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Using Isoconversional Methods to Study the Effect of Antioxidants on the Oxidation Kinetics of Milk Fat

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USING ISOCONVERSIONAL METHODS TO STUDY THE EFFECT OF ANTIOXIDANTS ON THE OXIDATION KINETICS OF MILK FAT

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This thesis is dedicated to my father, mother, wife, brothers, sisters, and all friends for their support and encouragement throughout my study.

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ABBREVIATIONS

A_i – Pre-exponential factor corresponding to a given reaction model

A - Pre-exponential factor

ABTS - Radical scavenging capacity assay

AMF - Anhydrous milk fat

ARP - Antiradical power

BHA - Butylated hydroxy anisole

BHT - Butylated hydroxytoluene

BHT-CHO - BHT alcohol and aldehyde

DPPH - 2,2-Diphenyl-1-picrylhydrazyl radical assay

DSC - Differential scanning calorimetry

DTG - Derivative thermogravimetry

E_a - Activation energy

E_α – Activation energy at a given degree of conversion

E_i – Activation energy corresponding to a given reaction model

FAs - Fatty acids

FRAP - Ferric reducing antioxidant potential method

GPC - Gel permeation chromatography

GSH - Contain glutathione

HO• - Hydroxyl radical

HOO• - Hydroperoxyl radical

ICTAC – Kinetics Committee of the International Confederation for Thermal Analysis and Calorimetry

k - Rate constant

KAS - Kissinger-Akahira-Sunose method

MEC - Mammary epithelial cell

MF- Milk fat

MFGM - Milk fat globule membrane

MPC 85 - Milk protein concentrate 85%

MPC56 - Milk protein concentrate 56%

MPC70 - Milk protein concentrate 70%

OFW - Ozawa-Flynn-Well

ORAC - Oxygen radical absorbance capacity method

OSI - Oxidative stability index

PG - Propyl gallate

PV - Peroxide values

R^2 – Coefficient of determination

R^2_{adj} – Adjusted coefficient of determination

RO• - Alkyl radical

RRS – Residual sum of squares

SBO - Soybean oil

SMP - Skim milk powder

SOD - Superoxide dismutase method

T.S. - Total solid

T_f – Temperature where the oxidation is completed

TAGs - Triacylglycerols

TGA - Thermos gravimetrical analysis

T_{on} - Onset temperature of oxidation

T_p - Peak maximum temperature of oxidation

T_s - Start temperature of oxidation

WMP - Whole milk powder

α - Degree of conversion

α-Toc - α-Tocopherol

β-Car – Beta carotene

$B(T)$ – Heat flow signal proportional to the baseline

R - Universal gas constant

$S(T)$ - Heat flow signal at a given temperature

b – Regression parameter of the compensation effect

$f(\alpha)$ - Reaction model

$g(\alpha)$ – Integral form form of the reaction model

β – Heating rate

θ – Generalized time

y_{cal} – Calculated degree of conversion

y_{exp} – Experimental data of degree of conversion

ABSTRACT

USING ISOCONVERSIONAL METHODS TO STUDY THE EFFECT OF ANTIOXIDANTS ON THE OXIDATION KINETICS OF MILK FAT

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Milk fat is a versatile ingredient because of its nutritional value, functionality, and flavor. During processing and storage, milk fat may undergo oxidation resulting in many undesirable changes such as unpleasant flavor and aroma, and formation of toxic compounds. A common practice to prevent the oxidation of milk fat is by the addition of antioxidants. It is worth to mention that little is known on the effect of antioxidants on the oxidation kinetics. In this work, the effect of selected antioxidants on the oxidation kinetics of anhydrous milk fat (AMF) under non-isothermal conditions was investigated. AMF with an addition of either butylated hydroxytoluene (BHT), α-Tocopherol (α-Toc), or β-carotene (β-Car) at four different concentrations (0.02, 0.07, 0.2, and 0.4%) were oxidized using differential scanning calorimetry (DSC) at different constant heating rates (3, 6, 9, and 12°C min^{-1}) in a temperature range of 100-400°C. DSC spectra were analyzed according to the Kissinger-Akahira-Sunose (KAS) method, from which the kinetic triplet was obtained (pre-exponential factor (A), apparent activation energy (E_a), and reaction model ($f(\propto)$). In general, the concentration of antioxidant increased the onset temperature of oxidation (T_{on}) when comparing to the T_{on} of AMF without antioxidant. The *Ea* values were 82.73 ± 5.51, 93.14 ± 6.24, 68.06 ± 3.52, and 58.51 ± 7.51 kJ mol^{-1} for AMF, BHT, β-Car, and α-Toc, respectively. Remarkably, the addition of 0.2% of α-Toc inhibited the oxidation reaction by 9-fold, judging the constant rate. The obtained kinetic parameters

were interpreted in term of oxidation mechanism. The oxidation of milk fat was best described by the Avrami-Erofeev model. The obtained kinetic triplet (Ao, Eo, and $f(\propto)$) was used to systematically evaluate the role antioxidants. The addition of antioxidants significantly delayed the oxidation, being more effective α-Toc followed by β-Car and BHT. The outcomes of this study may enable off-line simulation and development of a databank.

Chapter 1

Introduction and objectives

1. Significance of the research

Milk fat (MF) has traditionally used as an ingredient because of its nutritional value, functionality, and flavor. Formulations of infant formula, ice-cream, bakery products, and confectionery products are examples of using MF. During processing and storage, MF may undergo oxidation which results in many undesirable changes such as unpleasant flavor and aroma, and formation of toxic compounds (Fränkel, 1980). The oxidation of fat is a complex set of chemical reactions taking place at different rates. For simplicity, the oxidation of fat is to categorize it in three stages: i) initiation, ii) propagation, and iii) termination (Saldaña and Martínez-Monteagudo, 2013).

Numerous factors contribute to the development of oxidation including presence of certain enzymes, exposure to light and metal ions, and presence of reactive oxygen species (Choe and Min, 2006). A common used strategy to reduce the oxidation is by the addition of antioxidants, compounds with the ability to inhibit or delay the initiation and propagation of the oxidation. The chemical structure of the antioxidants strongly determines their efficiency in preventing the oxidation, and therefore improve the oxidative stability of fats and oils (Oh and Shahidi, 2018).

Chemical and volumetric methods have been developed for quantifying and monitoring the oxidation of fats and oils including oxidative stability index (OSI), peroxide values (PV), derivative thermogravimetry (DTG), and gel permeation chromatography (GPC) (Gray, 1978; Kamal-Eldin and Pokorny, 2005). Advantages and limitations of the different methods for evaluating the oxidation have been reviewed elsewhere (Kamal-Eldin

and Pokorny, 2005). As mention earlier, the oxidation of lipids comprised a set of chemical reactions occurring simultaneously at different rates. Thus, it is commonly assumed that the oxidation of lipids cannot be evaluate using a single method (Kamal-Eldin and Pokorny, 2005).

Alternatively, the oxidation of lipids can be studied by thermal differential techniques such as thermogravimetrical analysis (TGA) and differential scanning calorimetry (DSC). In these techniques, the mass loss or the heat released as the oxidation proceed is recorded and subsequently related to kinetic parameters of oxidation. Information of lipid oxidation by means of DSC have been reported for a number of lipid systems, such as soybean/anhydrous milk fat blends, unsaturated fatty acids (oleic, linoleic, and linolenic acids), saturated fatty acids (lauric, myristic, palmitic, and stearic acids), vegetable oils (canola, corn, cottonseed, and soybean oils) and genetically modified vegetables oils.

This thesis hypothesizes that the improvement of oxidative stability of AMF and milk powder formulated after additional of antioxidants could describe using the isoconversional method. The main goal of this thesis was to systematically evaluate the effect of different antioxidants on the oxidation kinetics of AMF and milk powder under non-isothermal regime. In this work, oxidation was measured by non-isothermal DSC, while the oxidation of milk powder was measured by means of TGA. The specific objectives were to:

- Assess the oxidation kinetic of AMF at different heating rates (3, 6, 9 and 12°C min^{-1}) in a temperature range of 100 to 400°C (chapter 3).

- Assess the oxidation kinetics of AMF added with either BHT, α-Toc, and β-Car at four different concentrations (chapter 3).
- Assess the oxidation kinetics of milk powder formulated at different heating rates (3, 6, 9, and 12°C min^{-1}) in a temperature range of 100 to 300°C (chapter 4).
- Assess the oxidation kinetics of milk powder formulated with an addition of either 0.2% α-Toc, 0.2% α-Toc and hydrogen peroxide, and hydrogen peroxide (chapter 4).

The collected results afford important kinetic information on MF can help to new possibilities for product development of milk-based and food goods. The isoconversional method shows the kinetic triplets (Ao, Eo, and $f(\propto)$)) of each reaction that can be known as a fingerprint of the reaction. Using this information can help to improve the oxidative stability lipids products and decrease the speed of oxidation reactions.

1.2. References

Choe, E., and D.B. Min. 2006. Chemistry and reactions of reactive oxygen species in foods. Crit. Rev. Food Sci. Nutr. 46:1–22.

Fränkel, R. 1980. Lip seal training in the treatment of skeletal open bite. Eur. J. Orthod. 2:219–228.

Gray, J.I. 1978. Measurement of lipid oxidation: a review. J. Am. Oil Chem. Soc. 55:539–546.

Kamal-Eldin, A., and J. Pokorny. 2005. Analysis of Lipid Oxidation. AOCS Publishing.

Oh, W.Y., and F. Shahidi. 2018. Antioxidant activity of resveratrol ester derivatives in food and biological model systems. Food Chem. 261:267–273.

Saldaña, M.D.A., and S.I. Martínez-Monteagudo. 2013. Oxidative stability of fats and oils measured by differential scanning calorimetry for food and industrial applications. IntechOpen.

Chapter 2

Literature review

2.1 Chemistry of milk Fat

Cow's milk is an oil-in-water emulsion containing about 3.3 g of fat per 100 g of milk. Some factors can vary the level of fat such as, a stage of lactation, energy balance, plasma insulin concentration and chance reproductive state (Palmquist et al., 1969; Bachman et al., 1988; De Vries and Veerkamp, 2000). MF is one of the essential compounds in milk and uses as an ingredient in food manufacturing due to nutrition values and flavor. In milk, the fat is emulsified by the milk fat globule membrane (MFGM) (Elías-Argote et al., 2013). Inside the emulsion, fat is present in the form of triacylglycerols (TAGs), diacylglycerols, monoacylglycerols, cholesterol, free fatty acids, and phospholipids accounting for 97.5, 0.36, 0.02, 0.31, 0.02, and 0.6% of the total fat, respectively (Fox et al., 1998; Muehlhoff et al., 2013).

TAGs are a main molecular form of MF containing three fatty acids (FAs) esterified to a glycerol backbone (**Figure 1**), while diacylglycerols, monoacylglycerols, free fatty

acids, polar lipids and sterols, and trace amounts of vitamins are present in MF as fat soluble material.

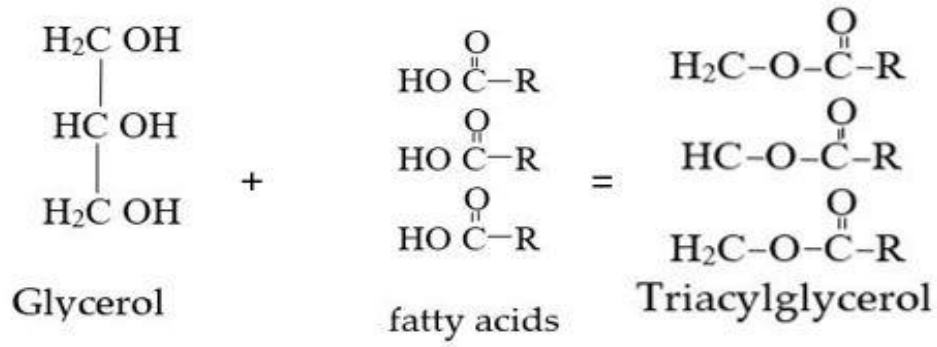


Figure 1. Schematic diagram of the triacylglycerol.

More than 400 individual FAs have been identified in MF, from which approximately fifteen FAs made up around 90% of the total MF (Lucey et al., 2017). **Table 1** shows a typical fatty acid profile of milk fat. FAs are characterized by the number of carbons and degree of saturation. **Table 1** shows FAs of carbon length ranging from 4 to 22.

Overall, FAs can be separated into two major categories: saturated and unsaturated. Saturated fatty acids are those which contain no double bonds as each carbon atom is surrounded by other carbon atoms and hydrogen atoms. These fatty acid molecules are joined in a zig-zag chain as there is freedom rotation about the carbon atoms due to the absence of double bonds. Unsaturated FAs contain carbon to carbon double bonds. These FAs are further classified as monounsaturated (one double bond), or polyunsaturated (more than one double bond). There are two possible configurations for the double bond. Double bonds for which the hydrogen atoms are on the same side of the chain are known as “cis

cursive:" double bonds and those where hydrogen atoms are at opposite sides of the bonds are known as "trans arise". The melting point of fatty acids depend on the chain length of the fatty acids and the degree of unsaturation of the molecule. Generally, the melting point of unsaturated *trans* fatty acids is greater than that of *cis* fatty acids of the same chain length. Milk fat approximately contains two-thirds of saturated FAs and a third of unsaturated FAs (30% monounsaturated and 4% polyunsaturated FAs) (Lucey et al., 2017). Some factors influence the concentration of FAs in MF including stage of lactation, energy balance, plasma insulin concentration, chance reproductive state, season of the year, genetics and cows nutrition (Palmquist et al., 1969; Bachman et al., 1988; De Vries and Veerkamp, 2000; Lock and Bauman, 2004).

The size of MF droplet is from 0.1 to 15 μm and around this droplet is a membrane which known as MFGM (Spitsberg, 2005). The MFGM is basically complex of lipids and protein with ratio 1:1 and some elements such as iron and copper (Kanno, 1990; Mather, 2000). MFGM is covered with three layers of phospholipid-protein which come from the endoplasmic reticulum and the apical membrane of the epithelial cell (Heid and Keenan, 2005).

Table 1. Typical fatty acid composition of milk fat (Martínez-Monteagudo et al., 2014).

Saturated Fatty acid	**Concentration (mg fatty acid g^{-1} fat)**	**Unsaturated Fatty acid**	**Concentration (mg fatty acid g^{-1} fat)**
C4:0	3.10	C18:1 C9	3.83
C6:0	13.72	C18:1 C11	18.91
C8:0	9.12	C18:1 n7	5.4
C9:0	0.22	C14:1	9.45
C10:0	20.44	C18:2	19.33
C11:0	2.64	C16:1 cis	3.23
C12:0	24.73	C18:3 n3	0.21

C14:0	88.80	C20:1 n12	1.33
C19:0	4.5	C20:1 n15	0.46
C15:0	10.51	C18:3 w3	3.94
C16:0	239.40	C18:2	5.30
C20:0	1.41	C20:2	0.31
C22:0	0.32	C16:1 trans	18.10
C18:0	91.90	C20:2 w6	0.94
		C20:4	1.10
		C20:5	0.33
		C22:3	0.21

2.2 Milk Powder

Milk has been used since antiquity and is still an extremely important food in many civilizations. Because milk is perishable, drying of milk is a common strategy for increasing its shelf-life for more than a year at room temperature while keeping its quality. Milk powder is obtained by removing water from milk, reaching a final moisture content between 3-5%. **Table 2** shows the approximate composition of some types of milk power (Skim milk powder (SMP), Buttermilk powder, Whole milk powder (WMP), Milk protein concentrate 56, 70, and 85 (MPC56, 70, and 85)). The main components of milk powder are carbohydrates (mostly lactose), protein, and fat. The concentration of these components is strongly influenced by the drying conditions, drying method, and storage conditions. Milk powder offers advantages during transportation, handling, processing, and product formulations (Sharma et al., 2012).

Table 2. Proximate composition (w/w %) of different types of milk powder. (United States Department of Agriculture-Agricultural Research Service, 2005; Singh and Singh, 2015).

Constituents	**Powder form**					
	SMP	**Buttermilk powder**	**WMP**	**MPC56**	**MPC70**	**MPC85**
Fat	0.6 – 1.25	4.5 - 7	26 – 28.5	1.3	1.4	1

Protein	34 - 37	32 – 34.5	24.5 – 27	57.1	70	85
Lactose	49.5 – 52	46.5 – 49	36 – 38.5	30.1	17	3
Moisture	3.5 – 4.5	8.3 – 8.8	2 – 4.5	3.8	4.4	4.4
Minerals	8.2 – 8.6	3 - 4	5.5 – 6.5	7.7	7.2	7.2

SMP – skim milk powder; WMP – whole milk powder; MPC – milk protein concentrate

2.3 Manufacture of milk powder

Milk powder is a versatile ingredient having numerous applications in food formulations. Ice cream, yogurt, cheese, infant formula, reconstituted dairy products, bakery, and confectionery goods are common examples of using milk powder. Depending on the intended use, the powder should meet specific requirements such as solubility, absence of cooked flavor, clotting properties, and satisfactory heat stability. One type of milk powder cannot reconcile all these desirable properties. Overall, the manufacture if milk powder involves numerous unit operations such as standardization, thermal treatment, evaporation and drying. The intensity of the heat treatment is used to classify the powders as low-, medium-, or high-heat. **Figure 2** illustrates a flow diagram for the manufacture of whole milk powder. The manufacturing involves numerous steps including pasteurization, concentration, homogenization (optional), and drying.

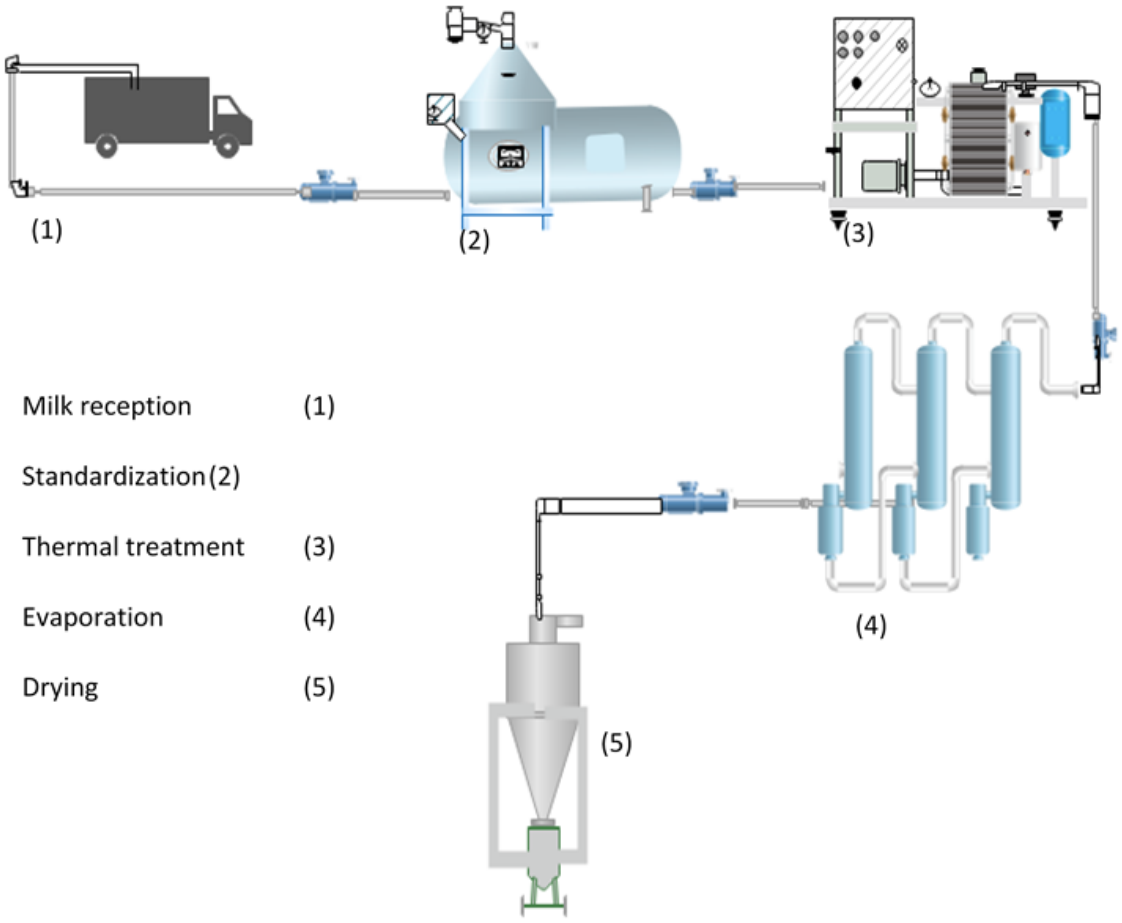


Figure 2. Schematic representation of the main manufacturing steps of Whole Milk Powder.

2.3.1. Clarification

After receiving the milk, it contains some impurities such as foreign particles, bacterial spores, dirt, hairs, and somatic cells that affect the quality of milk products. Thus, it is crucial to clarify the milk as soon the milk received to avoid any physical or chemical change on the milk. The milk clarification can be done by passing the milk through a rapidly rotating clarifier under pressure at 10°C using centrifugal separators (Singh and Singh, 2015). The main goal of clarification is to eliminate any foreign particles, bacterial

spores, dirt, hairs, and somatic cells in order to have milk powder with high microbiological quality. After that, the milk is cooled and storage at 4°C. In terms of milk powder, having milk with high bacteria counts can lowering the solubility of milk powder and increase the chance of oxidation reaction to occur (Carić, 1994).

2.3.2. Standardization

Numerous dairy industries are receiving milk from different sources that may have a different percentage of fat and non-fat compounds. This can lead to producing produces with different qualities. Thus, the standardization is a method that can be used to adjust total solids (T.S.) to achieve product specification. For manufacturing WMP, the primary purpose of standardization is to ration fat to TS or non-fat (Ipsen and Hansen, 1988). For manufacturing WMP, only protein is removed; however, fat, lactose, and salts are removed for manufacturing SMP (Singh and Singh, 2015). The MF should not contain more than 0.01 for manufacturing skim milk powder. The MF for SMP and WMP should be ≤ 1.5%, and 26 – 40 % in the final product, respectively.

2.3.3. Heat treatment

The preheating is a crucial step to destruct bacteria, inactivate enzymes (Ipsen, 1980). Moreover, the preheat treatment plays a vital role in the heat stability of milk that can increase after the treatment (Newstead et al., 1975). It also produces free SH groups that play as antioxidants (Farkye, 2006). During the preheating, the whey protein is denatured, formatted aggregates, and associated with casein micelles and MFGM (Singh and Creamer, 1991; Singh and Singh, 2015). Moreover, the soluble salts are transferred to

the colloidal phase, and pH is reduced (Singh and Singh, 2015). It can be either direct heating following by vacuum flash cooling or indirect heating (plat heating exchanger). Depending on the temperature and time was used in thermal treatment, the milk powder can be named: low heat milk powder (75 °C for 15 seconds), medium heat milk powder (75 °C for 1–3 minutes), or high heat milk powder (80 °C for 30 minutes or 120 °C for 1 minute) (Singh, 1992). The milk powder with low heat treatment is more likely to oxidize comparing to milk powder with medium and high heat treatments (Stapelfeldt et al., 1997). The common preheat temperature is from 80 to 90 C for 10 to 20 min (Singh and Singh, 2015). The preheating is known as the highest temperature that the milk achieved during milk powder manufacturing.

2.3.4. Evaporation

The evaporation is typically done under low pressure to decrease the boiling point temperature. The milk is evaporated between 50 to 70 °C to obtain 40 to 50% T.S for spray dryer and 30 to 35 % T.S for roller dryer (Singh, 1992). The temperature should be in this range in order to minimize the whey protein denaturation (Singh and Creamer, 1991). The concentrated milk should not keep it warm for a long time to avoid the microorganisms to growth (Walstra et al., 2005). During the increasing of T.S., the casein micelle size, colloidal salts are increased, which leads to a decrease in the pH (Singh et al., 2015). The pH of milk concentrated can be decreased from 6.7 to 6.3 at 45% T.S due to physical changing of calcium phosphate from soluble to colloidal state which increases the hydrogen ions (Singh and Singh, 2015). The primary purpose is to remove water to save the milk powder quality due to increasing of viscosity, which may make difficulty of

eliminating water from concentrated milk. It also helps to decrease the required energy of spray drying (Schuck, 2011).

On the other hand, the ultrafiltration is a method that can be used to concentrate milk which used in manufacturing high protein milk powders (EI-Gazzar and Marth, 1991). Thus, manufacturing MPC does not require heating treatment except pasteurization (Singh and Singh, 2015). The milk passes through the membrane that only allows water, lactose, non-protein compounds, and some soluble salt.

2.3.5. Homogenization

In 1899, the first homogenization in dairy industries was attributed to Augustus Gaulin of France. The homogenization plays a vital role in counteracting creaming, fat stability, and viscosity (Walstra et al., 2005). It can be done by pumping the milk concentrated through a small hole at a pressure of 103 to 124 bar (1500 to 1800 psi) (Partridge, 2008). The viscosity of milk concentrated is increased due to the movement of significant casein micelles to the fat globules confer the latter such an irregular shape as to raise the efficient volume fraction of fat globules plus casein micelles (Walstra et al., 2005). Moreover, the high viscosity may affect the efficiency of the drying step (Bloore and Boag, 1981). Thus, the viscosity of milk concentrated needs to be controlled. The concentrated milk is homogenized to achieve fat globules range between 0.2–2 μm (Singh et al., 2015). The size of fat globule effects on many factors such as type of homogenizer, homogenizing pressure, fat content, and temperature. The temperature of milk concentrated should be more than 37°C in order to ensure MF is in the liquid state (Partridge, 2008). The requirement of the homogenizing step depends on the fat content. It helps to increase the

number of fat globules. Also, it helps to increase the viscosity of concentrated milk (Walstra et al., 2005).

2.3.6. Drying

There are many types of dryers have been used in dairy, food, chemical and pharmaceutical industries, such as spray, roller, freeze, microwave, and steam dryer (Augustin and Smithers, 2013). The spray dryer is most commonly used for MP due to better solubility, flavor, and color. The spray dryer is involved atomize milk concentrated into hot air. The concentrated is dehydrated by transferring the concentrated milk into a spray of small droplets and exposed to a flow of hot air (inlet 80 – 90 °C outlet 180–220 °C) (Pisecky, 1997). This can be either a pressure nozzle or a centrifugal disc. The small droplet size helps to evaporate very fast at low temperature and less damage to the powder (Refstrup, 2003). The powder collects from the base of the cyclone. It is essential to concentrate the milk in order to decrease the drying energy as this method is an expensive method. The spray dryer can be either single stage or two stages. The single stage system requires a shorter time, higher outlet temperature comparing to the two stages system (Augustin and Smithers, 2013).

On the other hand, the roller dryer is involved drying the milk concentrated by contact to the hot surface of rotating rollers. A thin film of milk concentrated (1.1 mm) is dried by either a rotary metal cylinder or a drum at (<100 °C). This method is not preferred and rarely used due to the cost and undesirable changing on milk powder such as lactose caramelization, and protein denaturation. Also, the freeze dryer is involved

freeze a thin layer of milk under vacuum at -20 °C (Wang et al., 2004). This method is expensive, not for large quantities, and causing coalescence of fat globules.

2.4. Fundamentals Oxidation Reaction of Lipid

The oxidative deterioration of lipid-based foods is of great economic importance. Oxidation of unsaturated fatty acids not only produces off-flavor compounds but also decrease the nutritional quality and safety of the final product. The fundamentals of lipid oxidation date back to the 1940s (Farmer, 1946), where the free radical chain reaction was established. Years later, breakdown pathways of oxidation products under various conditions were included in the understanding of the lipid oxidation (Fränkel, 1980). The classical representation of lipid oxidation is illustrated in **Figure 3**.

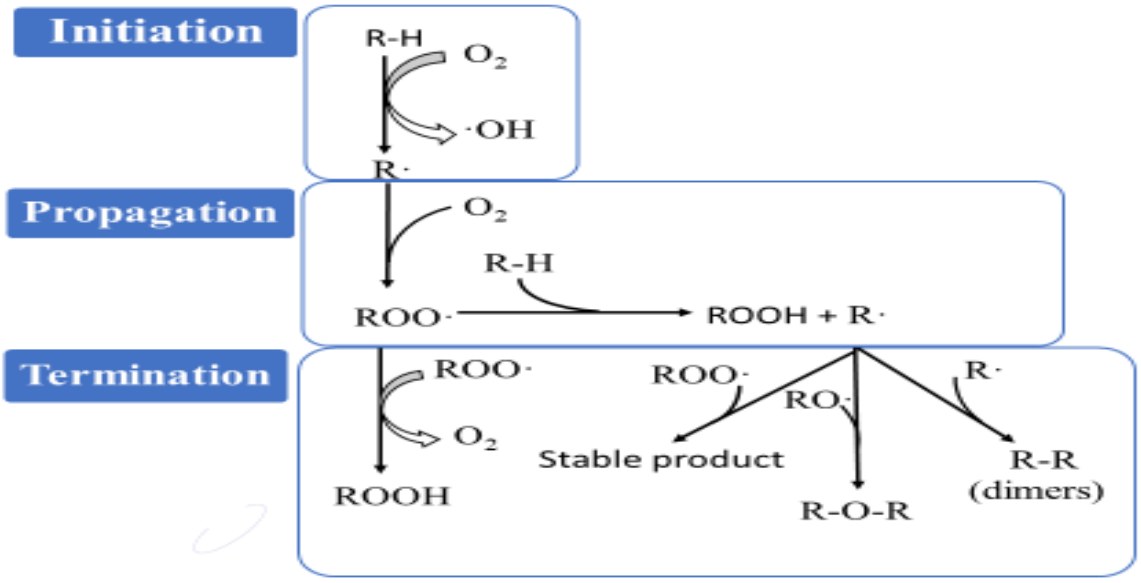


Figure 3. Illustration of the different stages of lipid oxidation.

The first step of the oxidation of lipids is the removal of an electron or hydrogen atom to form the free radical. This reaction is not spontaneous, and it requires an initiator.

The exposure to light and the presence of enzymes and metal ions (Ca^{2+} and Fe^{3+}) are common initiators of lipid oxidation (Choe and Min, 2006). In the presence of oxygen, these radicals react pseudo-instantaneously forming peroxyl radicals, which then abstract hydrogen atoms (H) from nearby lipid molecules to form a hydroperoxide and generate a new carbon-centered radical. It means that the addition of air, light, and warmth can increase the chance of oxidations to occur (Browne, 1899). This process is known as propagation, and it repeats itself indefinitely to expand the oxidation to other lipid molecules. In the propagation stage, a single initiating event can end up oxidizing hundreds of lipid molecules. Furthermore, the formed hydroperoxides decompose to form alkoxyl radicals by reduction or peroxyl radicals by oxidation (Privett and Blank, 1962).

The rates of H abstraction by peroxyl radicals are relatively slow, while the abstraction of H atoms by alkoxyl radicals is much faster than the rates of peroxyl radicals. Thus, the oxidation is strongly accelerated when hydroperoxides decompose and alkoxyl radicals dominate the main chain propagating species (Pryor, 1986). A common assumption within the oxidation theory is the relatively long periods at the beginning of the oxidation between the initiation and propagation. Recent evidence suggests that hydroperoxides follow a different pathway sometimes were not detectable at all while secondary reactions. However, current practice for measuring lipid oxidation involves the determination of conjugated dienes and hydroperoxides by chemical assays, and selected volatile secondary products by gas chromatography.

2.5. Antioxidants

Antioxidants are food additives that delay the oxidation of lipids, which help in extending the shelf life of oils and fatty foods during storage and processing (McClements and Decker, 2000). Antioxidants within the food materials provide a degree of protection against oxidation. Thus, dairy and food processors routinely formulate food products with antioxidants in order to delay the onset of oxidation. Nowadays, the consumption of antioxidant has been associated with many health benefits. The European Commission Concerted Action on Functional Food proves that there is a relationship between the human's disuses and free reduces thus the antioxidants have been added to decrease the free reduces effects (Diplock et al., 1998). Antioxidants can delay the oxidation in many ways, depending on their structure and mechanisms of the antioxidant itself (Quinchia et al., 2011). The antioxidants can primary or secondary antioxidants. The primary antioxidant is reacted with peroxy radicals that produce form the initiation stage in order to inhibit the oxidation reaction (Wanasundara and Shahidi, 2005). On the other hand, the secondary antioxidant is catching prooxidant and catalyst metal ions which helps to inhibit the oxidation reaction to occur (Wanasundara and Shahidi, 2005).

The content of antioxidants in foods emerges in three ways: i) naturally present; ii) added during formulation, and iii) created during food processing. Milk and milk products contain vitamins and bioactive compounds that react as antioxidants (Saxelin et al., 2003). Moreover, a study shows that using some culture such as *pediococcus pentosaxeus* during processing yogurt product increasing the activity of antioxidant (Balakrishnan and Agrawal, 2014). The addition of antioxidants can be either natural or synthetic antioxidants

that can be added during processing food. The antioxidants should be added at low concentrations to avoid per-oxidant actions (Wanasundara and Shahidi, 2005). Consumers prefer natural antioxidants due to emotional reasons (Pokorný, 2007). There are some antioxidants such as α-tocopherol, and D-ascorbic acid are synthetic antioxidants; however, they are considered and discussed under natural antioxidants (Wanasundara and Shahidi, 2005). This may be the main structures of them are built according to the natural structure, and they give the same activities to delay the oxidation reaction.

Dairy products contain different active antioxidants, such as vitamins and enzymes (Pihlanto, 2006). For example, natural antioxidants in milk are low molecular weight thiols (Niero et al., 2014), ascorbate (Nielsen et al., 2001), tocopherol, retinol and carotenoids (Jensen and Nielsen, 1996; Nozière et al., 2006). Sindhi et al., 2013 has classified the antioxidants into three groups. First, antioxidants that contain superoxide dismutase, catalase, glutathione reductase, and minerals like Se.

Second, antioxidants which contain glutathione (GSH), vitamin C, albumin, and vitamin E. Third, antioxidants that contain a complex group of enzymes for the repair of damaged DNA, damaged proteins, oxidized lipids and peroxides as lipase. Moreover, the antioxidants can be classified into two groups: enzymatic (primary and secondary enzymes) and non-enzymatic antioxidants (Bunaciu et al., 2012). He classifies the non-enzymatic antioxidant into seven groups that include vitamins, minerals, carotenoids, organosulfur compounds, low molecular weight, antioxidant cofactors, and polyphenols.

Furthermore, synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) are chemical compounds that have been created and tested by scientists in order to improve the oxidative stability of products and ensure they are saved and will not affect the consumers' health. The synthetic antioxidants could be dangerous to human health if used in high concentrations (Wanasundara and Shahidi, 2005). In terms of the food and dairy industries, synthetic antioxidants have potent effects, and a small quantity of them are needed in comparison to natural antioxidants.

2.4.1. Butylated hydroxytoluene (BHT)

BHT is a synthetic phenolic antioxidant and derives from the corresponding alcohol and aldehyde (BHT-CHO) (Fujisawa et al., 2004) (Figure 4). It was patented in 1947 (Stecher, 1968); however, the FDA was proved in 1954 as a save food additive (Federal, 1977). It can delay cancer induction with different chemical carcinogens (Slaga, 1995). The synthetic antioxidants have been used as primary antioxidants (Shahidi, 2015). It is one of the most additive food that can be found in butter, oil, meat, and animal feed. According to the Expert Committee on Food Additives, and the Codex Alimentarius (EFSA) list, BHT number is E320 under the butylates group with the low molecular weight, which is formula $C_{15}H_{24}O$. The melting and boiling points of BHT are 158°F (70°C) and 509°F (265°C) respectively. It widely used as a food additive since the 1950s (International Agency for Research on Cancer,1988) to maintain the physical and chemical properties of food (Fries and Püttmann, 2002). According to the U.S. Food and Drug Administration, the concentration of BHT ranges from 0.01 to 0.02% in most foods.

It is mostly insoluble in water and can be dangers on human health if it takes at a high concentration (Leng and Gries, 2002; Sicińska, 2008).

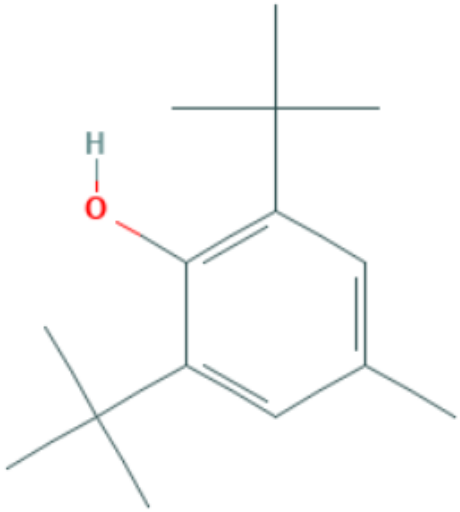


Figure 4. Chemical structure of 3,5-Di-tert-4-butylhydroxytoluene (BHT) from pubchem.ncbi.nlm.nih.gov

2.4.2. β-carotene

Dairy and food industries have used the carotenoids as a food additive because they are fat-soluble antioxidants and natural value (Dutta et al., 2005; Fernández-García et al., 2012). A naturally, there are more than 600 identified carotenoids that can be divided to two groups: carotenes contain carbon and hydrogen such as α-carotene and β-carotene, and xanthophylls contain carbon, hydrogen, and oxygen such as lutein and zeaxanthin (Olson and Krinsky, 1995; Failla et al., 2008). Even though carotenoids have oxygen on their structures, they are water hydrophilic and dissolve in lipids (Britton, 1995). β-carotene is the most popular carotenes that used as an antioxidant (**Figure 5**). β-carotene is a C_{40} H_{56}

that has 11 double bonds in its structure (Shahidi, 2015). According to the EFSA list, the β-carotene number is E161b under Lutein. A good source of β-carotene is orange, yellow, and red fruits and vegetables such as orange, carrots, pumpkin, and sweet potato.

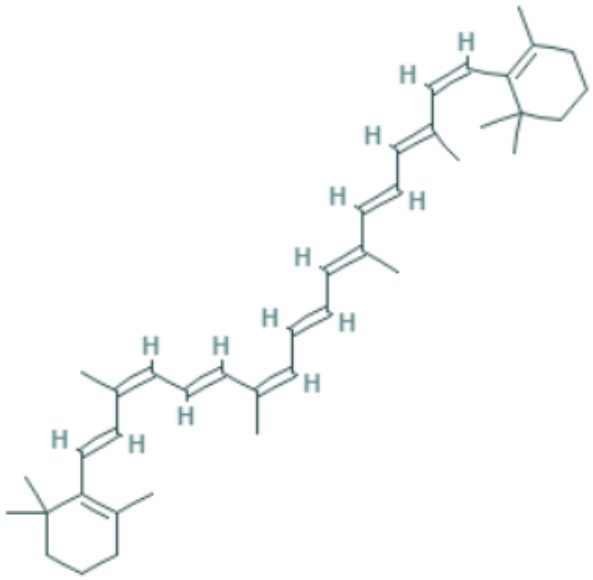


Figure 5. Chemical structure of β-carotene (β-Car) from pubchem.ncbi.nlm.nih.gov.

2.4.3. D-alpha-Tocopherol

Vitamin E is a natural antioxidant that can found naturally or additive in some food. It is a fat-soluble that is a group of compounds: tocopherols and tocotrienols. In terms of tocopherols, the side chain is saturated; however, the side chain in tocotrienols is unsaturated (Shahidi, 2015). A good source of Vitamin E is nuts, seeds, and oil. They can be found in polyunsaturated phospholipid-rich domains (Bernstein et al., 2016). The tocopherols can be named marinic acid and caffeic acid (Barbosa-Pereira et al., 2013). α-, β-, γ-, and δ- are forms of tocopherols and tocotrienols that can be found in foods (Ross et

al., 2007). The structure of these compounds has a chroman ring and a phytyl chain (Carocho et al., 2018). The activity of these form is decreased from delta to alpha (Dziezak, 1986). According to the EFSA list, the tocopherols number are range from E 306 to E 309. According to the Food and Nutriton Board (FNB), the recommended dietary allowances (RDAs) for α-tocopherols are 4-5 for infants, 6-11 for children, and 15 mg for adults. The α-tocopherol is the most effective form of vitamin E that can natural or synthetic sources (**Figure 6**) (Krueger et al., 2014).

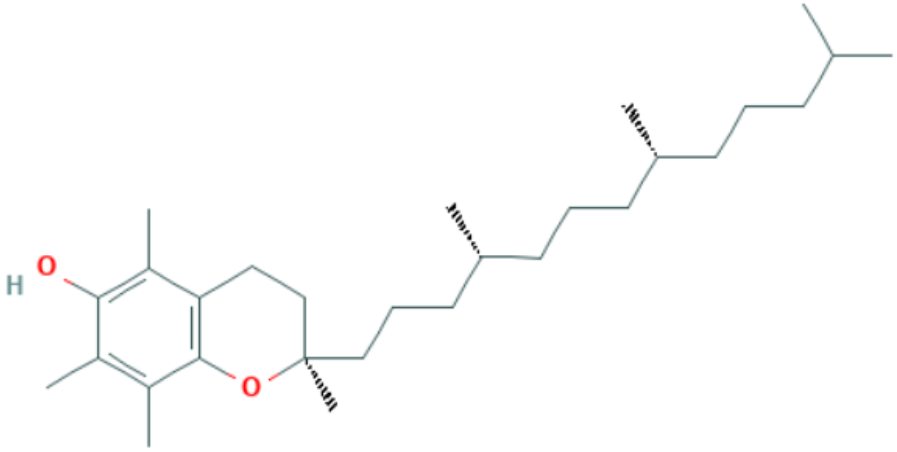


Figure 6. Chemical structure of d-α-Tocopherolβ-carotene (α-Toc) from pubchem.ncbi.nlm.nih.gov.

2.5. Estimation of antioxidants Capacity

Numerous methods have been applied to study the capacity of antioxidants such as ABTS radical scavenging capacity assay, oxygen radical absorbance capacity (ORAC) method, superoxide dismutase (SOD) method, ferric reducing antioxidant potential (FRAP) method (Dudonne et al., 2009), protective, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical assay, and factor (PF). The DPPH radical assay is a method that can be used for

both solid and liquid antioxidants (Brand-Williams et al., 1995). Also, it is not specific to any particular antioxidant component but applies to the overall antioxidant capacity of the sample. It is an easy technique compared with other methods. From previous studies, it observed that the most widely used one is the antiradical activity (EC_{50}), defined as the amount of antiradical necessary to decrease the initial DPPH• concentration by 50%, as well as its reciprocal the antiradical power (ARP), ARP = $1/EC_{50}$ (Brand-Williams et al., 1995). EC_{50} for any antioxidant can be affected by the solvent that uses and the concentration of antioxidant. For example, DPPH• with a polar solvent will be more effective with low concentration polar antioxidants compound such as Ascorbic Acid and Gallic Acid; however, it may less effective with nonpolar antioxidants compound such as BHT. The remaining DPPH % was studied by this equation:

Equation (1)

$$\text{Remaining DPPH } (\%) = \frac{A_t}{A_o} \times 100$$

Where: A_t and A_o are the absorbance of the mixture at the steady state and the absorbance of the mixture at the beginning, respectively.

Another mothed to evaluate the effect of antioxidants is through the PF which used the start temperature of oxidation (Ts), PF $\leq$ 1 means that the antioxidant has a pro-oxidant effect; PF $\geq$ 1 the antioxidant can be considered as a measurement of antioxidant effectiveness.

Equation (2)

$$PF = \frac{T_s \; with \; antioxidant}{T_s \; without \; antioxidant}$$

2.6. Fundamentals on the use of DSC and TGA to study lipid oxidation

The oxidation reaction of lipid is a complicated reaction that cannot be measured by a single method. Nevertheless, numerous chemical and volumetric methods have been developed for quantifying and monitoring the oxidation of fats and oils including oxidative stability index (OSI), peroxide values (PV), derivative thermogravimetry (DTG), and gel permeation chromatography (GPC) (Gray, 1978; Kamal-Eldin and Pokorny, 2005). All these methods either measure primary or secondary oxidation products (Kamal-Eldin and Pokorny, 2005). Alternatively, the oxidation reaction can be studied by thermal differential techniques such as differential scanning calorimetry and (DSC) and thermo-gravimetrical analysis (TGA). Thermodynamically, the oxidation of lipids is an exothermic reaction where heat is released as the oxidation progressed. The released heat from the oxidized lipid is compared to the heat flowing from an inert reference (empty pan) both heated at the same rate. During DSC oxidation, the recorded heat shows a peak which area is proportional to the amount of heat released by the sample. **Figure 7** illustrates the released heat during the oxidation and the relationship with the three consecutive reaction stages of initiation, propagation and termination.

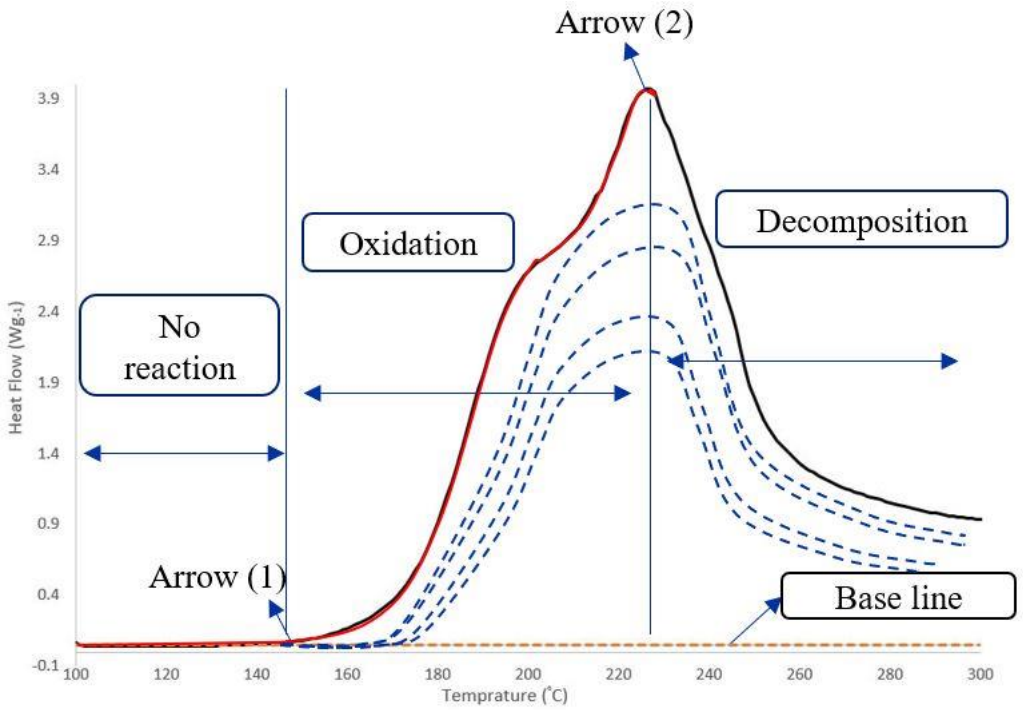


Figure 7. Illustration of the differential scanning calorimetry oxidation. Figure obtained from Saldaña & Martinez-Monteagudo (2013).

In the schematic illustration presented in **Figure 7**, the heat flow signal (y-axis) is recorded as a function of temperature (x-axis). At the beginning of the oxidation curve, there is no change in the heat flow signal which is commonly known as the induction time. The length of the induction time is often considered as a measurement of lipid stability since no chemical reaction occurs (Šimon, 2006). As the oxidation takes place, the heat flow signal starts to separate from the baseline (straight line) and it is considered the end of the induction time (arrow (1)). Further increase in the temperature results in initiation of oxidation, which is theoretically interpreted as the reaction between the radicals and the unsaturated fatty acids. The resulting products of this reaction are unstable hydroperoxides that further react propagating the oxidation. A sudden increase in the heat flow signal is related to the propagation stage. The blue dashed lines illustrate oxidation reactions that

occur and cannot be detected by the DSC because they are less exothermal. Finally, arrow (2) illustrates the termination stage, where stable products are formed. The red line is the actual heat flow recorded by the DSC (Saldaña and Martínez-Monteagudo, 2013).

Thermoanalytical techniques are gaining popularity in research and quality laboratories because they can determine the quality and oxidative stability of lipids. Both DSC and TGA have been used to determine the physical or chemical changing of materials during heat changes (Biliaderis, 1983). Moreover, they have been used to determine the oxidative stability of oils (Vittadini et al., 2003) and fat (Bentz and Breidenbach, 1969; Miller et al., 1969; Md. Ali and Dimick, 1994; Thurgood et al., 2007; Wirkowska-Wojdyła et al., 2014).

2.6.1. Factors affecting the DSC oxidation

The existing literature suggests that DSC is a convenient and fast technique to measure the oxidation of lipids. However, there are numerous factors and variables that strongly influence the reproducibility of the results. Pressure use, temperature range, and sample characteristics (mass and particle size) are experimental factors and they contribute to the overall quality and reproducibility of the results (Vyazovkin, 2001). The temperature should set at lower than the self-ignition temperature for the sample to capture the oxidation event (Saldaña and Martínez-Monteagudo, 2013). For example, the self-ignition temperature for fats and oils is 350 °C. In terms of non-isothermal study, there is an inverse relationship between heat flow and speed of oxidation. This may be using high heating rates can help to evaporate the liquid lipid before they can react (Adhvaryu et al., 2000). Perhaps, the most significant factor is the amount and thickness of sample to be used for

the oxidation measurements that should be between 1 to 4 mg and 1 mm respectivly. Tsang and Walker (1985) argue that there is no sigificant different on the DSC thermogrms using sample between 1 to 4 mg. In a typical DSC experiment, a liquid sample is loaded into the DSC pan using a syringe or a Pasteur pipette.

The DSC oxidation can be carried out using an open aluminum pan or in a hermetic sealed pan with a pinhole (**Figure 8**). The principal difference between an open pan and a sealed pan with a pinhole is the diffusion of oxygen and the amount of oxygen that is in contact with the sample. Due to the thermal conductivity of the air is smaller than that of the metal of the pan. The heating rate plays a vital role to study the oxidation of lipids. It is recommended that to equilibrium period between 3 to 5 minutes to improve the baseline and it should be no more than 25 °C min^{-1} (Martínez-Monteagudo, 2011).

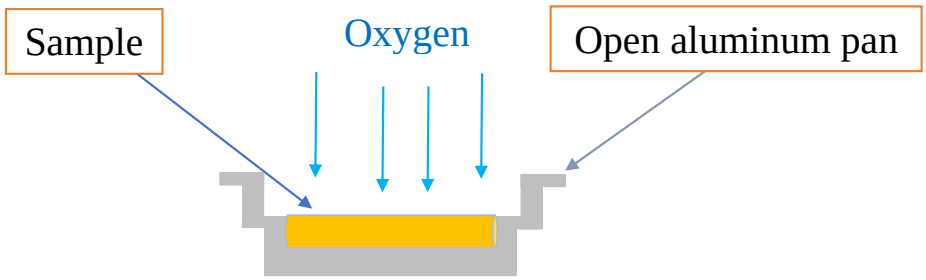


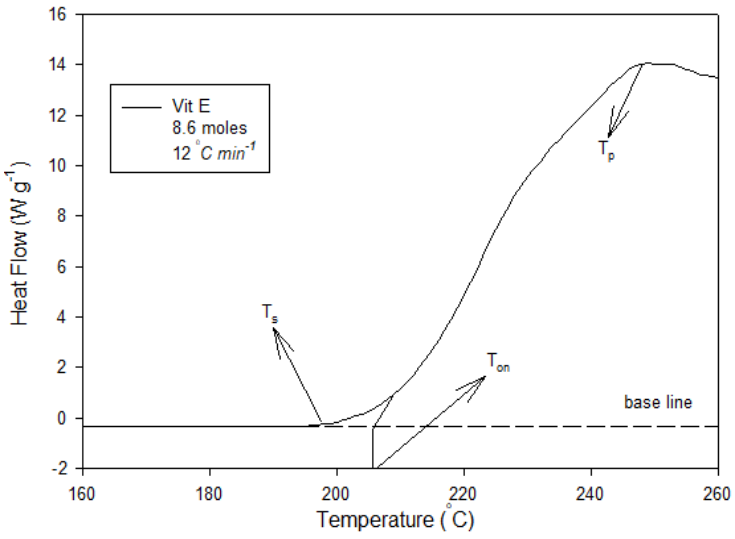
Figure 8. Illustration of an aluminum open DSC pan (Saldaña and Martínez-Monteagudo, 2013).

On the other hand, the TGA is a thermal analysis technique that measures the mass loss of a sample at a controlled temperature program. It is studied the mass loss from the sample because of the gas formation using a function of temperature and/or time in a particular temperature program (Vyazovkin and Wight, 1998). The differential

thermogravimetric (DTG) curve can obtain by the differentiation of sample weight to temperature or time that can be used to determine the physical and chemical properties of samples. There are two types of kinetic studies: isothermal using a constant temperature, and non-isothermal using different flow rates.

2.6.2. Analysis of the DSC oxidation curves

The analysis of the generated DSC spectra consists in identifying the start temperature (T_s), onset temperature (T_{on}), and peak temperature (T_p). The start temperature of oxidation is calculated using the first derivative of the signal when an inflexion point between a maximum and a minimum point of the signal, and the heat flow signal is greater than the departure value. The peak temperature (T_p) is obtained when the first derivative of the signal intersects with the x-axis and the second derivative reached a maximum point on the signal. The onset temperature (T_{on}) was obtained extrapolating the tangent drawn on the steepest slope of T_p. A detailed explanation of the methodology can be found elsewhere (Saldaña and Martínez-Monteagudo, 2013; Martínez-Monteagudo et al., 2011). Using the



T_s, T_{on}, and T_p, the DSC spectra can be analyzed with the aid of TA Universal Analysis software (TA Instruments, New Castle, DE) (**Figure 9**).

Figure 9. Representative DSC thermogram of non-isothermal oxidation.

2.6.3. Isothermal oxidation

The oxidation of DSC under isothermal regime was first reported in 1905 (Lewis, 1905). He studied the oxidation of silver at a constant temperature 335 ˚C for several hours. In the isothermal mode, the oxidation occurs using the same temperature during the entire test. Normally, the time required to detect the start, or the onset temperature of oxidation is recorded and used as the main parameter for ranking the oxidative stability. This temperature should be lower than the self-ignition temperature for fats and oils to record the lipid oxidation rather than combustion (Saldaña and Martínez-Monteagudo, 2013). It is recommended that the starting temperature should be in range of 50 to 70 ˚C (Vyazovkin et al., 2011). A sample will be heated under argon flow until achieved a thermal equilibrium, and then oxidized after the modification of the oxygen atmosphere (Saldaña and Martínez-Monteagudo, 2013). There are some limitations for this study such as, adjusting the temperature range and reaching the complete conversion (Vyazovkin et al., 2011). Mathematically, the oxidation kinetics under isothermal regime can be expressed as follows:

Equation (3)

$$\frac{d\alpha}{dt} = k(T) \cdot f(\alpha)$$

Equation (4)

$$\int_0^{\alpha} \frac{d\alpha}{f(\alpha)} = k \int_0^{t_\alpha} dt$$

2.6.4. Non-isothermal oxidation

Non-isothermal oxidation is using different temperatures against time. The non-isothermal study was ignored until 1969 when Flynn studied and developed the principle of work in non-isothermal kinetics (Flynn et al.,1969). When applied in lipid oxidation, the non-isothermal mode shows valuable analyses and kinetic information concerning the measurement of heat flow versus temperature at different heating rates (Martínez-Monteagudo et al., 2011). The kinetic methods of this study can convent into differential and integral methods (Vyazovkin and Wight, 1998)It is recommended that to use at least three to five heating rates to determine the kinetic parameters (Vyazovkin et al., 2011).

Equation (5)

$$\frac{d\alpha}{dt} = k(T) \cdot f(\alpha) = A \cdot exp\left[-\frac{E_a}{R \cdot T}\right] f(\alpha)$$

Equation (6)

$$\int_0^{\alpha} \frac{d\alpha}{f(\alpha)} = A \int_0^{t_\alpha} exp\left[-\frac{E_a}{R \cdot T}\right] dt$$

2.6.5. Comparison of isothermal and non-isothermal

There are two types of kinetic studies: isothermal using a constant temperature, and non-isothermal using different flow rates. The first kinetic study was completed using isothermal methods (Lewis, 1905). However, the non-isothermal study was ignored until

1969 when Flynn studied and developed the principle of work in non-isothermal kinetics (Flynn et al., 1969). When applied in lipid oxidation, the non-isothermal mode shows valuable analyses and kinetic information concerning the measurement of heat flow versus temperature at different heating rates (Martínez-Monteagudo et al., 2011). There are some advantages and disadvantages of each one. First of all, the non-isothermal mothed requires a large number of experiments compared to the isothermal method (Lucey et al., 2017). Second of all, the temperature range of the non-isothermal experiment typically higher than an isothermal experiment, which may lead to an increase in the values of Ea and α (Lucey et al., 2017).

On the other hand, a sample of the isothermal method is required a time to reach the temperature requirement of an experiment (Vyazovkin, 1996). During this time, some reactions could occur in the sample, which may affect the accuracy of the result. Also, it cannot cover all the reaction stages. Typically, the isothermal method started with high temperature while the temperature of the non-isothermal method is increasing gradually, which gives more kinetics information (Vyazovkin and Wight, 1998). In this case, the conversions of the isothermal method can be seen as significant before reaching the isothermal regime profile (Vyazovkin and Wight, 1998). For these reasons, the non-isothermal method comes more popular.

2.7. Kinetic analysis

2.7.1. Degree of conversion

The degree of conversion (α) in chemical reactions or range of reaction of a specific compound is known by moles at a specific time divided by the initial moles is range

between 0 to 1 ($0 \leq \alpha \leq 1$) (Vyazovkin, 1996). In terms of thermal analysis, α can be defined using the heat flow at a specific time or temperature where the highest heat flow signal is achieved (Saldaña and Martínez-Monteagudo, 2013).

Equation (7)

$$\alpha = \frac{\int_{T_s}^{T}(S(T) - B(T))dT}{\int_{T_s}^{T_f}(S(T) - B(T))dT}$$

Where T_s is the initial temperature of oxidation, and T_f is the final temperature when the oxidation has been completed. $S(T)$ is the heat flow signal at a given temperature. $B(T)$ is the heat flow signal proportional to the baseline.

2.7.2. Isoconversional methods

The isoconversional is a method that has been used to describe the thermal kinetics using multiple single-step (Vyazovkin and Sbirrazzuoli, 2006). For example, Friedman, Ozawa-Flynn-Well (OFW) and Kissinger-Akahira-Sunose (KAS) method have been used to collocate activation energy (Achilias et al., 2011). It can be used different heating rates program to gain data of different rates at a stable extent of conversion (Achilias et al., 2011). It can be study whole kinetics of substances during experimental temperatures. These methods can evaluate the dependency of E_α on α.

Conclusions

The milk fat plays a vital role in terms of nutritional value and flavor. It has used as an ingredient in the food and dairy industries. The main limitation of this is that it can be oxidized during processing and storage produces, which causes unpleasant flavor.

Addition of antioxidant is one way to inhibit or delay the oxidation reaction. The antioxidants can be either natural or synthetic antioxidants. The use of DSC and TGA to determine the oxidation is reliable, convenient, and straightforward techniques. They provide qualitative and quantitative information and offer unique advantages; for example, the small sample is needed, and easy methods. These methods can set either isothermal or non-isothermal study. From the data obtained from DSC and TGA, the degree of conversion can be collected which can be used to collect the kinetic triplet: pre-exponential factor (A), activation energy (Ea), and reaction model (f(α)).

References

Achilias, D.S., E. Panayotidou, and I. Zuburtikudis. 2011. Thermal degradation kinetics and isoconversional analysis of biodegradable poly (3-hydroxybutyrate)/organomodified montmorillonite nanocomposites. Thermochim. Acta 514:58–66.

Augustin, M.A., and G.W. Smithers. 2013. Advances in Dairy Ingredients. John Wiley & Sons.

Bachman, K.C., M.J. Hayen, D. Morse, and C.J. Wilcox. 1988. Effect of pregnancy, milk yield, and somatic cell count on bovine milk fat hydrolysis. J. Dairy Sci. 71:925–931.

Balakrishnan, G., and R. Agrawal. 2014. Antioxidant activity and fatty acid profile of fermented milk prepared by Pediococcus pentosaceus. J. Food Sci. Technol. 51:4138–4142.

Barbosa-Pereira, L., J.M. Cruz, R. Sendón, A.R.B. de Quirós, A. Ares, M. Castro-López, M.J.

Barbosa-Pereira, L., J.M. Cruz, R. Sendón, A.R.B. de Quirós, A. Ares, M. Castro-López,

M.J. Abad, J. Maroto, and P. Paseiro-Losada. 2013. Development of antioxidant active films containing tocopherols to extend the shelf life of fish. Food Control 31:236–243.

Bernstein, P.S., B. Li, P.P. Vachali, A. Gorusupudi, R. Shyam, B.S. Henriksen, and J.M. Nolan.

2016. Lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. Prog. Retin. Eye Res. 50:34–66.

Bentz, A.P., and B.G. Breidenbach. 1969. Evaluation of the differential scanning calorimetric method for fat solids. J. Am. Oil Chem. Soc. 46:60–63.

Biliaderis, C.G. 1983. Differential scanning calorimetry in food research—a review. Food Chem. 10:239–265.

Bloore, C.G., and I.F. Boag. 1981. Some factors affecting the viscosity of concentrated skim milk. New Zeal. J. Dairy Sci. Technol.

Brand-Williams, W., M.-E. Cuvelier, and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28:25–30.

Britton, G. 1995. Structure and properties of carotenoids in relation to function.. FASEB J. 9:1551–1558.

Brown, M.E., D. Dollimore, and A.K. Galwey. 1980. Reactions in the Solid State. Elsevier.

Browne, C.A. 1899. A CONTRIBUTION TO THE CHEMISTRY OF BUTTER-FAT.. J. Am. Chem. Soc. 21:975–994.

Bunaciu, A.A., H.Y. Aboul-Enein, and S. Fleschin. 2012. FTIR spectrophotometric methods used for antioxidant activity assay in medicinal plants. Appl. Spectrosc. Rev.

47:245–255.

Carić, M. 1994. Concentrated and Dried Dairy Products. VCH Publishers Inc.

Carocho, M., P. Morales, and I.C.F.R. Ferreira. 2018. Antioxidants: Reviewing the chemistry, food applications, legislation and role as preservatives. Trends food Sci. Technol. 71:107–120.

Choe, E., and D.B. Min. 2006. Chemistry and reactions of reactive oxygen species in foods. Crit. Rev. Food Sci. Nutr. 46:1–22.

Diplock, A.T., J.-L. Charuleux, G. Crozier-Willi, F.J. Kok, C. Rice-Evans, M. Roberfroid, W. Stahl, and J. Vina-Ribes. 1998. Functional food science and defence against reactive oxidative species. Br. J. Nutr. 80:S77–S112.

Dudonne, S., X. Vitrac, P. Coutiere, M. Woillez, and J.-M. Merillon. 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J. Agric. Food Chem. 57:1768–1774.

Dutta, D., U.R. Chaudhuri, and R. Chakraborty. 2005. Structure, health benefits, antioxidant property and processing and storage of carotenoids. African J. Biotechnol. 4.

Dziezak, J.D. 1986. Preservatives: antioxidants. Food Technol 40:94–102.

EI-Gazzar, F.E., and E.H. Marth. 1991. Listeria monocytogenes and listeriosis related to milk, milk produets and dairy ingredients: A review. List. monocytogenes 14–19.

Elías-Argote, X., A. Laubscher, and R. Jiménez-Flores. 2013. Dairy ingredients containing milk fat globule membrane: description, composition, and industrial potential. Adv. dairy ingredients 71–98.

Failla, M.L., T.-Y. Huo, and S.K. Thakkar. 2008. In vitro screening of relative bioaccessibility of carotenoids from foods. Asia Pac. J. Clin. Nutr. 17:200–203.

Farkye, N.Y. 2006. Significance of milk fat in milk powder. Springer.

Farmer, E.H. 1946. Peroxidation in relation to olefinic structure. Trans. Faraday Soc. 42:228–236.

Federal Register. 1977. Food and Drug Administration, Butylated hydroxytoluene. Use restrictions. U. S. Government Printing Office. Washington. D. C. 42 (104):27603-27607.

Fernández-García, E., I. Carvajal-Lérida, M. Jarén-Galán, J. Garrido-Fernández, A. Pérez-Gálvez, and D. Hornero-Méndez. 2012. Carotenoids bioavailability from foods: From plant pigments to efficient biological activities. Food Res. Int. 46:438–450.

Fox, P.F., P.L.H. McSweeney, and L.H. Paul. 1998. Dairy chemistry and biochemistry. Springer.

Fränkel, R. 1980. Lip seal training in the treatment of skeletal open bite. Eur. J. Orthod. 2:219–228.

Fries, E., and W. Püttmann. 2002. Analysis of the antioxidant butylated hydroxytoluene (BHT) in water by means of solid phase extraction combined with GC/MS. Water Res. 36:2319–2327.

Fujisawa, S., Y. Kadoma, and I. Yokoe. 2004. Radical-scavenging activity of butylated hydroxytoluene (BHT) and its metabolites. Chem. Phys. Lipids 130:189–195.

Gray, J.I. 1978. Measurement of lipid oxidation: a review. J. Am. Oil Chem. Soc. 55:539–546.

Heid, H.W., and T.W. Keenan. 2005. Intracellular origin and secretion of milk fat globules. Eur. J. Cell Biol. 84:245–258.

Jensen, S.K., and K.N. Nielsen. 1996. Tocopherols, retinol, β-carotene and fatty acids in fat globule membrane and fat globule core in cows' milk. J. Dairy Res. 63:565–574.

Kamal-Eldin, A., and J. Pokorny. 2005. Analysis of Lipid Oxidation. AOCS Publishing.

Kanno, C. 1990. Secretory membranes of the lactating mammary gland. Protoplasma 159:184–208.

Kowalski, B. 1991. Thermal-oxidative decomposition of edible oils and fats. DSC studies. Thermochim. Acta 184:49–57.

Krueger, L.A., D.C. Beitz, K. Onda, M. Osman, M.R. O'Neil, S. Lei, F.H. Wattoo, R.L. Stuart,

H.D. Tyler, and B. Nonnecke. 2014. Effects of d-α-tocopherol and dietary energy on growth and health of preruminant dairy calves. J. Dairy Sci. 97:3715–3727.

Ipsen, R., and P.S. Hansen. 1988. Factors affecting the storage stability of whole milk powder. Beret. Statens Mejeriforsoeg.

Leng, G., and W. Gries. 2002. Butylhydroxytoluol (BHT)[Biomonitoring Methods in German language, 2010]. MAK-Collection Occup. Heal. Saf. Annu. Threshold. Classif. Work. 1–14.

Lewis, G.N. 1905. Zersetzung von silberoxyd durch autokatalyse. Zeitschrift für Phys. Chemie 52:310–326.

Lock, A.L., and D.E. Bauman. 2004. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids 39:1197–1206.

Lucey, J.A., D. Otter, and D.S. Horne. 2017. A 100-Year Review: Progress on the chemistry of milk and its components. J. Dairy Sci. 100:9916–9932.

Martínez-Monteagudo, S.I., M. Khan, F. Temelli, and M.D.A. Saldaña. 2014. Obtaining a

hydrolyzed milk fat fraction enriched in conjugated linoleic acid and trans-vaccenic acid. Int. Dairy J. 36:29–37.

Martínez-Monteagudo, S.I., M.D.A. Saldaña, and J.J. Kennelly. 2011. Kinetics of non-isothermal oxidation of anhydrous milk fat rich in conjugated linoleic acid using differential scanning calorimetry. J. Therm. Anal. Calorim. 107:973–981.

Mather, I.H. 2000. A review and proposed nomenclature for major proteins of the milk-fat globule membrane. J. Dairy Sci. 83:203–247.

McClements, D.J., and E.A. Decker. 2000. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J. Food Sci. 65:1270–1282.

Md. Ali, A.R., and P.S. Dimick. 1994. Thermal analysis of palm mid-fraction, cocoa butter and milk fat blends by differential scanning calorimetry. J. Am. Oil Chem. Soc. 71:299–302.

Miller, W.J., W.H. Koester, and F.E. Freeberg. 1969. The measurement of fatty solids by differential scanning calorimetry. J. Am. Oil Chem. Soc. 46:341–343.

Muehlhoff, E., A. Bennett, and D. McMahon. 2013. Milk and Dairy Products in Human Nutrition.

Food and Agriculture Organization of the United Nations (FAO).

Newstead, D.F., W.B. Sanderson, and A.G. Baucke. 1975. effects of heat treatment and pH on the heat stability of recombined evaporated milk. New Zeal. J. dairy Sci. Technol.

Nielsen, J.H., G. Hald, L. Kjeldsen, H.J. Andersen, and H. Østdal. 2001. Oxidation of ascorbate in raw milk induced by enzymes and transition metals. J. Agric. Food Chem. 49:2998–3003.

Niero, G., A. Sturaro, A.R. Trentin, A. Masi, M. De Marchi, and M. Cassandro. 2014. Effect of cheesemaking with microparticulated whey proteins on the concentration of low molecular thiols in cheese. Acta Agrar. Kaposváriensis 18:103–108.

Nozière, P., P. Grolier, D. Durand, A. Ferlay, P. Pradel, and B. Martin. 2006. Variations in carotenoids, fat-soluble micronutrients, and color in cows' plasma and milk following changes in forage and feeding level. J. Dairy Sci. 89:2634–2648.

Oh, W.Y., and F. Shahidi. 2018. Antioxidant activity of resveratrol ester derivatives in food and biological model systems. Food Chem. 261:267–273.

Olson, J.A., and N.I. Krinsky. 1995. Introduction: the colorful, fascinating world of the carotenoids: important physiologic modulators.. FASEB J. 9:1547–1550.

Palmquist, D.L., C.L. Davis, R.E. Brown, and D.S. Sachan. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of d (−) β-hydroxybutyrate. J. Dairy Sci. 52:633–638.

Partridge, J. 2008. Fluid Milk Products. Dairy Process. Qual. Assur. Iowa John Wiley Sons, Inc 203–209.

Pihlanto, A. 2006. Antioxidative peptides derived from milk proteins. Int. dairy J. 16:1306–1314.

Pisecky, J. 1997. Handbook of Milk Powder Manufacture Copenhagen Niro A.

Pokorný, J. 2007. Are natural antioxidants better–and safer–than synthetic antioxidants?. Eur. J. Lipid Sci. Technol. 109:629–642.

Privett, O.S., and M.L. Blank. 1962. The initial stages of autoxidation. J. Am. Oil Chem. Soc. 39:465–469.

Pryor, W.A. 1986. Oxy-radicals and related species: their formation, lifetimes, and

reactions. Annu. Rev. Physiol. 48:657–667.

Quinchia, L.A., M.A. Delgado, C. Valencia, J.M. Franco, and C. Gallegos. 2011. Natural and synthetic antioxidant additives for improving the performance of new biolubricant formulations. J. Agric. Food Chem. 59:12917–12924.

Refstrup, E. 2003. Drying of milk. Encycl. Dairy Sci. 860–871.

Saldaña, M.D.A., and S.I. Martínez-Monteagudo. 2013. Oxidative stability of fats and oils measured by differential scanning calorimetry for food and industrial applications. IntechOpen.

Saxelin, M., R. Korpela, and A. Mäyrä-Mäkinen. 2003. Introduction: classifying functional dairy products. Elsevier.

Schuck, P. 2011. Milk powder: physical and functional properties of milk powders.

Ŝesták, J. 1984. Thermodynamical properties of solids. Prague Acad.

Shahidi, F. 2015. Handbook of Antioxidants for Food Preservation. Woodhead Publishing.

Sharma, A., A.H. Jana, and R.S. Chavan. 2012. Functionality of milk powders and milk-based powders for end use applications—a review. Compr. Rev. Food Sci. Food Saf. 11:518–528.

Sicińska, E. 2008. Przeciwutleniacze w żywności. Przem. Spożywczy 62:36–38.

Šimon, P. 2006. Induction periods. J. Therm. Anal. Calorim. 84:263–270.

Sindhi, V., V. Gupta, K. Sharma, S. Bhatnagar, R. Kumari, and N. Dhaka. 2013. Potential applications of antioxidants–A review. J. Pharm. Res. 7:828–835.

Singh, H. 1992. Aspects of proteins in milk powder manufacture. Milk Proteins; Mol. Physico-Chemical Biol. Asp.

Singh, H., and L.K. Creamer. 1991. Denaturation, aggregation and heat stability of milk protein during the manufacture of skim milk powder. J. Dairy Res. 58:269–283.

Singh, P.K., and H. Singh. 2015. Dry Milk Products. Dairy Process. Qual. Assur. 333.

Spitsberg, V.L. 2005. Invited review: Bovine milk fat globule membrane as a potential nutraceutical. J. Dairy Sci. 88:2289–2294.

Stapelfeldt, H., B.R. Nielsen, and L.H. Skibsted. 1997. Effect of heat treatment, water activity and storage temperature on the oxidative stability of whole milk powder. Int. Dairy J. 7:331–339.

Stecher, P.G. 1968. Merck index. Merck & Co. Inc. Rahway. N.J. p. 179.

Thurgood, J., R. Ward, and S. Martini. 2007. Oxidation kinetics of soybean oil/anhydrous milk fat blends: a differential scanning calorimetry study. Food Res. Int. 40:1030–1037.

Tsang, W., and J.A. Walker. 1982. Characterization of RDF properties through high pressure differential scanning calorimetry. Resour. Conserv. 9:355–365.

Vittadini, E., J.H. Lee, N.G. Frega, D.B. Min, and Y. Vodovotz. 2003. DSC determination of thermally oxidized olive oil. J. Am. Oil Chem. Soc. 80:533–537.

De Vries, M.J., and R.F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83:62–69.

Vyazovkin, S., A.K. Burnham, J.M. Criado, L.A. Pérez-Maqueda, C. Popescu, and N.

Sbirrazzuoli. 2011. ICTAC Kinetics Committee recommendations for performing kinetic computations on thermal analysis data. Thermochim. Acta 520:1–19.

Vyazovkin, S., and C.A. Wight. 1998. Isothermal and non-isothermal kinetics of

thermally stimulated reactions of solids. Int. Rev. Phys. Chem. 17:407–433.

Vyazovkin, S. 1996. A unified approach to kinetic processing of nonisothermal data. Int. J. Chem. Kinet. 28:95–101.

Vyazovkin, S. 2001. Two types of uncertainty in the values of activation energy. J. Therm. Anal. Calorim. 64:829–835.

Vyazovkin, S., and N. Sbirrazzuoli. 2006. Isoconversional kinetic analysis of thermally stimulated processes in polymers. Macromol. Rapid Commun. 27:1515–1532.

Vyazovkin, S., and C.A. Wight. 1998. Isothermal and non-isothermal kinetics of thermally stimulated reactions of solids. Int. Rev. Phys. Chem. 17:407–433.

Walstra, P., P. Walstra, J.T.M. Wouters, and T.J. Geurts. 2005. Dairy Science and Technology. CRC press.

Wanasundara, P., and F. Shahidi. 2005. Antioxidants: Science, technology, and applications. Bailey's Ind. oil fat Prod.

Wang, Y.-C., R.-C. Yu, and C.-C. Chou. 2004. Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. Int. J. Food Microbiol. 93:209–217.

Wirkowska-Wojdyła, M., J. Bryś, A. Górska, and E. Ostrowska-Ligęza. 2014. Oxidation kinetics and melting profiles of the structured lipids used in infant cookies. Eur. J. lipid Sci. Technol. 116:1546–1552.

Chapter 3

Advanced Isoconversional Kinetics: Role of Antioxidants during Milk Fat Oxidation [1]

3.1. Introduction

Milk fat is a key ingredient in many food formulations because of its nutritional value, functionality, and flavor. During processing and storage, milk fat may undergo oxidation resulting in many undesirable changes such as unpleasant flavor and aroma, and formation of toxic compounds (Lindmark-Månsson & Åkesson, 2000). Indeed, the main limiting factor for food formulations containing milk fat is the development of oxidation. The oxidation of milk fat occurs via free radical chain reaction, and it consists of an initiation, a propagation, and a termination stage. In the first stage, free radicals or starters compounds are formed due the break of covalent bonds. Numerous factors are involved in the breakage of such bonds including thermal homolytic process, presence of certain enzymes, exposure to light and metal ions, and presence of reactive oxygen species (Choe & Min, 2005). In the presence of molecular oxygen, these radicals form primary oxidation products (hydroperoxides) that further react through free radical mechanisms, propagating the oxidation (propagation stage). As the oxidation proceeds, other reactions take place such polymerization leading to the formation of viscous products, and they represent the termination stage of oxidation (Saldaña & Martínez-Monteagudo, 2013).

A common practice for preventing the oxidation of milk fat is by the addition of compounds that can prevent or delay the formation of radicals. These type of compounds

[1] A version of this chapter is to be submitted to Journal of Food Process Engineering

are known as antioxidants, and they can function by a number of mechanisms including physical and chemical quenching of singlet oxygen and radicals (Min & Lee, 1999). Tocopherol (α-Toc), butylated hydroxytoluene (BHT), and β-carotene (β-Car) are example of compounds used to prevent oxidation during processing and storage of food formulations containing milk fat.

Overall, the oxidation of lipids involves complex sequence of elementary reactions such as homolysis, radical formation, ionization, and polymerization (Yin, Xu, & Porter, 2011). In case of milk fat, the velocity at which some of these reactions occur is influenced by the food matrix, concentration, heating protocol, and form of fatty acids (Xia & Budge, 2017). Due to its complexity, the oxidation of milk fat needs to be measured with a battery of tests (Martínez-Monteagudo, Saldaña, & Kennelly, 2012). Nevertheless, the oxidation of milk fat is commonly evaluated and monitored by measuring either primary or secondary oxidation products, where the concentration of such compounds is used to rank the development of oxidation.

An alternative method for the analysis of the oxidation of lipids is the use of differential scanning calorimetry (DSC), where the heat released as the oxidation proceed is recorded and related to the level of oxidation (Martínez-Monteagudo, 2018). Analysis of lipid oxidation using DSC has been reported in blends of soybean/anhydrous milk fat (Thurgood, Ward, & Martini, 2007), anhydrous milk fat (Martínez-Monteagudo, Saldaña, & Kennelly, 2012), unsaturated and saturated fatty acids (Litwinienko, 2001), and selected vegetable oils (canola, corn, cottonseed, and soybean oil) (Adhvaryu, Erhan, Liu, & Perez,

2000). The existence literature suggests that DSC under non-isothermal regime is an effective method for ranking the oxidative stability of edible fats and oils. However, the associated kinetic parameters are obtained through a fitting procedure under questionable assumptions. Vyazovkin (2018) exemplified the limitations of such an approach. Instead, advanced kinetics methods are needed for the estimation of the kinetic triplet: pre-exponential factor (A), activation energy (E_a), and reaction model ($f(\alpha)$). Computational kinetics by means of isoconversional methods consist of a set of computational techniques involving DSC data that links kinetic equations and their parameters with a given degree of conversion (α). The Kinetics Committee of the International Confederation for Thermal Analysis and Calorimetry (ICTAC) has developed a set of guidelines for the estimation of the kinetic triplet from the data obtained by means of DSC (Vyazovkin et al., 2011). The determination of the kinetic triplet of the oxidation of milk fat provides insight into the reaction mechanisms as well as to predict the oxidative stability of milk fat. In the present investigation, different antioxidants (α-Toc, BHT, and β-Car) at four concentrations (0.02, 0.07, 0.2, and 0.4%) were added into anhydrous milk fat with the aim at performing a detailed kinetic analysis of the oxidation process using isoconversional kinetics.

3.2. Materials and methods

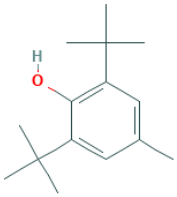
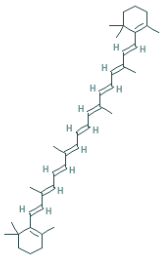
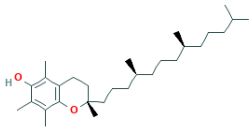
3.2.1. Materials

3,5-Di-tert-4-butylhydroxytoluene (99%, Sigma-Aldrich), β-carotene (99%, Alfa Aesar), and D-α-tocopherol (99%, Fisher Scientific) were purchased from commercial supplier. All antioxidants were stored at -18°C until the further use.

3.2.2. Anhydrous milk fat

Anhydrous milk fat (AMF) was prepared using the methodology reported by Martínez-Monteagudo, Saldaña, Kennelly, and Calorimetry (2012) with some modifications. Briefly, raw cream obtained from the Davis Dairy Plant at South Dakota State University (Brookings, SD) was churned at 4°C to obtain butter using a laboratory scale churner (TM 31 Thermomix, Vorwerk LLC, Thousand Oaks, CA). The resulting butter was separated from the buttermilk using a cheesecloth. Then, the butter was washed with cold water to remove the excess of buttermilk. After that, the butter was heated at 60°C until the visual appearance of two layers. The AMF was obtained after removing the top layer, and it was used within 24 h of manufacturing. One hundred grams of AMF was placed into an Erlenmeyer flask and heated at 60ºC. Then, three antioxidants (α-Toc, BHT, and β-Car) were studied individually at four concentrations. **Table 3** shows the concentration used and the chemical structure of the antioxidants.

Table 3. Concentration and chemical characteristics of the antioxidants used for the oxidation kinetic analysis.

Name	Concentration (mole per g of AMF)	Empirical formula	Molecular weight	Chemical structure
2,6-Di-tert-butyl-4-methylphenol (BHT)	4.4, 16, 44, and 88	$C_{15}H_{24}O$	220.35	
Beta-carotene	11, 44, 88, and 107	$C_{40}H_{56}$	536.88	
(-)-alpha-tocopherol	8, 16, 44, and 86	$C_{29}H_{50}O_2$	430.71	

3.2.3. Differential scanning calorimetry

The guidelines proposed by Saldaña and Martínez-Monteagudo (2013) on the use of DSC for oxidation of edible fats and oils were followed. The oxidation of the AMF added with different antioxidants under non-isothermal regime was conducted in a Q100 Differential Scanning Calorimeter (TA Instruments, New Castle, DE, USA). The oxidation experiments were conducted using an open aluminum pan. A glass Pasteur pipette was used to transfer samples (2-3 mg) into an aluminum pan. Then, the pan was placed in the DSC furnace, where it was equilibrated at 100°C for 1 min, and subsequently heated to 350°C at four different heating rates (β = 3, 6, 9, and 12ºC min^{-1}). An empty aluminum pan was used as a reference. Oxygen (dry 99% pure, Praxair, Sioux Falls, SD, USA) at a

flow rate of 20 mL min^{-1} at 20 psig was used as oxidizing agent. The DSC spectra were analyzed by a TA Universal Analysis software (TA instruments). The oxidation experiments were performed in triplicates.

3.2.4. Kinetic analysis

The analysis of the oxidation kinetics was performed following the recommendations developed by the ICTAC (Vyazovkin et al., 2011). The methodology described in the following sections allows the independent estimation of the kinetic triplet.

3.2.5. Kinetic equations

According to the law of mass action, the rate of a reaction is parameterized in terms of the reaction temperature and the degree of conversion, according to:

Equation (8)

$$\frac{d\alpha}{dt} = k(T) \cdot f(\alpha)$$

where $k(T)$ is the rate constant, which in turns obeys the Arrhenius law (Equation (9)), and $f(\alpha)$ is the associated reaction model.

Equation (9)

$$k(T) = A \cdot exp^{\left(\frac{-E_a}{R \cdot T}\right)}$$

Where A is the pre-exponential factor; E_a is the apparent activation energy (kJ mol^{-1}); and R is the universal gas constant. Under non-isothermal conditions using constant linear heating rate ($\beta = {}^{dT}/_{dt}$), Equation (8) can be written as follows:

Equation (10)

$$\frac{d\alpha}{dT} = \frac{A}{\beta} \cdot exp^{\left(\frac{-E_a}{R \cdot T}\right)} \cdot f(\alpha)$$

3.2.6. Determination of the degree of conversion

The DSC spectra were analyzed with a TA Universal Analysis software (TA Instruments, New Castle, DE) to calculate the degree of conversion ($0 \leq \alpha \leq 1.0$), according to Equation (11).

Equation (11)

$$\alpha = \frac{\int_{T_s}^{T} (S(T) - B(T))dT}{\int_{T_s}^{T_f} (S(T) - B(T))dT}$$

Where T_s is the start temperature of oxidation and T_f is the temperature where the oxidation is completed. $S(T)$ is the heat flow signal at a given temperature, and $B(T)$ is the heat flow signal proportional to the baseline. The baseline was determined using sigmoidal tangents at the beginning and at the end of the DSC signal. This approach compensates the changes in the amount of sample and their temperature dependence.

3.2.7. Isoconversional method

The Kissinger-Akahira-Sunose (KAS) method was used to evaluate the dependency of activation energy on the degree of conversion (E_α), Equation (12).

Equation (12)

$$ln\left(\frac{\beta_i}{T_{\alpha,i}^2}\right) = ln\frac{A \cdot R}{E_\alpha \cdot g(\alpha)} - \frac{E_\alpha}{R \cdot T_{\alpha,i}}$$

Where the subscript α denotes value at a specific degree of conversion and the subscript i denotes different heating rates. The KAS method involves the computation of E_α at selected regular α increment covering the range of 0.05 to 0.95. The E_α values are obtained through linear regression by plotting $ln\left(\frac{\beta}{T^2}\right)$ against $^1/_{T_\alpha}$ using Athena Visual Software.

3.2.8. Reaction models

The term f(α) of Equation (8) is the differential form of the reaction model, which can take many mathematical forms. Upon integration, the following equations are obtained:

Equation (13)

$$g(\alpha) = \frac{A}{\beta} \cdot \int_o^T exp^{\left(\frac{-E_a}{R \cdot T}\right)} dT$$

Equation (14)

$$g(\alpha) \equiv \int_o^\alpha \frac{d\alpha}{f(\alpha)}$$

Where $g(\alpha)$ is the integral form from of the reaction model. **Table 4** shows the algebraic expression of the integral form of the different tested reaction models for the kinetic analysis of non-isothermal oxidation. The integral of the right side of Equation (13) was solved using the fourth-degree solution of Senen and Yang approximation (Pérez-Maqueda & Criado, 2000), according to:

Equation (15)

$$g(\alpha) = \frac{A \cdot E_a}{R \cdot T} \cdot exp^{(-x)} \cdot \left[\frac{\pi(x)}{x}\right]$$

$$\pi(x) = \frac{x^3 + 18 \cdot x^2 + 86 \cdot x + 96}{x^4 + 20 \cdot x^3 + 120 \cdot x^2 + 240 \cdot x + 120}$$

Where x is the dimensionless activation energy. A first screening of the different reaction models was performed by fitting the different reaction models. For each tested model, a set of kinetic parameters were obtained using Athena Visual Workbench (www.athenavisual.com). The fitting ability of the different models were evaluated using the coefficient of determination (R^2), the adjusted coefficient of determination (R^2_{adj}), and residual sum of squares (RRS).

Equation (16)

$$RSS = \sum \left(y_{exp} - y_{cal}\right)^2$$

Where y_{exp} and y_{cal} are the experimental and calculated degree of conversion, respectively.

Table 4. Integral form of the different reaction models used in the kinetic analysis of oxidation of milk fat added with different antioxidants.

Reaction model	Code	$g(\alpha)$
Power law	P4	$\alpha^{1/4}$
Power law	P3	$\alpha^{1/3}$
Power law	P2	$\alpha^{1/2}$
Power law	P2/3	$\alpha^{3/2}$
One-dimensional diffusion	D1	α^2
First-order	F1	$-\ln(1-\alpha)$
Avrami-Erofeev	A4	$[-\ln(1-\alpha)]^{1/4}$
Avrami-Erofeev	A3	$[-\ln(1-\alpha)]^{1/3}$
Avrami-Erofeev	A2	$[-\ln(1-\alpha)]^{1/2}$
Three-dimensional diffusion	D3	$\left[1-(1-\alpha)^{1/3}\right]^2$
Contracting sphere	R3	$1-(1-\alpha)^{1/3}$
Contracting cylinder	R2	$1-(1-\alpha)^{1/2}$
Two-dimensional diffusion	D2	$(1-\alpha)\ln(1-\alpha)+\alpha$

3.2.9. Selection of reaction model

The identification of a suitable model was conducted by finding the best match between theoretical and experimental data, according to the Master plot method. A theoretical set is obtained for each reaction model using the concept of generalized time (θ) and a reference point at $\alpha = 0.5$, according to:

Equation (17)

$$\frac{g(\alpha)}{g(0.5)} = \frac{\theta}{\theta_{0.5}}$$

Under non-isothermal regime, the right side of Equation (17) is estimated by:

Equation (18)

$$\frac{\theta}{\theta_{0.5}} = \frac{p(x)}{p(x_{0.5})}$$

$$p(x) = \frac{exp^{(-x)}}{x} \cdot \pi(x)$$

For those models that closely match the theoretical and experimental data were further discriminated using the methodology reported by Pérez-Maqueda and Criado (2000). Such method involves plotting the experimental data points in the form of $ln\left[\left({}^{d\alpha}/_{dt}\right)/_{f(\alpha)}\right]$ against ${}^{1}/_{T}$. When the correct kinetic model ($f(\alpha)$) is assumed, a straight line is obtained from which the slope and intercept provide similar values of activation energy and pre-exponential factor than those initially estimated.

3.2.10. Pre-exponential factor

The pre-exponential factor (A) was calculated through the compensation effect (Vyazovkin et al., 2011). A pair of A_i and E_i is obtained for each tested reaction model, and these parameters are used to calculate a and b according to Equation (19). Then, a and b are further used in Equation (20) to calculate A.

Equation (19)

$$\ln A_i = a \cdot E_i + b$$

Equation (20)

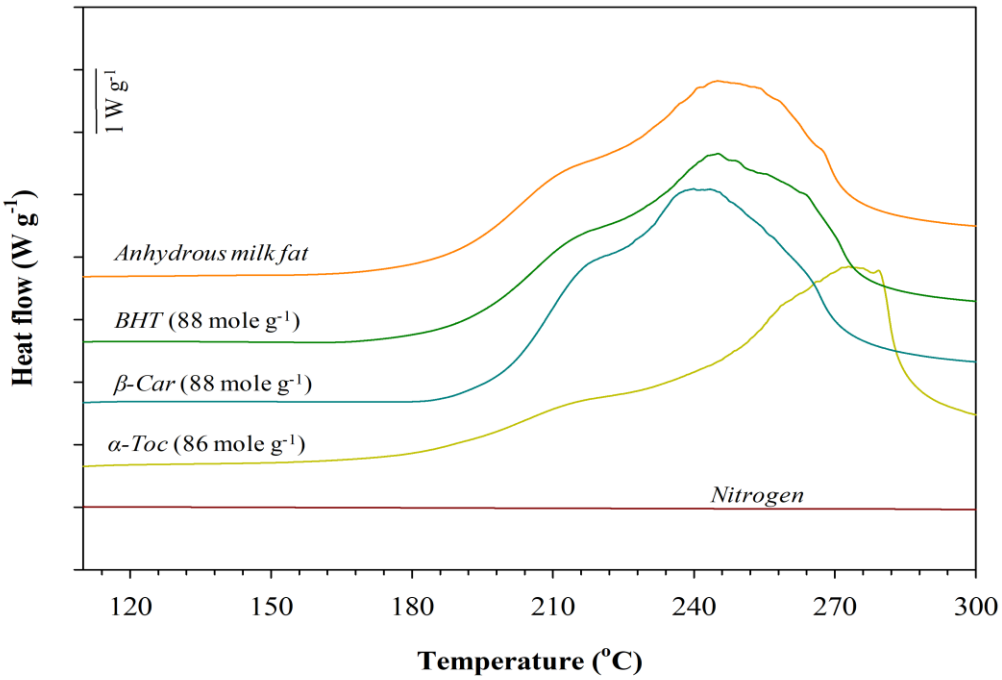
$$\ln A_o = a \cdot E_\alpha + b$$

3.3. Results and discussion

3.3.1. Oxidative profile

Figure 10 shows representative DSC oxidative curves (heat flow vs temperature) of milk fat added with antioxidants. The temperature range used in this study captures the oxidation event. Two main reasons can explain such a claim. Firstly, the maximum heat flow temperature (226-280°C) is lower than the self-ignition temperature of edible oils (350°C) (Martínez-Monteagudo, 2018). Secondly, the same temperature range was evaluated under nitrogen flow, and no thermal event was recorded. A consistent parameter to compare the oxidative curves between samples is the determination of the start temperature of oxidation (T_s). The T_s of milk fat ranged from 145 to 170°C increasing with the heating rate. Similarly, Martínez-Monteagudo, Saldaña, and Kennelly (2012) reported values of T_s from 164-180ºC within a heating rate range of 3-15°C min^{-1}.

Figure 10. Representative oxidative profiles of anhydrous milk fat added with different concentrations. The oxidation curves were obtained at 6°C min^{-1}. BHT – Butylated hydroxytoluene; β-Car – Beta-carotene; α-Toc – Alfa-tocopherol.



Further assessment of the effect of antioxidant during non-isothermal oxidation was performed considering T_s as a point of comparison. In general, the values of T_s increased with the heating rate, concentration and type of antioxidant (**Figure 11**). It is kwon that increasing the heating rate shifted to higher values the threshold of heat flow signal (Adhvaryu et al., 2000). This is because the intermediate oxidation products may evaporate from the liquid lipid before they can react (Martínez-Monteagudo, Saldaña, & Kennelly, 2012). Contrary, at slow heating rates, such compounds remain in the solution accelerating the oxidation. For BHT, the T_s increased asymptotically with the concentration (**Figure 11a**), and such increment was more notorious at higher heating rates. In the case of β-Car (**Figure 11b**), the T_s initially shifted to higher values with the concentration of 11 mole g^{-1}, and further increase in the concentration marginally increased the T_s values, regardless

of the heating rate. On the other hand, the T_s increased exponentially with the concentration of α-Toc reaching a maximum value of 202-207°C at 44 mole g^{-1}. Another way to evaluate the effect of antioxidants is through the protective factor (PF, T_s with antioxidant added divided by the T_s without antioxidant added) (Litwinienko, Kasprzycka-Guttman, & Jamanek, 1999). Values of PF lower than 1 means that the antioxidant has a pro-oxidant effect. On the other hand, PF values greater than one can be considered as a measurement of antioxidant effectiveness. The highest PF (1.34, 1.26, and 1.42) for BHT, β-Car, and α-Toc was obtained using a concentration of 88, 107, and86 mole g^{-1}, respectively. These values of concentration were further used to determine the kinetic triplet of milk fat with antioxidant added.

3.3.2. Degree of conversion

Figure 12 shows the degree of conversion as a function of temperature of anhydrous milk fat with added antioxidants. BHT – Butylated hydroxytoluene; β-Car – β-carotene; α-Toc – α-tocopherol. The numbers in parenthesis correspond to a specific heating rate: (1), (2), (3), (4) – 3, 6, 9, 12°C min^{-1}. In the case of anhydrous milk fat (**Figure 12a**), the oxidation curves were sigmoidal in shape regardless of the heating rate.

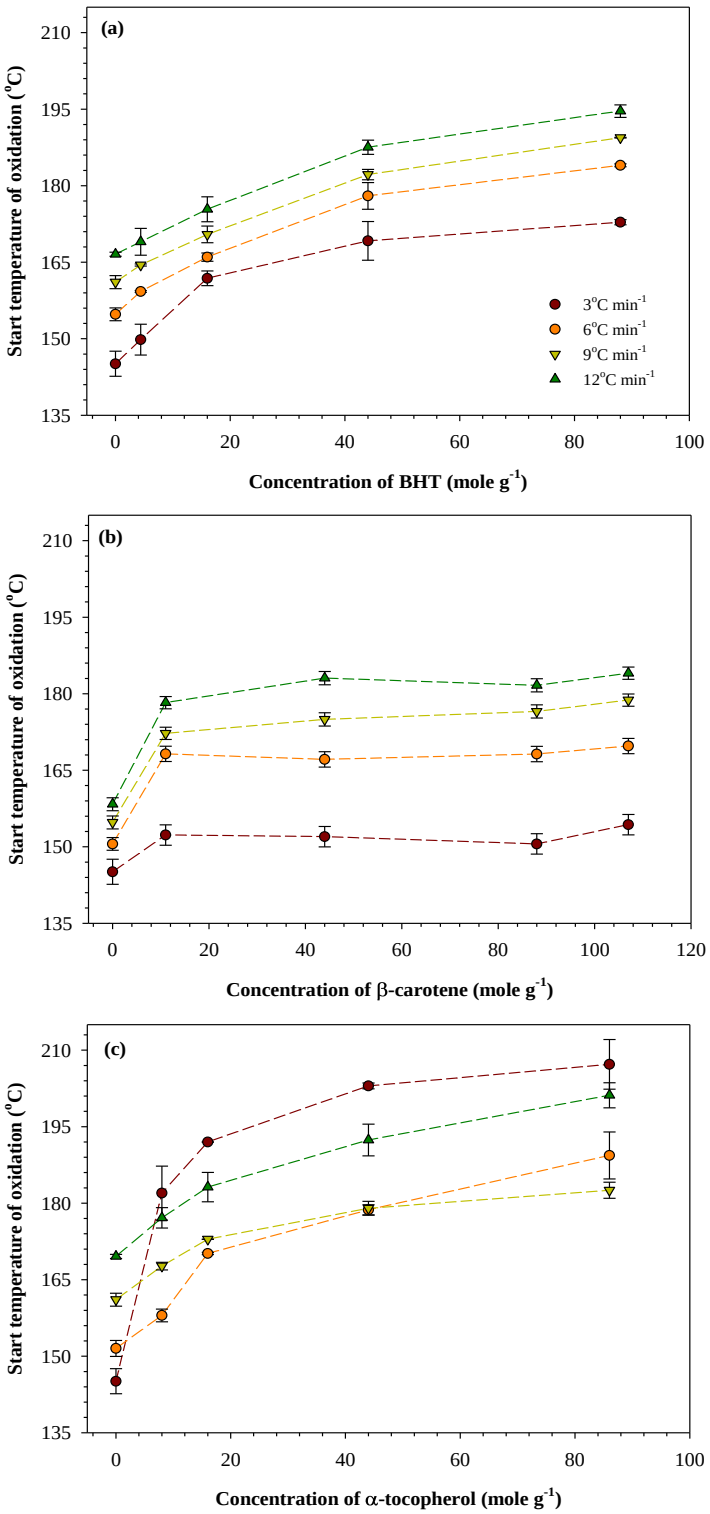


Figure 11. Influence of antioxidants on the start temperature of oxidation of milk fat: (a) 2,6-Di-tert-butyl-4-methylphenol (BHT); (b) β-carotene; (c) α-tocopherol.

A sigmoidal curve is often related to an autocatalytic reaction (Agrawal, 1992), and it is characterized by an initial stage where the oxidation is accelerated exponentially, and a final stage where the oxidation reached its maximum conversion (Vyazovkin et al., 2011). Similar curves has been reported for the oxidation of anhydrous milk fat enriched in conjugated linoleic acid and hydrolyzed milk fat (Martínez-Monteagudo, 2018; Martínez-Monteagudo, Saldaña, & Kennelly, 2012).

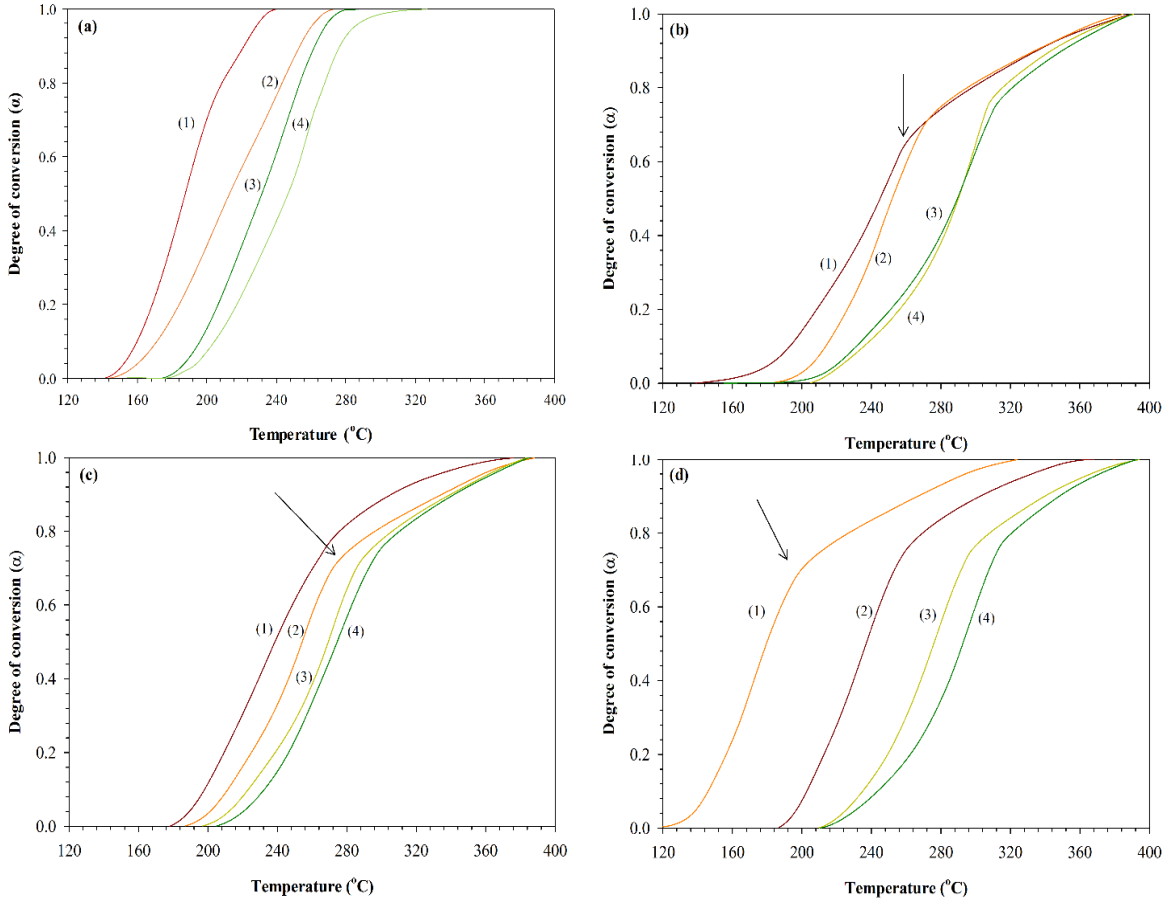


Figure 12. Degree of conversion as a function of temperature at different heating rates for: (a) anhydrous milk fat; (b) anhydrous milk fat added with BHT (88 mole g^{-1}); (c) anhydrous milk fat added with β-Car (107 mole g^{-1}); and (d) anhydrous milk fat added with α-Toc (86 mole g^{-1}).

The addition of antioxidants not only shift the T_s to higher values but also considerable altered the shape of the sigmoidal curve (indicated by arrows in **Figure 12b-d**). For all antioxidants, a clear change in the sigmoidal pattern was observed at a conversion of >0.7. Furthermore, the range of temperature at which the oxidation spanned increased considerable depending on the antioxidant used (144-270, 140-384, 179-383, and 190-390°C for AMF, BHT, β-Car, and α-Toc, respectively). These observations suggest a change in the oxidation mechanism occurring at advanced conversion. The analysis of isoconversional kinetic was performed to obtain insights into the role of antioxidants in changing the oxidation mechanism.

3.3.4. Determination activation energy

The activation energy (E_α) as a function of α for all samples was evaluated through linear regression according to the KAS method. **Figure 13** displays the changes in E_α with respect to the degree of conversion. For AMF, the values of E_α were within the range of 50-60 kJ mol^{-1}, and they remained constant over the entire range of oxidation. This observation indicates that the oxidation of AMF is likely controlled by multiple single-step reactions. When the antioxidants were added (BHT, β-Car, α-Toc), the dependency of E_α on the degree of conversion exhibited two distinctive regions (indicated by an arrow in **Figure 13**). In the first region ($0.05 \leq \alpha \leq 0.70$), the values of E_α varied from 56-65, 76-81, and 57-61 kJ mol^{-1} for BHT, β-Car, and α-Toc, respectively, without showing any particular trend. Within the first region, the addition of the antioxidants did not substantially change the oxidation mechanism when comparing with AMF. In the second region ($0.75 \leq \alpha \leq 0.95$), the values of E_α increased linearly with increasing α. The E_α is a

reflection of all reactions occurring during the progression of the non-isothermal oxidation, and their relative contribution varies as the oxidation proceeds (Martínez-Monteagudo, 2018). Increase of E_α at this stage of oxidation (α>0.70) could be related to the continuous changes in the composition during oxidation.

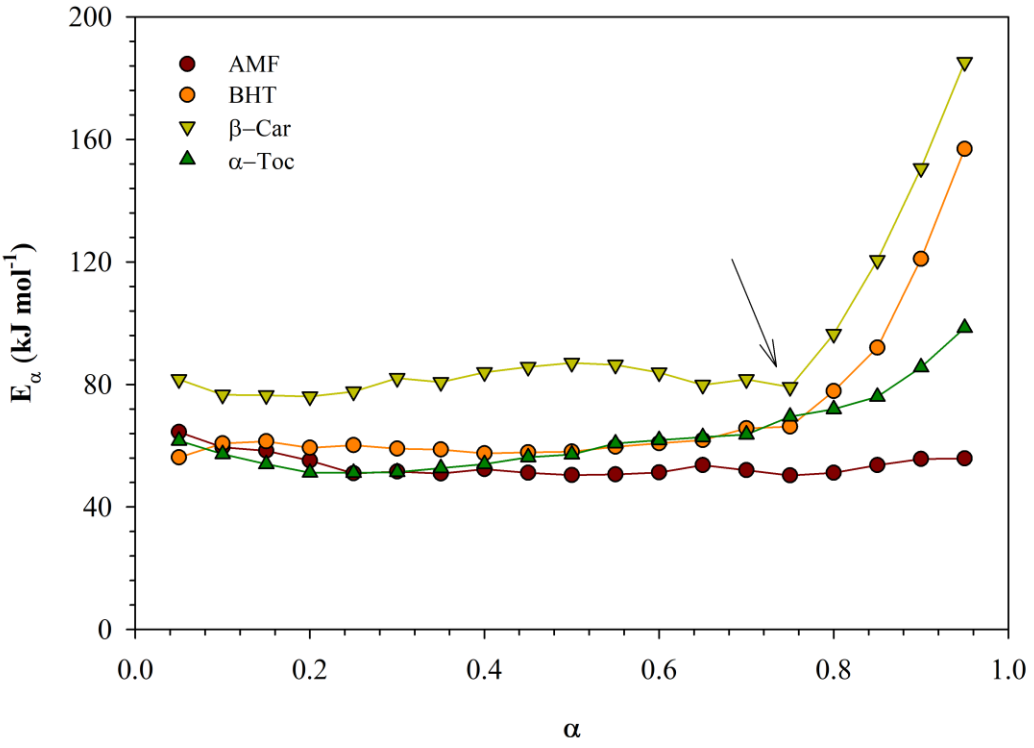


Figure 13. Activation energy (E_α) as a function of degree of conversion (α) according to the Kissinger-Akahira-Sunose method for anhydrous milk fat (AMF), butylated hydroxytoluene (BHT, 88 mole g^{-1}), β-carotene (β-Car, 107 mole g^{-1}), and α-tocopherol (α-Toc, 86 mole g^{-1}).

3.3.5. Determination of the reaction model

For all samples, the reaction model for the different samples was initially screened by fitting 13 different models (**Table 4**). The fitting was performed by non-linear regression using the parameters obtained from KAS method (ln A and E_α) as initial guess. The values of coefficient of determination (R^2) ranged from 0.40 to 0.99, depending on the

reaction model and sample. The fit was notably poor for some models, which were discarded for further analysis. Only those models whose values of R^2 were >0.97 were considered as statistically appropriate for describing the oxidation. The models P4, P2, A4, and A3 provided satisfactory fitting for AMF, while the oxidation of AMF added with BHT was satisfactorily represented by P3, P23, A4, A3, A2, and R3. Similarly, the oxidation of AMF added with β-Car was described by P4, P23, A4, A3, and A2. Finally, the addition of α-Toc followed the models of P23, D1, A4, A3, A2, and R3. These models were further investigated using the master plot method. **Figure 14** shows the reference theoretical curves dependent on the reaction model for AMF, BHT, β-Car, and α-Toc.

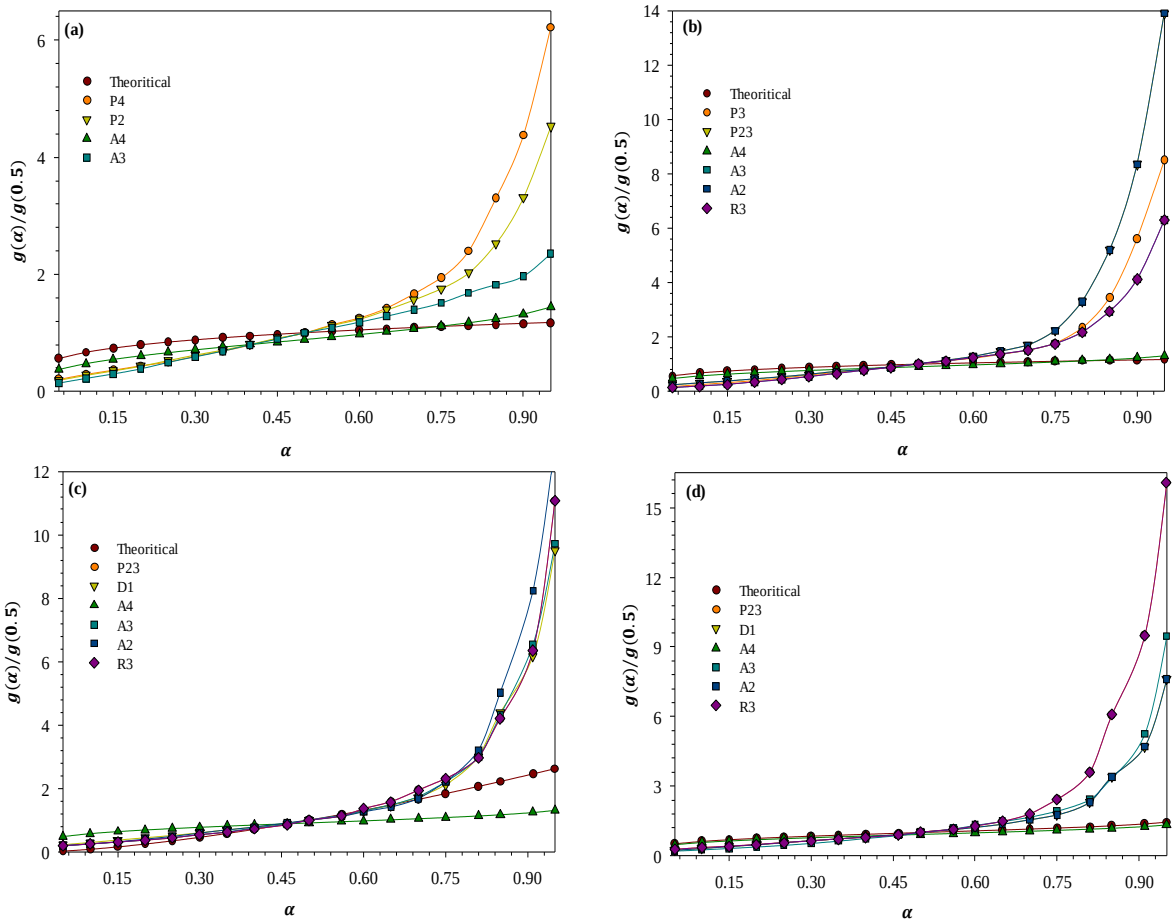


Figure 14. Theoretical master curves for the different kinetic models and the theoretical data for: (a) anhydrous milk fat (AMF); (b) butylated hydroxytoluene (BHT, 88 mole g^{-1}); (c) β-carotene (β-Car, 107 mole g^{-1}); (d) α-tocopherol (α-Toc, 86 mole g^{-1}).

For all samples, the theoretical plot corresponding to the A4 model closely matched the theoretical data over the entire range of α (0.05-0.95). Thus, the oxidation of AMF with and without added antioxidants was most probably described with the Avrami-Erofeer model (A4). This observation contradicts the first-order assumption commonly used during the analysis of milk fat oxidation. Deviations from the first-order model are expected during the oxidation of triacylglycerol since the fatty acids are distributed throughout different triacylglycerol, resulting in different combinations of molecular weights, chain lengths, and degree of saturation (Martínez-Monteagudo & Saldaña, 2015).

3.3.6. Computation of pre-exponential factor

The pre-exponential factor was calculated using the compensation theory (**Figure 15**). A linear relationship existed between A and E_α for the different models, and all data fell onto a master linear curve, from which the regression parameters were calculated from the slope (parameter a) and intercept (parameter b). The pre-exponential factor was then calculated by substituting a and b into their respective equation using the value of E_α within the range of 0.2-0.8, where $E_a \approx E_\alpha$. **Table 4** shows the regression analysis of the compensation theory. The parameters (a and b) were much higher than their respective 95% confidence interval (95% CI), meaning that these parameters can be used for predictions. In addition, the R^2 values (>0.97, **Table 5**) indicate that the pre-exponential factor can be represented within the tested conditions.

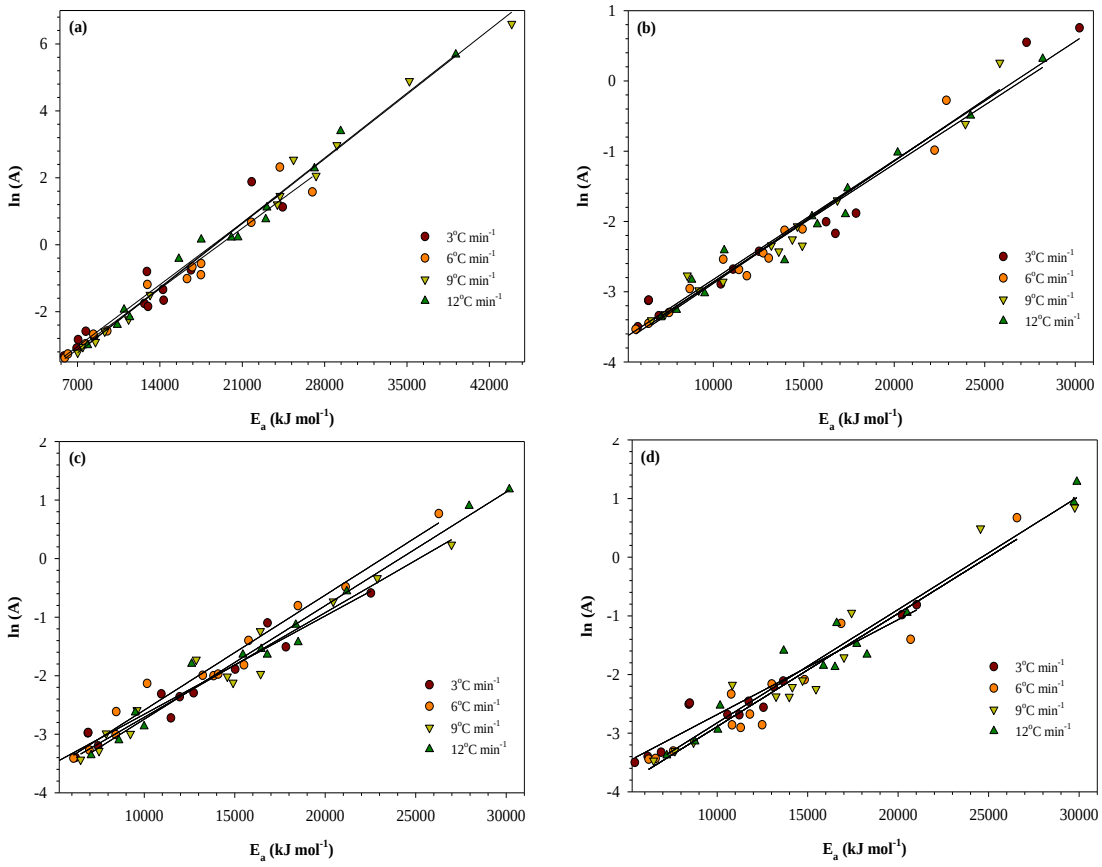


Figure 15. The compensation effect (lnA vs. Ea) obtained during the oxidation of: (a) anhydrous milk fat (AMF); (b) butylated hydroxytoluene (BHT, 88 mole g^{-1}); (c) β-carotene (β-Car, 107 mole g^{-1}); (d) α-tocopherol (α-Toc, 86 mole g^{-1}).

3.3.4. Role of antioxidants

Table 6 summarizes the calculated kinetic triplet obtained for AMF, BHT, β-Car, and α-Toc. The information presented in **Table 6** was further used for modelling the oxidation of milk fat by substituting the kinetic triplet. The activation energy can be seen as the energetic barrier that fat molecules need to overcome in order to be able to react. Reactive molecules collide with a certain frequency (A), providing the energy needed to react or overcome the energetic barrier. The frequency or pre-exponential factor (A) is also interpreted as the reaction rate when there is no energetic barrier (E = 0). Although milk

fat is a complex system, the obtained kinetic triplet is of great relevance to describe the influence of temperature during oxidation. A new set of data was generated using the calculated kinetic triplet (**Table 6**), and the resulting values of the constant rate (k) were plotted as a function of the temperature (**Figure 16)**. As the temperature increased, the k values for all samples exponentially increased, being greater for AMF. Importantly, the oxidation of milk fat was significantly delayed by the addition of antioxidants within the entire range of temperature. At 200°C, the oxidation was reduced 3.33-, 6.85-, and 34.98-fold for BHT, β-Car, and α-Toc, respectively.

Table 5. Regression parameters obtained from the compensation theory during the oxidation of milk fat added with antioxidants.

Heating rate (β, °C min^{-1})	**AMF**				
	a	**CI95%**	**b**	**CI95%**	**R^2**
3	2.64 x10^{-4}	7.14 x10^{-5}	-4.88	0.75	0.989
6	2.58 x10^{-4}	7.52 x10^{-5}	-4.92	1.01	0.987
9	2.76 x10^{-4}	3.17 x10^{-5}	-5.16	0.75	0.997
12	2.77 x10^{-4}	4.33 x10^{-5}	-5.15	0.93	0.998
Heating rate (β, °C min^{-1})	**BHT (88 mole g^{-1})**				
	a	**CI95%**	**b**	**CI95%**	**R^2**
3	1.69 x10^{-4}	4.45 x10^{-5}	-4.53	0.68	0.987
6	1.77 x10^{-4}	4.52 x10^{-5}	-4.58	0.59	0.998
9	1.71 x10^{-4}	4.46 x10^{-5}	-4.62	0.66	0.989
12	1.68 x10^{-4}	3.93 x10^{-5}	-4.53	0.64	0.986
Heating rate (β, °C min^{-1})	**β-Car (107 mole g^{-1})**				
	a	**CI95%**	**b**	**CI95%**	**R^2**
3	1.61 x10^{-4}	5.01 x10^{-5}	-4.33	0.63	0.987
6	1.92 x10^{-4}	4.55 x10^{-5}	-4.55	0.67	0.987
9	1.79 x10^{-4}	5.11 x10^{-5}	-4.49	0.79	0.979
12	1.94 x10^{-4}	3.62 x10^{-5}	-4.68	0.64	0.998
Heating rate (β, °C min^{-1})	**α-Toc (86 mole g^{-1})**				
	a	**CI95%**	**b**	**CI95%**	**R^2**
3	1.62 x10^{-4}	5.95 x10^{-5}	-4.31	0.73	0.984

6	1.93 x10^{-4}	7.37 x10^{-5}	-4.85	1.01	0.995
9	1.98 x10^{-4}	6.41 x10^{-5}	-4.85	1.03	0.978
12	1.93 x10^{-4}	5.35 x10^{-5}	-4.75	0.94	0.997

a, b – regression parameters; CI95% – 95% confidence interval; R^2 – coefficient of determination.

Table 6. Summary of the obtained kinetic triplets for the oxidation anhydrous milk fat, anhydrous milk fat added with BHT (88 mole g^{-1}), anhydrous milk fat added with β-Car (107 mole g^{-1}), and anhydrous milk fat added with α-Toc (86 mole g^{-1}).

Sample	k (min^{-1})	E_a (kJ mol^{-1})	$g(\alpha)$
AMF	0.0066 ± 0.0003	53.59 ± 4.57	A4
BHT (88 mole g^{-1})	0.0105 ± 0.0005	60.18 ± 5.41	A4
β-Car (107 mole g^{-1})	0.0112 ± 0.0004	63.21 ± 2.14	A4
α-Toc (86 mole g^{-1})	0.0092 ± 0.0004	68.98 ± 3.98	A4

k – constant rate; E_a – activation energy; $g(\alpha)$ – reaction model; BHT – Butylated hydroxytoluene; β-Car – β-carotene; α-Toc – α-tocopherol.

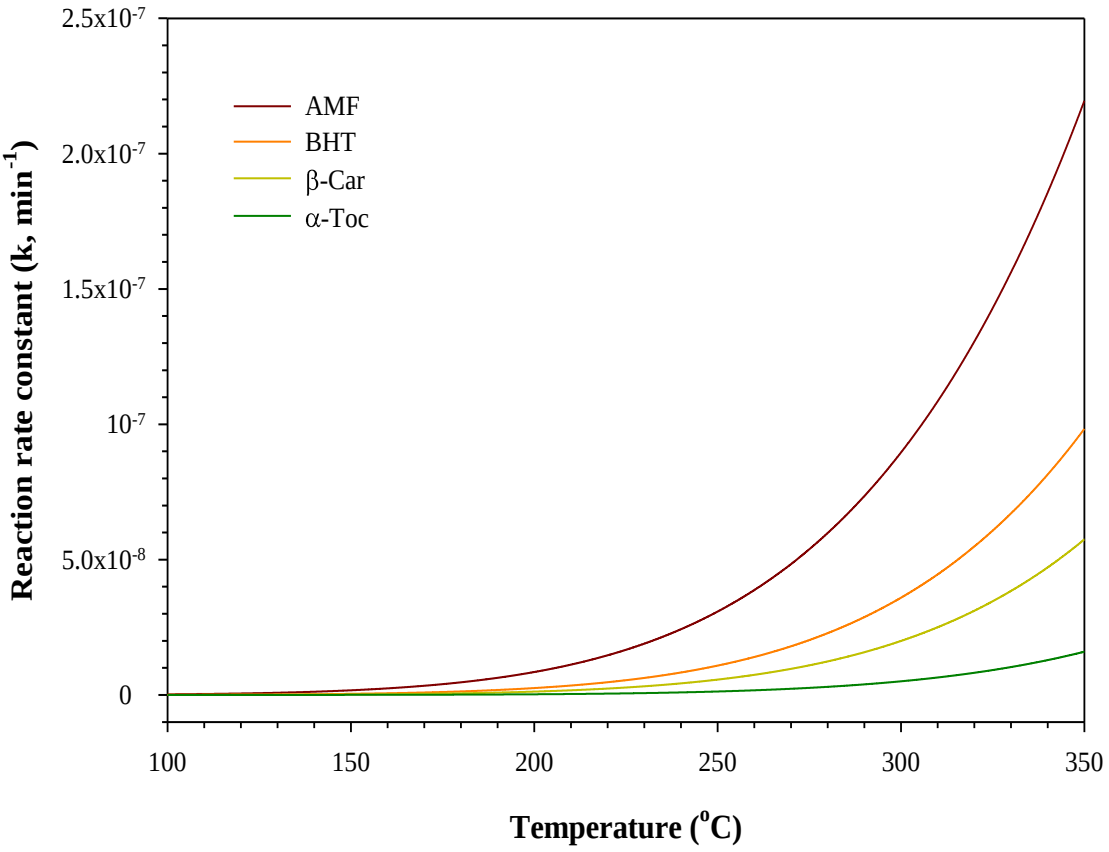


Figure 16. Reaction rate constant (k) for the oxidation of milk fat added with antioxidants. AMF – anhydrous milk fat; BHT – butylated hydroxytoluene (88 mole g^{-1}); β-Car – β-carotene (107 mole g^{-1}); α-Toc – α-tocopherol (86 mole g^{-1}).

4. Conclusions

The isoconversional method was used to study the oxidation of milk fat added with antioxidants. The kinetic analysis revealed that the oxidation of milk fat was mainly controlled by single-step reaction model. On the other hand, the addition of antioxidants changes the reaction kinetics according the values of the kinetic triplet. The dependence of activation with respect to the degree of conversion was established. The oxidation of milk fat was best described by the Avrami-Erofeev model. The obtained kinetic triplet (Ao, Eo, and reaction model) was used to systematically evaluate the role antioxidants. The addition of antioxidants significantly delayed the oxidation, being more effective α-Toc followed by β-Car and BH T. The outcomes of this research may enable online simulation and development of a databank.

References

Adhvaryu, A., Erhan, S. Z., Liu, Z. S., & Perez, J. M. (2000). Oxidation kinetic studies of oils derived from unmodified and genetically modified vegetables using pressurized differential scanning calorimetry and nuclear magnetic resonance spectroscopy. *Thermochimica Acta, 364*(1), 87-97. doi:https://doi.org/10.1016/S0040-6031(00)00626-2

Agrawal, R. K. (1992). Analysis of non-isothermal reaction kinetics: Part 1. Simple reactions. *Thermochimica Acta, 203,* 93-110. doi:https://doi.org/10.1016/0040-6031(92)85187-Z

Choe, E., & Min, D. B. (2005). Chemistry and Reactions of Reactive Oxygen Species in Foods. *70*(9), R142-R159. doi:10.1111/j.1365-2621.2005.tb08329.x

Lindmark-Månsson, H., & Åkesson, B. (2000). Antioxidative factors in milk. *British Journal of Nutrition, 84*(S1), 103-110. doi:10.1017/S0007114500002324

Litwinienko, G. (2001). Autooxidation of Unsaturated Fatty Acids and Their Esters. *Journal of Thermal Analysis and Calorimetry, 65*(2), 639-646. doi:10.1023/a:1017974313294

Litwinienko, G., Kasprzycka-Guttman, T., & Jamanek, D. (1999). DSC study of antioxidant properties of dihydroxyphenols. *Thermochimica Acta, 331*(1), 79-86. doi:https://doi.org/10.1016/S0040-6031(99)00058-1

Martínez-Monteagudo, S. I. (2018). Analysis of thermoxidation kinetics of milk fat. *Revista Mexicana De Ingeniería Química, 17*(2), 587-602.

Martínez-Monteagudo, S. I., & Saldaña, M. D. A. (2015). Retention of bioactive lipids in heated milk: Experimental and modelling. *Food and Bioproducts Processing, 94*, 290-296. doi:https://doi.org/10.1016/j.fbp.2014.03.008

Martínez-Monteagudo, S. I., Saldaña, M. D. A., & Kennelly, J. J. (2012). Kinetics of non-isothermal oxidation of anhydrous milk fat rich in conjugated linoleic acid using differential scanning calorimetry. *Journal of Thermal Analysis and Calorimetry, 107*(3), 973-981. doi:10.1007/s10973-011-1649-8

Martínez-Monteagudo, S. I., Saldaña, M. D. A., Kennelly, J. J. J. J. o. T. A., & Calorimetry. (2012). Kinetics of non-isothermal oxidation of anhydrous milk fat rich in conjugated linoleic acid using differential scanning calorimetry. *107*(3), 973-981. doi:10.1007/s10973-011-1649-8

Min, D. B., & Lee, H.-O. (1999). Chemistry of Lipid Oxidation. In R. Teranishi, E. L. Wick, & I. Hornstein (Eds.), *Flavor Chemistry: Thirty Years of Progress* (pp. 175-187). Boston, MA: Springer US.

Pérez-Maqueda, L. A., & Criado, J. M. J. J. o. T. A. (2000). The Accuracy of Senum and Yang's Approximations to the Arrhenius Integral. *Journal of Thermal Analysis and Calorimetry, 60*(3), 909-915. doi:10.1023/a:1010115926340

Saldaña, M. D. A., & Martínez-Monteagudo, S. I. (2013). Oxidative Stability of Fats and Oils Measured by Differential Scanning Calorimetry for Food and Industrial Applications. In A. A. Elkordy (Ed.), *Applications of Calorimetry in a Wide Context - Differential Scanning Calorimetry, Isothermal Titration Calorimetry and Microcalorimetry*.

Thurgood, J., Ward, R., & Martini, S. (2007). Oxidation kinetics of soybean oil/anhydrous milk fat blends: A differential scanning calorimetry study. *Food Research International, 40*(8), 1030-1037. doi:https://doi.org/10.1016/j.foodres.2007.05.004

Vyazovkin, S. (2018). Chapter 4 - Modern Isoconversional Kinetics: From Misconceptions to Advances. In S. Vyazovkin, N. Koga, & C. Schick (Eds.), *Handbook of Thermal Analysis and Calorimetry* (Vol. 6, pp. 131-172): Elsevier Science B.V.

Vyazovkin, S., Burnham, A. K., Criado, J. M., Pérez-Maqueda, L. A., Popescu, C., & Sbirrazzuoli, N. (2011). ICTAC Kinetics Committee recommendations for performing kinetic computations on thermal analysis data. *Thermochimica Acta, 520*(1), 1-19. doi:https://doi.org/10.1016/j.tca.2011.03.034

Xia, W., & Budge, S. M. (2017). Techniques for the Analysis of Minor Lipid Oxidation Products Derived from Triacylglycerols: Epoxides, Alcohols, and Ketones.

Comprehensive Reviews In Food Science And Food Safety, 16(4), 735-758. doi:10.1111/1541-4337.12276

Yin, H., Xu, L., & Porter, N. A. (2011). Free Radical Lipid Peroxidation: Mechanisms and Analysis. *Chemical Reviews, 111*(10), 5944-5972. doi:10.1021/cr200084z

Chapter 4

Using Isoconversional kinetics to study the effect of α-Tocopherol on the Oxidation of milk powder[2]

4.1. Introduction

Whole Milk powder is one of the most important dairy products because it is used as an essential ingredient and it is exported to developing countries. The fat content in whole milk powder is relatively high, no less than 26 % (USDEC, 2005). During processing and storage, fat in milk powder can be oxidized that may develop some changing on the produces, which decrease the quality and cause health problems (Lindmark-Månsson and Åkesson, 2000). For example, unpleasant flavor, aroma, and toxic compounds can be formed on the products. The oxidation reaction is a complex chemical reaction that happens on three stages: an initiation, a propagation, and a termination (Saldaña and Martínez-Monteagudo, 2013).

Numerous factors are involved in the breakage of such bonds including thermal homolytic process, presence of certain enzymes, exposure to light and metal ions, and presence of reactive oxygen species (Choe and Min, 2006). Over the last years, scientific evidence has proved that the addition of naturally or synthetically antioxidants can be inhabited or delayed the oxidation reaction (McClements and Decker, 2000; Jacobsen et al., 2008). These antioxidants have obtained popularity in nutritional studies because of its known antioxidative properties and potential health benefits in several biological functions, including immunity, metabolism, and tissue longevity. Examples of antioxidants are

[2] A version of this chapter is to be submitted to Thermochimica Acta

Butylated hydroxytoluene (BHT), D-alpha-Tocopherol, and β-carotene. Chemical and volumetric methods have been developed for quantifying and monitoring the oxidation of fats and oils, inducing differential scanning calorimetry (DSC), oxidative stability index (OSI), derivative thermogravimetry (DTG), gel permeation chromatography (GPC), and peroxide values (PV) (Gray, 1978; Kamal-Eldin and Pokorny, 2005). Due to its complexity, the oxidation of milk fat needs to be measured with a battery of tests (Martínez-Monteagudo et al., 2011).

Nevertheless, the oxidation of milk fat is commonly evaluated and monitored by measuring either primary or secondary oxidation products, where the concentration of such compounds is used to rank the development of oxidation. Alternatively, the oxidation reaction can be studied by thermal differential techniques such as thermosgravimetrical analysis (TGA) where mass lost during the oxidation is measured and subsequently related to the kinetic parameters of oxidation. The existing literature suggests that TGA under the non-isothermal regime is an effective method for ranking the oxidative stability of edible fats and oils. A general future of TGA for determining oxidative stability is the determining of the kinetic triplet: activation energy (E_a), pre-exponential factor (A), and reaction model ($f(\alpha)$). The isoconversional methods can collect the kinetic triplet and describe the reaction. The Kinetics Committee of the International Confederation for Thermal Analysis and Calorimetry (ICTAC) has developed a set of guidelines for the estimation of the kinetic triplet from the data obtained by using TGA (Vyazovkin et al., 2011). In this study, the oxidation of milk powder formulated added with α-Tocopherol, hydrogen peroxide and α-Tocopherol, or hydrogen peroxide measuring by Thermogravimetry (TGA) at linear

heating rates (3, 6, 9 and 12 °C min^{-1}) in a temperature range of 100 to 400°C was studied. It was found that the oxidative stability of samples were different in terms of the start and once the temperature of samples. The goal of this study was calculated the kinetic triples E_a, A, and $f(\alpha)$ to describe the thermoxidative behavior of milk powder formulated with an addition of either α-Tocopherol, hydrogen peroxide, and α-Tocopherol, or hydrogen peroxide.

4.2. Materials and methods

4.2.1. Materials

D-alpha-Tocopherol was purchased from Tokyo Chemical Industry (OR, USA) and stored at 4 C until the further analysis. Aluminum hermetic pans were obtained from TA Instruments (New Castle, DE), unsalted butter (Land O Lakes Inc., Arden Hills, MN, USA), whey protein concertation 80% (WPC, Milk Specialties Global, Eden Prairie, MN, USA) and D-alpha-Tocopherol was purchased from Tokyo Chemical Industry (OR, USA).

4.2.2. Anhydrous milk fat

Anhydrous milk fat (AMF) was prepared using the methodology reported by Martínez-Monteagudo et al., 2011, with some modifications. Briefly, raw cream obtained from the Davis Dairy Plant at South Dakota State University (Brookings, SD) was churned at 4°C to obtain butter using a laboratory scale churner (TM 31 Thermomix, Vorwerk LLC, Thousand Oaks, CA). The resulting butter was separated from the buttermilk using a cheesecloth. Then, the butter was washed with cold water to remove the excess of buttermilk. After that, the butter was heated at 60°C until the visual appearance of two

layers. The AMF was obtained after removing the top layer, and it was used within 24 h of manufacturing. One hundred grams of AMF was placed into an Erlenmeyer flask and heated at 60ºC.

4.2.3. Preparation of milk powder formulated

The milk powder formulated was prepared using the anhydrous milk fat and whey protein concertation (WPC80). Thirty percent of total solids was prepared using whey protein concertation (WPC80) and anhydrous milk fat (AMF), and was processed using ultrasound to be emulsified. The samples were divided into four groups and named: control, D-alpha, hydrogen peroxide, and D-alpha and hydrogen peroxide as are given in **Table 7**. The samples were dried using a single stage of spray dryer.

Table 7. Chemical characteristics of the four different milk powder formulated used for the oxidation kinetic analysis

Treatment	D-alpha-Tocopherol 0.2%	Hydrogen peroxide (three drops)
Sample 1		
Sample 2	x	
Sample 3	x	x
Sample 4		x

4.2.4. Thermogravimetric analysis

The guidelines proposed by Simon and Polavka (2006), on the use of TGA for the oxidation of milk powder formulated were followed. TGA experiments were accomplished with a (TGA, Q50, TA Instruments, New Castle, DE, USA) using TA Instrument Explorer software. The study was processed under an oxygen atmosphere. An open aluminum pan

was used for the oxidation experiments with an air flow of 50 mL/min at 20 psi supplied from a cylinder. A four to six mg of sample was transferred into an open aluminum pan then the samples were equilibrated at 100°C and heated to 300°C at linear heating rates (3, 6, 9 and 12°C min^{-1}). The TGA spectra were analyzed by a TA Universal Analysis software (TA instruments). The oxidation experiments were performed in duplicates.

4.2.5. Kinetic analysis

The analysis of the oxidation kinetics was performed following the recommendations developed by the Kinetics Committee of the International Confederation for Thermal Analysis and Calorimetry (Vyazovkin et al., 2011). A detailed explanation of the kinetic analysis is described in Chapter 3 (Section3.2.4).

4.3. Results and discussions

4.3.1 Oxidative profile

A representative TGA curve (normalized weight loss (%) against temperature) of the different samples are illustrated in **Figure 17**. The mass loss was normalized to account for a gradual and slight weight increase at the beginning of the heating program. The weight gain was observed at low temperatures (>120°C) and it was attributed to oxygen chemisorptions. The TGA curves exhibited three main exothermic events (indicated by arrows in **Figure 17**) during the entire heating program. The first exothermic event (arrow (1)) spanned from 150-225°C, and it is characterized by a slight decreased (>9%) in the values of normalized mass. Such reductions are associated to the evaporation of volatiles compounds derived from protein and lactose rather than oxidation. In the second event (arrow (2)), a sharp reduction in the values of normalized mass was detected within the

temperature range of 225-245°C. The temperature corresponding to the start of the sharp reduction in the mass was 225, 232, 234, and 245°C for the sample added with the prooxidant agent (H_2O_2), control, sample with added α-Toc, and α-Toc, respectively. The temperature of oxidation shifted to higher values for α-Toc, indicating an enhancing of the oxidative stability of the powder. The last exothermic event (arrow (3)) was recorded at a temperature of 238°C, and it associated with decomposition of protein and lactose. The exception of this generalization was obtained in the powder formulated with α-Toc, where the exothermic event was detected at 242°C.

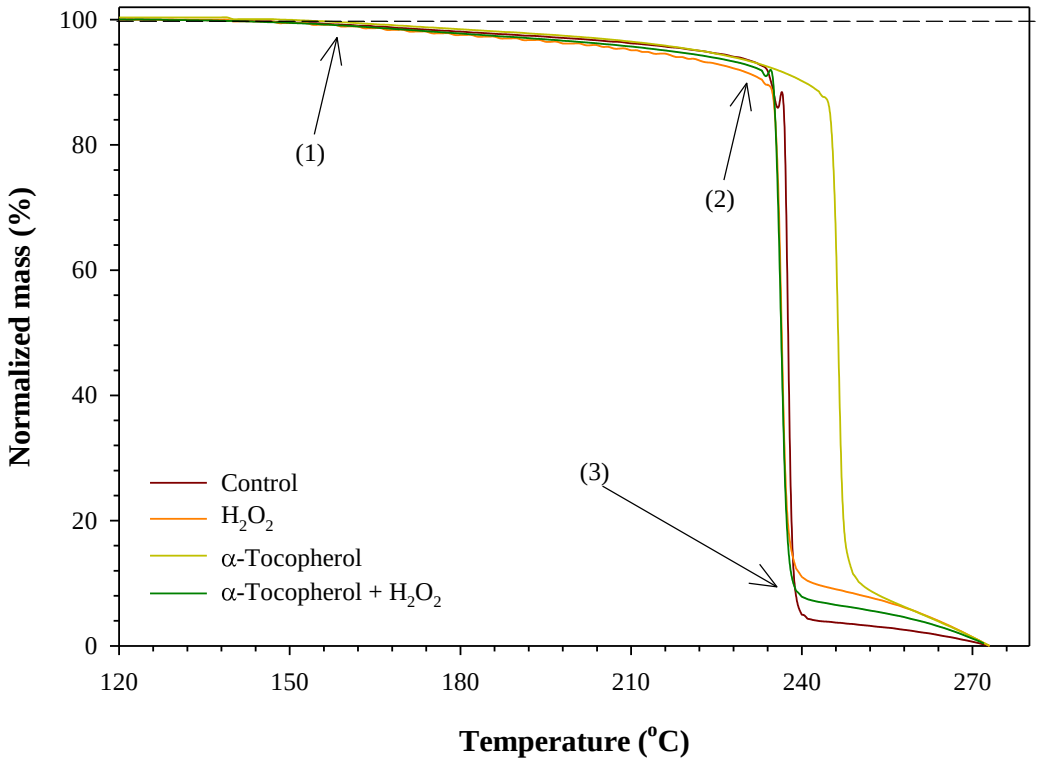


Figure 17. Representative thermogramimetrical curve of normalized mass loss against temperature for the different samples. Arrows indicate three distinctives exothermic events.

The onset temperature of oxidation was calculated according to the methodology described by Martinez-Monteagudo et al. (2012). A common way to evaluate the rank the oxidative stability of fat and oils is through the onset temperature (**Figure 18**). As expected, the values of the onset increased with the heating rate. The onset values ranged from 210 to 224°C increasing with the heating rate. Simon and Polavka (2006) reported values of onset temperature in the rage of 150-190ºC using a heating rate in the range of 1-15°C min^{-1}. The difference between Simon and Polavka's values can be explained by the amount of fat. Due to low molecular weight compounds that can generate in solution during the oxidation at low heating rates play a role to increase the speed of oxidation; however, they can be evaporated before they react with a solution when using high heating rates (Martinez-Monteagudo, 2018). In general, the Ts values were increased after α-Tocopherol was added. Further assessment of the effect of antioxidant during non-isothermal oxidation was performed considering Ts as a point of comparison. In general, the values of Ts increased with the heating rate, adding α-Tocopherol (**Figure 18**). During slow heating, compounds of low molecular weight may remain within the sample, and they serve as starters propagating the oxidation. On the other hand, these compounds evaporate before the can react with the sample. Consequently, higher temperature is required for the oxidation of the sample.

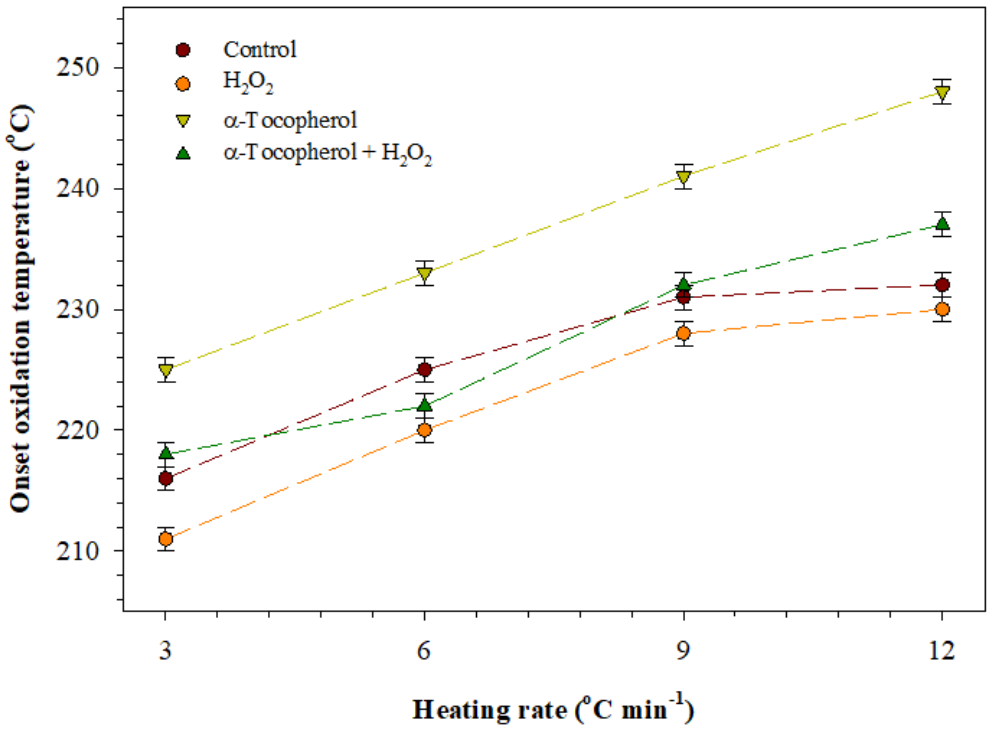


Figure 18. Onset temperature of oxidation of powder formulated with anhydrous milk fat (control), hydrogen peroxide (H_2O_2), α-tocopherol (α-Toc).

4.3.2. Degree of conversion

The degree of conversion of the different powder formulated with of either α-tocopherol, hydrogen peroxide and α-Tocopherol, or hydrogen peroxide are shown in **Figure 19**. The degree of conversion was calculated within the temperature range of 150-250°C, corresponding to the non-isothermal oxidation of edible fat and oils. For the powder formulated with addition of control, H_2O_2, α-Toc, and α-Toc+H_2O_2, the temperature span ranged from 100-286, 100-246, 100-290, and 100-247°C, respectively. The temperatures were below the self-ignition of edible oils, which means they can be considered as oxidation ranges (Martinez-Monteagudo, 2018).

The curves for all samples were sigmoidal in shape, which is a characteristic of an autocatalytic reaction (Agrawal, 1992). An autocatalytic reaction consists of an initial stage where the oxidation is accelerated exponentially, and a final stage where the oxidation reached its maximum conversion (Vyazovkin et al., 2011). The oxidation range was considered as the converted fraction of the span of temperature corresponding ($0 \leq \alpha \leq 1.0$). Moreover, these curves represent reaction steps that take place during the non-isothermal oxidation. One important characteristics of the non-isothermal oxidation is that several reactions occur having comparable values of activation energy and the heating rate influences the general pattern of degree of conversion curves. This behavior is explained by two reasons. The first is that the heat flow signal detected by the DSC is a result of those reaction having high values of activation energy. The second reason is due to structural changes induced by the heating rate, resulting in a diffusion-controlled reaction rather than a kinetic-controlled.

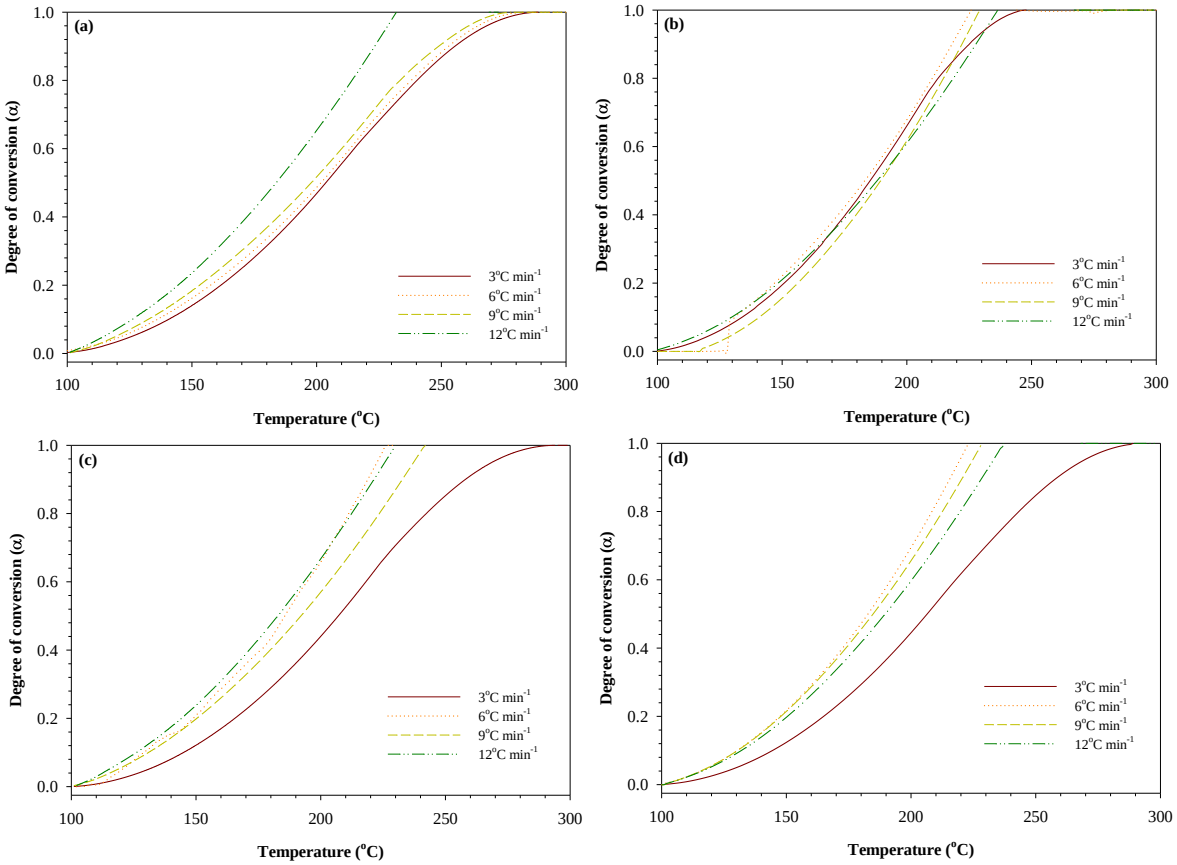


Figure 19. Degree of conversion as a function of temperature for the powder formulated without additives, control (a); powder formulated with H_2O_2 (b); powder formulated with α-Tocopherol (c); and powder formulated with α-Tocopgerol+H_2O_2.

4.3.3. Relationship Between E_α and α

The KAS method was used to estimate the activation energy (E_α) at a given value of α. The relationship between E_α and α for the control, H_2O_2, α-Toc, and α-Toc+ H_2O_2 is shown in **Figure 20**. For clarity, the influence of α on the E_α was divided into three stages:

- Stage I (0.05-0.20) – it shows a linear decreased of E_α from 239 to 68, 222 to 47, 117 to 78, and 143 to 66 kJ mol^{-1} for the control, H_2O_2, α-Toc, and α-

Toc+ H_2O_2, respectively. Such decrease in the values of E_α is due to the initiation of oxidation, where several reactions take place including formation of radicals and primary oxidation products. In this stage, the oxidation of powder is likely dominated by multi-step reactions.

- Stage II (0.20-0.80) – the values of E_α remained unchanged constant with α over 0.2 to 0.80. This is an indication that the oxidation of all samples is controlled by a single-step reaction, possible break down of primary oxidation products, and it can be adequately described by a single-step reaction model (Vyazovkin *et al.,* 2011).

- Stage III (0.80-0.95) – the values of E_α increased with α from 53-93, 43-143, 75-196, and 66-99 kJ mol^{-1} for the control, H_2O_2, α-Toc, and α-Toc+ H_2O_2, respectively. Such changes in the E_α might correspond when the oxidation approaching to the termination stage, where stable products are formed (Martínez-Monteagudo *et al.,* 2011). An increasing behavior of E_α with respect to α have been associated with thermal decomposition of polymers such poly (imide siloxane) (Shi, 1990). Thermal decomposition of polymers is characterized by competition among individual molecules and intermocular complex (Vyazovkin, 1996).

Despite the three distinctive stages, the overall behavior of E_α toward α did not resemble the generalized behavior for multi-step reactions such competitive, consecutive,

reversible, or diffusion-controlled reactions (Vyazovkin and Wight, 1997). Thus, it is reasonable to assume that the oxidation of the different samples can be described by a single-step model.

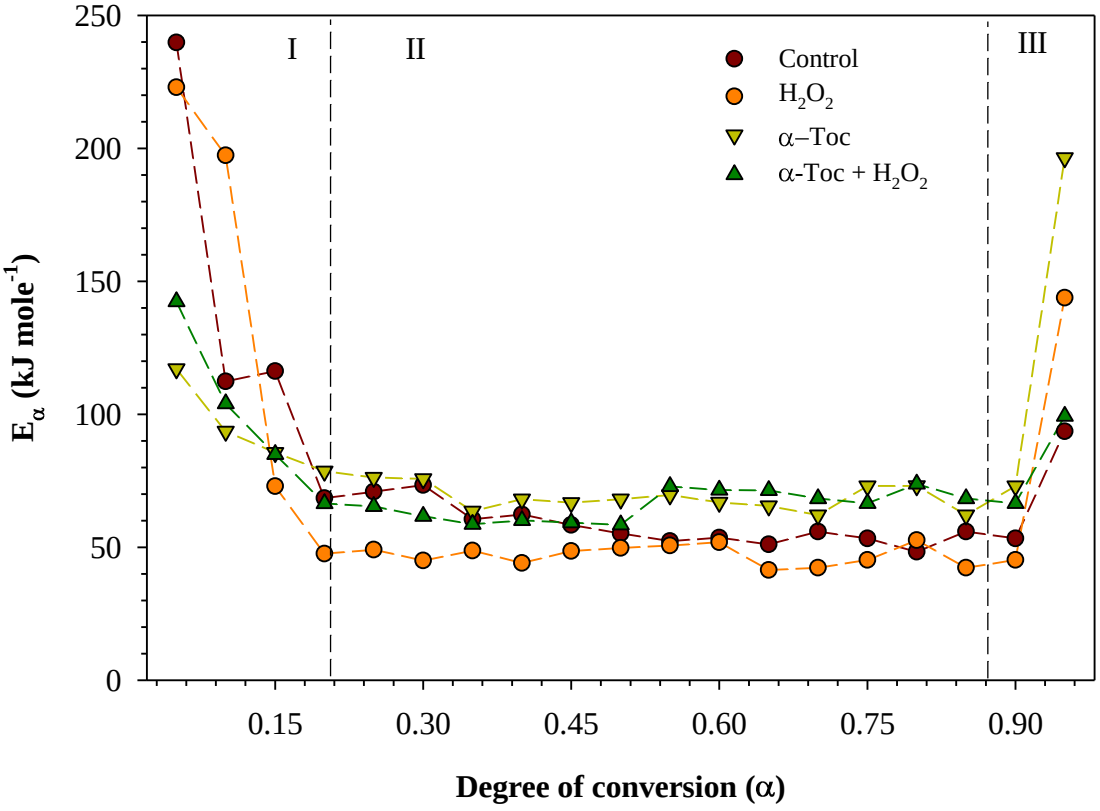


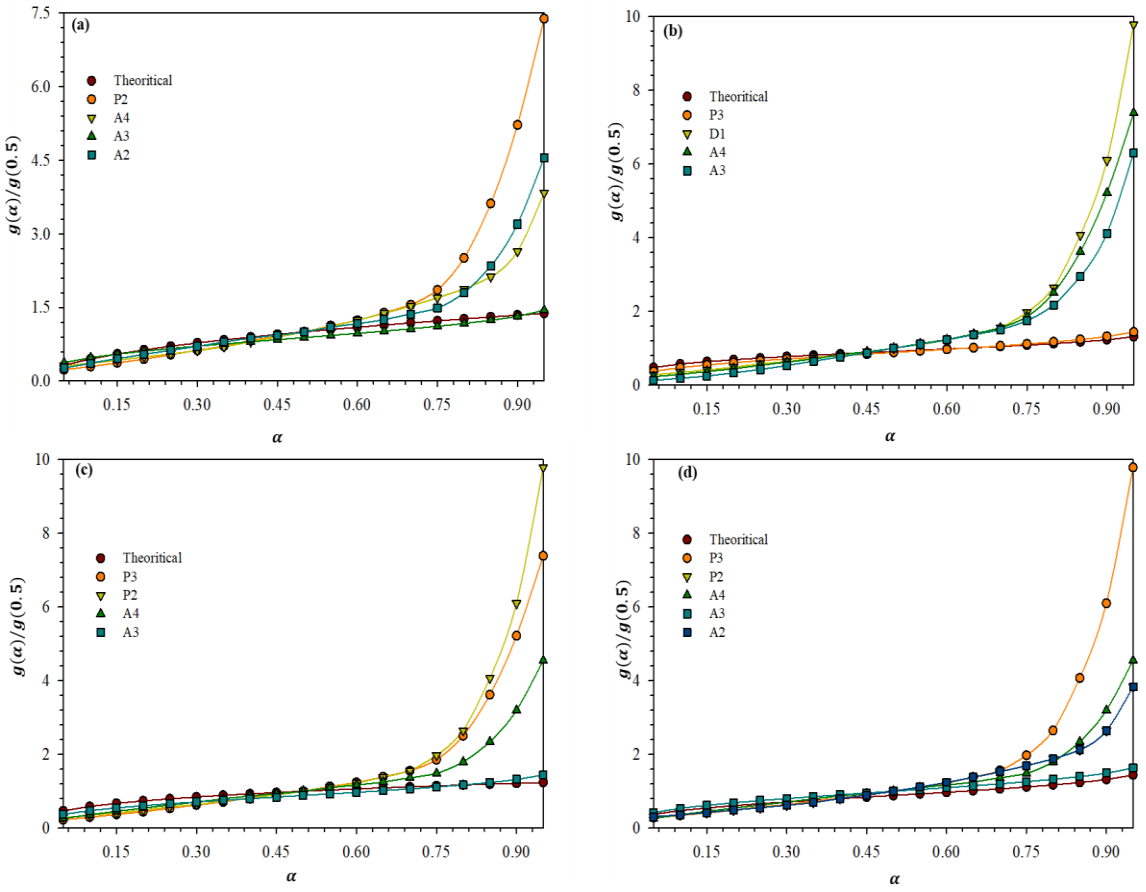
Figure 20. Values of activation energy (E_α) as a function of degree of conversion (α) for the powder formulated without additives, control (a); powder formulated with H_2O_2 (b); powder formulated with α-Tocopherol (c); and powder formulated with α-Tocopgerol+H_2O_2.

4.3.4. Determination of reaction model

The reaction model for (α) for the powder formulated without additives, powder formulated with H_2O_2, powder formulated with α-Tocopherol, and powder formulated with

α-Tocopgerol+H_2O_2 was determined by fitting 13 different models (**Table 4**). The testing model was completed by non-linear regression using $\ln A$ and E_α that were obtained from KAS method. Overall, the coefficient of determination (R^2) ranged from 0.40 to 0.99, depending on the reaction model and sample. For those models were the fit was notably poor were discarded for further analysis. Contrary, those models whose values of R^2 were >0.97 were considered as statistically appropriate for describing the oxidation. The models P2, A4, A3, and A2 provided satisfactory fitting for the powder formulated without additives, while the oxidation of powder formulated with H_2O_2 was satisfactorily represented by P3, P2, A4, and A3. On the other hand, the powder formulated with α-Tocopherol was described by P3, P2, A4, and A3. The models P3, P2, A4, and A2 satisfactorily described the powder formulated with α-Tocopgerol+H_2O_2. All these models were further investigated using the master plot method (**Figure 21**).

Figure 21. Theoretical master curves for the different kinetic models and the theoretical data for: the powder formulated without additives, control (a); powder formulated with H_2O_2 (b); powder formulated with α-Tocopherol (c); and powder formulated with α-Tocopgerol+H_2O_2.



For all samples, the theoretical plot corresponding to the A3 model closely matched the theoretical data over the entire range of α (0.05-0.95). Thus, the oxidation of AMF with and without added antioxidants was most probably described with the Avrami-Erofeer model (A3). This observation contradicts the first-order assumption commonly used during the analysis of milk fat oxidation. Deviations from the first-order model are expected during the oxidation of triacylglycerol since the fatty acids are distributed throughout different triacylglycerol, resulting in different combinations of molecular weights, chain

lengths, and degree of saturation (Martínez-Monteagudo & Saldaña, 2015). An ideal model would be one that not only having adequate statistical indexes but also providing insights into the kinetic scheme.

4.3.5. Determination of pre-exponential factor

The pre-exponential factor was calculated using the compensation theory (**Figure 22**). A linear relationship existed between A and E_α for the different models, and all data fell onto a master linear curve, from which the regression parameters were calculated from the slope (parameter a) and intercept (parameter b). The pre-exponential factor was then calculated by substituting a and b into their respective equation using the value of E_α within the range of 0.2-0.8, where $E_a \approx E_\alpha$. **Table 8** shows the regression analysis of the compensation theory. The parameters (a and b) were much higher than their respective 95% confidence interval (95% CI), meaning that these parameters can be used for predictions. In addition, the R^2 values (>0.98, **Table 8**) indicate that the pre-exponential factor can be represented within the tested conditions.

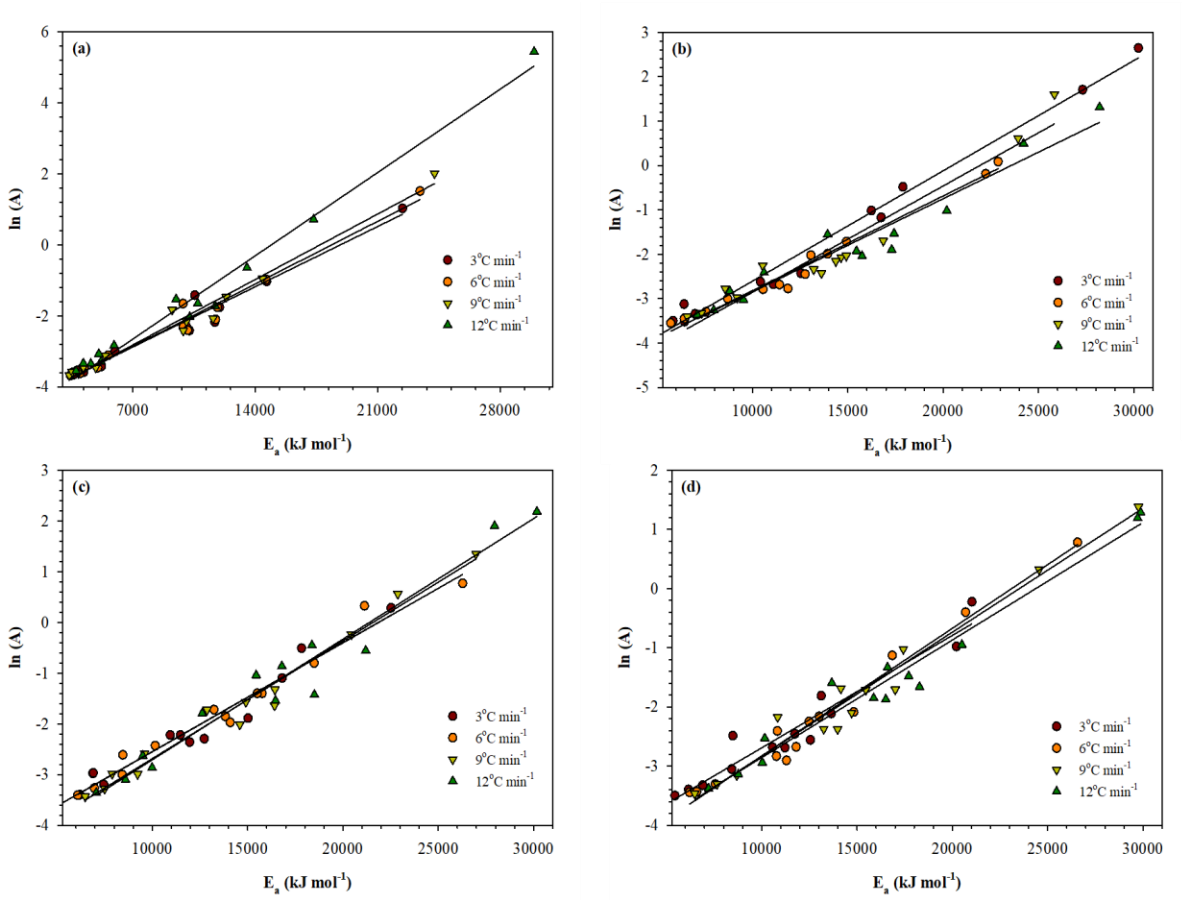


Figure 22. The compensation effect (lnA vs. Ea) obtained during the oxidation for: the powder formulated without additives, control (a); powder formulated with H_2O_2 (b); powder formulated with α-Tocopherol (c); and powder formulated with α-Tocopgerol+H_2O_2.

Table 8. Regression parameters obtained from the compensation theory during the oxidation of different powders.

Heating rate (β, °C min^{-1})	**Powder formulated without additives**				
	a	**CI95%**	**b**	**CI95%**	**R^2**
3	2.43 x10^{-4}	6.07 x10^{-5}	-4.58	0.63	0.987
6	2.52 x10^{-4}	5.54 x10^{-5}	-4.63	0.58	0.985
9	2.64 x10^{-4}	5.33 x10^{-5}	-4.67	0.56	0.995
12	3.35 x10^{-4}	5.75 x10^{-5}	-5.09	0.71	0.997
Heating rate (β, °C min^{-1})	**Powder formulated with H_2O_2**				
	a	**CI95%**	**b**	**CI95%**	**R^2**
3	2.49 x10^{-4}	3.91 x10^{-5}	-5.08	0.61	0.993
6	2.13 x10^{-4}	4.07 x10^{-5}	-4.95	0.54	0.989
9	2.40 x10^{-4}	8.81 x10^{-5}	-5.25	1.01	0.986
12	2.10 x10^{-4}	6.91 x10^{-5}	-4.93	0.97	0.987
Heating rate (β, °C min^{-1})	**Powder formulated with α-Tocopherol**				
	a	**CI95%**	**b**	**CI95%**	**R^2**
3	2.15 x10^{-4}	5.83 x10^{-5}	-4.69	0.74	0.989
6	2.16 x10^{-4}	4.63 x10^{-5}	-4.70	0.84	0.988
9	2.32 x10^{-4}	4.78 x10^{-5}	-5.07	0.74	0.984
12	2.38 x10^{-4}	6.58 x10^{-5}	-5.08	1.05	0.987
Heating rate (β, °C min^{-1})	**Powder formulated with α-Tocopgerol+H_2O_2**				
	a	**CI95%**	**b**	**CI95%**	**R^2**
3	1.90 x10^{-4}	6.79 x10^{-5}	-4.59	0.84	0.988
6	2.16 x10^{-4}	4.68 x10^{-5}	-4.98	061	0.986
9	2.09 x10^{-4}	5.19 x10^{-5}	-4.92	0.83	0.986
12	1.99 x10^{-4}	4.98 x10^{-5}	-4.84	0.89	0.982

a, b – regression parameters; CI95% – 95% confidence interval; R^2 – coefficient of determination.

4.3.6. Oxidation rate

Table 9 summarizes the calculated kinetic triplet obtained for Control, H_2O_2, α-Tocopherol, and α-Tocopherol+ H_2O_2. The information presented in **Table 9** was further used for modelling the oxidation of oxidation of the different powders by substituting the kinetic triplet. A new set of data was generated using the calculated kinetic triplet (**Table 9**), and the resulting values of the constant rate (k) were plotted as a function of the

temperature (Figure 16). As the temperature increased, the k values for all samples exponentially increased, being greater for H_2O_2, control, α-Tocopherol+ H_2O_2, and α-Tocopherol. Remarkably, the oxidation of a powder was significantly delayed by the addition of α-Tocopherol within the entire range of temperature. At- 200°C, the oxidation was reduced 97-fold by the addition of α-Tocopherol with respect to the intentionally oxidized sample (H_2O_2), while 7.84-fold with respect to the control sample.

Table 9. Summary of the obtained kinetic triplets for the oxidation of different powders.

Sample	k (min^{-1})	E_a (kJ mol^{-1})	$g(\alpha)$
Control	0.0088 ± 0.0004	58.12 ± 3.21	A3
H_2O_2	0.0064 ± 0.0003	46.91 ± 2.75	A3
α-Tocopherol	0.0076 ± 0.0003	69.43 ± 3.05	A3
α-Tocopgerol+H2O2	0.0081 ± 0.0004	65.89 ± 5.12	A3

k – constant rate; E_a – activation energy; $g(\alpha)$ – reaction model; Control – powder formulated without additives; H_2O_2 – powder formulated with H_2O_2; α-Tocopherol – owder formulated with α-Tocopherol; α-Tocopgerol+H_2O_2 – powder formulated with α-Tocopgerol+H_2O_2.

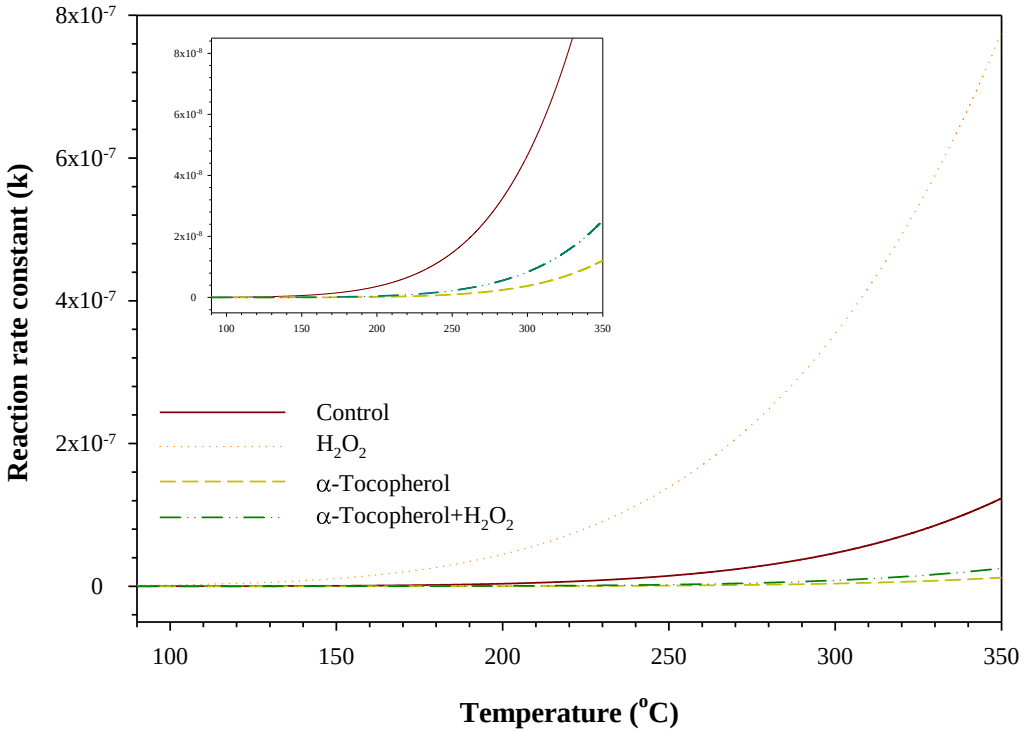


Figure 23. Reaction rate constant (k) for the oxidation of the different samples. Powder formulated without additives, control; powder formulated with H_2O_2; Powder formulated with α-Tocopherol; Powder formulated with α-Tocopgerol+H_2O_2. The insets is zoom into the reaction rate constant.

4. Conclusion

There is no doubt that milk powders are of high significance in terms of nutritional values and economical. In this study, the oxidation reaction of milk powder formulated with the addition of α-Tocopherol, hydrogen peroxide, and α-Tocopherol, or hydrogen peroxide was studied using thermosgravimetrical analysis (TGA) method. The isoconversional method was used to obtain the kinetic triplets (Ea, A, and reaction model) using a single-step reaction model. The dependence between Ea and α was confirmed using the KSA method. The best reaction model to describe the milk powder formulated with the addition of α-Tocopherol, hydrogen peroxide, and α-Tocopherol, or hydrogen peroxide

was Avrami-Erofeev (A3). The addition of α-Tocopherol delayed the speed of oxidation of milk powder. The outcomes of this study may use continue processing for milk powder and different dairy product.

References

Agrawal, R.K. 1992. Analysis of non-isothermal reaction kinetics: Part 1. Simple reactions. Thermochim. Acta 203:93–110.

Choe, E., and D.B. Min. 2006. Chemistry and reactions of reactive oxygen species in foods. Crit. Rev. Food Sci. Nutr. 46:1–22.

Gray, J.I. 1978. Measurement of lipid oxidation: a review. J. Am. Oil Chem. Soc. 55:539–546.

Jacobsen, C., M.B. Let, N.S. Nielsen, and A.S. Meyer. 2008. Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated lipids: a comparative evaluation. Trends food Sci. Technol. 19:76–93.

Kamal-Eldin, A., and J. Pokorny. 2005. Analysis of Lipid Oxidation. AOCS Publishing.

Lindmark-Månsson, H., and B. Åkesson. 2000. Antioxidative factors in milk. Br. J. Nutr. 84:103–110.

Martinez-Monteagudo, S.I. 2018. Analysis of thermoxidation kinetics of milk fat. Rev. Mex. Ing. Química 17:587–602.

Martínez-Monteagudo, S.I., M.D.A. Saldaña, and J.J. Kennelly. 2011. Kinetics of non-isothermal oxidation of anhydrous milk fat rich in conjugated linoleic acid using differential scanning calorimetry. J. Therm. Anal. Calorim. 107:973–981.

McClements, D.J., and E.A. Decker. 2000. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J. Food Sci. 65:1270–1282.

Pérez-Maqueda, L., and J. Criado. 2000. The accuracy of Senum and Yang's approximations to the Arrhenius integral. J. Therm. Anal. Calorim. 60:909–915.

Polavka, P.Š.-J. 2006. Thermooxidative degradation of dried milk studied by non-isothermal thermogravimetry. J. Food Nutr. Res. 45:166–170.

Saldaña, M.D.A., and S.I. Martínez-Monteagudo. 2013. Oxidative stability of fats and oils measured by differential scanning calorimetry for food and industrial applications. IntechOpen.

Vyazovkin, S., A.K. Burnham, J.M. Criado, L.A. Pérez-Maqueda, C. Popescu, and N. Sbirrazzuoli. 2011. ICTAC Kinetics Committee recommendations for performing kinetic computations on thermal analysis data. Thermochim. Acta 520:1–19.

Chapter 5

Summary and conclusions

A vast amount of literature correlates milk products with health benefits. More importantly, milk fat is used as an ingredient because of its nutritional value, functionality, and flavor. During processing and storage, milk fat may undergo oxidation resulting in many undesirable changes such as unpleasant flavor and aroma, and formation of toxic compounds. Thus, obtaining milk fat with high oxidative stability is highly desirable for the dairy and food industries. A common method aims at minimizing the oxidation is the use of antioxidants, compounds with the ability to inhibit or delay the oxidation.

Throughout the chapters of this thesis, the oxidative stability of milk fat with an addition of different antioxidants was extensively evaluated by means of kinetics studies which allowed identifying the best conditions to improve the oxidative stability of milk fat. Chapter 3 reported a kinetic study of AMF with an addition of either D-alpha-Tocopherol, BHT, or Beta-carotene at four different concentrations. The samples were oxidized using non-isothermal DSC at linear heating rates (3, 6, 9 and 12 °C min^{-1}) in a temperature range of 100 to 400 °C. DSC spectra were analyzed according to KAS method, from which A and Ea were obtained. Remarkably, the addition of 0.2% of α-Toc inhibited the oxidation reaction by 9-fold, judging the constant rate. One of implications of this study was addressed in Chapter 4, where the oxidation kinetics of milk powder formulated with an addition of either D-alpha-Tocopherol, hydrogen peroxide, and D-alpha-Tocopherol and hydrogen peroxide was studied using non-isothermal TG at linear heating rates (3, 6, 9 and 12 °C min^{-1}) in a temperature range of 100 to 300°C. The results obtained showed that the

oxidation of AMF, and milk powder formulated started at high temperature after added D-alpha-Tocopherol.