Application of Superheated Steam in Sample Preparation (Chicken Sausage) for Determination of Total Fat, Fatty Acid and Lipid Oxidation

Asmaa A. Abdulhameed1,2, Wahidu Zzaman1, Tajul A. Yang1,*

1Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, Minden 11800 Pulau Pinang Malaysia
2Al-Adel Health Center, Al-Karkh Health Directorate, Ministry of Health, Bab Al-Mudam Area, Baghdad, Iraq
*Corresponding Author: taris@usm.my

Abstract In this study, the variations in fat, fatty acid composition, and lipid oxidation of chicken sausage during superheated steam and conventional drying oven (hot air oven) were investigated. Different superheated steam drying temperature (140-170°C) was applied at various time domains (22-40) min. The hot air oven temperature was 105°C, and the drying time was between 16-18 h. Statistical analysis was performed to precisely identify the efficacy in the fat, fatty acid, and lipid oxidation. The results revealed that at a temperature below 170°C, there was no significant difference in fat content when superheated steam was applied in drying. Applying the hot air drying was producing the highest amount of SFA as compared to their values in the superheated steam. While, the proportion of PUFA and MUFA values were maximized in superheated steam drying especially at temperatures between (160-170°C). Conventional drying oven significantly had higher value of lipid oxidation as indicated by peroxide value, p- anisidine value and Totox value than superheated steam oven. The results indicated superheated steam drying oven has efficiently improved the quality of the analysis as well as it decreased the drying time.

Keywords Fat, Fatty Acid, Superheated Steam Drying, AOAC Drying, Chicken Sausage

1. Introduction

Meat and meat products are considered vital sources of B vitamins and trace ingredient, which considerably contributes to the daily intakes of numerous nutrients that are essential for optimal development and growth. Chicken meat and its products have experienced increasing popularity and become widely spread all over the world. Chicken sausage is considered as one of the popular foodstuffs among these products. It was found to be a good source of polyunsaturated fatty acid (PUFA) compared to pork and beef sausage. This is because of chicken meat has great nutritional value and high level of PUFA compared to red meat, which has a remarkable effect on health [1,2].

To quantify the amount of fat, the drying of meat/meat product is essential. Removing water from food can prevent or inhibit the growth of microorganisms [3]. Drying can contribute to a formation of porous structure, shrinkage and crack formation. The effect of a drying method on the physical properties and structure of food products has been reported by [4]. In addition, the quality of a dried food and its cost are greatly influenced by the drying technique. Currently, a wide range of drying techniques and equipment are available for use. Depending on the process and product needs, the selection ranges from contact and convection dryers, fluidized beds, spray dryers, freeze or vacuum dryers, to microwaves and radio-frequency dryers, in which most of the industrial dryers use hot air as a drying medium.

Normally, AOAC method considered one of the most common methods for drying food and determining moisture content, in which the hot air oven is applied as a drying medium. However, it has a significant limitation of uneconomical energy consumption and long drying time (need around 16-18 hours) resulting in product quality degradation [5, 6]. This technique employs a flow of heated air stream to supply heat to the food and remove its moisture, as well as, the moist air must be replaced by fresh air. During drying, the food contacts with oxygen in the air oven and may be exposed to high temperature for a long time. Such exposure to heat decreases the content of some valuable components which readily undergo oxidation at elevated temperature [7].

However, with growing concerns to higher food quality with shortening drying time, an emerging is needed for an alternative technique. Superheated steam has been used in drying instead of hot air oven. It was generated from the addition of sensible heat to water, this lead to increase in its temperature over the boiling point or saturation temperature.
at the given pressure. However, a drop in temperature (when superheated steam is applied) will not happen in condensation of the steam as long as the temperature is still higher than the saturation temperature at the processing pressure [8, 9]. In one hand, this technique might also improve the product quality such as, low shrinkage, high porosity and high vitamin C retention. On the other hand, it decreases the energy consumption and process emissions, eliminates the possibility of fire and explosion hazards due to the absence of oxygen and nitrogen. The lack of oxygen can also eliminate oxidative reactions (lipid oxidation) from occurring within the product [7, 10-13].

Bórquez and co-workers stated that the dried mackerel press-cake using superheated steam resulted in a product with very low losses of the valuable omega-3 fatty acids in comparison with hot air oven [7]. It has been studied the effect of superheated steam dryer on the stability of n-3 fatty acid for fish particles, in which the lower losses in n-3 fatty acid during drying in superheated steam were found [10]. Additionally, the effect of superheated steam drying on the fat content of beef was extensively investigated by Speckhahn et al. [2010]. They observed that the fat extraction efficiency of superheated steam is much faster than that for the hot air oven [14].

As such, to decrease drying time and increase the accuracy of fat and fatty acid analysis, the novel approach attained in this study was the implementation of superheated steam drying during the chemical analysis of chicken sausage. Therefore, the aim of this study was investigate the effect of superheated steam drying on the total fat, fatty acid composition, and lipid oxidation for chicken sausage.

2. Material and Method

2.1. Sample Preparation

Ready-to-cook commercial chicken cocktail sausage supplied from a local hypermarket was used in this study. The chicken cocktail sausage was held under frozen conditions (-18 to -20°C) in a freezer prior processing. Then the chicken sausage was thawed for 2 h, then cut in longitudinal section to the size of (2 * 2 * 0.5) cm and put in aluminium tray for drying steps.

2.2. Hot Air Drying Oven

Chicken sausage was placed in the hot air drying oven and dried at 105°C for overnight until reached the constant weight in according to the method of AOAC [15].

2.3. Superheated Steam Drying Oven

Superheated steam drying was carried out by placing the tray containing chicken sausage samples in the processing chamber of superheated steam processing system and exposed to different temperatures (140, 150, 160, and 170°C for (40, 30, 25, and 22) min respectively until reach the constant weight. Sharp AX-1500 was used as superheated steam equipment with a steam generation capacity of 16 cm³/min, oven capacity of 31 L, and team engine heater of 900 W. The steam was supplied by the boiler at the beginning of the drying process (at a pressure of approximately 1 bar) and it was heated up by the electric heater until it reached the superheated state.

2.4. Determination of Fat Content

Fat content in dried chicken sausage was determined in according to the method of AOAC [16] using soxhlet extraction with petroleum ether as a solvent.

2.5. Determination of Fatty Acid Composition

Fatty acid methyl ester (FAME) was prepared according to the method of Mondello et al., (2006). 0.2 ml of crude oil was extracted from chicken sausage samples and trans-esterified in a Pyrex tube by using 2 ml of boron trifluoride-methanol (20% BF3) reagent then heated for 30 min at 100°C. After cooling down, 2 ml of n-hexane and 8 ml of distilled water were added to the mixture, which was then mixed manually for 1 min and centrifuged for 2 min [17]. Approximately 1 ml of the upper n-hexane layer was transferred to a 1.5 ml glass insert for 2 ml vials after diluting the extracted hexane to obtain a suitable chromatographic response. Fatty acids were identified by comparing the retention times of FAME mixture with the standard Myristic acid palmitic acid, stearic acid, oleic acid, linoleic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA). The results of fatty acid composition were expressed in GC as area percentage. The fatty acid composition of chicken sausage was directly analyzed using Gas Chromatography (GC) after methylesterification. One µL of each fatty acid methyl ester (FAME) sample was injected (split ratio 15:1) into a GC 17 A-SHIMADZU Gas Chromatography (GC-MS, Shimadzu Scientific Inc., USA) with flame ionization detector. A BPX 70 (SGE, Australia) column consisting of a 30 m x 0.32 mm fused silica capillary coated with 70 % cyanopropylpolysilphenylene-siloxane of 0.25 µm film thickness was used, with Hydrogen as the carrier gas at a constant linear velocity (28 cm/s). The injector temperature was 250°C and the detector temperature 280°C. The oven was programmed as follows: 80°C for 2 min, 5°C/min to 200°C for 10 min and 10°C/min to 230°C for a further 10 min. Fatty acid were grouped as follows: saturated(SFA), mono (MUNA) and poly (PUFA) fatty acids.

2.6. Determination of Lipid Oxidation

Peroxide value (POV) was evaluated according to standard AOAC method [18]. 5 gram of fat was weighed into a conical flask and mixed with 30 ml acetic acid-chloroform
Then 0.5 ml of saturated potassium iodide (KI) solution was added into the mixture and shaken for 1 minute. 30 ml of distilled water (H₂O) was added into the mixture. Slowly titrate with 0.01M of sodium thiosulfate (Na₂S₂O₃) until the yellow color disappeared. 0.5 ml of 1% starch solution was added to the mixture. Continue titrating, shaking the flask vigorously until the blue color disappeared. All samples were measured in triplicate. Peroxide value was calculated as equation below:

\[
Peroxide \text{ value (millequivalent peroxide/kg of oil)} = \frac{(S * M * 1000)}{\text{weight of sample in grams}} \tag{1}
\]

\[S = \text{ml of sodium thiosulfate (Na₂S₂O₃) used}
\]

\[M = \text{molarity of sodium thiosulfate (Na₂S₂O₃) used}
\]

P-anisidine value (PAV) was determined by using spectrophotometric method of IUPAC (1989) [19]. 2 gram of fat was weighed and dissolved with 25 ml of iso-octane. Then, absorbance of the solution was measured at 350 nm. 5 ml of this mixture solution was then mixed with 1 ml of 0.25% p-anisidine reagent and stored in dark condition for 10 minutes at room temperature. Then, absorbance of this mixture was read at 350 nm using UV-160A spectrophotometer (Shimadzu Corp.). All samples were measured in triplicate. p-anisidine value all samples were calculated using equation below;

\[
p\text{-anisidine value} = \frac{(25 * (1.2 \text{ As} – \text{Ab}))}{(\text{weight of sample})} \tag{2}
\]

\[\text{As} = \text{Absorbance of fat solution after reaction with the p-anisidine reagent}
\]

\[\text{Ab} = \text{Absorbance of fat solution before reaction with the p-anisidine reagent}
\]

While the Totox value (Total oxidation) was determined as twice of peroxide value plus p-anisidine value.

### 2.7. Statistical Analysis

Statistical analysis was performed using analysis of variance (ANOVA). The Duncan’s new multiple range test was used to determine the differences among means at a 5% significance level.

### 3. Result and Discussion

#### 3.1. Fat Content

To investigate the behavior of superheated steam at various treatments, the fat content was plotted against the temperature and shown in Fig. 1. There was a slight increase in fat content with temperature. As seen from the figure, the increase in fat at temperatures between (140-150) °C is only 0.07%, while it shows an increase of 0.66% between (150-160) °C. However, the statistical analysis exhibited no significant difference (p > 0.05) in fat content within superheated steam treatments.

The fat content of chicken sausage that dried using superheated steam and air oven were listed in Table 1. At superheated steam drying temperature below 170°C, there was no significant difference (p > 0.05) in fat content as compared to the hot air dryer.

![Figure 1. Effect of superheated steam drying temperature on fat (g/100 g) content for chicken sausage](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drying time</th>
<th>Fat content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional drying</td>
<td>16-18 h</td>
<td>38.13±0.37a</td>
</tr>
<tr>
<td>Superheated steam</td>
<td>140°C</td>
<td>39.06±0.68ab</td>
</tr>
<tr>
<td>Superheated steam</td>
<td>150 °C</td>
<td>39.09±1.06ab</td>
</tr>
<tr>
<td>Superheated steam</td>
<td>160 °C</td>
<td>39.35±0.39ab</td>
</tr>
<tr>
<td>Superheated steam</td>
<td>170 °C</td>
<td>39.43±0.41b</td>
</tr>
</tbody>
</table>

Results are expressed as means ± standard deviations. 
**a-b** Average with different letters in the same column indicate significant differences (p < 0.05). (n=3)

For the purpose of illustration, the increase in fat content when superheated steam was applied was evaluated using Eq. 1

\[
\% \text{ F}_{\text{change}} = \left(\frac{F_{\text{SHS}} - F_{\text{HA}}}{F_{\text{SHS}}}\right) * 100 \tag{1}
\]

where, \(\% \text{ F}_{\text{change}}\) represents the percentage change in the fat. \(F_{\text{SHS}}\) and \(F_{\text{HA}}\) represent the amount of fat for superheated steam and hot air dryer, respectively. Fig. 2 explained the increase in fat percent when superheated steam was compared to the hot air dryer. The highest percent (p<0.05) was obtained at a temperature of 170 °C and 22min of resident time.

There are two parameters might be responsible for fat losses during the dying process; the presence of oxygen and its residence time. Both of these parameters were found to affect the fat loss in hot air, in which it might be responsible for the deviation in fat content as that of in the superheated steam. Similar observations were found by Wu and Mao, (2008) in which they stated that the losses in fat content for...
Application of Superheated Steam in Sample Preparation (Chicken Sausage) for Determination of Total Fat, Fatty Acid and Lipid Oxidation

Grass carp fillets during drying with hot air oven were higher than microwave drying oven, due to the higher retention of fat in a microwave oven than hot air drying [5]. This result is in contrast with other researchers that acquired the fat losses due to lipid oxidation and lipolysis [20]. However, it has found in another research that the fat content of beef samples dried using SHS drying is less than that of air dryer at similar drying conditions (temperature and time) [14].

3.2. Changes in fatty acid composition during drying

Fatty acid composition of chicken sausage that was dried in both hot air and superheated steam drying oven are shown in Table 2. The MUFA which represent in higher amounts and were dominated by oleic acid (C18: 1) and palmitoleic acid (C16: 1). The PUFA was dominated by linoleic acid (C18: 2n-6) as the most abundant omega-6 fatty acid and α-linoleic acid (C18: 3n-3) as the most abundant omega-3 fatty acid. The SFA was dominated by palmitic acid (C16: 0) and stearic acid (C18: 0).

For better illustration of the result, the SFA, PUFA, MUFA, PUFA/SFA, and n-6/n-3 were presented in Fig 3. At various temperatures and different drying time, the fatty acid analysis of superheated steam showed a slight difference. As such, the SFA proportion at all temperatures was approximately constant. While, for PUFA and MUFA fatty acid the behaviors of them were approximately identical at the examined conditions.

Figure 2. Increase of fat percent when superheated steam was applied at different drying temperatures

Table 2. Fatty acid composition (% of total fatty acids) of hot air-dried and superheated steam-dried chicken sausage

<table>
<thead>
<tr>
<th>FATTY ACID (%)</th>
<th>Conventional drying</th>
<th>Superheated steam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(105 °C,(16-18 hr))</td>
<td>(140 °C,40min)</td>
</tr>
<tr>
<td>C14:0</td>
<td>2.17±0.13 a</td>
<td>1.74±0.12 b</td>
</tr>
<tr>
<td>C15:0</td>
<td>1.50±0.37 a</td>
<td>1.31±0.24 a</td>
</tr>
<tr>
<td>C16:0</td>
<td>24.51±0.58 a</td>
<td>22.33±0.06 b</td>
</tr>
<tr>
<td>C18:0</td>
<td>8.53±0.23 a</td>
<td>7.34±0.12 a</td>
</tr>
<tr>
<td>C20:0</td>
<td>3.255±0.17 b</td>
<td>2.70±0.19 a</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.269±0.03 a</td>
<td>0.396±0.01 b</td>
</tr>
<tr>
<td>C15:1</td>
<td>0.145±0.06 a</td>
<td>0.625±0.06 b</td>
</tr>
<tr>
<td>C16:1</td>
<td>4.11±0.09 b</td>
<td>5.19±0.11 b</td>
</tr>
<tr>
<td>C18:1n9</td>
<td>35.367±0.83 a</td>
<td>37.260±0.52 b</td>
</tr>
<tr>
<td>C18:3n6</td>
<td>0.102±0.05 a</td>
<td>0.132±0.04 b</td>
</tr>
<tr>
<td>C18:3n3</td>
<td>0.930±0.06 a</td>
<td>1.28±0.05 b</td>
</tr>
<tr>
<td>C18:2n6</td>
<td>15.15±0.07 a</td>
<td>15.4±0.05 b</td>
</tr>
<tr>
<td>C20:3n3</td>
<td>1.197±0.07 a</td>
<td>1.396±0.03 b</td>
</tr>
<tr>
<td>C20:3n6</td>
<td>0.16±0.05 a</td>
<td>0.21±0.05 b</td>
</tr>
<tr>
<td>C20:5(n3) EPA</td>
<td>0.103±0.05 a</td>
<td>0.127±0.04 b</td>
</tr>
<tr>
<td>C22:6(n3) DHA</td>
<td>0.082±0.04 a</td>
<td>0.140±0.09 b</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard deviation.

a-dAverage with different letters in the same row indicate significant differences (p < 0.05). (n=3)
As indicated in Fig. 3, the amount of SFA for chicken sausage dried using the hot air oven is higher than that of the superheated steam. However, the values of MUFA and PUFA were found to decrease in hot air oven. It is generally accepted that the unsaturated fatty acid was found to decrease in hot air dryer as shown in pioneering study reported by Phatanayindee et al. (2012). In which, they found that the unsaturated fatty acid decreased significantly in hot air drying oven due to lipid oxidation that initiated by the presence of oxygen in hot air oven, which attacked the double bond of unsaturated fatty acid [21]. Similar finding was observed in the study of Wu and Mao (2008), in which they observed that microwave oven dried grass carp fillet, had the lower value of SFA, and higher PUFA and MUFA than that of hot air drying oven (Wu and Mao, 2008[5]. Additionally, Sampels et al. (2004) and Cosgrove et al., (1987) were ascribed the increase in SFA and the decrease in PUFA fractions to the oxidation process [22, 23].

The results revealed that the ratio PUFA/SFA in hot air oven is lower than that of the superheated steam dryer. While, the reverse behavior were observe for the ratio of n-6/n-3 fatty acid. The decreasing in the content of PUFA after drying and at a lower ratio of PUFA/SFA, are also an indications of an oxidation process. This is because of the unsaturated fatty acid undergo oxidation more easily than SFA [22]. The highest losses in PUFA were also observed during drying kaffir lime leaves using hot air drying oven [6].

One of the possible ways to decrease the fatty acid oxidation is by working in oxygen-free medium. As the superheated steam is normally working in the absence of oxygen, in which this condition could prevent or reduce the oxidation of unsaturated fatty acid (Huang et al., 2004). Furthermore, other researchers believed that the short resident time during superheated steam drying could reduce the losses in the unsaturated fatty acid. They found that the superheated steam reduces the losses of the valuable omega-3 fatty acids in comparison with hot air oven due to short resident drying time [7, 10].

The improvement in the analysis of fatty acid (when superheated steam was applied rather than the hot air dryer) might be evaluated using Eq.2

\[ \% F_{\text{change}} = \left( \frac{F_{\text{ASHS}} - F_{\text{AHA}}}{F_{\text{ASHS}}} \right) \times 100 \]  

where, \( F_{\text{change}} \) represents the percentage change in the fatty acid, \( F_{\text{ASHS}} \) and \( F_{\text{AHA}} \) represent the amount of fatty acid for superheated steam and hot air dryer, respectively. Using equation 1, the variations of SFA, PUFA, and MUFA were represented in Fig. 4. In which, the positive values for this equation refer to the increasing in the FA content, while the negative values indicate the decreasing of their FA. In this figure, the x-axis refers to the superheated steam temperatures, and the y-axis indicates the change in the fatty acid percentage. It might conclude from the figure that there was a decreasing in SFA with temperature. Their values reached the maximum values at 170 °C (14.94 %). At drying temperatures between (140-160 °C), the PUFA and MUFA were found to increase when superheated steam was applied. However, their values (relative to that of 160 °C) were shown to decrease slightly at a drying temperature of 170 °C.

Based on the results presented in this section, there was an enhancement in the fatty acid amount that obtained using superheated steam in drying of chicken sausage samples. The analysis of the data proved that the improvement in the fatty acid is mainly due to the short residence time (as compared with hot air oven). The short drying residence time (between 22-30 min), and at a temperature between 160-170 °C, the values of PUFA and MUFA showed the highest values.

3.3. Determination of Lipid Oxidation

Peroxide value (PV), p-Anisidine (PAV) value and Totox value of fat samples that were extracted from dried samples and evaluated to determine the quality of the food products. The results of above mentioned parameters are detailed in Table 3.
Lipid peroxidation is a free radical chain reaction that may be described in the terms of initiation, propagation, and termination processes. Oxygen species and activated catalysts seem to be the key factor of polyunsaturated fatty acid oxidation through three different pathways: hydrogen abstraction, free radical attack on double bonds, and singlet oxygen "ene reaction" [24]. In the present study, the peroxide value in all samples ranged between (4.92-2.25 meq/kg), were below 25 meq of active O2/kg, which is considered as limit of acceptability in fatty food [25]. Peroxide value for hot air oven was significantly (p<0.05) higher than superheated steam oven. This may be due to the presence of oxygen with long drying time in hot air oven could accelerate the lipid oxidation. Ahn et al., (1992) also reported that oxygen contact with meat was the most important factor in the development of lipid oxidation [26]. However, there is no significant difference (p>0.05) in peroxide value during drying with superheated steam oven. This may explain by the absence of oxygen inside superheated steam which minimizes or prevent the lipid oxidation reaction in dried samples. Huang et al., (2004) found that the superheated steam fried Zousoon had superior lipid stability to that prepared by the conventional pan-frying method [24]. While, Speckhahn et al., (2010) noted that the curve of peroxide value during drying in superheated steam were almost constant and it lower than hot air drying, this due to the absence of oxygen in superheated steam consequently hardly any peroxides develop in the meat during exposure to superheated steam [14]. P-anisidine value was analyzed to determined secondary product of lipid oxidation. P-anisidine value is mainly a measure of 2-alkenals and 2,4-dienals in animal fat and vegetable oil. The result of this study showed that the p-anisidine value in hot air oven significantly (p< 0.05) higher than in superheated steam oven and it ranged between (6.31-3.80). This is may be attributed to the presence of oxygen in hot air oven and the long resident drying time which resulted in a greater degree of lipid oxidation. However the p-anisidine value of the sampled dried with superheated steam had constant line due to exclusion of molecular oxygen in superheated steam and the short drying time. Total oxidation value, the so-called Totox value, calculated from twice the peroxide value plus the p-anisidine value, is another useful indicator of measuring the onset of progressive deterioration in oil and provides information regarding progression of the formation of primary and secondary oxidation products [27].

As indicated in Table 3, the Totox value was ranged between (16.16-7.86). Similarly to changes in peroxide and p-anisidine, the sample dried in hot air oven had higher Totox values than in superheated steam oven. While, the Totox value showed constant level during drying in superheated steam at different temperature and drying times. The stability in peroxide value, p-anisidine value, and Totox value during superheated steam drying confirm the advantages of utilization the superheated steam as a drying medium to reduce the degree of oxidation, and thus reduction in another undesirable quality changes affecting aroma and flavor of the meat.

### 4. Conclusions

During the analysis of fat and fatty acid, superheated steam drying technique and the conventional drying methods were implemented to dry chicken sausage samples. At a temperature below 170 °C, statistical analysis exhibited no significant difference in fat content when superheated steam was applied in drying. However, at 170 °C, there was a significant increase in fat content (3.49%) higher than that of the hot air dryer. Applying the superheated steam in drying leads to a decrease in SFA content (between 13.08-14.94%). As obtained, the PUFA and the MUFA values were increased by (5.18-7.51 %) and (7.46-10.98 %), respectively. The highest increases in PUFA and MUFA (FAchange %) were found at superheated steam drying temperatures between (160-170 °C). Superheated steam drying oven exhibited stability toward lipid oxidation at different time and temperature. However, conventional drying oven had higher value of lipid oxidation. Therefore it can conclude that the utilization of superheated steam in drying of chicken
sausage resulted in a remarkable enhancement in the quality of the analysis. Also, the time required in this method is much lower than that used in the conventional drying technique.

REFERENCES


