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# The Effect of Frying Process on the Level of Malondialdehyde in Different Meat Products

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***ABSTRACT***

**Background:** Frying is one of the popular cooking methods for the preparation of food especially meat products. However, this process has some adverse effects, such as lipid oxidation that results in deterioration and rancidity of food during preparation and storage. Malondialdehyde (MDA) as a secondary product of oxidation is commonly used as an index of rancidity in food products. However, the level of MDA produced during the frying process varies depending on the type of food. **Methods**: This study was performed to evaluate the levels of MDA before and after the frying process in different products, including chicken, fish, pan kebab, beef, sausage, and hamburger. Also, the effect of meat content in hamburger was evaluated on the MDA level. The spectrophotometric thiobarbituric acid reactive substances (TBARS) test was used to determine MDA in the food products. **Results:** The concentration of MDA in the products increased significantly ($P < 0.01$) after frying in oil. The highest amount of MDA between the products was detected in fried fish (1.24 µM/g). By increasing the percentage of meat in hamburgers, the content of MDA increased in this product. In the hamburger containing 90% of meat, the MDA level was significantly ($P < 0.05$) higher than others (0.98 µM/g). **Conclusion:** The results of the present study can provide proper knowledge about the levels of lipid peroxidation and the safety of different fried meat products.

**Keywords:** *Malondialdehyde; Oxidation; Meat; Frying.*

## Introduction

Meat is very important in human nutrition due to its proper content of protein, B vitamins, and trace elements. It is usually supplied in the raw form in the markets; however, consumers are highly interested in pre-cooked and ready-to-eat products (Demirezen and Uruç, 2006, Pandey *et al.*, 2014). Among the various cooking processes, frying is one of the common methods for the preparation of food especially meat varieties and meat products that is performed using different types of oils and fats (Suomela *et al.*, 2004). The overall quality, flavor, and texture of foods are usually improved using this process (Chukwu, 2009). Despite its popularity, this processing method has some harmful effects on food composition. During the frying process, frying oils and fats are exposed to the moisture and air, resulting in the physical and chemical deterioration

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of food. It also can affect the frying efficiency shelf life of fried products (Fauziah and Aini, 2000, Pandey *et al.*, 2014). The frying oil has three main adverse reactions with food ingredients, including oxidation, hydrolysis, and thermal decomposition (Dana and Saguy, 2001). The fatty acids and triglycerides of food are oxidized during the frying process. Lipid oxidation is the major cause of chemical spoilage of meat products which results in texture modification, rancidity, loss of nutrients, production of unfavorable odors, and toxic compounds (Broncano *et al.*, 2009, Papastergiadis *et al.*, 2012). Oxidation of unsaturated fatty acids results in the formation of odorless and tasteless compounds called hydroperoxides. These compounds are then decomposed to flavorful products, such as aldehydes, ketones, alcohols, acids, and hydrocarbons (de las Heras *et al.*, 2003, Mendes *et al.*, 2009, Nassu *et al.*, 2003, Papastergiadis *et al.*, 2012). These products can affect the quality of food in terms of color, texture, flavor, and odor (Mendes *et al.*, 2009). It has also been reported that lipid oxidation products are associated with several adverse effects on the body health, such as atherosclerosis, Alzheimer's disease, cancer, inflammation, or aging processes (Broncano *et al.*, 2009, Nassu *et al.*, 2003, Rahman *et al.*, 2007).

In the products like fish and meat, determination of malondialdehyde (MDA) level is often chosen as an index of rancidity. The structure of MDA is composed of a three-carbon dialdehyde that is attached to carbonyl groups at the positions of C-1 and C-3. The mutagenic and carcinogenic effects of MDA have been reported for human body because of the formation of adducts with proteins and DNA (Papastergiadis *et al.*, 2012, Zeb and Ullah, 2016).

In recent years, a variety of analytical methods have been proposed for determination of MDA (Bauer-Plank and Steenhorst-Slikkerveer, 2000, Zeb, 2012, Zeb and Ullah, 2016). Given the spectrophotometric method has a simple and low-cost procedure, it is usually used for the analysis of a large number of samples (Papastergiadis *et al.*, 2012). The 2-thiobarbituric acid (TBA) test is a widely used spectrophotometric method to measure lipid oxidation in meat products which can determine MDA content (Broncano *et al.*, 2009, de las Heras *et al.*, 2003, Labropoulos *et al.*, 2013, Papastergiadis *et al.*, 2012). This method is precise, sensitive, and highly reproducible (Zeb and Ullah, 2016). At low pH and high temperature, the active methylene groups of TBA react with the monoenolic form of MDA. This reaction produces a pink-colored complex that gives a maximum absorbance at the wavelength of 532−535nm (Broncano *et al.*, 2009, de las Heras *et al.*, 2003, Papastergiadis *et al.*, 2012, Seljeskog *et al.*, 2006). The intensity of color is used for the measuring of MDA concentration (Mendes *et al.*, 2009). Given TBA may react with some other active compounds; a more general term is used that is called thiobarbituric acid reactive substances (TBARS) (Sun *et al.*, 2001, Zeb and Ullah, 2016).

Fried foods, such as pan kebab (Kebab Tabei), fried hamburger, sausage, chicken, fish, and beef are known as the most consumed meat products in Iran. Therefore, the purpose of present study was to determine the effect of frying process on levels of MDA in these products before and after frying.

## Materials and Methods

*Chemicals and materials:* MDA tetrabutylammonium salt (≥ 96%) and TBA (≥ 98%) were purchased from Sigma-Aldrich (Steinheim, Germany). Glacial acetic acid (glacial) (≥ 99.8%) was also provided from Merck Company (Darmstadt, Germany). Other used chemicals had an analytical grade.

*Samples of meat products:* During the spring of 2020, chicken, fish and meat, commercial hamburgers (containing 30%, 55%, 70%, and 90% of meat), and sausage were bought fresh from the local markets (Tabriz city, Iran). Samples were placed into a box containing ice packs and they were immediately transferred to the laboratory.

Breast muscles of the chicken were excised from each carcass, and cut into 1 cm-thick slices using a scalpel. Cow muscles were also cut into small pieces (1 cm thickness) and all visible fat was removed from them. Fresh rainbow trout were




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washed with potable water to remove the slime and blood on its surface. To yielding the fillets, the skin, head, tail, and bones were removed from each fish. Minced meat and onion were used for the preparation of pan kebab. The main ingredients of these products are presented in **Table 1**. The frying process was performed in a pan containing 5 mL of sunflower oil. The temperature of frying process was about 220°C. One sample from each product was used as raw control. Others were subjected to the frying process in three replicates.

*Measurement of MDA in the samples:* In order to prepare a stock solution (1 mM, w/v), 31.35 mg of MDA was dissolved in a flask containing glacial acetic acid (100 ml). Different concentrations of MDA (0.1, 0.2, 0.4, 0.6, and 0.8 mM) were prepared from the stock solution. Also, 11.532 mg of TBA was dissolved in 20 ml of glacial acetic acid to prepare the standard solution of TBA (4.0 mM).

One ml of the standard TBA solution was taken to a test tube containing 1 ml of MDA standards. The tubes were heated in a water bath at 95°C for 60 min. The mixtures were cooled at room temperature and absorbance of them was measured at the wavelength of 532 nm using a UV-visible spectrophotometer (Beckman, Germany). Acetic acid was used as a blank sample. Different concentrations of MDA (ranged between 0.1 mM and 1.0 mM) were used for drawing a standard curve.

The level of MDA in the samples was determined before and after frying. Only completely fried portions of the products were used for the analysis. One gram of each homogenized sample was completely dissolved in a test tube containing 5 ml of acetic acid. The mixture was shaken for 1 h and filtered using a filter paper. The filtrates were centrifuged for 10 min and the supernatant was used for the analysis. The supernatant (1 ml) was mixed in a tube containing TBA (1 ml).

The tubes were placed in a water bath for 1 h at 95°C. They were cooled at room temperature. The absorbance of mixtures was measured by a spectrophotometer in three replicates. The level of TBARS (µM MDA/g) was calculated in the extracts using the standard curve (Pandey *et al.*, 2014).

*Data analysis:* Statistical analysis of the data was carried out using SPSS 18 statistics package (IBM, SPSS, Inc.). The differences between the mean values were determined significant when the p-value was lower than 0.05. The one-way analysis of variance (ANOVA) was used for this purpose.

## Results

The calibration curve was prepared using different concentrations of MDA in the range of 0.1 to 1.0 $\mu$M. The stock solution of MDA gave an accurate standard curve with high repeatability in the spectrophotometer. Linear regression in the calibration curve of MDA showed a correlation coefficient of 0.9969 (**Figure 1**).

The TBARS values in the raw products were in the range of 0.03 to 0.160 µM MDA/g (**Figure 2** and **Figure 3**). The highest and lowest levels of MDA in raw products were detected in chicken (0.03 µM/g) and pan kebab (0.16 µM/g), respectively.

It was found that the frying process significantly ($P < 0.05$) increased TBARS content. The highest amount of MDA in fried products was detected in fried fish (1.24 µM/g). The results showed that the pan kebab contained a higher MDA level in comparison to other products made from cow meat.




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**Table 1.** Ingredients of the evaluated meat products in the study

| Products | Ingredients |
|---|---|
| Hamburger 90% | Minced meat (90%), Soya powder, vegetable oil, spices, onion |
| Hamburger 70% | Minced meat (70%), Soya powder , vegetable oil, spices, onion |
| Hamburger 55% | Minced meat (55%), Soya powder, vegetable oil, spices, onion |
| Hamburger 30% | Minced meat (30%), Soya powder, vegetable oil, spices, onion |
| Pan Kebab (kebab Tabei) | Minced meat and onion |
| Chicken | Chicken breast fillet |
| Beef | Cow muscle |
| Fish | Rainbow trout fillet |
| Sausage | Minced meat (30%), soya powder, starch, casein, milk powder, vegetable oil, spices, onion |

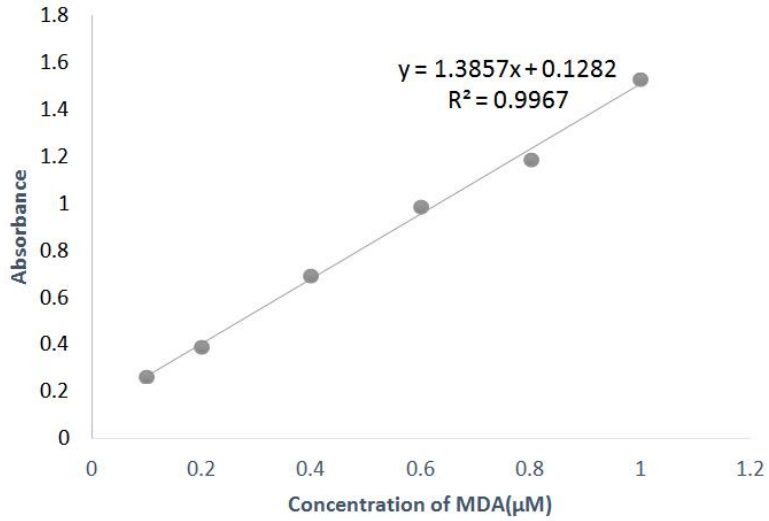


**Figure 1.** The linear regression curve of MDA in the range of 0.1–1.0 $\mu$M

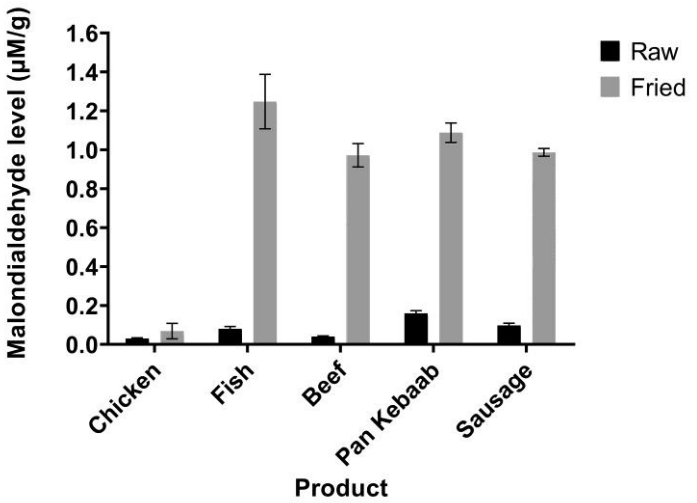


**Figure 2.** Comparison of MDA level (μM/g) in meat products before and after frying in sunflower oil. Results are presented as the mean of three replicates ± standard error.





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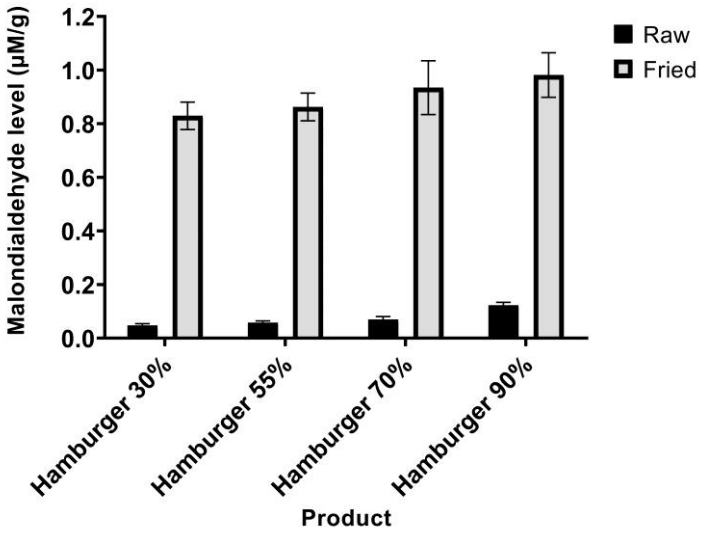


Figure 3. Comparison of MDA level (µM/g) in hamburgers containing 30%, 70%, and 90% of meat before and after frying in sunflower oil. Results are presented as the mean of three replicates ± standard error.

In the hamburger, the MDA content also increased by the frying process. By increasing the percentage of meat in the raw and fried hamburgers, the content of MDA also increased. In the hamburger containing 90% of meat, the level of MDA was significantly ($P < 0.05$) higher than the other ones (0.98 µM/g).

## Discussion

Lipid oxidation is one of the important indicators that are used for determination of the quality and acceptability level of meat products (Pandey *et al.*, 2014). Oxidation of unsaturated fatty acids particularly the polyunsaturated fatty acids (PUFA) is mainly responsible for the oxidative spoilage of meat lipids. MDA is produced after the breaking down of the hydroperoxides during the oxidation of lipids (Hernández *et al.*, 1999). In the present study, the TBARS value as an index of MDA level was evaluated. Six types of foods (pan kebab, beef, sausage, hamburger, chicken and fish) which have wide popularity in Iran were selected for the present study. It was found that the frying process significantly ($P < 0.05$) increased TBARS content. Similar results have also been reported in other studies, showing higher levels of TBARS in the fried meat in comparison to the raw meat (Broncano *et al.*, 2009, Hernández *et al.*, 1999, Serrano *et al.*, 2007).

The extent of lipid oxidation and the formation of MDA can be affected by the proportion and the degree of unsaturation of the fatty acids (Hernández *et al.*, 1999, Labropoulos *et al.*, 2013, Milijašević *et al.*, 2017). The PUFA, such as eicosapentaenoic (EPA) and docosahexaenoic (DHA), are known to be highly sensitive to oxidation during heating processes (Weber *et al.*, 2008). Moreover, the oxidized PUFAs with more than two double bonds have a major role in the production of MDA (Papastergiadis *et al.*, 2012). The highest amount of MDA in fried products was detected in fried fish. High values of TBARs in fried fish samples may be associated with the fact that fish contains a high content of PUFA and a large amount of metals which make it highly susceptible and easily oxidizable than meat during manipulation, processing, and cooking (Mendes *et al.*, 2009, Sakai *et al.*, 1999).

Pan kebab is a popular food in Iran that is usually made of minced meat and onion and it is fried in a pan using vegetable oil. In the present study, the pan kebab contained a higher MDA level in comparison to other products made from cow meat. It has been known that processed




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products, which are minced, mixed and/or heated, are very sensitive to lipid oxidation (Eburne and Prentice, 1994, Nassu *et al.*, 2003).

The MDA content also increased in hamburger samples by the frying process. Also, by increasing the percentage of meat in the raw and fried hamburgers, the content of MDA increased. It was also reported that the secondary oxidation products can increase by frying hamburgers in the pan (Rodriguez-Estrada *et al.*, 1997). When the raw meat is exposed to heat in the presence of oxygen, myoglobin is denatured and converted to chemical compounds resulting in the oxidation of lipids (Labropoulos *et al.*, 2013).

In the frying process, oil transfers the heat to the meat. Therefore, the oxidative changes occur in the meat and oil following the contact of hot oil with the product surface. The oils also can change the antioxidant content and the composition of fatty acid that can enhance the oxidation in the meat products (Broncano *et al.*, 2009, Ramírez *et al.*, 2004, Saghir *et al.*, 2005, Serrano *et al.*, 2007).

## Conclusion

Based on the previous studies, long-term use of the frying process has harmful effects on the quality of meat products and the safety of human. In the present study, oxidation levels increased in all products and there was a direct association between the food product and lipid oxidation. Therefore, the determination of MDA content in food can be used as a proper tool for monitoring the levels of lipid peroxidation and the safety of fried meat products.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Authors' contribution

All authors contributed to data gathering, analyzing, writing the manuscript, and final revision.

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