EFFECT OF SUPERHEATED STEAM HEATING ON FAT RELATED CHANGES IN RECONSTITUTE MILK AND FRESH MILK

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EFFECT OF SUPERHEATED STEAM HEATING ON
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AND FRESH MILK

By

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<td>Microgram</td>
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<td>Redness</td>
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<td>AOAC</td>
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<tr>
<td>b*</td>
<td>Yellowness</td>
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<tr>
<td>CCD</td>
<td>Central Composite Design</td>
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<td>DOE</td>
<td>Design Of Experiment</td>
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<td>DPPH</td>
<td>1,1-Diphenyl-2-Picrylhydrazyl</td>
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<tr>
<td>FAME</td>
<td>Fatty Acid Methyl Ester</td>
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<tr>
<td>FOMP</td>
<td>Reconstituted fortified milk powder</td>
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<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>GC</td>
<td>Gas Chromatography</td>
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<tr>
<td>GC-MS</td>
<td>Gas Chromatography–Mass Spectrometry</td>
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<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
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<td>International Unit</td>
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<tr>
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<td>Kilogram</td>
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<tr>
<td>Kcal/mol</td>
<td>Kilocalorie per mole</td>
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<td>L*</td>
<td>Lightness</td>
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<td>meq</td>
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KESAN PEMANASAN STIM PANAS LAMPAU KE ATAS PERUBAHAN BERKAITAN LEMAK DALAM SUSU CAMPURAN DAN SUSU SEGAR

ABSTRAK

Susu serta produk tenusu memainkan peranan penting bagi keperluan nutrisi manusia, dan menjadi sumber nutrisi utama di kebanyakan negara. Lemak dan protein merupakan kandungan penting di dalam susu, dan ianya boleh dipengaruhi oleh beberapa faktor seperti bahan makanan, usia lembu dan pemprosesan haba. Susu merupakan elemen penting yang digunakan dalam penyediaan pelbagai jenis makanan contohnya pastri, pai, coklat dan kek serta ia berupaya meningkatkan ciri-ciri makanan seperti tekstur, warna, dan rasa. Dalam kajian ini, mod stim panas lampau (SHS) telah digunakan untuk memanaskan tiga jenis susu lembu iaitu, susu tepung campuran semula penuh (RWMP), susu tepung campuran semula diperkukatan omega 3 & 6 (FOMP), dan susu mentah, pada suhu dan tempoh masa tahanan yang berbeza. Kondisi suhu SHS untuk RWMP dan FOMP diselaraskan pada 120-180 °C selama 5-15 min manakala susu mentah adalah 130-150 °C untuk 30-60 saat. Kajian dijalankan terhadap perubahan warna \((L^*, a^* dan b^*)\), kelikatan, ketumpatan, pH, indeks pengoksidaan lemak, profil asid lemak, pengisomeran asid lemak, vitamin, antioksidan, mikrobiologi dan sebatian merup. Pemerhatian juga dilakukan terhadap kondisi optimum pemanasan oleh SHS terhadap susu mentah, dan dibandingkan dengan kaedah gelombang mikro serta kaedah pemanasan konvensional. SHS mempengaruhi perubahan yang signifikan terhadap warna dan ciri-ciri fizikal RWMP dan FOMP. Walaugaimanapun, indeks pengoksidaan lemak adalah stabil pada 120-150 °C selama 5 minit dengan sedikit perubahan terhadap
EFFECT OF SUPERHEATED STEAM HEATING ON FAT RELATED CHANGES IN RECONSTITUTE MILK AND FRESH MILK

ABSTRACT

Milk and dairy products play main role in human nutrition, which places it first in human nutrition in many countries. Fat and protein are the most important ingredients in milk and can be affected by several factors such as feeding, cow’s age and heat processing. Milk is used as a main ingredient to prepare many types of foods, such as pastries, pies, chocolates and cakes, and can help to improve the overall food characteristics such as texture, colour, and flavour. In this study, superheated steam (SHS) mode was used to heat three types of cow’s milk reconstituted whole milk powder (RWMP), reconstituted omega-3&6 fortified milk powder (FOMP) and raw milk using different parameters temperature and holding time. The SHS heat condition of the RWMP and RFMP were 120-180 °C for 5-15 min, and the raw milk was 130-150 °C for 30-60 second. The changes in colour ($L^*$, $a^*$ and $b^*$), viscosity, density, pH, lipid oxidation index, fatty acid profile, isomerization fatty acids, vitamins, antioxidant activity, microbiological and volatile compounds were investigated. The study also included optimizing the best conditions of raw milk, heated by SHS and comparison with different heat treatment (microwave and conventional) methods. SHS significantly affected on colour and physical properties of RWMP and FOMP. However, the lipid oxidation index showed stable to degradations at 120-150 °C for 5 min with slight changes at high temperatures and long holding time. From the changes in fatty acids profile, SHS indicated stability relevant to the degradation MUFA and PUFA at the same condition mention above with no isomerization was detect in the SHS heat treatment.
to the both of RWMP and RFMP. The vitamins (A retinol, C ascorbic acid and E α-tocopherol) and antioxidant activity (% inhibition of DPPH radical) showed decrease the values with increase the temperatures and holding time. The volatile compounds responsible for the off-flavour such as hexanal, 2-hexanal were not detected in SHS. Effect of SHS heat treatment of the raw milk also indicates no significant effect on the colour and physical properties except slight changes in viscosity. However, the result did not show any significant differences with respect to lipid oxidation, fatty acid profile, antioxidant activity and volatile compounds. On the milks microbiological quality, SHS fully inactivated pathogenic bacteria (total coliform and *Staphylococcus aureus*) with reduction in the total bacterial count from $15.33\times10^4$ - 63 (cfu. ml$^{-1}$). Response surface methodology was used to optimize the best conditions of SHS, the result indicate SHS 130 °C for 10 Sec to be the best optimal heating conditions of raw cow’s milk. The comparison between SHS optimum condition with microwave and convention methods was also investigated. The study showed the best with SHS heat treatment to be better than the convention and microwave methods.
CHAPTER 1
GENERAL INTRODUCTION

1.1 Background information

Milk and other dairy products play important role in the human nutrition. They are used as the raw products (milk) or supporting with other foods like cakes, pastries, pies, cookies, etc to improve the characterization of the food (such to flavour, texture, colour and nutrition) (Meshref, 2008). Milk is nutritive rich product, general composition of which includes all the essential minerals needed for human nutrition. However, cow’s milk cannot be consumed directly as the raw product due to contain high level of bacteria such as coliform and Staphylococcus aureus. This makes it necessary to adopt processing to inactivate the pathogenic bacteria and reduce the total bacteria count to the acceptable number for the safety of consumers (Brinez et al., 2007; Topeka et al., 2006). On the other hand, using milk for preparing foods also subjected to the high temperatures and long holding time, which induce changes in the physiochemical properties, especially in milk lipids, thus resulting in unacceptable product (Aguilar and Ziegler, 1995). Milk fat, is one of the main composition in the milk include 400 types of fatty acids. However milk fat also includes anticarcinogenic compounds like conjugated linoleic acid (CLA), sphingomyelin and butyric acids. Degraded milk fat by heat processing can be present undesirable physiochemical changes especially during the oxidation steps resulting undesirable flavoured and less nutritional value (Herzallah et al., 2005).

The oxidative stability of milk and dairy products its very important in dairy industry. Oxidation processes in milk can present strong off-flavour and deterioration the nutritional quality of milk (Smet et al., 2008). Martinez-
Monteagudo and Saldana (2015) demonstrated heating fatty acid during conventional heating can be directly affect especially on the conjugated Linoleic acid (CLA) which can reduce to 80 % when treated the milk at high temperatures at long holding time. On the other hand, Martinez-Monteagudo et al (2015) found increase in the oxidative indicators in UHT milk at temperatures between 120-145 °C (with holding time of 2-20 second). The increase in the hydroperoxides value was also found owed to the increase in free radicals. Pasteurization and UHT milk can provide extended shelf life estimated to be about 15 days at 6 °C and 180 days at room temperature, respectively. During this holding time the primary lipid oxidation which can increase in the storage due to decompose the hydroperoxides to the secondary oxidation products responsible on the off-flavour. The thermal convention heating type can also decrease the nutritional value of milk, thus compromising the purpose of adding milk to the food (Hotchkiss et al., 2006). High temperature ranges can break the double bond between carbon atoms and can produce free radicals; and these free radicals can combine with the oxygen in the propagation step of oxidative reaction to produce hydroperoxide, which are unstable and can decompose into simpler off-flavours causing compounds (e.g., aldehydes, ketones, or alcohol) that are volatile (Botosoa et al., 2013). Reactive oxygen species can produce hydroxyl and peroxy radicals, which are considered essential in the oxidation process of lipids (Karppi et al., 2007). Fatty acids consist of hydrocarbon chains and high degrees of unsaturation and carbon chain length; various classifications of fatty acids include saturated fatty acids (SFA), cis monounsaturated fatty acids (MUFA), and cis polyunsaturated fatty acids (PUFA) (–n-3 and –n-6 fatty acids) (Bansal et al., 2009; Liu et al., 2007; Priego-Capote et al., 2007). Even exposure of lipids to high heat treatment between 150 °C to 180 °C by cooking with other foods in the presence of
oxygen and water may lead to several chemical reactions (such as hydrolysis, oxidation, polymerization, isomerization, and cyclization), which can change the composition of fatty acids and produce volatile and non-volatile compounds affecting the sensory, functional, and nutritional values (Ackman, 1988). Deep exposure to heating in the presence of oxygen can lead to loss of unsaturated fatty acids during the oxidation process (Chen et al., 2001). Enriched polyunsaturated fatty acid such as C18:2 causes decrease in iodine value by thermo-oxidation leads to distortion of double bonds by oxidation, scission, and polymerization (Choe and Min., 2007). Polyunsaturated fatty acid (PUFA) can be significantly higher than saturated fatty acid (SFA) in oxidation when treated at high temperatures in the presence of oxygen because many double bonds can attach with oxygen molecules to produce hydroperoxide, a basic compound in fatty acid degradation (Wolff and Nourooz-Zadeh., 1996). Previously, Penumetcha et al. (2000) reported that oxidized linoleic acid can be absorbed efficiently inside the intestine leading to problems in the human body. Oxidative stress is primarily dependent on the presence of oxygen that can be produced by hydroperoxide, which is one of the important products from fat degradation. Hydroperoxide is produced by secondary oxidation, which is responsible for the development of off-flavours (Coupland and Mcclements., 1996). The mechanism of lipid oxidation involves the deterioration of the original structure of fatty acids, particularly unsaturated fatty acids. Oxygen, temperature, and light are the catalysts of lipid peroxidation. Oxygen is important in lipid peroxidation because it is responsible for the production of free radicals that break the double bonds of unsaturated fatty acid, which leads to primary lipid oxidation, secondary lipid oxidation, and off-flavour development (Stapelfeldt et al., 1997).
The most important vitamin affected by heat treatment in milk and milk product is vitamin C and its can be decreased to the half by during the heat process followed by its vitamin B₂ (Flavin) and can be distraction among the 30 % (Oamen et al., 1989). While some of the authors reported loss in vitamin A, B₂ and C in cow’s milk processed by microwave oven (Sieber et al., 1996). The conventional heat treatment of milk has been affected on the soluble water, vitamin especially vitamin C which loss between 20-25 % of pasteurization, milk with whole destroy in sterilization milk and this distraction occur by dehydroascorobic acid action between the vitamin C and the oxygen (Rolls and Porter., 1973). Superheated Steam is a clear, colourless gas obtained by heating ordinary steam at 100°C to a higher temperature under normal pressure (Mujumdar., 1995; Zzaman et al., 2014). In temperatures above the boiling point, steam becomes unsaturated steam or the superheated steam (Karimi., 2010). Superheated steam was discovered before 100 years ago, but not developed until 30 years ago especially in energy efficient (Anke et al., 2010; Li et al., 1999). In industries, superheated steam is applied because of its numerous advantages and its ability to develop desirable characteristics in food. Features, which is characterized by superheated steam made it widely used for dehydration and processing foods such as herbs, lumber, paper, coal, sugar-beet, pulp, tortilla, chips, shrimp, vegetables, potato and spices (Pronyk et al., 2004). One of the important advantage in superheated steam is that there is no oxygen available during the drying or processing unlike that of other thermal methods such as hot air drying or direct heat treatment which results in non-oxidative or combustion reactions that occur during the processing. Hence, lack of oxygen in the processing can play an important role to improve the product quality making it widely used to dry many products continent high fat (Abdulhameed et al., 2014; Zzaman et al., 2013; Zzaman and
Yang, 2013a, 2013b). Addition, less of oxygen can be reduce risks resulting from explosions (Head et al., 2010). Advantage of superheated steam application in many process is documented, such as those in cooking, sterilization and drying (Karimi., 2010; Prachayawarakorn et al., 2002; Zzaman and Yang, 2013). The aim of the present study was to study effect of superheated steam on physical and lipid stability of reconstituted whole milk powder, fortified milk with omega-3&6 and raw milk. This study also focused on fat related changes such as vitamins and antioxidant activity during the heat processing. On the other hand, diagnosis changes in side reaction that’s can be occur in heat processing such as the changes in isomerization of unsaturated fatty acids and volatile compounds to preview the quality and nutritional value of the end product. For the raw milk also investigated total bacteria count, coliform and Staphylococcus aureus. Moreover, Apply the response surface methodology to optimum the best condition of SHS to process the raw milk and compare the result with microwave and convention heating methods base on physcochemecal changes, lipid oxidation index, vitamins changes, antioxidant activity, microbial and volatile compound changes.

1.2 Problem statement

1- Heating milk by conventional methods affects the physicochemical properties of milk sample resulting in undesirable reactions and less acceptable to the consumers.

2- Heating milk using conventional methods can increase the lipid oxidation indicators by increasing the hydroperoxides owed to the presence of oxygen.

3- Conventional heating of milk can present undesirable volatile compounds such as aldehyde and ketone groups that can develop even during the storage conditions resulting in shorter self-life as well as produce off-flavour.
4- Conventional methods can affect vitamins especially those related with lipid oxidation.

5- The changes in fatty acids can occur in conventional heating which can affect the milk quality.

1.3 Objectives

The main objective of this study was to study the effects of superheated steam on fat and fat related changes in different types of cow’s milk. Hence the specific objective of this study are as follows:

1- To evaluate the changes in physicochemical analysis, oxidation stability, fatty acids composition, vitamins, antioxidant properties and volatile compounds of reconstitute whole milk powder.

2- To evaluate the changes in physicochemical analysis, oxidation stability, fatty acids composition, vitamins, antioxidant properties and volatile compounds of reconstitute omega 3 & 6 enriched milk powder.

3- To evaluate the changes in physicochemical analysis, oxidation stability, fatty acids composition, antioxidant properties, microbial properties and volatile compounds of raw cow’s milk.

4- To optimize the SHS heating conditions of raw milk based on the lipid oxidation, antioxidant activity and total microbial counts, on using superheated steam.

5- To compare the SHS optimize condition with two heating methods, microwave and convention based on fat related changes and microbiological quality.
CHAPTER 2
LITERATURE REVIEW

2.1 Cow’s milk

2.1.1 Cow’s milk history

Milk is an important product produced by mammalians. As it is the substance produced to feed the mammalian infant. All types of mammals from the human to the whales can produce milk for this purpose. Many centuries ago, perhaps as early as 6000-8000 BC, ancient man learned to domesticate species of animals for the provision of milk to be consumed by them. These included cows (genus Bos), buffaloes, sheep, goats, and camels, all of which are still used in various parts of the world for the production of milk for human consumption. The role of milk in the traditional diet has varied greatly in different regions of the world. The tropical countries have not been traditional milk consumers, whereas the more northern regions of the world, Europe (especially Scandinavia) and North America, have traditionally consumed far more milk and milk products in their diet. In tropical countries where high temperatures and lack of refrigeration has led to the inability to produce and store fresh milk, milk has traditionally been preserved through means other than refrigeration, including immediate consumption of warm milk after milking, by boiling milk, or by conversion into more stable products such as fermented milks (Goff, 2011).

2.1.2 Milk composition

Cow’s milk is one of high nutrition sources in the nature that includes the all essential compounds required for daily human nutrition. The composition of milk
can vary based on the cow’s breed, climate and stage of lactation, which can give a different concentration of milk compounds. The cow’s milk is an emulsion and contain fat as the globules form taken range between 1–10 micron. The nature temperatures affected on the fat globules such as 45 °C, the fat take the liquid phase, but if the temperatures decrease, the fat will be starting to crystallize but this process it’s not make changes in characterization milk fat where quickly returning to normal face when exposed to heat again. The second main compound in cow’s milk its proteins and its play main roll to the nutrition. Milk protein can be divided into the two faction: (a) the casein fraction and (b) the whey protein fraction. The casein fraction is complex, and exists in micelles with a size range of 30–300 nm and is very sensitive to the heat treatment (Lewis and Deeth., 2008). Casein fraction can be affected by several factors include heating, ionized calcium and pH. In general, the casein is more stable to the heat treatment of the whey protein and the high quality of milk can be with stand to 140 °C for 10 min.

Casein milk can be classified into the various types such as alpha s1-casein, alpha s2-casein, beta casein, kappa casein and gamma casein and its take different concentration in the milk. The highest concentration its alpha s1 casein followed by beta casein make up 32.4% and 26.1 % of the total protein. However, the second part of the milk protein could whey protein and its also can be classified into two types (Lewis and Deeth., 2008). Alpha lactalbumin and beta lactoglobulin make up 3.6% and 9.8 % from the total protein in milk. The third important compound in milk is lactose and it is the disaccharide made up by link two units of monosaccharides glucose and galactose. The proportion of Lactose in cows milk range between 4.8-5.2 %. Milk lactose is not sweet as sucrose and its can be hydrolyzed by β -D-galactosidase (lactase). Milk Lactose can be also fermented to the lactic acid by
lactic acid bacteria which considered as beginning of many fermented dairy products. Vitamins are organic substances that help in many metabolic processes and its can be found in many products in the nature.

Milk has two type of vitamins soluble fat vitamins include A, D, E, K and water soluble vitamins includes B1 – thiamine, B2 – riboflavin, B6 – pyridoxine, B12 – cyanocobalamin, niacin and pantothenic acid. However, vitamin C found in small amounts in milk estimate 20 mg per litter milk and is an insignificant amount relative to human needs while 20 % is destroyed by pasteurization process (Gutierrez, 2014). Cows milk also has essential minerals to the human diets. The 22 of minerals detected in cows milk included Sodium, Potassium, Chloride, Calcium, Magnesium, Phosphorus, Iron, Zinc, Copper, Manganese, Iodine, Fluoride, Selenium, Cobalt, Chromium, Molybdenum, Nickel, Arsenic and Aluminum (Goff., 2011; Lewis., 1994).

2.1.3 Milk fat

Milk fat is one of the distinctive types of fat in nature and has a unique flavour that distinguish it from the rest of the fat (Buldo., 2012). Milk fat consist range from most of cow’s milk 3-6% (Janssen and Macgibbon., 2007). Milk fat has been found as globule in milk. Milk fat globule distribution between 0.1-12 μm with 4.5 μm as average in the many type of milk and this distribution depends on the breed of animal as shown in Fig 2.1 (Wiking., 2005). Triacylglycerol is the basic formation of milk fat which consists ester derived glycerol with three fatty acids. The fat globules in milk consist of a triglyceride core, surrounded by a thin membrane, called the milk fat globule membrane (MFGM). This membrane, about 10–20 nm in cross-section, acts as an emulsifier and protects the globules from coalescence and enzymatic
degradation. The fat milk globule membrane consist approximately 90% from polar lipid with protein and the other things includes cholesterol, glycoprotein, enzymes and other compounds as showed in Fig 2.2 (Dewettinck et al., 2008). One of the most important protein in MFGM is mucin, xanthine oxidase/dehydrogenase, butyrophilin, adipophilin and periodic acid schiff glycoprotein (Dewettinck et al., 2008; Keenan and Mather, 2006). The polar lipid in fat milk globule membrane consist from glycerophospholipids, such as, phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine(Sanchez-Juanes et al., 2009).

Figure 2.1 Milk fat globule size distribution from Danish Holsteins and Jersey cows (Source: Wiking, 2005).

Figure 2.2 Milk fat globule membrane (Buldo, 2012).
Milk fatty acids originate either from microbial activity in the rumen, and transported to the secretory cells via the blood and lymph, or from synthesis in the secretory cells. The main milk lipids are a class called triglycerides which are comprised of a glycerol backbone binding up to three different fatty acids. The fatty acids are composed of a hydrocarbon chain and a carboxyl group. Milk fat contains approximately 400 different types of fatty acid that makes it the most complex of all natural lipids. The structural composition of fatty acids are composed of a hydrocarbon chain and a carboxyl group and the highest amount of fatty acids in milk approximately 70% as saturated bonds and 11% of milk fat its short chain fatty acid. Mono-unsaturated fatty acids consist 25% and poly unsaturated fatty acids consists 2.3% include omega-3 and omega-6. Saturated fatty acids such as myristic, palmitic and stearic acid bonds between the carbon chain saturated type and mono-unsaturated fatty acids such as oleic acid has is the most one double bond. While, the poly unsaturated fatty acids such as Linoleic acid and Linolenic acid consist more than two unsaturated bonds between carbon chain of these fatty acids. From the total saturated fatty acids in cows milk palmitic acid (16:0) is most important fatty due to increase its percentage in milk fatty acids approximately 30% of total fatty acids follow up the stearic acid (18:0) and myristic acid (14:0) acid which consist 11 and 12% from total saturated fatty acids. From the mono-unsaturated fatty acids in cows milk the oleic the major one with 23% of the total mono-unsaturated fatty acids. The remaining ratio about 2.3% its unsaturated fatty acids and it is distributed between linoleic acid (18:2) and α-linolenic acid (18:3) accounting to 1.6 and 0.7% by weight of the total fatty acids (Mansson, 2008; Goff, 2011). Milk fat has a wide melting range, and is fully melted at 104°F (40°C). Normally pasteurization conditions does not have an affect on the functional and nutritional properties of milk fat but increase
heat treatment may stimulate oxidation reactions and cause fat deterioration and off-flavours specially with present the oxygen. The high heat treatments such as ultra-high temperature UHT can disrupt the milk fat globule membrane proteins and destabilize the globules resulting deterioration milk fat (Herzallah et al., 2005).

2.2. The heating operation

2.2.1 Milk fat heat operation
In the process of cooking, many food requires temperatures higher than the boiling point of water in range between 120-180 °C depend type of food (Farkas et al., 1996). This temperature can causes changes in physical and chemical properties in composition of food prepare that may cause undesirable flavour in food prepared that outline up by Fritsch (1981) in the mechanism of heat treatment on lipids as shown in Fig 2.3. When expose a food to heating, the heat transfers to middle of food hyphen to all its parts, including fat in this case the many reaction will be do in the fat which was the need to heat as an adjunct to complete the interaction which associated with many other factors such as presence air and oxygen absorption which lead to breakdown the fatty acids from the triglycerides and decrease the nutrition value of fats such as oxidation steps (Blumenthal, 1991).

2.2.2 Chemistry of thermal processing
Although the use of heat is an easy way to prepare food, it may be possible reason for the changes, it is desirable or undesirable. The degradation of fat and less nutritional value of food normally occur by high heat processing with presence molecule oxygen due to many chemicals reaction even the process (Gertz, 2001). During processing under the high temperature many changes occurs and this can be classified as three steps oxidative changes (this step can due by atmospheric oxygen
that be found around the food even food processing). Hydrolytic changes (this step can be due by water vapors from the food under food processing by high temperature). Thermal changes (this step can be due by keep the food for a long time under high temperature).

Even in this three steps many changes in lipid compounds occur and which can be related to changes in colour, texture, test, and flavour, all of these changes are responsible for off-flavour in milk fat (Gertz, 2001; Takeoka et al., 1997). Oxidation of unsaturated fatty acids starts to form free-radical, producing hydroperoxides (RO$_2$H), which are the primary oxidation products. The hydroperoxides are very unstable and quickly decompose to form secondary oxidation products, such as aldehydes, alcohols or hydrocarbons (Toledo, 2005).
Figure 2.3: Principle events of fat during exposure to heat. (Adapted from Source Fritsch, 1981).
The second reaction that is also responsible of off-flavour with decrease the nutrition value of fat milk is hydrolytic reaction cause cleavage of ester bound in the fat milk and lead to formation free fatty acids with di and mono acyl glycerides. The products of three reactions steps are shown in Table 2.1:

Table 2.1 Changes in lipids during thermal processing. Adapted from (Toledo, 2005)

<table>
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<th>Steps of changes</th>
<th>Causative</th>
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<td>Atmospheric oxygen</td>
<td>• Oxidized monomers, dimers and polymers</td>
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<tr>
<td></td>
<td></td>
<td>• Non polymer, dimers and polymers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Volatile compounds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Aldehyde, ketone, alcohol, acids, etc.)</td>
</tr>
<tr>
<td>Hydrolytic reaction</td>
<td>Moisture</td>
<td>• Free fatty acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Monoglycerides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Diglycerides</td>
</tr>
<tr>
<td></td>
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<td>• Glycerol</td>
</tr>
<tr>
<td>Thermal reaction</td>
<td>Temperature</td>
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<td></td>
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<td>Dimers and polymers</td>
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2.3 Oxidative reaction

2.3.1 Oxidative reaction

Even during heat processing, the temperature range is one of the main factors that play a important role to accelerate the reaction between oxygen and lipids leading to the formation of various degradation product. The Thermo-oxidation process is an autoxidation can be proceeds by mechanism of free radical which divided to the three main steps initiation, propagation, and termination step. During heat processing to the any foods include high concentration of fat there are some changes will occur in the strictures of fatty acids make it undergo to the external changes and produce different compounds. As mention earlier, during heat processing in the initiation step alkyl radical will form by remove one hydrogen from carbon atom in the fatty acid. Many catalyzed such as temperature, light, and metal effected in initiation step as energy carrier (St. Angelo et al., 1996). The free radicals formation required amount of energy to remove hydrogen atom which its take different energy depend it position on the carbon chain. For example, remove one hydrogen from C11 of the linoleic fatty acid required amount of energy estimate as 50 (Kcal/mol) from its position of fatty acids while remove same hydrogen atom but from C8 or C14 to the seam fatty acid require 75 Kcal/. However, the energy required to remove one hydrogen atom from saturated carbon bonds such as C17 and C18 in linoleic acid about 100 (Kcal/mol) (Aladedunye, 2011). On propagation step, the free radicals formation in initiation steps can be react with oxygen to form the peroxy radical which it is unstable also rapidly abstract hydrogen atom from another free radicals to produce the hydroperoxides (Choe and Min, 2006). The lipid hydroperoxides are considered primary products to the oxidation reaction rapidly decompose by heat processing to the other product such as aldehydes, alcohols, ketones, lactones, esters,
acids, hydrocarbons and acids which considered volatile compounds responsible on the off-flavour in oil and lipids Fig 2.4. Lipid oxidation products as mentioned above produces ‘off-favour’ via volatile oxidation products, while the saturated and unsaturated aldehydes such as hexanal, 2-hexanal, 2,4-decadienal, 2,4-octadienal, 2,4-nonadienal, 2-heptenal and 2-octenal, are the major compounds responsible on the off-flavour in oil and lipids. For instance, the grass-like off-flavour in oxidized soybean oil has been attributed to hexanal and 2t-hexenal responsible on the grassy flavour while the 1-octen-3-one and 2t,4c,7t-decatrienal are responsible on fish off-flavour (Min and Bradley, 1992). For the further study on the volatile compounds responsible on the off-flavour the hexanal, pentanal, pentane and 2,4-decadienal were used as indicators to follow up the oxidation degradation of oils and lipids (Toyosaki et al., 2008). These researchers found an excellent correlation between PV, peroxide value, and flavour. Volatile oxidation compounds are responsible on different off-flavour in food processing which corresponding characteristic flavour as the listed in Table 2.1 can be impacted on food acceptable form the consumers (Aladedunye, 2011).
Figure 2.4 Oxidative reaction and representative products (Aladedunye, 2011).

The secondary oxidation products is more important to indicate the off-flavours in milk samples due to the presence of aldehydes and ketone volatile compounds responsible on the off-flavours in foods. The main reason that makes aldehydes and ketones groups are responsible on the off-flavour return to have low sensory threshold can be ranged between 0.002 - 1.50 (mg/kg) to the aldehyde and 0.4-80
(mg/kg) to the ketone in milk (Badings, 1984). Homolitic cleavage of the hydroperoxides can be identifies the type of aldehyde to be saturated or unsaturated aldehydes. The saturated aldehyde has flavour can descript as pungent, fatty, tallow and green while the unsaturated aldehyde featuring scents as fatty, fruity, sweet and green flavours (Gutierrez, 2014). However, the aldehyde compounds are responsible for the metallic and mushroom flavours in oxidized Table 2.2 (Gutierrez, 2014).

Table 2.2 Characteristics of individual volatiles formed from oxidative degradation of oils and fat (Source: Aladedunye, 2011).

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Reported odor descriptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentane</td>
<td>-</td>
</tr>
<tr>
<td>Hexane</td>
<td>-</td>
</tr>
<tr>
<td>Butanal</td>
<td>-</td>
</tr>
<tr>
<td>Pentanal</td>
<td>Painty, herbal</td>
</tr>
<tr>
<td>Hexanal</td>
<td>Fatty, green, fruity, cut grass, herbal, rancid, painty, crushed weeds.</td>
</tr>
<tr>
<td>Heptanal</td>
<td>Weeds, green, sour, sweaty, herbal, painty, rancid.</td>
</tr>
<tr>
<td>Octanal</td>
<td>Lime, grassy, citrus, sharp, heavy, candle like, crushed weeds.</td>
</tr>
<tr>
<td>Nonanal</td>
<td>Green, soapy, rubbery, beany.</td>
</tr>
<tr>
<td>Decanal</td>
<td>Fruity, candle-like.</td>
</tr>
<tr>
<td>2-pentenal</td>
<td>-</td>
</tr>
<tr>
<td>3-Hexenal</td>
<td>Green, apple-like.</td>
</tr>
<tr>
<td>2-Heptenal</td>
<td>-</td>
</tr>
<tr>
<td>2-Nonenal</td>
<td>Green, fatty, tallow.</td>
</tr>
<tr>
<td>2-Decenal</td>
<td>Metallic.</td>
</tr>
</tbody>
</table>
Hydroperoxides are formed in propagation steps of lipid oxidation, and these are unstable compounds which can be readily broken down to form nonvolatile and volatile products. Hydroperoxides decomposition includes many products such as aldehydes, ketones, hydrocarbons, lactones, alcohols, esters, acids and furans. During the propagation oxidation step hydroperoxides forms by alkoxy and hydroxy radical through homolytic cleavage of (OOH) group. However, the alkoxy radical can be undergoes to β-scission between the carbon atoms C-C bond resulting aldehyde and alkyl with vinyl radical (Azzara and Campbell, 1992). The alkyl or vinyl radical can then react with aldehydes, ketones, or alcohols in a general sense to produce volatile off-flavour compounds. The mechanism of decomposition of hydroperoxides are of numerous types, but the general form can be summarizing in Fig 2.5 (Azzara and Campbell, 1992).
Figure 2.5. Formation of aldehydes, alcohols, and ketones from the decomposition of hydroperoxides (Source: Azzara and Campbell, 1992).

From the hydroperoxides products, the aliphatic aldehydes which considered the most important due to take highest contributors for unpleasant odors and flavours in food products. The polyunsaturated acids such as oleic, linoleic, linolenic, and arachidonic are the most important sources to formation the aldehyde compounds in milk products and lipids (Forss and Sugisawa, 1981; Shiota, Takahashi et al., 2004). Aldehydes formation from the autoxidation can also undergo further reactions and contribute to the flavour of dairy products. However, saturated aldehydes such as nonanal can be oxidize to the acid Table 2.3 present off favour compounds from the origin sources (fatty acids) (Shiota et al., 2004; Shipe and Charalambous, 1980).
Table 2.3 Possible origin to some aldehydes obtained from the oxidation of oleic, linoleic, linolenic, and arachidonic acids.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Hydroxide Position</th>
<th>Aldehyde Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>C11</td>
<td>Octanal</td>
</tr>
<tr>
<td></td>
<td>C8</td>
<td>2-undecenal</td>
</tr>
<tr>
<td></td>
<td>C9</td>
<td>2-decenal</td>
</tr>
<tr>
<td></td>
<td>C10</td>
<td>Nonanal</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>C13</td>
<td>Hexanal</td>
</tr>
<tr>
<td></td>
<td>C9</td>
<td>2,4-decadienal</td>
</tr>
<tr>
<td></td>
<td>C11</td>
<td>2-octenal</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>C16</td>
<td>Propanal</td>
</tr>
<tr>
<td></td>
<td>C14</td>
<td>2-pentenal</td>
</tr>
<tr>
<td></td>
<td>C12</td>
<td>2,4-heptadienal</td>
</tr>
<tr>
<td></td>
<td>C13</td>
<td>3-hexenal</td>
</tr>
<tr>
<td></td>
<td>C11</td>
<td>2,5-octadienal</td>
</tr>
<tr>
<td></td>
<td>C9</td>
<td>2,4,7-decatrienal</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>C15</td>
<td>Hexanal</td>
</tr>
<tr>
<td></td>
<td>C13</td>
<td>2-octenial</td>
</tr>
<tr>
<td></td>
<td>C12</td>
<td>3-nonenal</td>
</tr>
<tr>
<td></td>
<td>C11</td>
<td>2,4-decadienal</td>
</tr>
<tr>
<td></td>
<td>C10</td>
<td>2,5-undecadienal</td>
</tr>
<tr>
<td></td>
<td>C7</td>
<td>2,5,8-tridecatrienal</td>
</tr>
</tbody>
</table>
2.4 Main factors affecting on fat oxidation

Fat stability of an food can be affected by several factors but the important factors recorded significantly affected on fats or lipids oxidation in foods during the processing can be divides to two factors oxygen and temperatures.

2.4.1 Oxygen

Oxygen plays an important role in degradation of the fat during oxidation steps. As previously stated, free radicals (alkyl radical) formed in the initiation step of oxidation can be rapidly reacts with molecular oxygen resulting form the peroxy radicals. Oxidation reaction it depends on the concentration of oxygen available during the processing. Min and Wen (1983) mentioned that the oxidation reaction of oil take increase with increase the oxygen dissolving in oil sample. On the other hand, Przybylski and Eskin (1988) reported that the increase the amount of oxygen can be providing format peroxide value estimate by 10 (meq/kg). However, Fujisaki et al (2002) reported 22 volatile aldehyde compounds to be detected in sunflower oil during heat processing and the volatile concentration increase with increase the dissolving oxygen. Present concentration of oxygen with food processing also can be affected on the products format by fat degradation. Fujisaki et al (2002) have reported acetaldehyde to be the main compound from the carbonyl group formatted with oxygen concentration 2%. However, the nonanal and hexanal were formation with increase the oxygen concentration to 20 % of oil. Despite the poor solubility of oxygen at frying temperatures and steam blanketing, several factors are known to increase the availability of oxygen in the frying medium. Introduction of fresh food and oil into a frying medium increases the level of oxygen in the oil (Warner, 2004). During the thermal treatment the surface tension of liquid take decrease which can
lead to enhances accessibility of atmospheric oxygen by enlarging the surface area (Mezouari and Eichner, 2006). On the other side, free fatty acid can also decrease the surface tension resulting increase the propagation rate atmosphere oxygen in liquid food to such as oil (Aladedunye, 2011). Many applications were used in order to reduce the amount of oxygen during the food processing, Shyu et al (1998) work about reduced oxygen level to carrot slices fried by soybean oil, palm oil and lard by use vacuum system at 105 °C to 20 the fatty acid composition, Peroxide value, viscosity, acid value and total polar components are used to follow up the degradation of thermo-oxidative process. The results concluded lower thermo-oxidation by vacuum frying compare with typical atmospheric frying. The conclusion of them study was reduce the concentration of oxygen with processing food can help to maintain the quality of products from degraded by thermo-oxidation and deterioration fatty acids with produce undesirable flavour effected on acceptability product from consumers.

2.4.2 Temperature

Heat processing have a direct affect on the oxidation reaction though effected on the fatty acids even the oil or lipids. For instance the total energy estimates by 50 (Kcal/mol) needed to breakdown the carbonhydrogen bond in linoleic acid on the carbon atom C11. However, the oxygen bond of alkyl hydroperoxides need energy estimated to be 44 (Kcal/mol) to break it (Min and Boff, 2002). Increase the temperature during heat processing of foods can be enhances degradation of the hydroperoxides formation by oxidation reaction, from the other hand degradation of hydroperoxides resulting volatile and unvolatile compounds responsible on the off-flavours in foods (Aladedunye, 2011). Nawar (1984) reported that a peroxide value