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EFFECT OF ROSEMARY AND THYME EXTRACTS ON THE FATTY ACID PROFILE, LIPID OXIDATION, QUALITY AND SOME FAT HEALTH RELATED INDICES OF CHICKEN BURGER

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ABSTRACT

The current study aimed to include natural ingredients (rosemary and thyme extracts, as well as a combination of them) in the formulation of chicken burgers in an attempt to improve their fatty acid profile, lipid oxidation, quality and some fat health related indices. Four batches of chicken burgers were manufactured: product 1: control burger, product 2: burger fortified with rosemary extract, product 3: burger fortified with thyme extract, product 4: burger fortified with rosemary and thyme extracts. The samples were analyzed for their chemical characteristics (moisture, ash, fat, protein, and carbohydrates), fatty acid profile, thiobarbituric acid reactive substances (TBARS) to determine lipid oxidation, and nutritional quality of chicken burger by calculating the atherogenic and thrombogenic indices, polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) ratio, and omega-6 / Omega-3 (ω 6/ω 3) fatty acid ratio. The results showed that the incorporation of extracts in the chicken burger caused a significant decrease in the percentage of trans-fat from 0.06 to 0.02 and atherogenic (AI) and thrombogenic (IT) indices from 0.30 to 0.26 and from 0.79 to 0.72, respectively. A non-significant decrease in saturated fatty acid (SFA) from 27.33 to 27.23was noted. However, a significant increase in the levels of polyunsaturated fatty acid (PUFA) from 15.36 to 19.67, monounsaturated fatty acid (MUFA) from 47.28 to 48.88, ω 6/ω 3 ratio from 13.49 to 15.85, and PUFA/SFA ratio from 0.56 to 0.72 in chicken burger was observed. Whereas addition of the extracts had a variable influence on the sensory characteristics of the freshly prepared and stored burgers. It was concluded that the fortification of chicken burger with rosemary and thyme extracts improved the nutritional and quality properties and gives a desirable change in sensory evaluation.

Key words: Rosemary extract, Thyme extract, Fatty acid profile, Health indices

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INTRODUCTION

Researchers and health organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have demonstrated that changes in human eating and living patterns are the primary cause of the rise in diseases such as obesity, cancer, and cardiovascular problems [1, 2]. People are increasingly interested in meals that contain bioactive or functional components that can boost their health [3]. Food products are suitable and required conveyances for people to take in and absorb key nutrients that may improve their wellbeing [4].

Poultry meat is a popular food commodity due to its low production cost, low fat level, high nutritional value, and distinct flavor [5]. Burgers are one of the most popular applications due to their high nutritious content and appealing sensory features. However, the grinding process disrupts cellular components, making minced muscle more prone to peroxidation and microbial development [6].

As such, antioxidant bioactive compounds can be added to product formulation, coated on a product's surface, or included in the packaging material to limit the growth of unwanted bacteria and minimize lipid oxidation in ready to eat meat-based meals [7], Moringa oleifera leaves and edible Polymers and Secondary Bioactive Compounds, such as polysaccharides, proteins, and lipids, can be used as a thin layer to the surface of food or inside the package [8]and onion Peel it can be used as a functional food ingredient and in various applications, including as a packaging material and meat quality improver , enhancer food quality and prolong shelf-life, due to its antioxidant properties [9].

Synthetic antioxidants such as butylated hydroxytoluene (BHT) are used to delay or prevent lipid oxidation by scavenging chain-carrying peroxyl radicals or limiting the generation of free radicals. Research is being conducted to uncover novel, naturally occurring molecules that maintain the sensory and microbiological integrity of meat products. More so, consumers dislike synthetic antioxidants due to their carcinogenicity and therefore, customer demand for natural food additives has prompted the food industry to seek natural replacements [7].

The use of natural preservatives has increased due to customer tastes for minimally processed foods and consumer concerns about synthetic preservatives [10]. Studies have shown that adding phytogenic additives or essential oils, or extracts to animal and poultry diets improves live body weight, feed conversion

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ratio, immune response, antioxidant status, carcass traits and quality, and reduces morbidity and mortality rates [11].

Rosemary and thyme are two Mediterranean herbs that are commonly used in cooking due to their antibacterial, antiseptic, and antioxidant properties [12]. The two main polyphenols included in rosemary extract are carnosic acid and rosmarinic acid [13]. They are also abundant in molecules with biological activity, such as polyphenols and other compounds with antioxidant properties. These compounds have the potential to be used as nutraceuticals to boost the effects of other substances and as substitute food additives to inhibit the growth of pathogenic bacteria [7, 14].

The existence of fatty acids in various foods is due to the nature of their food matrix. Fatty acids are essential components of fats and oils found in both plant and animal-based foods. The composition of fatty acids in food depends on the type of food and its source. For example, fish and nuts are rich in omega-3 fatty acids, while vegetable oils contain predominantly omega-6 fatty acids [15].

The presence of fatty acids in different foods contributes to their nutritional value and impacts human health. The food matrix plays a crucial role in determining the bioavailability and absorption of these fatty acids in the human body. Therefore, the existence of fatty acids in foods is intricately linked to the nature of their food matrix [16].

This study was performed to monitor the effect of thyme and rosemary extracts and their mixture and on the fatty acid composition, presence of any harmful trans-fatty acids and the nutritional indices of chicken burgers.

MATERIALS AND METHODS

Raw Material

Fresh plants (thyme, rosemary) were used, whereas, rosemary was collected from the University of Jordan, Amman, in the winter period (January, 2022), and thyme was purchased from a local market (January, 2022).

Chicken burgers were prepared at a local Meat factory in Amman, Jordan, by grinding and mixing the chicken forequarter pieces in a mincer.
Product Formulation

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The edible parts of the collected plants (leaves of thyme and rosemary), Four kg were taken, washed with tap water to remove any adhering soil on the surface, rinsed several times with water, and dried for 16h to 24h in a 40°C oven. The dried plants were ground using home-style mixer. Five grams of ground plant material were soaked in 100 ml of distillated water and placed in a closed container in a water bath for 2 hours at 50°C, followed by 30 minutes agitation in a sonicator at 40°C, and left overnight for 24hat room temperature in a dark place before filtering through filter paper the next day to obtain extract-1.

To achieve homogeneity, the chicken forequarter pieces with 12% fat were ground and mixed in a mincer, using a plate with 8 mm-diameter holes. It was then divided into four distinct batches, each weighing 4 kg. Table 1 shows the formulations of the created burger samples in detail. Product 1: Chicken, NaCl, water, control burger (CBR.C). Product 2: Chicken, NaCl, water and extract of rosemary (CBR.R). Product 3: Chicken, NaCl, water and extract of thyme (CBR.T). Product 4: Chicken, NaCl, water and extract of thyme and rosemary (CBR.RT).

Following the additions, each batch of mince was manually blended for 2 minutes. After that, the chicken was minced once more and combined to create a finer-grained product for burgers. A meat patty making machine (Wimpex, London, U.K.) was used to form the 120-gram-per-burger patties between two sheets of paper. The burgers were immediately placed in a -20°C freezer overnight. They were then moved in groups to polythene bags and kept for analysis. All analysis was done on raw chicken burger and sensory evaluation on cooked chicken burger.

PREPARATION OF THE EXTRACT FOR ANALYSIS

The same procedures as for extract-1 were used to obtain extract-2 for laboratory analysis, but the plants were soaked in 100 ml 80:20 (v/v) ethanol/ distillated water.

DATA COLLECTION

All the laboratory analyses were done in triplicate. Initial analysis included crude moisture, crude protein, crude fat and crude ash contents, fatty acid profile, TBARS (all the products were examined for 0-3-5 days to evaluate stability. General laboratory precautions were taken to obtain reliable and valid data".

Moisture Content

Moisture content was determined by oven drying method according to AOAC (2011) number 925.09 [17], where 10 grams sample were heated in an air oven (Memmert, Germany) for 6 hours at 105°C. Until, constant weight was obtained. Moisture percent was calculated using the following equation:

Moisture% = (Loss in weight/Sample weight) x 100

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Ash content

Crude ash was determined using dry ashing method according to AOAC (2011) number 923.03[17]. Five grams of the food sample was added in crucible and placed in a muffle furnace at 550°C for 3 hours until they became light gray or white color ash). Then, the crucible (with its content) was cooled in a desiccator at room temperature and weighed. Ash contents were calculated as follows:
Ash % = (Weight gain by the dish /Weight of the sample) ×100.

Protein content

Nitrogen content was determined by using the Kjeldahl method with a factor of 6.25 to determine crude protein content following AOAC (2011) number 920.87 [17]. DK20 heating digester and UDK129 distillation unit were used to determine the Nitrogen content. Titration was carried out using 0.1N HCl, and the protein content was calculated according to the following equation:

$$\text{protein } \% = \frac{(\text{V} - \text{B}) \times \text{N} \times 14.007 \times 6.25}{10 \times \text{sample weight}}$$

where V: volume of titration acid consumed by sample, B: volume of titration acid consumed by Blank, N: normality of acid used in the titration.

Fat content determination

Crude fat was determined according to AOAC, 2011 number 930.09 [17] using Soxhlet method. Ten grams of dried sample was weighed into a filter paper and wrapped in such a fashion as to prevent escape of the product. The wrapped sample was put into a thimble then placed in a soxhlet extractor and then attached to a pre-weighed extraction flask containing 150 ml of diethyl ether. After 6 hours of extraction, the flask was disconnected and the diethyl ether was evaporated using a rotary evaporator at 35°C. The flask containing the crude fat was cooled in a desiccator at room temperature and weighed. The crude fat content was calculated as follows:

Crude fat % = (Weight of fat extracted / Weight of sample) X 100
Carbohydrate Content Determination (Nitrogen-Free Extract (NFE)
Carbohydrate content was determined by differential method. Basically, it was done by subtracting the sum of moisture content, protein, fat, fiber and ash from 100 AOAC (2011).

Nitrogen free extract% = 100 – (%Moisture + % Protein+ % Fat+ %Ash)

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FATTY ACID PROFILE

Fatty acid methyl esters (FAMEs) were produced in accordance with EC Regulation no. 2568/91[18]. In brief, 50 mg of lipid extract was weighed, dissolved in 2 mL of GC grade hexane, and vortexed for 1 minute. After adding 200 µl of 2 M-potassium hydroxides prepared in anhydrous methanol and mixing for 30 seconds until the solution became clear, 200 µl of acetic acid was added and mixed for 30 seconds. The prepared methyl esters were analysed using capillary GLC column (Restek, Rtx-225, USA, cross bond 50%- cyanopropylmethyl 50%-phenylmethyl polysiloxane, 60 m, 0.25 mm/D, 0.25 µm df) immediately after esterification by injection 1.00 µl of the hexane layer (model GC-2010, Shimadzu. Inc., Koyoto, Japan). After adjusting the GLC conditions., the initial chamber temperature was 165°C, held for 4 minutes, increased at a rate of 2°C/min to 180°C, increased at a rate of 5°C/min to 230°C, and then held for 10 minutes, for a total program time of 36 minutes. The injector temperature was 250°C, the FID temperature was 260°C, the flow rate was 1 ml/min Helium, and the split ratio used was 80. The fatty acids methyl esters (FAMEs) were identified using chromatogram of a fatty acid standard.

THIOBARBITURIC ACID REACTIVE SUBSTANCES VALUES (TBARS)

As described by Lehaçani [19] twenty five mL of 20% trichloroacetic acid (TCA), 10 g of food sample, and 20 mL of warm distilled water were mixed together, then homogenized for two minutes using a stomacher. After filtering the homogenate through Whatman No. 1 filter paper, 2 mL of the filtrate and 2 mL of 0.02 M aqueous 2-thiobarbituric acid (TBA) were addedand mixed together to a test tube. The tubes were kept in the dark for 20 hours at 22°C. The solution's absorbance was finally determined at 532 nm using a spectrophotometer (lambda 3b, Perkin Elmer, USA). The TBARS number was reported as mg of malondialdehyde/kg (MDA) of sample using a conversion factor of 7.8.

HEALTH NUTRITIONAL INDICES

Nutritional quality was determined by calculating the omega6 / omega3 ratio, PUFA/ SFA ratio, and Index of atherogenicity and thrombogenicity.

Determination of Atherogenicity Index (IA)

Ulbricht and Southgate [20] introduced a new index called the "Atherogenic Index" owing to the fact that the PUFA/SFA ratio is too wide and unsuitable for estimating

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the atherogenicity of foods. IA indicates the relationship between the sum of the major saturated and unsaturated FA classes, and was calculated according to the following formula:

IA = [(C16: 0 + (4 × C14: 0) + C18: 0)]/ (ΣMUFA + Σω6+ Σω3)

Determinations of Thrombogenicity Index (IT)

In addition, Ulbricht and Southgate [20] developed the index of thrombogenicity (IT) with IA to demonstrate a proclivity to form clots in blood vessels. This is the interaction of pro-thrombogenic (SFA) and anti-thrombogenic (MUFA and PUFA) fatty acids [21]. The formula below was used:

IT = (C14: 0 + C16: 0 + C18: 0)/ [(0.5 × ΣMUFA) + (0.5 x ω6 + (3 × ω3) + (Σω3/Σω6)]

SENSORY EVALUATION

Cooked burgers were assessed by 30 trained panelists. Both sexes were represented on the panelists from the sensory team at the department of Food Science and Technology, the University of Jordan, Amman, Jordan. Each sample was tasted independently. The samples were assessed for desirability in appearance, color, tenderness, flavor, juiciness, and overall acceptability using a9-point hedonic scale as defined by Larmond [22], ranging from 9 (like extremely) to 1 (dislike extremely). Water and breadcrumbs were utilized to mask the taste differences between samples.

STATISTICAL ANALYSIS

All measurements were done in triplicate except sensory evaluation, and the mean values were reported. To determine any significant differences among the study's parameters, an analysis of variance (ANOVA, One Way) was performed using JMP version 10 (SAS institute, Cary, NC). To distinguish differences in the properties of the different chicken burgers, the least significant difference (LSD) at a 5% level of probability was determined.

RESULTS AND DISCUSSION

Proximate composition of the burgers

The approximate composition of chicken burgers is presented in Table 2. The moisture content of samples ranged between 64.66% and 65.13%, where CBR.R had the highest moisture content, followed by CBR.T, CBR.RT, and CBR.C. There

was a significant difference between the control and CBR.RT. However, no significant difference existed between the other samples.

Comparing with the literature, the USDA [23] mentioned that the moisture content of chicken burger was in the range of 42.5-46%, which is lower than the obtained results. Ramadhan *et al.* [24] reported that moisture content varied between 46.72-69.37% which is consistent with our results. However, Unzil *et al.* [25] found that the water content of chicken burger ranged between 45%-60% which is inconsistent with our study.

Table 2 depicts the ash content of chicken burger samples, which ranged between 2.15 to 2.31%. The ash content was determined based on its wet weight and expressed as the percentage of its dry weight. Ashes are sum of the total minerals presented in food such as sodium, phosphorus and iron that can be contributed by the meat as raw material, salt and spices added [26].

Chicken burger that contained rosemary extract (CBR.R) had the highest amount of ash content followed by CBR.RT, CBR.C, and CBR.T. There was no significant difference between all samples.

According to Al-Bahouh [27] and Unzil [25] the high amount of ash could possibly be due to soft bone and other chicken parts in the patty, as well as the presence of calcium and other macro minerals.

The USDA [23] and Ramadhan *et al.* [24] reported ash contents that are in agreement with the data obtained in the present study (2.09-2.46% and 1.50-2.96%, respectively).

The protein content of the burger samples is shown in Table 2. The protein content was calculated as a percentage of dry weight based on its dry weight. Values ranged from 21.18 to 21.62%, where CBR.RT had the highest protein content, followed by CBR.R, CBR.C, and CBR.T. There were significant differences between CBR.C and CBR.RT.

The protein content found was higher than that reported by the USDA [23] (11.9-12.4%), and Ramadhan *et al.* [24] (11-19%).

Generally, the protein content of the samples in this study was within the ranges reported in the literature. In Malaysia, Al-Bahouh *et al.* [27] indicated that local and imported chicken burgers from Kuwait had high levels of protein that met the

Kuwait Standard (1187/1999), with amounts corresponding to the minimum level of protein in burgers of 15% or higher.

In this study, the fat content of chicken burger samples was determined using dry weight and expressed as a percentage of dry weight (Table 2). The fat content in the burger samples ranged from 5.40% to 5.93%. The highest fat content was found in the CBR.C sample, while the lowest was found in the CBR.R sample. The fat content of the remaining burger samples was 5.66%for CBR.T, and 5.88% for CBR.RT. There were significant differences between samples.

The fat content of the examined samples was within the ranges reported in the literature. Al-Bahouh *et al.* [27] found that the fat content of chicken burgers from Kuwait ranged from 3.0% to 16%; based on their findings, the low-fat content in the burger could be due to excessive being fat removed during processing.

The findings obtained contradict those of the USDA [23] (13.5-16.9%) and Ramadhan *et al.* [24], who found that fat content in uncooked commercial chicken patties ranged from 9 to 21%.

Total available carbohydrate of the burger samples is presented in Table 2. The carbohydrate contents ranged between 5.54 and 6.09%, where CBR.T had the highest value (6.09%), followed by CBR.C (5.95%), CBR.R (5.75%), and CBR.RT (5.54%). There were significant differences between CBR.T and CBR.RT. According to the ANOVA results, there were significant differences in the percentage of total carbohydrate between the burger samples. Unzil *et al.* [25] found that the carbohydrate content of the burger samples ranged from 7.0 to 19.0%, which is higher than our findings. However, Ramadhan *et al.* [24] indicated that the carbohydrate content of uncooked commercial chicken patties ranged from 2 to 22%. Aside from these findings, the carbohydrate content of Kuwaiti chicken burgers ranged from 3-25% [27].

THIOBARBITURIC ACID REACTIVE SUBSTANCES VALUE (TBARS)

Thiobarituric acid analysis identifies secondary lipid oxidation products, primarily malondialdehyde (MDA), which may contribute to the off-flavor, rancid odor, and undesirable taste of oxidized fat. Figure 1 depicts the antioxidant effects of rosemary, thyme, and (rosemary+ thyme) extracts on the TBARS values of chicken burgers during 5 days of storage (4°C). The TBARS levels in all chicken samples increased significantly as the storage period extended from 0 to 3 days. After 3 days, the TBARS values of treated samples showed lower concentrations of MDA

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in comparison to the control with the chicken burgers lacking antioxidants having the highest concentration of MDA, which is particularly important, since high levels of TBAR are known to be toxic, carcinogenic, and mutagenic [28]. However, the decrease in TBARS, according to Ninan *et al.* [29], is most likely due to the interaction of lipid oxidized products (as MDA) with proteins.

The extracts of rosemary, thyme, and their combination had significant decrease in lipid oxidation in chicken burgers. At all storage times, the inhibition effect was stronger in (rosemary+ thyme) samples than in thyme and rosemary samples (individually).

Analysis of variance showed that the TBARS values were significantly affected ($P<0.05$) by both storage and treatment. These results suggest that these antioxidants delayed lipid oxidation during storage.

El-Fakhrany *et al.* [30] reported that when the TBA value exceeds 1 mg MDA/kg, off-odors and lipid oxidation may occur. TBA values of 0.02-2.5 mg MDA/kg have been established in several studies as the accepted limit for no rancidity in meat and meat products [31].

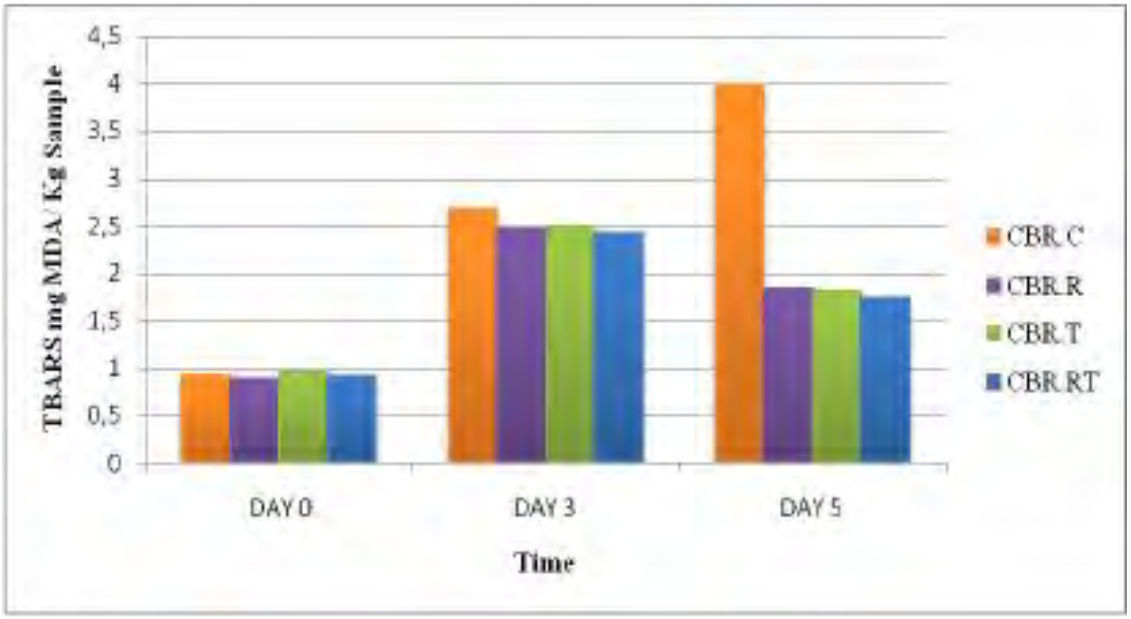

Figure 1: TBARS values (mg MAD/kg sample) of chicken burgers (made with rosemary, thyme and combination of them) during a storage period of 5 days at 4°C

CBR.C: chicken burger control, CBR.R: chicken burger with rosemary extract, CBR.T: chicken burger with thyme extract, CBR.RT: chicken burger with rosemary + thyme extracts
TBARS = Thiobarbituric-acid-reactive-substances, MDA= Malondialdehyde

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FATTY ACID PROFILE

Table 3 shows the levels of SFA found in the chicken burger treatments. The CBR.C treatment had the highest content of total SFA (27.33%), whereas CBR.R had the lowest SFA content (25.23%), where treatments ranked in descending order of SFA content as follows: CBR.C, CBR.T, CBR.RT, and CBR.R (27.33%, 27.27%, 27.27%, and 27.23%, respectively).

Palmitic acid (C16:0) contents varied on average between 25.10 and 25.16% of the total acids, was the most abundant SFA in all evaluated samples, followed by Stearic acid (C18:0) 1.37–1.45 %, Myristic acid (C14:0)0.46–0.49%, Arachidic acid (C20:0) 0.11–0.16%, and Heptadecanoic acid (C17:0) 0.10–0.11%.

Similarly, Daili *et al.* [32] reported that Palmitic acid (C16:0) was the predominated SFA and the main contributor to increasing the SFA% in selected fast foods. This fatty acid account is responsible for increased cholesterol activity and cardiovascular disease.

The highest amount of Myristic acid (C14:0), Heptadecanoic acid (C17:0), Stearic acid (C18:0) and Arachidic acid (C20:0) were found in CBR.C. High amount of Stearic acid (C18:0) is responsible for increased cholesterol activity and cardiovascular disease [33].

Mohamed *et al.* [34] found that high levels of SFA in chicken burgers may be attributed to the fiber content of some treatments. This absorbs more fats during frying and reduces the amount of oil used. Rapeseed oil, animal fat, and palm fat are also commonly used in poultry feed. In addition, Palmitic acid and Myristic acids have been linked to the etiology of heart disease by increasing plasma low density lipoprotein cholesterol [35].

Table 4 presents the MUFA content of the investigated chicken burger treatments, which ranged from 47.28% in CBR.C, to 48.88% in CBR.RT. Oleic acid (C18:1) was found to be predominant in all samples; where CBR.RT showed the highest content of 42.61%, and CBR.C showed the lowest level of 41.43%. The main unsaturated FA that contributed to the increase in total MUFA was Oleic acid. Similarly, Alagawany *et al.* [11] reported that Oleic acid was the most abundant FA in all chicken abdominal fat. However, the remaining MUFAs, such as palmitoleic acid (C16:1), ecosonic acid (C20:1) and cis-heptadecenoic acid (C17:1) were found in lower concentrations.

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Epidemiological evidence suggests that consuming more MUFA, particularly Oleic acid, is linked to a lower risk of coronary heart disease (CHD) [36]. Monounsaturated fatty acid has been shown to reduce LDL cholesterol while possibly increasing HDL cholesterol. In contrast to PUFAs, which protect against insulin resistance, Oleic acid may promote insulin resistance while also lowering systolic and diastolic blood pressure in susceptible individuals and decreasing the risk of stroke [37].

Table 5 reveals the PUFA content of the samples examined. As can be seen, the PUFA detected in the highest amounts was linoleic acid (C18:2), which ranged from 14.30 to 18.50%, while linolenic acid (C18:3) was detected in very low amounts (1.05-1.16%).

Chicken burger control had the lowest levels of both linoleic and linolenic fatty acids and thus, the lowest amount of PUFA (15.36%). However, CBR.R, CBR.T, and CBR.RT had higher levels of linoleic acid and linolenic acid of 18.50%, 17.98%, and 19.67%, respectively, and thus, experimental burgers were more PUFA-rich than the control. According to Mozdziak [38], chicken meat is a low-fat source of healthy nutrition high in unsaturated fat. Chicken meat has fewer SFA and more unsaturated FA than beef.

Table 5 depicts the levels of Trans-fatty acids (TFA) in the investigated chicken burger treatments. The percentages ranged from 0.02 to 0.06%. Chicken burger control had the highest percentage of TFA (0.06%) among all samples, followed by CBR.RT (0.04%), CBR.R (0.04%), and CBR.T (0.02%).

Trans -fatty acid is naturally present in meats, milk, and dairy products. They are also in foods that contain hydrogenated or partially hydrogenated oils [39]. The addition of chicken skin may increase PUFA, allowing for an increase in TFA. This might indicate that the chicken burgers contained skin, which was high in TFA. Trans-fatty acid consumption should be limited to less than 1% of total energy intake (about 2 g/day) [40].

Trans-fatty acid has been shown to raise LDL cholesterol while decreasing HDL cholesterol, potentially increasing the risk of heart disease. Its excessive consumption has been linked to other health issues such as breast cancer and diabetes [40].

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The World Health Organization (WHO) movement [41], in particular the "REPLACE TRANS FAT" program, offers a tactical method for getting industrially produced Trans-fat out of national food supplies, with a worldwide elimination target of 2023.

THE NUTRITIONAL INDICES OF CHICKEN BURGERS

In this study, several important nutritional indices were used to describe the FA composition of chicken burgers, to evaluate the nutritional value of FAs, and to inspect their potential use in disease prevention and treatment.

The nutritional indices of the studied chicken burgers are shown in Table 6. Linoleic acid (C18:2 ω- 6) was the most abundant and dominant PUFA in all chicken burger samples. However, linolenic acid (C18:3 ω-3) was present in very low amounts. Because omega-6 and omega-3 FAs cannot be synthesized in the human body, they must be obtained through diet [42]. The ratio of omega-6 to omega-3 FAs determines the beneficial effects of these PUFAs [35].

The most important factor in maintaining a healthy dietary pattern is the ω-6/ω-3 ratio [37]. The ideal ω-6 to ω-3 FA ratio is generally accepted to be around 4:1, which means that a healthy diet should contain one to four times as much omega-6 as omega-3 FAs [35]. The ratio of ω-6/ω-3FAs in the brain is between 1:1 and 2:1 and should be the target ratio for health [43].

Omega-3 and omega-6 FAs are important in human nutrition, with benefits including brain development, structural integrity, coronary heart disease, cancer, inflammatory bowel disease, rheumatoid arthritis, psoriasis, mental health, and neurodegenerative diseases [43].

According to Table 6, all chicken burger samples had anomega-6/ omega-3 ratio generally higher than those reported in the literature, where values in the present study ranged from 13:1 in the control, to 16:1in CBRT sample (containing rosemary and thyme extracts); which was much higher than the recommended value.

The PUFA/SFA ratio is one of the most important parameters for assessing nutritional quality in the case of available lipid fraction in food products. According to Afshari [44], the "healthy" ratio should be greater than 0.4, where the FAO [2] recommends a ratio of 0.85 for human diet. According to Simopoulos [45], the recommended PUFA/ SFA ratio is 1:5. Increasing this ratio may result in a decrease in plasma cholesterol.

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The PUFA/SFA content of the examined chicken burgers is shown in Table 6. Values ranged from 0.56 in CBR.C to 0.72 in CBR.RT. Chicken products have a higher PUFA/SFA ratio in general, which is consistent with Chen and Liu's [21] findings that the PUFA/SFA ratio of chicken ranges from 0.308 to 2.042 for various dietary treatments, which is to a certain extent consistent with our findings.

ATHEROGENIC AND THROMBOGENIC INDICES

The aforementioned FA ratio did not suffice to express the effects of each fatty acid on human health, therefore, they are replaced by atherogenic index (AI) and the thrombogenic index (TI). In a "Healthy" diet, very low levels of these indexes are recommended.

Table 6 shows that CBR.C had the highest IA and IT values among all chicken burgers studied (0.30 and 0.79, respectively), followed by CBR.T (0.27 and 0.75, respectively), and CBR.R (0.27 and 0.74, respectively). CBR.RT had the lowest IA and IT values, which were 0.26 and 0.72, respectively.

This is determined by the difference in saturated and unsaturated FAs between foods. The main SFAs that promote lipid adhesion to cells of the immune and circulatory systems are C14.0, C16.0, and C12.0, which are considered pro-atherogenic and pro-thrombogenic, respectively, whereas C18:0 is thought to be atherogenic but thrombogenic, and thus, the high IA and IT values in CBR.C and CBR.R were primarily attributed to high C14:0 and C16:0 SFA [46].

On the other hand, CBR.RT had the lowest IA and IT values due to their high MUFA and PUFA levels, which are anti-atherogenic and anti-thrombogenic because they inhibit plaque aggregation and lower levels of esterified FA, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro- coronary diseases [47]. As a result, it is recommended to consume foods or products with lower IA and IT levels, which can lower total cholesterol and LDL-C levels in human blood plasma [48]. Low atherogenic, thrombogenic and hypercholesterolemia foods are good for retarding atherosclerosis and thus the risk of cardiovascular disorders in human [49]. Burgers had an IA of around 1.6 and an IT of around 1.8, according to Afshari *et al.* [44] which is higher than our results.

SENSORY EVALUATION

Sensory profile of various chicken burgers is presented in Table 7. Mean scores showed significant differences ($p \leq 0.05$) between the control and experimental samples for all attributes except for the aroma.

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The CBR.R sample exhibited the highest scores in terms of all sensory attributes among the examined burger samples. This was possibly because of the incorporation of various ingredients such as herbs or spices in chicken burgers, as a valid strategy not only to improve certain textural properties of meat, but also to enhance the sensorial profile of the product itself [50].

A significant ($p \leq 0.05$) difference was found in color between CBR.C and CBR.R. Fernández-lópez [26] showed that calories the most important attribute among the sensory characteristics in which influence panelist choice. Regarding burgers' juiciness, scores were significantly higher ($p \leq 0.05$) in the treated samples compared to the control.

The evaluation of tenderness of meat and meat products by panelists is correlated mainly with juiciness; Abdullah [51] reported that juiciness is related to the type of meat used in the formulations.

Chicken burger control was characterized by a lower overall score compared to all other chicken burgers samples. There were significant ($p \leq 0.05$) differences in the overall acceptability between control and CBR.R sample. According to the panelists, CBR.R showed a significantly higher overall acceptability compared to CBR.T, CBR.RT and CBR.C.

LIMITATIONS OF THE DISCUSSION

It would have been better and more informative if we analyzed the fatty acid composition of the raw extract and of the cooked sample, analyzed the extract of water, and did shelf-life experiment of the final products.

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

This study provides a large database on the fatty acid composition and nutritional quality of the chicken burgers. The study based on replacing the use of synthetic preservatives with natural preservatives and produce a new product which is healthy and functional. The results indicated that the chicken burger fortified with a combination of rosemary +thyme has the better analysis result than rosemary or thyme. Separately, the control sample contains high level of tans–Fat which is not healthy in comparison with other samples, the control chicken burger had lower fatty acid quality in terms of P/S and omega-3/ omega-6. As a result, the addition of rosemary and thyme extracts to chicken burger was provided to achieve fatty acid modification, enhanced the PUFA/SFA ratio while increasing the omega 6/omega 3 ratio, exhibited higher sensory scores. And, therefore, improved the

nutritional (IA and IT) and sensory quality of this product. For further study, it is recommended to utilize different concentrations of the extract to achieve better result.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to disclose.

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Table 1: Formulations of the chicken burgers developed with the addition of thyme and rosemary extracts and their combination

Ingredients	Formulations (%)			
	CBR.C	CBR .R	CBR.T	CBR.RT
Chicken burger (g)	88	88	88	88
Rosemary (%)	-	0.1	-	0.1
Thyme (%)	-	-	0.1	0.1
Salt (g)	1.5	1.5	1.5	1.5
Water (ml)	10.3	10.2	10.2	10.1

CBR.C: chicken burger control, CBR.R: chicken burger with rosemary extract, CBR.T: chicken burger with thyme extract, CBR.RT: chicken burger with rosemary + thyme extracts

Table 2: Proximate Analysis (%) of chicken burger developed with the incorporation of the extracts of rosemary, thyme, and their combination

Treatment	Moisture (%) (g/100g)	Fat (%) (g/100g)	Protein (%) (g/100g)	Ash (%) (g/100g)	CHO (%) (g/100g)
CBR.C	64.66^{b}±0.28	5.93^{a}±0.06	21.21^{b}±0.13	2.23^{ab}±0.03	5.95^{ab} ±0.27
CBR .R	65.13^{a}±0.17	5.40^{c}±0.03	21.38^{ab}±0.13	2.31^{a} ±0.04	5.75^{ab} ±0.00
CBR.T	64.89^{ab}±0.12	5.66^{b}±0.04	21.18^{b}±0.17	2.15^{b}±0.06	6.09^{a} ±0.27
CBR.RT	64.68^{b}±0.24	5.88^{a}±0.15	21.62^{a}±0.08	2.25^{ab}±0.10	5.54^{b}±0.19

Values are means of triplicate determinations ±SD

^{a,b,c} lower case letters within each column indicate statistically significant differences (P<0.05)

CBR.C: chicken burger control, CBR.R: chicken burger with rosemary extract,

CBR.T: chicken burger with Thyme extract, CBR.RT: chicken burger with rosemary + thyme extracts

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Table 3: Saturated Fatty Acids (g/100g total FA) composition of chicken burgers treated with the natural extracts of rosemary, thyme, and their combination

Saturated FA (g/100g)	CBR.C	CBR.R	CBR.T	CBR.RT
C14:0Myristic acid	0.49^{a} ±0.01	0.49^{a} ±0.01	0.46^{b} ±0.01	0.46^{b} ±0.01
C16:0Palmitic acid	25.10^{a} ±0.61	25.12^{a} ±0.61	25.12^{a} ±0.61	25.16^{a} ±0.61
C17:0Heptadecanoic acid (Margaric)	0.11^{a} ±0.00	0.10^{b} ±0.00	0.11^{ab}±0.00	0.11^{ab} ±0.00
C18:0 Stearic acid	1.45^{a} ±0.03	1.37^{b} ±0.03	1.43^{ab} ±0.03	1.41^{ab} ±0.03
C20:0Arachidic acid	0.16^{a} ±0.00	0.14^{b} ±0.00	0.14^{b} ±0.00	0.11^{c} ±0.00
Σ SFA	27.33^{a} ±0.61	27.23^{a} ±0.69	27.27^{a} ±0.65	27.27^{a} ±0.68

Values are means of triplicate determinations ±SD

[a,b,c] lower case letters within each row indicate statistically significant differences (P<0.05)

CBR.C: chicken burger control, CBR.R: chicken burger with rosemary extract,

CBR.T: chicken burger with thyme extract, CBR.RT: chicken burger with rosemary + thyme extracts

Table 4: Monounsaturated fatty acids composition (g/100g total FA) of chicken burger incorporated with the natural extracts of rosemary, thyme, and their combination

MUFA	CBR.C	CBR.R	CBR.T	CBR.RT
C16:1 Palmitoleic acid	$\mathbf{5.53^{c}}$ ±0.13	$\mathbf{6.25^{a}}$ ±0.15	$\mathbf{5.96^{b}}$ ±0.14	$\mathbf{5.86^{b}}$ ±0.14
C17:1 cis-Heptadecenoic acid	$\mathbf{0.00^{d}}$ ±0.00	$\mathbf{0.09^{a}}$ ±0.00	$\mathbf{0.09^{b}}$ ±0.00	$\mathbf{0.08^{c}}$ ±0.00
C18:1(n-9)-Oleic acid	$\mathbf{41.43^{a}}$ ±1.01	$\mathbf{41.59^{a}}$ ±1.01	$\mathbf{41.81^{a}}$ ±1.01	$\mathbf{42.61^{a}}$ ±1.03
C20:1Eicosenoicacid	$\mathbf{0.31^{a}}$ ±0.00	$\mathbf{0.29^{b}}$ ±0.00	$\mathbf{0.32^{a}}$ ±0.00	$\mathbf{0.31^{a}}$ ±0.00
Σ MUFA	$\mathbf{47.28^{a}}$ ±1.15	$\mathbf{48.23^{a}}$ ±1.17	$\mathbf{48.16^{a}}$ ±1.15	$\mathbf{48.88^{a}}$ ±1.19

Values are means of triplicate determinations ±SD

[a,b,c,d] lower case letters within each row indicate statistically significant differences (P<0.05)

CBR.C: chicken burger control, CBR.R: chicken burger with rosemary extract

CBR.T: chicken burger with thyme extract, CBR.RT: chicken burger with rosemary+ thyme extracts

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Table 5: Polyunsaturated fatty acids (g/100g total FA) composition of chicken burger incorporated with the natural extracts of rosemary, thyme, and their combination

PUFA	CBR.C	CBR.R	CBR.T	CBR.RT
C18 :2 (n-6)-Linoleic acid	14.30^{c} ±0.34	17.34^{b} ±0.42	16.83^{b} ±0.41	18.50^{a} ±0.45
C18 :3 α-Linolenic acid	1.05^{b} ±0.02	1.15^{a} ±0.02	1.14^{a} ±0.02	1.16^{a} ±0.02
Σ PUFA	15.36^{c} ±0.37	18.50^{b} ±0.45	17.98^{b} ±0.43	19.67^{a} ±0.47
C18 :3 Trans	0.063^{a} ±0.0010	0.043^{c} ±0.0010	0.029^{d} ±0.0007	0.049^{b} ±0.0010
Unknown	12.45^{a} ±0.30	8.48^{c} ±0.20	9.02^{b} ±0.22	6.61^{d} ±0.16

Values are means of triplicate determinations ±SD

[a,b,c,d] lower case letters within each row indicate statistically significant differences (P<0.05)

CBR.C: chicken burger control, CBR.R: chicken burger with rosemary Extract

CBR.T: chicken burger with thyme Extract, CBR.RT: chicken burger with rosemary + thyme extracts

Table 6: Nutritional quality indexes (atherogenic and thrombogenic indices, omega 6 to omega 3 ratio, and polyunsaturated to saturated fatty acids ratio) of chicken burger incorporated with the natural extracts of rosemary, thyme, and their combination

Nutritional indices	CBR.C	CBR.R	CBR.T	CBR .RT
IA	0.30^{a} ±0.00	0.27^{bc} ±0.00	0.27^{b} ±0.00	0.26^{c} ±0.00
IT	0.79^{a} ±0.01	0.74^{bc} ±0.01	0.75^{b} ±0.01	0.72^{c} ±0.01
ω- 6 / ω-3	13.49^{d} ±0.00	14.98^{b} ±0.00	14.69^{c} ±0.00	15.85^{a} ±0.00
PUFA / SFA	0.56^{d} ±0.00	0.67^{b} ±0.00	0.65^{c} ±0.00	0.72^{a} ±0.00

Values are means of triplicate determinations ±SD

[a,b,c,d] lower case letters within each row indicate statistically significant differences (P<0.05)

IA: Atherogenic index; IT: Thrombogenic index; ω- 6 / ω-3: OMEGA 6 to omega 3 ratio; PUFA / SFA: Polyunsaturated to saturated fatty acids ratio

CBR.C: chicken burger control, CBR.R: chicken burger with rosemary extract, CBR.T: chicken burger with thyme extract, CBR.RT: chicken burger with rosemary + thyme extracts

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Table 7: Sensory characteristics of chicken burger incorporated with the natural extracts of rosemary, thyme, and their combination

Samples	Color	Taste	Aroma	Juiciness	Tenderness	Overall Acceptability
CBR.C	$7.03^{b} \pm 1.54$	$7.20^{b} \pm 0.99$	$7.13^{a} \pm 1.33$	$7.33^{b} \pm 1.06$	$7.70^{b} \pm 0.87$	$7.30^{c} \pm 0.95$
CBR.R	$7.83^{a} \pm 1.02$	$7.87^{a} \pm 0.97$	$7.73^{a} \pm 1.08$	$8.37^{a} \pm 0.80$	$8.23^{a} \pm 0.93$	$8.27^{a} \pm 0.78$
CBR.T	$7.40^{ab} \pm 1.19$	$7.73^{ab} \pm 0.82$	$7.50^{a} \pm 1.19$	$8.00^{a} \pm 1.08$	$8.23^{a} \pm 0.81$	$8.00^{ab} \pm 0.87$
CBR.RT	$7.23^{ab} \pm 1.52$	$7.53^{ab} \pm 1.19$	$7.67^{a} \pm 1.06$	$7.97^{a} \pm 0.92$	$8.07^{ab} \pm 1.01$	$7.73^{bc} \pm 1.01$

Values are means of triplicate determinations ±SD

^{a,b,c} lower case letters within each column indicate statistically significant differences ($P<0.05$)

CBR.C: chicken burger control, CBR.R: chicken burger with rosemary extract,

CBR.T: chicken burger with thyme extract, CBR.RT: chicken burger with rosemary + thyme extracts

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