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Korrespondenzadresse:
mframadan@zu.edu.eg

[1]) Bolu Abant Izzet Baysal University, Food Engineering Department, Golkoy Campus, Bolu, Turkey; [2]) Balikesir University, Engineering Faculty, Food Engineering Department, Balikesir, Turkey; [3]) Umm Al-Qura University, Deanship of Scientific Research, Makkah, Kingdom of Saudi Arabia; [4]) Agricultural Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt

Quality characteristics, lipid oxidation parameters and 3-monochloropropane-1,2-diol (3-MCPD) content of Doner kebab during the cooking process

Qualitätsmerkmale, Lipidoxidationsparameter und 3-Monochlorpropan-1,2-diol (3-MCPD)-Gehalt von Döner-Kebab während des Kochprozesses

Sevinc Gulen[1]), Semra Turan[1]), Mustafa Kiralan[2]), Mohamed Fawzy Ramadan[3,4])

Summary

Doner (döner) kebab (DK) is a traditional fast food in Turkey and several countries in the world. In the present study, the changes in lipid fraction due to thermal treatment applied during chicken DK production were investigated. To determine the oxidation levels of the lipid fraction extracted from DK samples during cooking, color *(L*, a*, b*, C*, h*),* conjugated diene (CD), K_{232}, K_{270}, peroxide value (PV), thiobarbituric acid value (TBA), free fatty acids (FFAs) content and polymer triglyceride content were determined. In addition, free and ester-bound 3-monochloropropane-1,2-diol (3-MCPD) levels were determined. The maximum center and surface temperatures of DK during cooking were 10.6°C and 80.3°C, respectively. During cooking, *L** values of DK samples were increased while *a** values decreased. As the moisture content of DK samples decreased with cooking, the lipids amounts increased. The FFAs contents of the lipids extracted from the cooked DK samples were 1.33–2.06%; peroxide values 2.0–3.2 meq O_2/kg; K_{232} values 2.39–4.33; K_{270} values 0.64–1.71; TBA values 0.74–2.24 mg MA/kg; and polymer triglyceride contents were 0.024–0.031 g/100 g. As the amount of ester 3-MCPD increased with the cooking process, free 3-MCPD levels decreased. Since the cooking was carried out at low temperatures, the amounts of free and ester-bound 3-MCPD and lipid oxidation products were low.

Keywords: Döner kebab, chicken meat, cooking, lipid oxidation, 3-monochloropropane-1,2-diol

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Introduction

Fast food restaurants have become important food consumption points. Doner (döner) kebab (DK) is a popular fast food worldwide (Kilic, 2003; Özsaraç et al., 2019). The consumption of DK highly increased due to its taste, nutritional value, and low price. Moreover, packed DK has started to be sold in the international market in the form of ready-to-eat products (Vazgecer et al., 2004; Öksüztepe and Beyazgül, 2014; Haskaraca and Kolsarici, 2019). DK name in Turkish means rotating kebab. In some countries, DK is known as gyros, dona-kebab, donair, donna-kebab, and chawarma (Vazgecer et al., 2004; Bingöl et al., 2013; Haskaraca and Kolsarici, 2019). The consumption rate of DK was 500 tons/day in Turkey in 2015, wherein the number of DK sale points was about 25.000 in Germany and 50.000 in Europe (Vazgecer et al., 2004; Kilic 2009; Ergönül et al., 2012; Özsaraç et al., 2019).

DK is composed of veal, lamb, beef, or chicken meat and seasoned with pepper, tomatoes, onion, and other spices. Chicken and turkey breast meat are preferred in DK production due to its ease digestibility, low levels of fats and cholesterol, and its cheap price (Kilic et al. 2001; Kilic 2009). The production process of DK includes; preparation and marination of meat, spitting the meat on a DK stick, cooking the meat block in front of an oven by rotating, and finally DK is cut into slices and served (Kayisoglu et al. 2003; Vazgecer et al., 2004; Kayaardi et al. 2006; Kilic 2009; Askin and Kilic 2009; Ergönül et al., 2012; Özsaraç et al., 2019).

Cooking and thermal processing used to prepare meat products, causes physicochemical changes in meat and produced desirable aroma and flavor constituents (Özsaraç et al., 2019). DK is rich in lipids, wherein poultry meats are susceptible to lipid oxidation due to its high content of polyunsaturated fatty acids (PUFA). Therefore, lipids are the main factor that affect the quality of chicken DK (Kilic and Richards 2003; Min et al. 2008). Some researchers studied lipid oxidation in DK during storage (Kilic and Richards 2003; Ergonuel and Kundakci 2007).

3-Monochloropropane-1,2-diol (3-MCPD) is a process contaminant that found in foodstuffs such as meat, poultry, fish, cereal, dairy, and miscellaneous products (Chung et al. 2008). The presence of 3-MCPD and glycidyl esters are widespread in refined or heated oils, and lipids-containing foods (Kuhlmann 2011; Pudel et al. 2011; Arisseto et al. 2015). In survey research, 3-MCPD was detected in some cooked and cured meat products (Crews et al. 2002). It was reported that smoked and fried fish, poultry and meat products contained free and ester bound 3-MCPD (Chung et al., 2008; Jira, 2010; Karl et al., 2015). Ilko and Dolezal (2013) reported that the content of 3-MCPD esters in bacon increased after dry frying for 3 min in the presence of precursors of both chlorides come from salt and partial glycerol esters. They showed that shallow frying caused more 3-MCPD and glycidyl esters compared to dry frying of bacon due to high amount chloropropanol esters and glycidyl esters found in the refined oil. Wong et al. (2017) studied the effects of frying temperature, frying time and salt content on the formation of 3-MCPD ester and glycidyl ester content in palm olein during frying of chicken breast meat. They showed that 3-MCPD ester content decreased, whereas glycidyl esters increased as the frying time increased during without salt. They claimed that 3-MCPD esters are not stable, therefore, they decreased as the heating time increased due to decomposition. Inversely, 3-MCPD esters increased as the salt content of chicken breast meat increased during frying at 160 and 180°C. However, the addition of different amount of salt to breast meat did not significantly affect the formation of glycidyl esters in palm olein. Schallschmidt et al. (2012) studied the effects of different grilling conditions on the 3-MCPD content of grilled meat. The highest 3-MCPD content was found in the steak pre-treated with an oily marinade cooked on a charcoal grill.

Considering the constituents of DK and direct contact with heat during cooking, the formation of oxides and 3-MCPD is expected. Some studies have been done on the lipid oxidation and 3-MCPD contents of some meat products. To the best of our knowledge, no studies have been carried out dealing with the effect of cooking time on lipid oxidation and 3-MCPD content of DK. The aim of the current work was to determine the changes in the lipid fraction of DK such as physicochemical properties, lipid oxidation, and the content of 3-monochloropropane-1,2-diol (3-MCPD) during different cooking periods.

Materials and methods

Material

Chicken DK samples were obtained from the Erpiliç Integrated Chicken Product Industry and Trade Limited Company (Bolu, Turkey). To prepare DK samples; butterfly boneless fillet with skin and leg meat were mixed with the chicken fat and seasoned with tomato paste, onion, yogurt, water, salt, spices and a special sauce. After placing chicken meat blocks, all sides of 19.0 kg meat block were cooked in front of a gas oven during rotating. Cooking and cutting processes of DK were completed during 6 h with automatic knives. Raw DK and 100 g sample were taken every 60 min. Samples were homogenized using homemade shredder, then stored at -18°C until analyses.

Diethyl ether, ethyl acetate, *n*-hexane, chloroform, methanol, ethanol, tetrahydrofuran, petroleum benzine, *tert*-butyl methyl ether, trichloroacetic acid, iron III chloride hexahydrate, sodium chloride, sodium hydroxide, hydrochloric acid (wt. 37%), sodium methylate solution (30% wt. in methanol) were obtained from Merck (Darmstadt, Germany). 2,2,4 trimethylpentane, diatomaceous earth, 1-(heptafluorobutyryl) imidazole (HFBI, >97%), 2-thiobarbituric acid (TBA, 98%), potassium bromide, anhydrous sodium sulfate, ammonium thiocyanate, 3-chloro-1,2-propanediol were purchased from Sigma-Aldrich (Buchs, Switzarland). 3-MCPD-d5 was supplied from Cambridge Isotope Laboratories, Inc. (USA).

Methods

Extraction of lipids

An adapted protocol of the cold extraction developed by Folch et al. (1957) was used to extract total lipids from chicken DK samples. Ten grams of DK sample was homogenized with 45 mL chloroform: methanol (2:1, v/v) at 10.000 rpm using a homogenizer (Heildolph Silent Crusher, Germany). The second extraction was carried out with 30 mL solvent mixture then 10 mL NaCl (1%) was added to the filtrate and left for 20 min. The lower phase was evaporated in a rotary evaporator under vacuum. Nitrogen gas was flushed into the oil, and then it was maintained at 50°C in an oven for 30 min to remove the retained solvent. Total lipids extracted were stored at -18°C until further analyses.

Physicochemical analysis

For each sample, the center and surface temperature values of DK were measured with a digital thermometer. CIE color values (*L*, a*, b*, C** and *h**) of samples were determined by Minolta colorimeter (Osaka, Japan). *L*, a*, b*, h** and *C** values indicate lightness, redness, yellowness, hue and chroma, respectively (Barbut, 2013). Moisture and lipids content of samples were determined according to AOAC (1990). Moisture contents of the DK samples were determined in an oven (Binder, Germany) at 102±2°C, and the lipid contents were determined using petroleum ether in a Soxhlet extraction apparatus. Free fatty acids (FFAs, as percentage oleic acid) were determined in lipids extracted from DK sample according to AOCS method Ca 5a-40 (1990) by titration with sodium hydroxide (0.01 N).

Lipid oxidation measurements

Oxidation of lipids extracted from chicken DK was monitored by conjugated diene (AOCS method Ti 1a-64, 2000), K_{232} and K_{270} values (AOCS method Ch 5-91, 2000), peroxide value (International Dairy Federation FIL-IDF method 74A, 1991), and thiobarbituric acid (TBA) value (Tarladgis et al. 1960; Huber et al. 2009).

The principle of the peroxide analysis was the determination of Fe III ions formed after oxidation of Fe II by hydroperoxides in the presence of the ammonium thiocyanate. According to the International Dairy Federation FIL-IDF method 74A (1991), lipid extracted by Folch method (30 mg) from DK sample was dissolved in chloroform (3 mL). 0.25 mL of the solution was completed to 4.9 mL using methanol: chloroform (70: 30, v/v) mixture. Ammonium thiocyanate (50 µL, 30%) and Fe III chloride (50 µL, 20 mM in 3.5% HCl) were added. After incubation for 20 min, absorbance was measured at 500 nm using UV-visible spectrophotometer (Shimadzu UV1200, Japan). Calibration curve was prepared using Fe III chloride standard solutions in the concentration range of 6.71-67.1 µg/mL. The equation of the calibration curve was y=0.0076x - 0.0178 (R^2=0.993). Peroxide value was calculated according to the following formula

$$Peroxide\ value\ \left(\frac{meq\ O2}{kg\ oil}\right) = \frac{(As - Ab)}{55.84\ \times w\ \times b}$$

Where, *w:* fat weight (g); *As:* sample absorbance; *Ab:* blank absorbance; 55.84: atomic weight of Fe III; *b:* the slope of the Fe III calibration curve

During the determination of TBA value, distilled water (25 mL) was added to 5 g of DK sample, and homogenized at 10.000 rpm (Heidolph Silent Crusher, Germany). It was centrifuged for 2 min at 2000 rpm and filtrated through Whatman No.1 filter paper. TBA solution containing trichloroacetic acid, TBA, HCl and distilled water (2 mL) was added to the filtrate (2 mL). The tubes were placed in a water bath for 60 min, then cooled rapidly with ice pieces. The tubes were centrifuged at 2000 rpm for 5 min. The upper phase was used for the absorbance measurements at 538 nm (Shimadzu UV 1200, Japan). Molar absorption constant was taken as $1.56*10^{-5}$ L/mol x cm for malondialdehyde and TBA value was calculated as mg of malondialdehyde per kg of meat sample (Tarladgis et al. 1960; Huber et al. 2009).

3-MCPD esters in the lipids extracted from DK samples

Quantities of ester bond forms of 3-MCPD were determined according to Deutsche Gesellschaft fur Fettwissenschaft (DGF) standard method C-VI 18 (2011) and Mogol et al. (2014). Lipids (0.1 g) extracted from DK was dissolved in *tert*-butyl methyl ether and spiked with 100 µL internal standard solution (10 µL/mL 3-MCPD-d_5). Steps of analysis were transesterification with sodium methylate, addition of acidified KBR solution, removing of fatty acid methyl ester with *n*-hexane, extraction of MCPD compounds with diethyl ether/ethyl acetate (60:40, v/v), evaporation to dryness under N_2 stream, dissolution in 2,2,4 trimethylpentane and derivatization with 1-(heptafluorobutyryl) imidazole (HFBI, >97%).

Standard solutions at different concentrations were prepared from the stock solution of 3-MCPD for the preparation of calibration curve. After the addition of internal standard and 2,2,4-trimethylpentane to each standard solution, the derivatization was done similarly using HFBI. The equation of the calibration curve was y= 0.0021x – 0.0071, R^2=0.997; where x is the amount of 3-MCPD in ng/2 mL, and y is the area ratio of 3-MCPD/3-MCPD-d5. LOD and LOQ were found as 2.6 ng/mL and 7.7 ng/mL, respectively. Recovery was above 90% for ester bound 3-MCPD and 75% for free 3-MCPD.

GC-MS (Thermo Scientific, Trace 1300 GC, ISQ QD MS) separation was performed on a DB-5MS column (30 m x 0.25 mm id x 0.25µm film thickness). The carrier gas was helium with a constant flow of 1.2 mL/min and splitless mode; while the injection volume was 1 µL. The analysis conditions were as follows: the initial column temperature was settled at 50°C for 1 min, then raised at a gradient of 2°C/min to 90°C, then raised at a gradient of 30°C/min to 150°C, and kept at 150°C for 3 min. The temperature of the injector was set at 250°C, and of the transfer line at 280°C (Abu-El-Haj et al. 2007). Mass spectrometer was operated in the EI mode at 70 eV. The ion source temperature was set at 230°C. For selected ion monitoring (SIM), following ions were chosen for 3-MCPD, m/z 149, 169, 253, 275 and 453, for MCPD-d_5, m/z 169, 257, 278, 294 and 456 (m/z). Retention time was 14.32 min for MCPD-d_5 and 14.51 min for 3-MCPD.

Free 3-MCPD in the defatted DK samples

Defatted DK samples were used in the determination of free 3-MCPD. Free 3-MCPD extraction and derivatization procedure were carried out according to Mogol et al. (2014). Briefly, NaCl (5 M) solution and internal standard (3-MCPD-d5, 10 µL/mL) were added to the defatted samples. Free MCPD were eluted with diethyl ether using a column (40 cm, 2 cm i.d.) containing diatomaceous earth and anhydrous sodium sulphate. The extract was filtered, and the solvent was evaporated under vacuum. The extract was dissolved in 2,2,4 trimethylpentane and after derivatization, and injected to GC-MS. Derivatization and the GC-MS conditions were the same as the described for the ester-bound 3-MCPD analysis in lipid fraction.

Polymer triglycerides in lipid fraction

The polymer triglycerides analysis was carried out according to Gertz (2001). A weighed sample of 0.3 g lipid was dissolved in 10 mL of petroleum ether: diethyl ether (90:10, v/v). SPE cartridge (Strata SI-1 Silica, 55 µm, 70A, 500 mg/6 mL, Phenomenex, USA) was activated using 6 mL of the same solvent mixture. One mL of test solution was

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added to SPE cartridge and eluted with 10 mL of the same solvent mixture. The non-polar phase was separated from the cartridge. The polar triglycerides in retained cartridge were washed using 10 mL diethyl ether and collected in a flask. The solvent was evaporated on a rotary evaporator, then dissolved in 1.5 mL tetrahydrofuran. This solution was injected into HPLC (Shimadzu Prominence LC 20A, Japan). Analysis was done using two gel permeation chromatography columns (Agilent PL-Gel 100 A°, 2 x 300 x 7.6 mm, 5 µm, USA). Oven temperature was allowed to stand at 35°C during analysis. The injection volume was 20 µL and the mobile phase was tetrahydrofuran at a flow rate of 0.7 mL/min. Refractive index detector was used. The ratio of polymerized triglycerides (dimeric and oligomeric) content in the samples was calculated using the formula:

$$W = [A_p / (F \times E_p)] \times [(E_v \times G_v) / A_{TG}]$$

where: W: polymerized triglyceride content (%); A_p: is the peak of the area of di- and oligo-meric triglycerides in the fractionated sample; F: is the dilution factor (F = 2); E_v: is the mass of the external standard (virgin olive oil) dissolve in tetrahydrofuran in gram per milliliter; E_p: is the mass of the test portion in grams per milliliter; G_v: is the percentage of monomeric triglycerides in the reference standard (virgin olive oil) (%); based in peak areas (about 99%).

The content of monomeric triglycerides in the virgin olive oil was calculated as follows:

$$G_v = A_{TG} \times 100 / \Sigma ALL$$

where: ΣAll: Sum of all peak areas (without FFAs); A_{TG}: Peak area of the monomeric triglycerides in the external standard; G_v: Percentage of mono triglycerides in the reference standard

Virgin olive oil was used as the reference standard. Olive oil (0.3 g) was dissolved in 10 mL tetrahydrofuran and injected into the HPLC under the same conditions. Heptadecanoic acid was used to determine the retention time of the FFAs, while waste frying oil was used to determine the retention time of the polymer triglycerides.

Statistical analysis

The cooking experiments were replicated four times in different DK blocks. The color values and temperature of DK samples were measured five times. K_{232}, K_{270}, conjugated diene value, TBA value and free fatty acid content of lipid samples extracted from DK samples were determined three times. Moisture and fat content of DK samples, peroxide value, polymer triglyceride content and 3-MCPD contents of DK lipids were analyzed duplicate. Data were subjected to analysis of variance (ANOVA). Duncan multiple comparison test was used to determine the significance of mean values for multiple comparison at ($p < 0.05$). Statistical analyses were performed using the Statistical Package of Social Service (SPSS, version 18.0).

Results and discussion

Changes in physicochemical properties during cooking

The mean values of the center and surface temperature of DK samples during cooking are given in Table 1. Center and surface temperatures of DK samples increased with cooking time significantly ($p<0.05$), and the maximum center and surface temperature were found as 10.6°C and 80.3°C, respectively. In a study, Pexara et al. (2006) mentioned that the center temperatures of gyros between 25-35°C promote microbial development. Similarly, the center temperatures of DK samples during cooking are suitable for the growth of microorganisms. The surface temperature of the DK might be not enough to kill the pathogen microorganisms. The surface temperatures of the DK were lower than the common frying temperatures of 160-180°C (Wong et al., 2017). Kayısoglu et al. (2003) reported that the DK sold in a province of Turkey had low hygienic quality and they contained undesired bacteria like coliforms and pathogens of *C. perfringens* and *Salmonella* spp. due to insufficient cooking.

The changes in color values of DK samples upon thermal treatments are given in Table 1. Among color values, especially *L**, *h** and *a** values of raw and cooked samples were significantly ($p<0.05$) different from each other. The *L** and *h** values increased with cooking, while the *a** values decreased in DK samples. The increase in *L** indicated that the meat discolored with cooking. The decrease in *a** was thought to be due to the thermal decomposition of the myoglobin pigment during cooking. Various studies have reported that the myoglobin changes its color with heat treatment (Hunt et al., 2008; Suman and Joseph, 2013). It was also reported that carbohydrates and proteins in the marinated meat products involved in the Maillard reactions are responsible for the color changes during heat treatment (Barbut, 2013). Similarly, Fernández-López et al. (2003) reported a decrease in *a** values and an increase in *L** values during cooking of pork meat. Similar results for the color values of DK samples were reported. The *L** values agree with the results of Kilic (2003) for chicken DK samples. Although *a** and *b** values were higher than the results of Kilic (2003), these differences could be explained by the formulation of chicken DK which contain different raw substances (Demircioğlu et al. 2013).

High ΔE values indicate that the color difference between raw

TABLE 1: *Physicochemical properties of DK during cooking.*

Parameter	Cooking time (h)*						
	Raw	1	2	3	4	5	6
Temperature (°C)							
Center	4.2±0.2E	5.3±0.3D	7.4±0.3C	7.6±0.3BC	7.9±0.1B	10.4±0.3A	10.6±0.2A
Surface	10.8±0.2E	66.2±0.7D	67.8±2.8D	72.0±2.2C	75.2±1.4B	78.7±1.2A	80.3±0.9A
Color							
L*	48.23±2.25C	73.26±2.00B	75.44±0.81AB	75.96±1.36A	75.08±1.24AB	75.58±0.79A	75.94±0.63A
a*	11.36±1.12A	5.35±1.41B	3.56±0.58C	4.00±0.75C	3.43±0.61C	4.28±0.56BC	4.55±0.11BC
b*	23.68±3.02AB	22.79±1.40AB	22.41±1.51AB	22.36±1.53AB	21.54±2.40B	23.90±2.57AB	25.22±0.98A
C*	26.32±3.19A	23.53±1.53ABC	22.71±1.59BC	22.75±1.57BC	21.83±2.41C	24.28±2.62ABC	25.65±0.95AB
h*	64.60±1.33C	77.07±2.69B	81.17±0.75A	80.02±1.52A	80.87±1.32A	79.85±0.45A	79.87±0.70A
ΔE	–	26.02A	28.58A	28.80A	28.13A	28.28A	28.77A
Moisture (%)	78.7±1.1A	62.9±2.7B	61.0±2.0C	60.5±1.7BC	58.0±2.2CD	56.1±2.8D	58.7±2.5CD
Fat (%)	10.9±5.2E	14.8±1.6DE	15.7±2.2DE	17.5±2.4CD	22.1±3.3BC	26.6±2.5B	33.1±5.0A
Free fatty acid (%, as oleic acid)	1.79±0.08A	1.96±0.21A	2.04±0.25A	2.06±0.54A	1.73±0.83A	1.52±0.65A	1.33±0.46A

* Mean ± SD. Number of the analyzed samples (n) was 28. A-E The values in a row with the different letters are significantly different ($p < 0.05$)

and cooked samples is large. The ΔE value was found to be 28.77 at the 6th hour, while the lowest value (26.02) at the 1st hour of cooking and the change in ΔE value was found insignificant (p>0.05) during cooking period.

The results of moisture and lipid content are given in Table 1. While the moisture content of DK samples decreased with cooking time, the lipids content increased. These results agreed with Ergonul and Kundakci (2007) who reported that moisture content of DK tended to decrease with cooking, while the lipids content in DK samples increased during cooking. As given in Table 1, FFAs content of DK (1.33%-2.06%) did not change significantly (p>0.05).

Lipid oxidation during cooking

Table 2 shows peroxide value (PV), conjugated diene (CD), K_{232}, K_{270} and TBA values in the lipid fraction of DK during the periods of cooking. The initial PV of DK was higher than those reported in the Turkish DK sample (0.62 meq/kg) reported by Ergonul and Kundakci (2007). However, the changes in PV of DK according to cooking time (h) were not significant (p>0.05), and there was a decreasing trend in PV of samples with increasing the cooking time. At the end of cooking, the PV declined from 3.2 meq O_2/kg to 2.0 meq O_2/kg. Generally, a decreasing trend was also observed in CD, K_{232} and K_{270} values of lipid samples extracted from DK after 1 h of cooking. The change in K_{270} value was not significant (p>0.05). Unlike other lipid oxidation parameters, TBA values of cooked samples were higher (1.46-2.24 g MA/kg meat) than raw samples (0.74 g MA/kg meat). According to Al-Kahtani et al (1996), meat products could be accepted as good, when TBA value was 3 mg MDA/kg sample. In terms of oxidation, it could be considered as a safe product. The fluctuating of TBA was probably due to the difference in cooking conditions. Especially, the surface temperatures of samples obtained each 1 h were different.

Polymer triglycerides and free and ester-bound 3-MCPD

In the present study, the content of polymerized triglycerides of the analyzed samples significantly increased after 2 h of cooking and increased up to 0.031 g/100 g at the end of 4 h cooking. As shown in Table 3, the amounts of polymerized triglycerides were quite low and the changes in polymer triglyceride content was found insignificant (p>0.05). The heat treatment applied during the cooking of DK did not cause the formation of high amounts of polymerized triglycerides.

The average amount of 3-MCPD esters in the lipid fraction of raw DK samples was 49.28 µg/kg, while in the lipid fractions of the cooked DK samples ranged between 86.41-205.7 µg/kg. The amount of 3-MCPD esters increased significantly (p<0.05) with cooking time. The average amount of free MCPD in lean meat samples was 26.0 µg/kg before cooking. The lower free MCPD contents were determined in the cooked DK samples and it changed between 15.7-20.7 µg/kg. The amounts of free MCPD in cooked DK samples decreased insignificantly (p>0.05) with cooking time.

3-MCPD generally occur in foods because of thermal processing, as well as other processing or storage conditions (Chung et al. 2008). However, as shown in Table 3, 3-MCPD found also in raw samples. This could be explained because DK composition contains sauce, tomato paste and spices. The higher standard deviation values resulted from the use of different chicken DK blocks. These differences might derive from the variations in chicken meat composition, lipid content, cooking temperature, cooking duration and marination process. The DK samples taken during cooking might contained the precursors such as mono- and diacylglycerols, organic and inorganic chlorine in different amounts due to the heterogeneity of the meat surface. The precursors of ester-bound 3-MCPD during cooking in the bacon was also mentioned by Ilko and Doležal (2013).

To the best of our knowledge, there is no literature on the amount of free and ester- bound 3-MCPD in DK samples during cooking. However, there are published researches on the amount of MCPD in meat, poultry, and their products. Chung et al. (2008) determined 3-MCPD in meat, poultry, and their products, wherein the values varied between 4 µg/kg and 22 µg/kg. These values are close to or lower than our results. Arisseto et al. (2015) determined the concentration of 3-MCPD esters was between 0.12 mg/kg and 0.14 mg/kg in chicken croquettes, which is comparable with the results reported in the present study. Ilko and Doležal (2013) found the dry-fried bacon had 0.32 mg/kg 3-MCPD esters, and 0.42 mg/kg glycidyl esters, while the sallow-fried bacon had 0.6 mg/kg 3-MCPD esters, 0.37 mg/kg 2-MCPD esters and 0.32 mg/kg glycidyl esters. Hamlet et al. (2002) reported the instability of 3-MCPD in aqueous solutions at temperatures above 80°C. Wong et al. (2017) also expressed the decomposition of 3-MCPD esters, while the glycidyl esters remained constant at high temperatures during repeated frying of chicken breast

TABLE 2: *Lipid oxidation in the lipid fraction of DK during cooking.*

Parameter	Cooking time (h)*						
	Raw	1	2	3	4	5	6
Peroxide value (meq O_2/kg)	3.2±0.5A	3.0±0.7A	2.4±0.5A	3.0±0.8A	2.2±1.2A	2.4±1.1A	2.0±1.2A
Conjugated diene (%)	0.24±0.11AB	0.30±0.09A	0.25±0.13AB	0.23±0.03AB	0.21±0.07AB	0.17±0.05AB	0.14±0.05B
K_{232}	3.59±1.27AB	4.33±1.10A	3.71±1.56AB	3.39±0.38AB	3.24±0.85AB	2.75±0.55AB	2.39±0.63B
K_{270}	1.49±0.96A	1.71±0.65A	1.68±1.20A	1.41±0.49A	1.34±0.72A	0.85±0.46A	0.64±0.45A
TBA (mg MA/kg meat)	0.74±0.34D	2.24±0.31A	1.64±0.12ABC	1.46±0.13C	1.58±0.20BC	2.16±0.52AB	1.84±0.69ABC

* Mean ± SD. Number of the analyzed samples (n) was 28. A-D The values in a row with the different letters are significantly different (p < 0.05)

TABLE 3: *Polymerized triglycerides and free and ester-bound 3-MCPD content of lipids extracted from DK during cooking.*

Parameter	Cooking time (h)*			
	Raw	2	4	6
Polymerized triglycerides (g/100g)	0.027 ± 0.008A	0.024 ± 0.006A	0.031 ± 0.007A	0.030 ± 0.006A
3-MCPD content (µg/kg)				
Ester-bound	49.28±33.98B	86.41±63.57AB	133.50±125.81AB	205.74±82.11A
Free	26.00±13.14A	20.78±4.83A	17.16±4.98A	15.77±4.33A

* Mean ± SD. Number of the analyzed samples (n) was 16. A-B The values in a row with the different letters are significantly different (p < 0.05)

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meat. In a study, it was suggested that the DAG content of palm oil was responsible for the formation of glycidyl esters during deodorization (Destaillats et al. 2012).

Conclusion

In the current study, the values of lipid oxidation markers including PV, CD, K_{232}, K_{270} and TBA in chicken DK samples were influenced by cooking time. TBA values of cooked DK were significantly higher than those of raw DK. The present values were within the normal limitations of PV and TBA parameters. The occurrence of 3-MCPD esters in lipid fraction extracted from DK during the cooking process was reported for the first time in the present study. Besides, free 3-MCPD in chicken meat was determined under the same conditions. Ester-bound 3-MCPD in the lipid fraction of chicken DK increased with cooking time, whereas the level of free 3-MCPD decreased. Because of the high consumption of chicken DK, the results of the present study are useful for the examination of the lipid oxidation products and 3-MCPD contaminants. The source of 3-MCPD should be controlled in the raw materials used in the manufacture of chicken DK. In a further study, the change in glycidyl esters during cooking of DK samples might be investigated in addition to 3-MCPD esters.

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Conflict of interest

The authors declare no conflict of interest.

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Address of corresponding author:
Prof. Mohamed Fawzy Ramadan
Agricultural Biochemistry Department
Faculty of Agriculture
Zagazig University
Zagazig 44519
Egypt
mframadan@zu.edu.eg