 0.29 ^{ab}	5.5 ± 0.25 ^{ab}	5.6 ± 0.23 ^{a}	5.4 ± 0.39 ^{a}	5.2 ± 0.29 ^{ab}
Sample with 1% RE	4.7 ± 0.29 ^{ab}	5.1 ± 0.27 ^{ab}	5.4 ± 0.29 ^{a}	4.9 ± 0.24 ^{a}	4.7 ± 0.24 ^{bc}
Sample with 2% RE	4.3 ± 0.37 ^{b}	4.8 ± 0.39 ^{b}	5.1 ± 0.36 ^{a}	5.4 ± 0.39 ^{a}	4.0 ± 0.29 ^{c}

Likewise, Banerjee et al., reported the addition of broccoli powder extract not to cause any significant changes in the appearance, flavor, texture, juiciness, and overall acceptability scores of nuggets [42]. Demirok and Kolsarici stated that the incorporation of green tea extract into chicken drumsticks and chicken wings did not negatively affect the sensory properties [20].

IV.CONCLUSION

According to our findings, adding rosemary and thyme extracts did not significantly change pH and AnVs, but enhanced the maintenance of moisture and significantly decreased POV and TOTOX values in the studied samples. Addition of rosemary and thyme extracts could effectively reduce the amounts of acrylamide in fried beef. Several studies have reported the carcinogenicity of acrylamide and its role in damaging the nervous system in both humans and animals. Therefore, acrylamide content of foods should be minimized. In this study, although treatment with 2% rosemary extract led to optimal inhibition of acrylamide and oxidative indexes in fried beef, it failed to provide high sensory acceptance. Therefore, adding both 1% thyme and 1% rosemary extracts was introduced as the best treatment. In conclusion, adding natural antioxidants such as thyme and rosemary extracts can be an effective strategy to control acrylamide formation in fried foods and meat products. However, further research is required to clarify the effects of treatment with these two antioxidants on acrylamide reduction in other fried or baked food products.

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