A study of the fat component's quality and quantity and their effect on the oxidative stability of beef and chicken meat burgers and shawarma in Amman area

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This study attempted to track the lipid oxidation in meat, on one hand, by giving a considerable attention to fatty acids (FAs) composition, cholesterol oxidation product (COPs) levels as well as fat and cholesterol content, and on the other hand, by measuring the peroxide value (PV), p-anisidine (PA), and TOTOX values. The investigation was carried out on one kilogram of meat from each of the products (beef burger (BB), chicken burger (CB), beef shawarma (BS) and chicken shawarma (CS). Cholesterol, COPs, and FAs composition were analysed by gas chromatography. Results showed that all of CB, CS, BB and BS were in an oxidised status and presented toxicologically significant levels of COPs.

Keywords: Burger, Shawarma, Beef, Chicken, Lipid oxidation, Fatty acids, Cholesterol oxidation products

1. INTRODUCTION

The rich nutritional composition of meat makes it very susceptible to chemical and bacterial deterioration. Lipid oxidation is the major cause of chemical deterioration in meat [1]. It is an inevitable and spontaneous process which depends mainly on the degree of unsaturation of fatty acids, level of oxygen, metals and on other factors such as storage conditions, processing methods, types of ingredients, as well as the presence and the concentrations of pro or antioxidants. The three major substrates for lipid oxidation in meat are triacylglycerols, phospholipids and cholesterol. The free radicals and cholesterol oxidation products (COPs) produced by lipid oxidation contribute to cell cytotoxicity, leading to several human pathologies, such as cancer [1].
Several scientific studies have demonstrated that meat-based fast foods, such as burgers and shawarma, are susceptible to lipid oxidation [1, 2, 3, 4, 5]. Therefore, this study aims at understanding what makes the meat in burgers and shawarmas susceptible to lipid oxidation, on one hand, by giving a considerable attention to fatty acid composition, COPs levels as well as fat and cholesterol content of the meats, and on the other hand, by tracking the degradation using peroxide value (PV), p-anisidine (PA) and TOTOX values.

2. MATERIALS AND METHODS

2.1. FOOD SAMPLE COLLECTION AND PREPARATION

One kilogram of meat from each of the products (beef burger, chicken burger, beef shawarma and chicken shawarma) was collected randomly from different restaurants in Amman. The collected samples were kept in an insulated ice box and were transported to the department of Nutrition and Food Technology laboratory to be analysed. Sampling was performed weekly over a period of 3 months from December to February 2021. The meat samples

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of both products were collected from the restaurants and immediately transferred to the laboratory of the department, prepared, and the fat was extracted and kept in glass vials and then the various analysis of fat were carried out immediately on the same or next day.

2.2. DETERMINATION OF FAT AND MOISTURE CONTENTS

Cold extraction of fat was carried out according to [6]. Briefly, 10 grams of meat sample were mixed with 150 ml of chloroform and 150 ml of methanol in a Mixer (Scovil, Hamilton Beach, Model NO. 936-1S, USA) and another 150 ml of chloroform was added during grinding after which the mixture was filtered using Whatman NO. 1 filter paper and transferred into a separatory funnel. Thereafter, potassium chloride (47 g in 500 ml water) was added to the mixture to facilitate phase separation. The lower layer (containing chloroform and fat) was withdrawn and sodium sulphate anhydrous was added to remove traces of water and then stored in closed vials at -18°C until further analysis. Moisture was determined following the method of [7]. Briefly, an aluminium empty plate was weighted (W1). Then, 10 grams of the meat samples were placed in the aluminium plate, weighted, and reported as (W2). The samples were then placed in an air-oven (Mermert, 854, West Germany), and dried at 105°C until a constant weight was obtained. Then, the samples were removed from the oven, cooled in a desiccator at room temperature and weighted (W3), and finally, the moisture content of the samples were calculated as follows:

Moisture content % = [Loss in moisture weight (W2 – W3) / weight of sample (W2 – W1)] × 100

2.3. FATTY ACID METHYL ESTER PREPARATION

Analysis of fatty acids involved 3 steps: extraction of lipids (as explained in the previous section); then the conversion of the extracted lipids to fatty acid methyl esters (FAMEs); which was carried out according to the procedure reported by [8]. In summary, 2 ml of hexane was added to 5 drops of the extracted fat (5 drops = approximately 100 mg of the sample), followed by the addition of 2 µl from KOH in methanol (2 N) with shaking for 1 minute. After that, 2 µl of acetic acid was added followed by shaking for 1 minute. One microliter (µl) was taken from the upper layer by a micro syringe and injected into the injection port of the gas chromatography (GC) (Shimadzu Corporation, Japan), at which fatty acids composition was analysed using a flame ionisation detector. Helium was used as a carrier gas. The fatty acids peaks were identified by comparing with the retention time of the reference standards. The quantification of the methyl ester fatty acids was then done by calculating: Area of the fatty acid% / total area of fatty acids.

2.4. DETERMINATION OF PEROXIDE VALUE

Peroxide value was determined according to the AOAC [9] method, where 5 g of the extracted fat was dissolved in 50 ml of chloroform-acetic acid solution (2:3) and agitated until the fat was dissolved after which 0.5 ml of freshly prepared and saturated potassium iodide (KI) was added. The mixture was agitated for 1 minute followed by addition of 50 ml of distilled water and few drops of starch indicator (1%). Afterwards, 0.01 N sodium thiosulfate was used to titrate the mixture with vigorous agitation until the blue colour vanished. The peroxide value was calculated using the following equation:

Peroxide value= V× N × 1000/ sample weight (g)

Where:
V = volume of the titrant,
N = normality of sodium thiosulfate.
Unit of peroxide value: milliequivalents (meq) of active oxygen per kilogram of oil.

2.5. DETERMINATION OF p-ANISIDINE VALUE

The determination of p-anisidine value was carried out according to [10]. Briefly, 0.5 g of extracted fat was dissolved in isooctane in a volumetric flask (25 ml capacity) and diluted up to the mark (solution 1). The optical density of solution I was measured at 350 nm using a spectrophotometer (Spectro, Model NO. 2000RS, USA) and isooctane as a blank (Ea). 5 ml of solution 1 was pipetted and transferred into a 10 ml test tube. Then 1 ml of p-anisidine was added, vigorously shaken, and allowed to stand for 10 minutes (solution2). The optical density of solution 2 was read at 350 mm using the same spectrophotometer and isooctane as a blank (Eb). P-anisidine index was calculated according to the following equation:

p-anisidine index = 25 (1.3 Eb-Ea)/ m

Where:
Ea= the optical density of solution 1,
Eb= optical density of solution 2,
M = weight of the sample in grams.

2.6. DETERMINATION OF TOTOX INDEX

TOTOX index was calculated using the following equation [42]:
TOTOX = 2 Peroxide Value + p-anisidine

2.7. DETERMINATION OF CHOLESTEROL OXIDATION PRODUCTS

2.7.1. Saponification of fat

Saponification of fat was conducted according to [11]. Briefly, 0.5 g of fat sample was mixed with 20 µl of α cholestane standard and 10 ml of KOH in ethanol (95% w/v), and then the mixture was stored in the dark for 24 hours after which it was placed in a water bath (Thermo Fischer Scientific, Model NO. H950D,

Massachusetts, USA) at 40°C for 1 hour to dissolve traces of unsaponifiable material. Afterwards, 5 ml of water was added to the mixture to dissolve the soap formed followed by addition of 10 ml of hexane to dissolve unsaponifiable materials. The mixture was shaken for 30 seconds, and the upper phase was separated followed by addition of sodium sulphate anhydrous to remove traces of water. Ten millilitres of hexane was added to the upper phase and the steps were repeated. Hexane was evaporated using a rotary evaporator (Heidolph, Heizbad WB, Model NO. 517-01000-00-0, Schwabach, Germany) and the dried sample was transferred into a test tube, flushed with nitrogen, and stored at -18°C.

2.7.2. Derivatisation of cholesterol and cholesterol oxides

Derivatization was carried out according to method described by [12]. In brief, the dried sample (non-saponified) was mixed with 0.5 g of trimethylsilyl derivatives (TMS) solution, prepared by mixing one volume of Trimethylchlorosilane, two volumes of hexamethyldisilazane and five volumes of pyridine, for 1 minute with shaking and then the mixture was placed in a water bath (Thermo Fischer Scientific, Model NO. H950D, Massachusetts, USA) at 40°C for 20 minutes followed by evaporating the TMS solution using nitrogen gas. Then, 100 µl of hexane was added to the dried sample followed by another 100 µl, mixed for 30 seconds and centrifuged (Hermle, Model NO. Z 326 K, Germany) for 5 minutes. After centrifugation, 10 µl was withdrawn from the supernatant using a micro syringe and injected into the injection port of the GC (Shimadzu Corporation, Japan). Cholesterol and COPs peaks were identified by comparing with the retention time of the reference standards. The quantification of COPs was done by using an internal standard: 5-alpha-Cholestane.

2.8. STATISTICAL ANALYSIS

Statistical analysis was carried out using statistical analysis system package (SAS Inc, 2000). Analysis of variance (ANOVA) was used following a Complete Randomised Design. Least Significant difference (LSD) test was used to test differences between the means. Data was presented as the mean ±standard deviation (±SD), and differences were considered significant at $P \leq 0.05$. All samples were performed in triplicates.

3. RESULTS AND DISCUSSION

3.1. FAT AND MOISTURE CONTENT OF MEAT SAMPLES

The fat and moisture percentages of all chicken shawarma (CS), chicken burger (CB), beef shawarma (BS), and beef burger (BB) samples are presented in Table I. The chemical analysis of fat showed that the main significant difference was between the type of meat (chicken and beef) rather than the type of food (shawarma and burger). Both BB and BS contained the highest fat levels (41.34±7.03% and 36.39±11.73, respectively) compared to CB and CS (32.03±15.23% and 26.63±2.98%, respectively). This is probably due to the generally lower fat composition in chicken carcasses when compared to beef. Moreover, fat levels of chicken and beef shawarma were lower than that of chicken and beef burgers, this could be due to the higher fat loss, since shawarma grilling may last for as long as a day [13]. Moisture content of CB (61.12±3.53) was significantly higher than all of CS, BB, and BS. A similar moisture range of chicken burger was reported by [14]. Moreover, CB exhibited greater moisture and lower fat contents than BB. A similar trend was observed by [15], who reported that moisture content of meat is inversely related to its fat content. Data collected on fat content in this study agreed with [16] who reported an average fat content of beef sausages of 37.75%. However, fat contents were higher than those observed by [2,17,18,19, 20, 21]. These differences are expected and could be attributed to the effect of dietary factors, age, sex, and source of animals, as well as the proportion of water added mainly in beef and chicken burger, in addition to the animal skins used in preparation of different types of shawarma and burger. Moreover, cooking losses of meat products, which are mainly affected by the ability to retain fat and moisture throughout thermal treatment, vary according to the cooking procedure implemented and its characteristics such as heating rate [22].

3.2. FATTY ACID COMPOSITION OF MEAT SAMPLES

Twelve fatty acids were identified and quantified in CS, BS, CB, and BB samples. Individual percentages of each fatty acid are presented in Table II. The investigated shawarma and burgers were mainly rich in monounsaturated fatty acids (MUFAs) as well as saturated fatty acids (SFAs). This agrees with [15] who

Table I - Measured fat and moisture content* of the examined chicken shawarma (CS), beef shawarma (BS), chicken burger (CB) and beef burger (BB) samples

Parameter	CS	BS	CB	BB
Fat (%)	26.63^{c} ±2.98	36.39^{ab} ±11.73	32.03^{bc} ±15.23	41.34^{a} ±7.03
Moisture (%)	49.30^{b} ±4.75	49.01^{b} ±7.20	61.12^{a} ±3.53	49.82^{b} ±6.29

*Values are means of triplicate determinations ±SD.
^{a,b,c} Superscripts within the same row indicate significant differences ($p<0.05$).
CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.

Table II - Fatty acid composition* of the examined chicken shawarma (CS), beef shawarma (BS), chicken burger (CB) and beef burger (BB) samples.

Fatty acid (g/100g total FA)	CS	BS	CB	BB
Myristic acid (C14:0)	0.53^{b} ±0.11	3.50^{a} ±2.27	1.58^{b} ±1.19	3.31±0.14^{a}
Palmitic acid (C16:0)	20.68^{b} ±0.93	27.27^{a} ±6.17	20.78^{b} ±4.35	24.79±1.68^{a}
Margaric acid (C17:0)	0.09^{c} ±0.10	2.50^{a} ±1.17	1.49^{b} ±1.51	1.69±0.69^{ab}
Stearic acid (C18:0)	6.90^{b} ±0.39	12.70^{a} ±4.68	9.40^{b} ±3.17	15.38^{a} ±4.15
Arachidic acid (C20:0)	2.69^{a} ±0.61	0.47^{b} ±0.28	2.06^{a} ±1.60	0.55^{b} ±0.12
Behenic acid (C22:0)	1.13^{ab} ±0.15	0.36^{bc} ±0.19	1.66^{a} ±1.80	0.25^{c} ±0.12
Palmitoleic acid (C16:1)	3.58^{a} ±0.50	3.14^{ab} ±1.33	2.21^{b} ±1.23	4.01^{a} ±0.86
10-cis-heptadecenoic acid (C17:1)	0.09^{c} ±0.10	1.57^{a} ±0.85	0.74^{b} ±0.73	1.34^{ab} ±0.77
Oleic acid (C18:1)	36.03^{bc} ±2.56	39.37^{ab} ±4.49	34.82^{c} ±6.76	41.53^{a} ±3.64
Linoleic acid (C18:2)	29.81^{a} ±2.72	6.62^{b} ±2.75	24.71^{a} ±16.44	4.59^{b} ±2.45
Linolenic acid (C18:3)	0.00^{d} ±0.00	0.39^{b} ±0.21	0.20^{c} ±0.25	0.55^{a} ±0.11
C18:1TRNS	0.05^{c} ±0.13	3.08^{a} ±1.00	1.08^{b} ±1.58	2.81^{a} ±0.84
Total saturated fatty acids (SFA)	32.04^{b} ±0.94	46.81^{a} ±6.94	37.00^{b} ±8.32	45.84^{a} ±5.15
Monounsaturated fatty acids (MUFA)	39.70^{bc} ±2.99	44.07^{ab} ±4.44	37.77^{c} ±8.36	46.89^{a} ±4.43
Polyunsaturated fatty acids (PUFA)	29.81^{a} ±2.72	7.00^{b} ±2.86	24.91^{a} ±16.29	5.14^{b} ±2.42
Trans fatty acids (TFA)	0.05^{b} ±0.12	3.08^{a} ±1.58	1.09^{b} ±0.98	2.80^{a} ±0.84

*Data are expressed as means of triplicate determinations ±SD.
^{a,b,c} Superscripts within the same row indicate statistically significant differences ($p<0.05$).
CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.
SFA is the sum of C14, C16, C17, C18, C20, and C22; MUFA is the sum of C16:1, C17:1 and C18:1; PUFA is the sum of C18:2 and C18:3; TFA is the amount of C18:1 TRNS.

reported that the fatty acid profile mainly demonstrate a dominance of SFAs and MUFAs. The most ubiquitous fatty acid in all of BB, BS, CB, and CS were oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids. This was in accordance with previous studies [15,23,24,25]. The polyunsaturated fatty acids (PUFAs) detected represented the essential fatty acids, linoleic acid (C18:2) and linolenic acid (C18:3). Oleic acid (C18:1), was the most prevalent fatty acid in all the examined samples. This finding agrees with [22] and [26]. However, palmitic (C16:0), and stearic (C18:0) acids, were more abundant in BB and BS than in CB and CS. These results are supported by those reported by [27] who found that these fatty acids were the most predominant in the marbling of lean beef. Nevertheless, our results showed lower levels in CB (63.61%) and CS (65%) compared with those of [28] who have demonstrated that 76 to 82% of total fatty acids in hens came from palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1). Both CS and CB contained somewhat higher proportion of unsaturated fatty acids (69.51% and 62.68%, respectively), than BS (51.07%) and BB (52.03%). These results were nearly like those previously reported in literature [15,29,30,31]. Saturated fatty acids were found to be greater in BS and BB than in CS and CB. The proportion of MUFAs was higher in BS and BB than in CS and CB. However, when comparing the fatty acid composition of meat origin, a notable amount of PUFAs was found in CS (29.81%) and CB (24.91%) compared to BS (7.00%) and BB (5.14%), with linoleic acid (C18:2) being the predominant PUFA. These results correlated with the findings of [15]. However, BS and BB showed higher levels of trans fatty acids (TFA) of 3.08 and 2.80, respectively, compared to CS and CB (0.05 and 1.09, respectively). Our results were slightly lower than those found by [32]. Nonetheless, both results exceeded the Danish legal limit of trans-fat (i.e., 2g/100 g fat). The differences in the degree of unsaturation could be explained by the differences in the metabolic process taking place in ruminant and non-ruminant animals, since hydrogenation of unsaturated fatty acids occurs extensively in ruminant animals. Hydrogenation occurs by two systems: 18:2 and 18:3 are hydrogenated to 18:1, and 18:1 to 18:0 [33].

3.3. CHOLESTEROL CONTENT AND COPS LEVELS

Table III shows the cholesterol content and cholesterol oxidation products (COPS) levels in CS, BS, CB, and BB samples. Among the tested samples, the highest cholesterol contents were 96.42 and 80.79 mg/100g, and were observed in CS and CB, respectively. The lowest cholesterol contents (74.35 and 65.22 mg/100g) were detected in BS and BB, respectively. However, no significant difference was found except between CS and BB. This could be attributed, on one hand, to the type of meat since the main fat source in poultry meat is the chicken skin, which is known to contain high levels of cholesterol. On the other hand, this difference could be due to the higher use of skin or animal fats during the preparation of shawarma than in burger. Our results agree with de [34], who also found that chicken meat presented higher cholesterol levels than beef. However, the cholesterol content found in our study was

Table III - Cholesterol and cholesterol oxidation products (COPs) content in the examined chicken shawarma (CS), beef shawarma (BS), chicken burger (CB) and beef burger (BB) samples.

	CS	BS	CB	BB
Cholesterol (mg/100g food)	96.42^{a} ±28.95	74.35^{ab} ±38.55	80.79^{ab} ±25.37	65.22^{b} ±23.60
COPs (mg/100g food)	1.20^{b} ±0.52	2.48^{ab} ±2.19	4.69^{a} ±4.72	2.29^{ab} ±1.64

*Results are expressed as means of triplicate determinations ±SD.
^{a,b} Superscripts within the same row indicate statistically significant differences ($p<0.05$).
CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.
COPs: cholesterol oxidation products.

slightly higher compared to their results. Such differences could be attributed to variations in the parts of chicken and beef used for cholesterol determination. Nevertheless, our results were in accordance with those reported by [28], who found a cholesterol content of 75-98 mg/100g for broiler meat, as well as those previously reported in the literature which showed that the cholesterol content of meats varies between about 30 and 120 mg/100 g of food, being even higher in offals [23, 24, 25].

Table IV - Cholesterol and cholesterol oxidation products (COPs) content of the collected chicken shawarma, beef shawarma, chicken burger and beef burger samples.

Samples	Cholesterol (mg/ 100g food)	Cholesterol Oxidation Products (COPs) (mg/ 100g food)
CS1	84.48	0.95
CS2	142.02	2.16
CS3	53.25	0.55
CS4	116.51	1.49
CS5	81.59	0.92
CS6	100.71	1.16
MEAN	**96.42**	**1.21**
CB1	68.07	2.00
CB2	83.94	4.85
CB3	70.08	0.98
CB4	86.29	6.83
CB5	110.38	1.95
CB6	34.85	14.92
CB7	111.95	1.34
MEAN	**80.79**	**4.69**
BS1	33.84	0.65
BS2	47.81	2.26
BS3	117.64	1.46
BS4	30.55	1.33
BS5	65.13	7.06
BS6	93.93	0.71
BS7	131.63	3.94
MEAN	**74.36**	**2.48**
BB1	59.42	0.38
BB2	86.58	3.29
BB3	56.49	1.15
BB4	73.85	2.49
BB5	101.26	0.57
BB6	54.28	2.89
BB7	24.69	5.26
MEAN	**65.23**	**2.29**

CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.

The results of COPs content in the investigated shawarma and burger showed that the significant difference was between CS and CB. In fact, CB exhibited a COPs level of nearly four times bigger than that of CS. These results lead us to the assumption that the COPs levels were influenced mainly by the type of food (shawarma and burger) rather than the type of meat (chicken and beef). This could be possibly due to the presence of high levels of hydroperoxides derived from the oxidation of unsaturated fatty acid during the preparation of CB which increased the oxidation levels of cholesterol. Since, [35], indicated that cholesterol oxidation may develop both directly, if oxygen is present, and indirectly, if other oxidisers are present in food, such as unsaturated fatty acids in the lipid fraction particularly rich in PUFA. Our results were nearly like those found by [36]. However, [37] found very low levels of cholesterol oxidation compared to our study. COPs in foods are considered potential health risk but there is no regulation limiting their levels in foods [38]. However, according to [15], our results concerning COPs are considered toxicologically significant, since they indicated that an amount of COPs above 0.5 mg represent a risk from the toxicological stand point. Our results showed mean values with a wide scatter, this could be related to the heterogeneity of the analysed samples in terms of composition, formulation, and processing between the different restaurants from where our samples were collected. In addition, and to explain the high values of the standard deviation of the results shown in Table III, all the individual values of the cholesterol and cholesterol oxidation products results were presented in Table IV.

3.4. THE OXIDATION STATUS OF MEAT SAMPLES EVALUATED BY PV, P-ANISIDINE AND TOTOX

Means of peroxide value (PV), para-anisidine value (PA) and total oxidation value (TOTOX), as indicators of lipid oxidation, are demonstrated in Table IV. PV is useful in evaluating the initial step of oxidation of edible fats and oils. The PV of samples obtained ranged from 1.30 to 2.51 mEq/Kg lipid for BB and CB, respectively. Our results were consistent with

[15]. Meanwhile, the level of lipid peroxides found in the present study is considered acceptable according to [39] and [40], if following the standard AOCS methods, a Peroxide value (mEq Active O2 /kg Lipid) <5 is expected for a weight of 5 g lipid sample [41]. However, no significant differences were detected regarding PV between BB, BS, CS and CB. Moreover, PV is notoriously an empirical assay [42], which could cause a difficulty in visually distinguishing the colour change marking the actual end point determination. [43] concluded that, due to the rapid decomposition of hydroperoxides at temperature ≥100°C, the determination of PV is not applicable for all types of cooking methods. Therefore, to get an overall picture of lipid oxidation, [44] required testing of secondary products.

Para-anisidine values, which show the secondary oxidation products, ranged from 13.09 for CS, to 47.01 for CB, whereas the TOTOX values, in like manner, ranged from 14.76 for CS, to 48.45 for CB. Although, CS and BS showed lower PA and TOTOX values compared to chicken burger and beef burger, no significant differences were found except with CB. This could be because shawarma slices could have been cut from a recently renewed surface which was less exposed to heat (short cooking time) or cut from the inner parts of the meat cone where perhaps the oxygen was low or absent. The highest PV, PA and TOTOX values were detected in CB. This may be elucidated by the relatively elevated levels of unsaturated fatty acids and low levels of natural tocopherols in poultry meat [20]. It is generally agreed that lipid oxidation increases significantly with the increase of unsaturated fatty acids [1,45,46]. Therefore, the level of PUFA in meat usually determines the susceptibility to lipid oxidation in different species. [47] found that the susceptibility to oxidation decreases in the order chicken>pork>beef>lamb. Poultry and poultry products are particularly prone to oxidative processes in lipids and proteins, by virtue of the fact that they contain unsaturated lipids. However, this was not the case in CS which showed lower oxidation levels than that in beef shawarma and beef burger. This could be attributed to the antioxidant effect of spices, that is mainly related to the presence of flavonoids and phenolic compounds which actively participate in neutralizing free radicals [1, 48]. The relatively high oxidation levels in BB could be explained by the presence of considerably larger amount of iron and myoglobin in bovine muscle [49,50]. Moreover, it was stated by [20] that fats of animal origin are less stable than equally saturated vegetable fats, because they lack natural antioxidants. The high levels of PA and TOTOX values found in our study showed that all of CB, CS, BB, and BS were in an oxidised status. These results reflected an unacceptable quality of the samples, since [42,51,52] indicated that for a high-lipid food to still be acceptable the TOTOX value should be less than 10. These findings could be attributed to bad storage conditions; since TOTOX value was considered to combine evidence about the history of fat (as reflected in p-anisidine value) with its present state (as evidenced by the pV). In addition to the lipid composition of the meat, many other factors were found to influence lipid oxidation, such as processing methods [53]. In fact, [54] reported that cooked meats are even more susceptible to lipid oxidation than raw meats, because higher temperatures lead to the release of oxygen and heme iron, thereby, inducing the production of free radicals. Furthermore, the mechanical processes employed in meat processing such as chopping, mixing, and grinding, massaging was also found to promote lipid oxidation by increasing surface-to-volume ratio in contact to oxygen [55]. Beside the latter, Sodium chloride which is considered one of the most important additives in meat industry, since it is used for enhancing preservation, flavour and softness [56], was reported as a pro-oxidant by several investigators [53, 57]. Regardless of the different factors influencing our results concerning lipid oxidation, PA value may not have reflected the accurate status of oxidation since, the fat extracted from our samples was observed to be intensely-coloured (orangish colour). This pigment might have contributed to an additional radiation absorbance in the 350nm wavelength range which could have led to an overestimation of the reported data. Furthermore, PA value is neither quantitative nor specific. Thus, all aldehydes react but unsaturated aldehydes have higher colour response than saturated, so results can give only relative, not absolute findings. Additionally, of all these difficulties, meat is a complex matrix that generally causes the appearance of compounds, affecting the obtained results. So, the analyte extraction and isolation process

Table V - Physicochemical properties* of the examined chicken shawarma (CS), beef shawarma (BS), chicken burger (CB) and beef burger (BB) samples.

Physicochemical property	CS	BS	CB	BB
PV	2.01^{a} ±1.17	1.40^{a} ±0.69	2.51^{a} ±2.55	1.30^{a} ±1.11
PA	13.09^{b} ±3.81	14.11^{b} ±10.86	47.01^{a} ±48.64	22.27^{b} ±15.03
TOTOX	14.76^{b} ±3.91	15.31^{b} ±10.62	48.45^{a} ±48.74	23.39^{b} ±15.13

*Results are expressed as means of triplicate determinations ±SD.

^{a,b} Superscripts within the same row indicate statistically significant differences ($p<0.05$). CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.

PV: Peroxide value; PA: p-Anisidine value; TOTOX=2×PV+PA.

are the main challenge to ensure accurate results.

CONCLUSION

Our data demonstrated that all of CB, CS, BB and BS were in an oxidised status and presented toxicologically significant levels of COPs. CB was more susceptible to oxidation than the other samples, since it showed the highest level of PA and TOTOX. In addition, CB exhibited higher COPs levels compared to CS. These findings lead to the assumption that, on one hand, there is a strong relation between COPs and lipid oxidation and on the other hand, storage conditions, processing methods and the types of ingredients added, highly influences the lipid oxidation despite the type of meat.

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