CAUSE OF COLOR COMPONENT FORMATION IN OILS DURING FRYING

KELSEY LAZARICK
B.Sc., University of Lethbridge, 2009

A Thesis
Submitted to the School of Graduate Studies of
the University of Lethbridge
in Partial Fulfillment of
the Requirements for the Degree

MASTER OF SCIENCE

Department of Chemistry and Biochemistry
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

Abstract

Color formation in oils during frying is one of the most noticeable degradation reactions that occur in the frying oil. Degradation reactions cause formation of products that positively and negatively impact the nutritional and sensory qualities of both the food being fried and the frying oil. The origins of these pigment forming reactions in the oil and the factors affecting these reactions are not well understood. Assessments of the mechanisms, the components involved and external conditions affecting oil darkening were conducted.

The effect of basic food ingredients, commercially sold and laboratory formulated breading and battering, preformed lipid hydroperoxides and phospholipids on color formation and oil degradation of the frying oil were investigated.

Protein products, specifically whey protein, caused both the fastest darkening and thermo-oxidative deterioration of the frying oil. This breakdown was aided further through the addition of minor food materials such as glucose and amino acids as well as lipid hydroperoxides in concentrations greater than 5 % of the frying oil. Nonenzymatic browning is the main reaction causing color formation in the frying oil and utilizes carbonyls from the food product such as starches, sugars and lipid oxidation products as starting materials alongside amino groups from proteins and amino acids. Breading ingredients contributed to oil color formation due to particles from the food crust breaking off into the frying oil to further accelerate browning reactions.

Increasing the temperature of the frying oil provided additional stimulus for color forming and thermo-oxidative reactions to progress at a faster rate.

Acknowledgements

To my professor, Dr. Roman Przybylski, I appreciate the opportunity to be taught by someone with such an abundance of knowledge, experience, and patience. Your guidance and encouragement has helped lead me through the course of this study.

I would like to thank my other committee members, Dr. Paul Hayes and Dr. Roy Golsteyn, for their time, commitment, and interest.

For the financial support provided to me, I would like to thank the University of Lethbridge, the Keith and Hope Ferguson Memorial Scholarship and Dr. Przybylski.

I would like to express my appreciation to my lab mates, Felix Aladedunye, Ewa Sosinska, Eliza Gruczynska, Magdalena Rudzinska, Dominic Kmiecik, Ozan Ciftci, and Yohann Catel. They provided advice and assistance along with friendship and the ability for me to experience the world without leaving Canada.

Finally, I am forever grateful to my loving parents, family, and friends for their support and understanding. Thank you all for never leaving my side. You will forever be in my heart.

Table of Contents

List of Tables

List of Figures

List of Schemes

List of Abbreviations

1 % NP	1 % oxidized oil with peroxides reduced
ANOVA	single factor analysis of variance
AV	para anisidine value
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BM	commercial batter mixture
CF	Corn flake® breading
CO	canola oil
CPM	composition of polar materials
DSC	differential scanning calorimetry
ELSD	evaporative light scattering detector
FAME	fatty acid methyl esters
FFA	free fatty acids
FTIR	fourier transformed infrared spectroscopy
FTNIR	fourier transformed near-infrared spectroscopy
GC	gas chromatography
Glu 1 %	starch + glucose (99:1, wt/wt)
Glu 10 %	starch + glucose (90:10, wt/wt)
Gly	glycine
HLCO	high oleic, low linolenic canola oil
HNE	4-hydroxynonenal
HPLC	high performance liquid chromatography
LC	liquid chromatography
Lys	lysine
HPSEC	high performance size exclusion chromatography
MH	whey + starch + glucose + glycine (33:65.9:1:0.1, wt/wt/wt/wt)
MS	mass spectroscopy
MO	whey + starch + glucose + glycine (33:65.9:1:0.1, wt/wt/wt/wt) + oxidized oil
NIR	near infrared spectroscopy
NMR	nuclear magnetic resonance

Ox	oxidized oil
OxWG	whey + glycine (99.9:0.1, wt/wt) + oxidized oil
PCI	photometric color index
Phos	L-α-phosphatidylcholine or phospholipid
ppm	parts per million
PUFA	polyunsaturated fatty acids
PV	peroxide value
RCO	regular canola oil
RP	reduced peroxides
S&B	Shake and bake® breading
SGG	starch + glucose + glycine (98.9:1:0.1, wt/wt/wt)
SGly	starch + glycine (99.9:0.1, wt/wt)
SLys	starch + lysine (99.9:0.1, wt/wt)
SIM	selected ion monitoring
SLys	starch + lysine (99.9:0.1, wt/wt)
St	starch
TAG	triacylglycerols
TBHQ	*tert*-butylhydroquinone
THF	tetrahydrofuran
TPC	total polar components
WGG	whey + glucose + glycine (98.9:1:0.1, wt/wt/wt)
WGly	whey + glycine (99.9:0.1, wt/wt)
WLys	whey + lysine (99.9:0.1, wt/wt)

Chapter 1 – Literature Review

1.1 The frying industry

The frying of food can be performed in a variety of ways such as pan frying, deep fat frying, stir frying, and sautéing. Each type of frying can be modified to suit the needs of the individuals carrying out the frying process. Deep fat frying is an important food preparation process in the food industry because it is fast, convenient, and produces highly accepted foods amongst consumers. Unique properties of fats and oils provide a distinctive flavor, odor, and pleasing mouth-feel to fried food products (Orthoefer and List, 2006a). Properly cooked, fried food products have a golden brown color, crispy and non-greasy crust with a moist and tender inside. Commercial food processing, table service restaurants, and fast food restaurants all use deep fat frying to achieve desirable qualities in their foods (Lawson, 1995). Despite the present nutritional recommendations asking for reduced fat intake, the output of fried food products is continuously growing (Dobarganes et al., 2000a). In the United States, an estimated $83 billion is attributed to the economy of commercial deep fat frying, which is at a level twice as high as the amount in the rest of the world (Choe and Min, 2007). The Canadian Community Health Survey conducted by Statistics Canada asked more than 35,000 people to recall what they had eaten during the 24 hours before they were interviewed and a quarter of Canadians had dined at fast food restaurants and consumed fried foods (Garriguet, 2006). Among people aged 31 to 50, over a quarter of them had exceeded the recommended fat intake and consumed more than 35 % of their total calories from fat, the upper limit of the recommended value (Institute of Medicine, 2005). As a result of market trends, a rising interest in quality of fried food products and oils has occurred.

1.2 The frying process

Deep fat frying is a simple method of food preparation but the complex chemical reactions and physical changes that occur during frying are not fully understood. The process

of deep frying involves heating oil continuously or repeatedly at high temperatures with the complete immersion of food products (Choe and Min, 2007). Direct heat and mass transfer occurs between the three major constituents; the oil, atmosphere, and food. Heat transfer from the hot oil to the cold food causes moisture evaporation from within the food and cooking of the inside (Orthoefer and List, 2006a). This conductive heat exchange initiates extensive mass transfer when the internal moisture is released into the oil and air. Water from the food is transferred to the oil as steam and also helps to move components from the food into the frying oil and from the oil into the food. Initially, the formation of steam and its release from the food cools the oil. The heat from the fryer eventually warms the oil back to its frying temperature. This high temperature dehydrates the surface of the food and promotes reactions among and between the food components and the frying oil. Mass transfer during deep frying includes water loss and oil absorption into the food as well as numerous interactions of food ingredients with the frying oil including exchange of fat components between food and oil. Volatile components are released into the atmosphere and oxygen aerates the oil. All of these reactions result in positive changes to food characteristics but undesirable modifications occur alongside to the frying oil and are outlined in **Figure 1**. In the beginning, most frying oils are pure triacylglycerides (> 96 %) (Blumenthal, 1987). Due to oxygen absorption from the oil-air interface, leaching of food components, and the breakdown of oil constituents, a mixture of hundreds of chemical components is formed. The rapidly degrading frying oil can produce compounds that reduce oil quality, be absorbed by the fried food to create off-flavors, and cause a decrease in the nutritional value of the fried food (Orthoefer and Cooper, 2006). This can potentially become hazardous as highly oxidized oils have been linked to deleterious health effects in mice causing weight loss, liver and kidney damage, and intestinal tumours (Andia and Street, 1975; Paul et al., 1997; Stier, 2004).

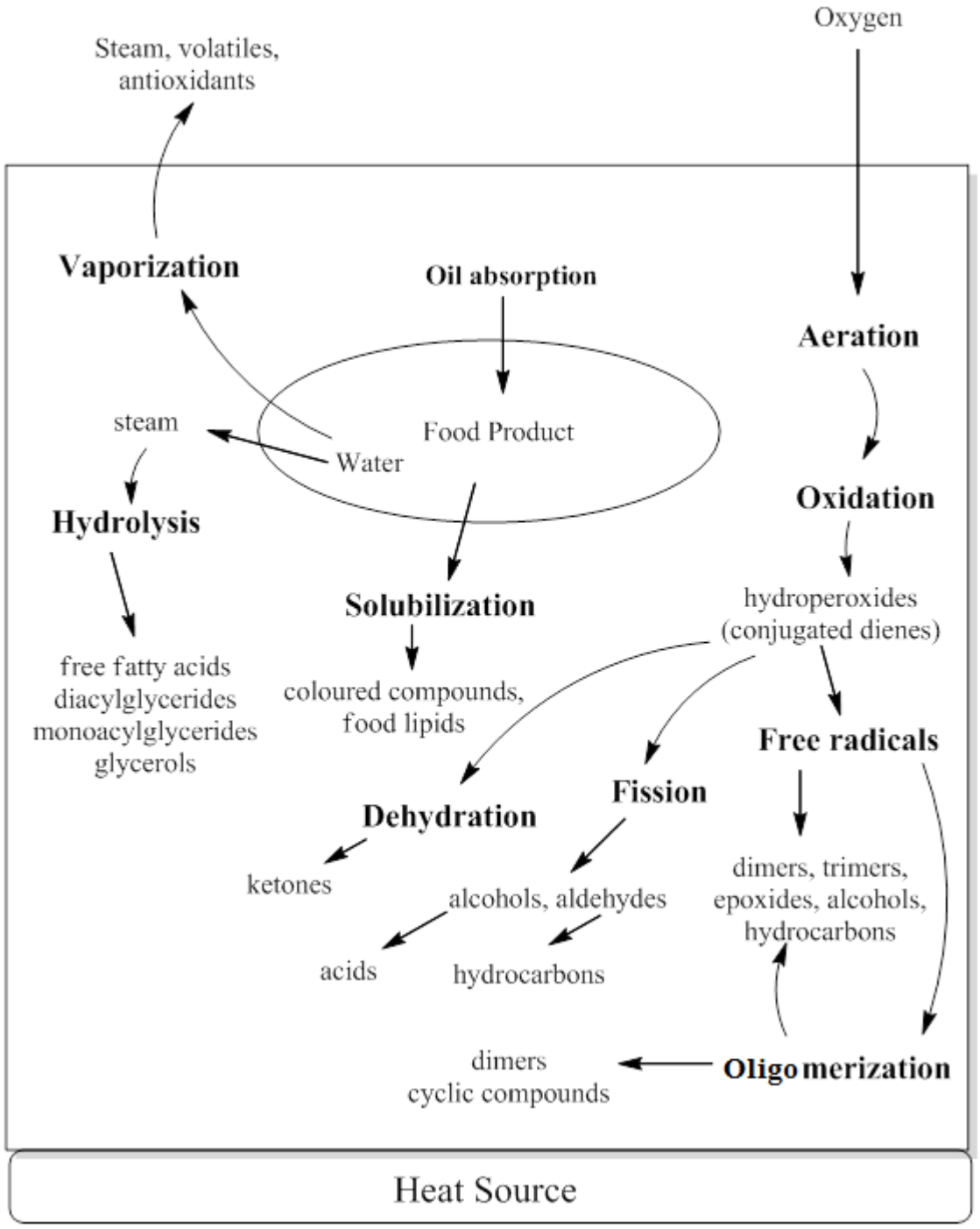


Figure 1: Physical and chemical reactions occurring during deep fat frying. Adapted from Fritsch (1981)

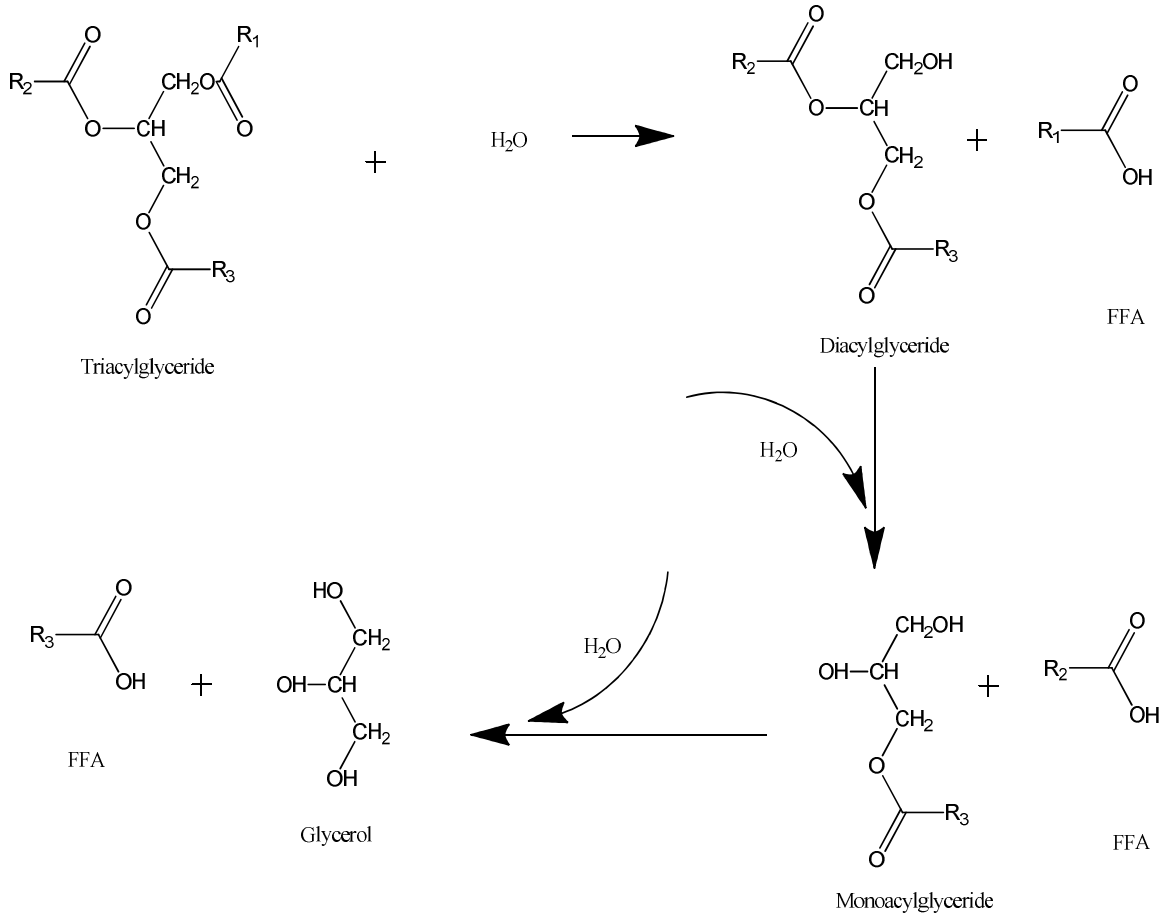
1.3 Major reactions in the frying oil

During deep fat frying at high temperatures with constant oxygen exposure and in the presence of water, the degradation of the frying oil and food components is encouraged through a series of chemical reactions and physical changes (**Figure 1**). Due to the absorption of oil by fried foods, the oil is often replenished. This combination of fresh and degraded oil along with the variety of foods fried and temperature fluctuations results in a steady increase in the number of chemicals forming and eventually the presence of over 500 different chemicals in frying oil (Gertz, 2001). The major chemical reactions that occur during frying include: hydrolysis, oxidation, oligomerization, and nonenzymatic browning reactions along with numerous physical changes (Warner, 2004). Altogether these reactions produce a complex mixture of compounds that are responsible for the unique, desirable or undesirable taste, flavor, texture, and color of the frying oil and food.

1.3.1 Hydrolysis

Hydrolytic reactions that occur in the frying oil and food are initiated by both water and steam (Choe and Min, 2007). Water is a weak nucleophile that attacks the ester bond of the triacylglycerides to produce di- and monoacylglycerides, glycerol and free fatty acids (**Scheme 1)**. Hydrolysis products have a lower molecular weight and higher polarity than the original triacylglycerides (Dobarganes and Marquez-Ruiz, 2006). Since glycerol evaporates, the equilibrium shifts towards the formation of hydrolysis products (Warner, 2004). The hydrolysis rate increases with rising oil temperatures, forming more hydrolysis products, and burning accumulated food particles. Sodium hydroxide or other alkali used for cleaning as well as the presence of cations and anions stimulates oil hydrolysis (Pokorný, 1989). The rate of hydrolysis can be lowered by increased oil turnover (Romero et al., 1998). The hydrolysis rate is also affected by the presence of liquid water, which hydrolyzes the frying oil faster than steam due to the closer proximity of liquid water molecules (Choe and Min, 2007). Thermal hydrolysis can simultaneously take place but only within the oil phase and not along the water-oil interface. Short chain and unsaturated fatty acids are more soluble in

water creating better access of water to the triacylglycerides that increases the efficiency of the hydrolytic reaction (Nawar, 1969).



Scheme 1: The hydrolytic reaction and its products. Adapted from Warner (2004)

Hydrolysis products decrease the fry life of oil. Free fatty acids are surface-active substances that lower the surface tension of the frying oil and increase the oxygen penetration, therefore increasing oxidative degradation of the frying oil (Choe and Min, 2006; Choe and Min, 2007). Off-flavor compounds are produced by free fatty acid oxidation and are less desirable in frying flavor formation. The hydrolysis products usually lack nutritional significance as they are also produced by small intestine lipases and are absorbed in this form (Dobarganes and Marquez-Ruiz, 2006).

Hydrolytic free fatty acids lower the smoking point of frying oil and may stimulate darkening of the frying oil (Yuki et al., 1961). According to Perkins (2006) hydrolytic products lack the appropriate functional groups, are volatile, are present in minute concentrations and do not offer any significant contribution to the formation of pigments in frying oil.

1.3.2 Oxidation

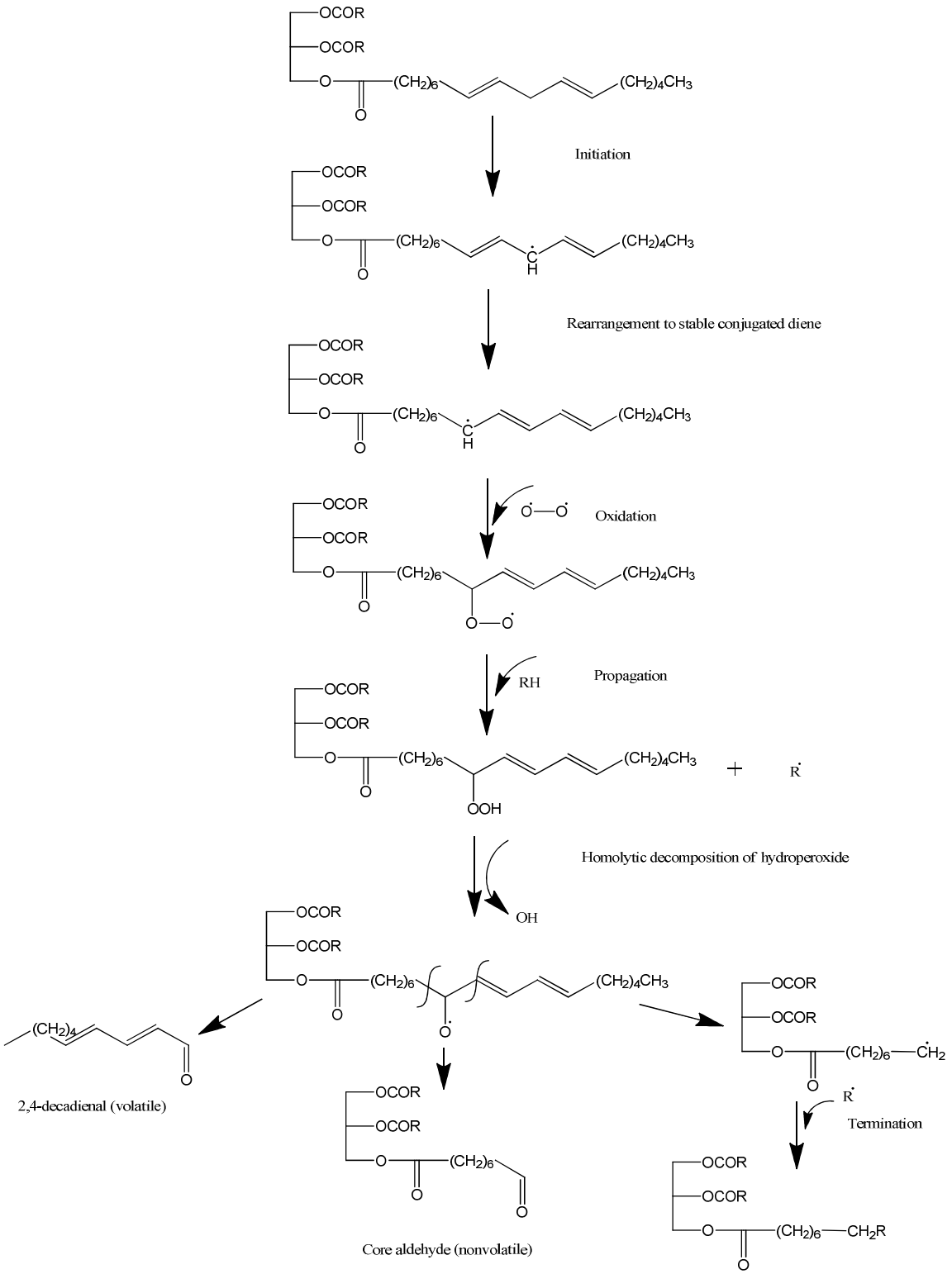
During deep fat frying, the surface of the oil is exposed to oxygen from the air. The oxidation reaction mechanism is the same regardless if it is auto-oxidation at room temperature or thermal oxidation at frying temperature (Choe and Min, 2007). **Scheme 2** shows the free radical mechanism that takes place in three steps; initiation, propagation, and termination. These steps often consist of a series of complex reactions (Frankel, 2005).

The initiation step begins with the removal of a hydrogen atom from the fatty acid to produce a lipid alkyl radical. This step is catalyzed by high temperatures, light, and metals (Artman, 1969; St. Angelo, 1996). The first hydrogen molecule is removed from the weakest bonded hydrogen to the carbon, usually from the methylene carbon in methylene interrupted double bond configurations. For example, to remove hydrogen from carbon 11 of linoleic acid requires 50 kcal/mol whereas from C8 or C14 - 75 kcal/mol (Min and Boff, 2002). On the saturated C17 or C18 carbon of linoleic acid, the homolytic dissociation energy is at 100 kcal/mol. Therefore, the strength of the carbon-hydrogen bond and the ease of free radical formation both determine how prone to oxidation the fatty acid is.

The propagation step starts when the lipid alkyl radical reacts with triplet oxygen to form peroxy radicals (Choe and Min, 2006). A hydrogen molecule is removed from another fatty acid by the lipid radical to produce a hydroperoxide and another lipid radical. This newly produced lipid alkyl radical starts chain reactions that can autocatalyze oxidation

further. The lipid alkyl radicals can form oligomers when they react with other alkyl radicals, alkoxy radicals, and peroxy radicals, thus terminating the oxidative reactions. Lipid hydroperoxides are the main primary products of oxidative degradation but decompose rapidly at frying temperatures into other secondary products. Steam evaporation, further decomposition, and reactions with other food components through oxidation and oligomerization remove most of the secondary volatile products (Choe and Min, 2007). Free fatty acids produced from hydrolysis have been shown to catalyze oxidation by increasing the solubility of oxygen and by transforming the free carboxyl group on the fatty acid into free radicals by homolytic decomposition (Kochhar, 2001).

The volatile oxidation products that stay in the oil can be absorbed by the food product and affect the flavor of the fried food (Warner, 2004). The typical deep fried flavors are associated with unsaturated aldehydes such as 2,4-decadienal, 2,4-nonadienal, 2,4-octenal, or 2-heptenal. A number of off-flavor compounds are formed such as saturated and unsaturated aldehydes and specifically include heptanal, octanal, nonanal, and 2-decenal which produces fruity and plastic off-odors in heated high oleic oils, and acrolein, which is responsible for the typical acrid odor in food products. A number of other volatile oxidation products providing distinctive flavors associated with fried foods are described by Malcolmson et al. (1996). The non-volatile end products formed as a result of oxidation include oxidized, oligomeric mono-, di-, and triacylglycerides (Perkins, 2006). Oxidized monomeric triacylglycerides are similar in molecular weight to the starting triacylglycerides and are polar because of an extra attached oxygen molecule. Both volatile oxidation products and oxidized monomeric triacylglycerides are present in insignificant amounts when compared to higher molecular weight products and therefore the contribution of these components to color formation in the frying oil are insignificant as well.



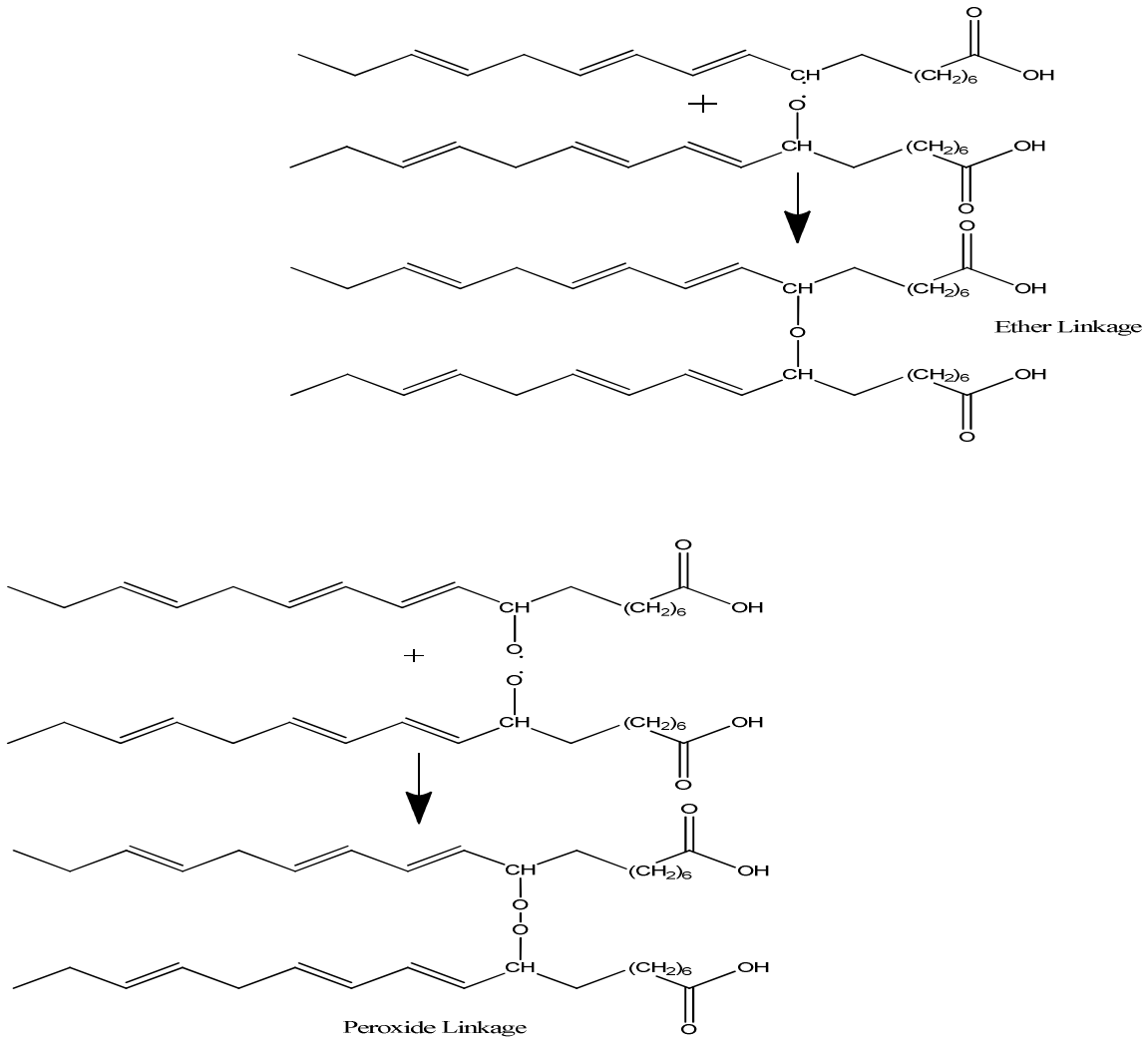
Scheme 2: The lipid oxidation reaction and selected products. Adapted from Warner (2004)

1.3.3 Oligomerization

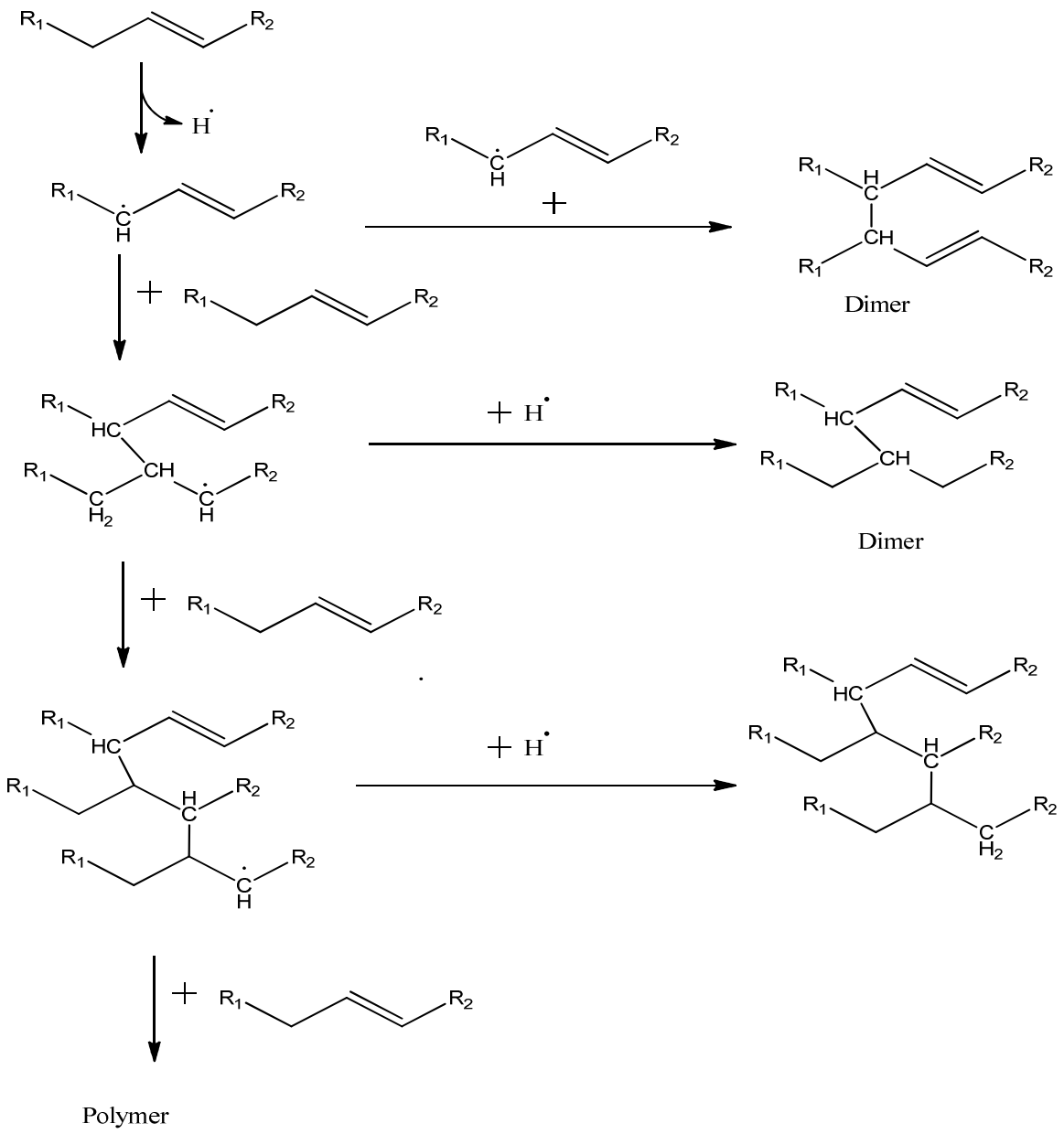
Excessive oxidation causes oligomerization to occur in the oil. These non-volatile triacylglyceride dimers and oligomers make up a majority of the decomposition products present in used frying oil (Choe and Min, 2007). Oligomerization is a radical reaction that can form acyclic or cyclic compounds. The lipid alkyl radical formed in the initiation step of oxidation, alkylperoxy radicals formed with the addition of oxygen, and alkoxy radicals formed by decomposition of hydroperoxides all are precursors of dimers and oligomers (Dobarganes and Marquez-Ruiz, 2006). Dimers are a result of bonds between two triacylglycerides (Orthoefer and List, 2006b). Further cross-linking produces oligomers of higher molecular weights which can be connected by –C–C–, –C–O–C– or –C–O–O–C– linkages that determine the polarity of the molecule (Choe and Min, 2007). Polar oligomers are formed by a free radical mechanism forming an ether or peroxide linkage between molecules as shown in **Scheme 3**. Nonpolar oligomers can be both acyclic (**Scheme 4**) and cyclic also formed through a free radical mechanism (**Scheme 5**) or through a Diels-Alder reaction (**Scheme 6**). A mechanism involving a non-radical cation initiated reaction for fatty acid dimerization has been proposed (**Scheme 7**).

Due to the difficulties in analyzing polymers in complex oil mixtures, the precise structures and their formation is not clear (Orthoefer and List, 2006b). Tricyclic and bicyclic dimers and cyclic monomers of linoleate in soybean oil during deep frying have been isolated (Choe and Min, 2007). Diels-Alder nonpolar dimers, acyclic dimers with –C–O–C– linkages and tetrahydrofuran substituted dimers from soybean oil have also been found (Ottaviani et al., 1979).

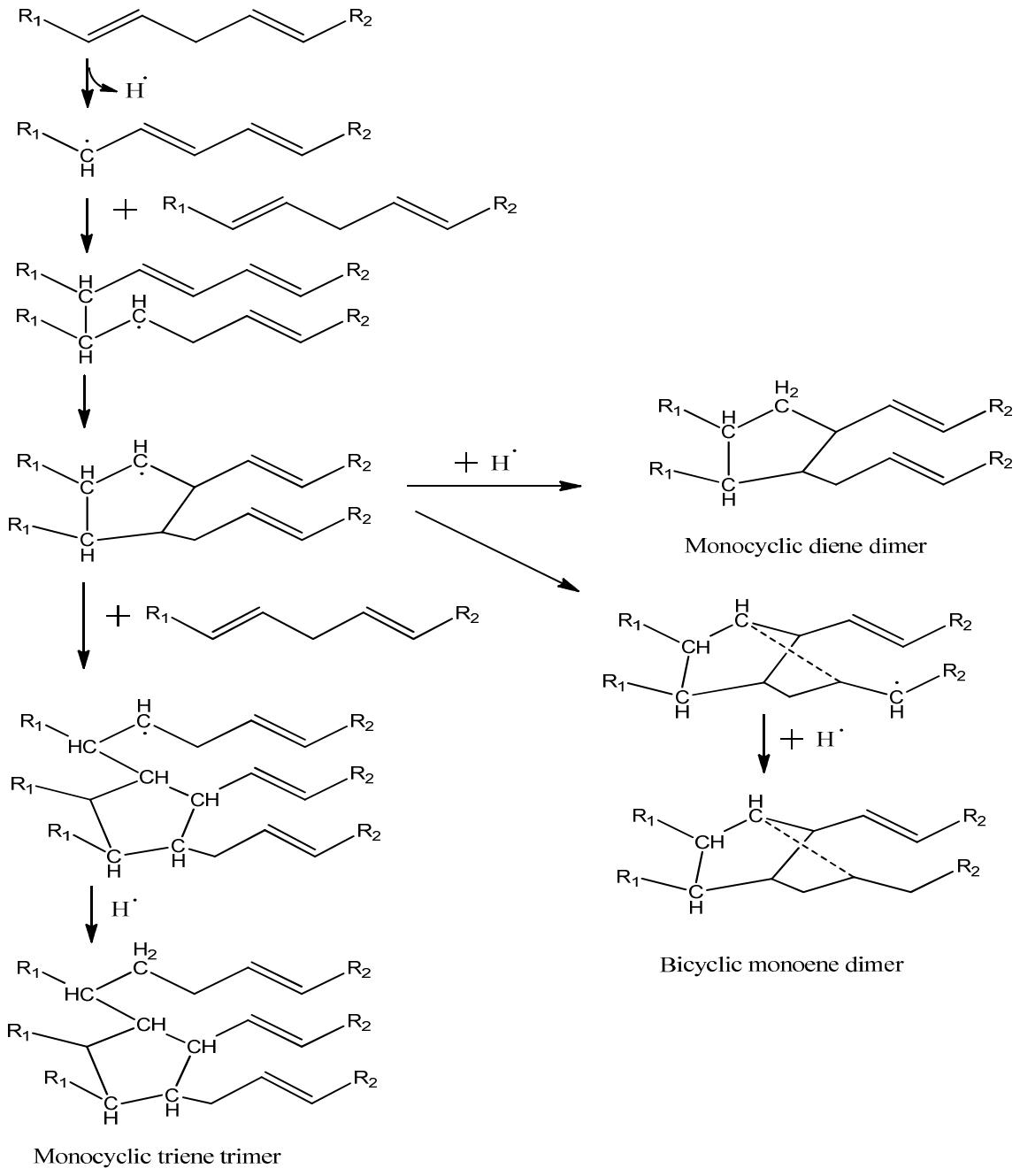
Oligomers vary in molecular mass and their production depends upon the type of oil, frying temperature, number of frying batches, and oxygen availability (Choe and Min, 2007). A larger number of frying food batches and higher oil temperatures increases the amount of oligomers formed during frying (Cuesta et al., 1993; Sanchez-Muniz et al., 1993a; Takeoka et al., 1997). As the contribution of linolenic acid in the frying oil increases, so does the formation of cyclic monomers and polymers. Oil containing higher amounts of linoleic acid has been found to polymerize faster than oil rich in oleic acid (Takeoka et al., 1997; Tompkins and Perkins, 2000). Increasing amounts of polymerization products in the oil creates important physical changes to the frying oil properties including higher oil foaming, viscosity, oil gumming, darkening and higher absorption of oil to the fried food product (Tseng et al., 1996). Polymers can produce a brown resin-like residue along the inside walls of the fryer by trapping moisture and air and causing an increase in oxidation, poor heat transfer and other factors causing faster oil degradation (Lawson, 1995; Moreira et al., 1999). Oligomerization directly leads to oil darkening as all oligomers are dark brown in color (Choe and Min, 2007). Since high molecular weight compounds are non-volatile, polar, and their amounts steadily increase with time, they can be used as a good indicator of fat abuse (Orthoefer and List, 2006b).



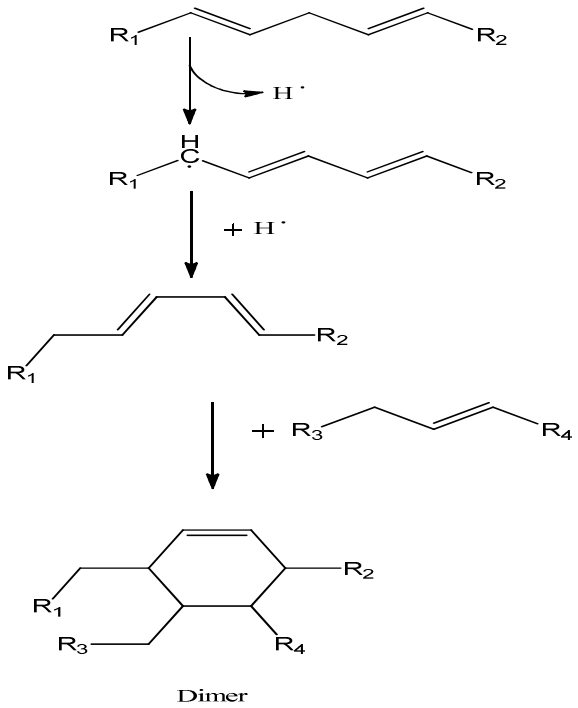
Scheme 3: Oligomer formation through an ether or peroxide linkage as a result of thermo-oxidation. Adapted from Choe and Min (2007)



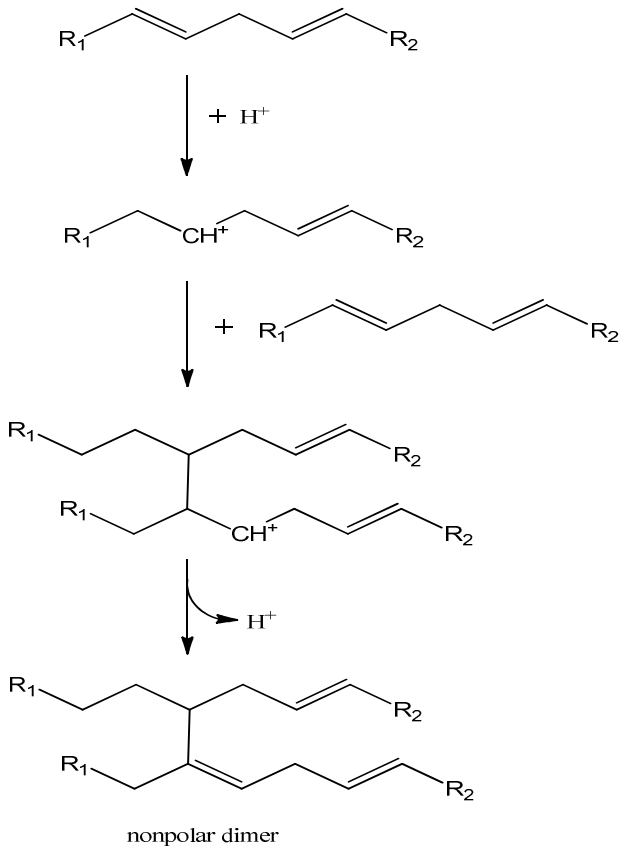
Scheme 4: Acyclic nonpolar polymer formation from oleic acid. Adapted from Choe and Min (2007)



Scheme 5: Free radical mechanism for cyclic nonpolar dimer and polymer formation from linoleic acids. Adapted from Choe and Min (2007)



Scheme 6: Cyclic nonpolar dimer formation through the Diels-Alder reaction as effect of linoleic acid oxidation. Adapted from Choe and Min (2007)



Scheme 7: Non-radical cation initiated unsaturated fatty acid dimerization. Adapted from Brutting and Spitteller (1994)

1.3.4 Nonenzymatic browning

There are primarily two types of nonenzymatic browning that happen during deep fat frying. The Maillard reaction between reducing sugars and amino groups is the main form of nonenzymatic browning. However, during deep fat frying, other reactive compounds with carbonyl groups arising from lipid oxidation can replace carbohydrates.

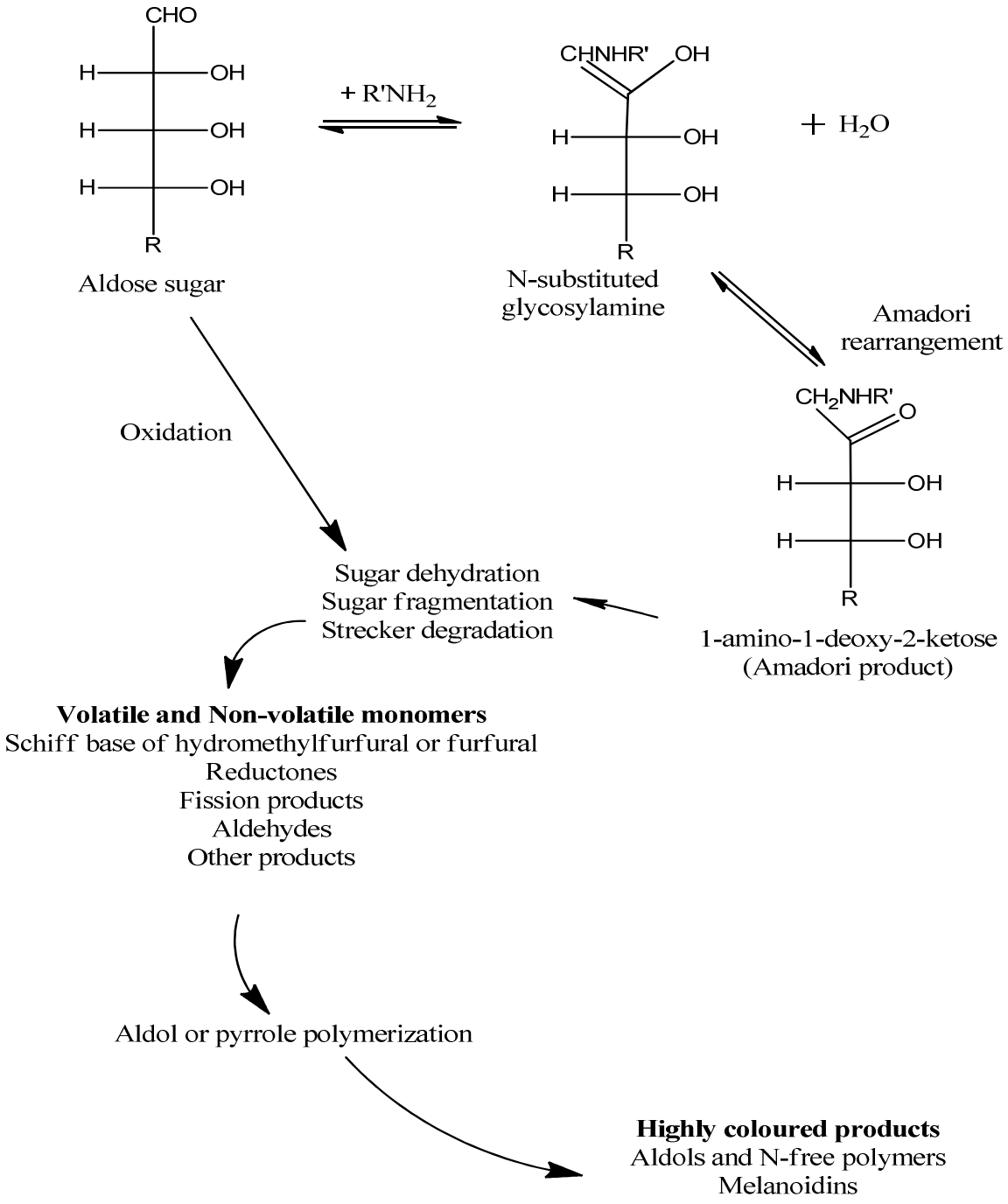
1.3.4.1 Maillard reaction

The Maillard reaction utilizes different precursors to produce a large variety of compounds through the nonenzymatic glycosylation of amino groups within amino acids

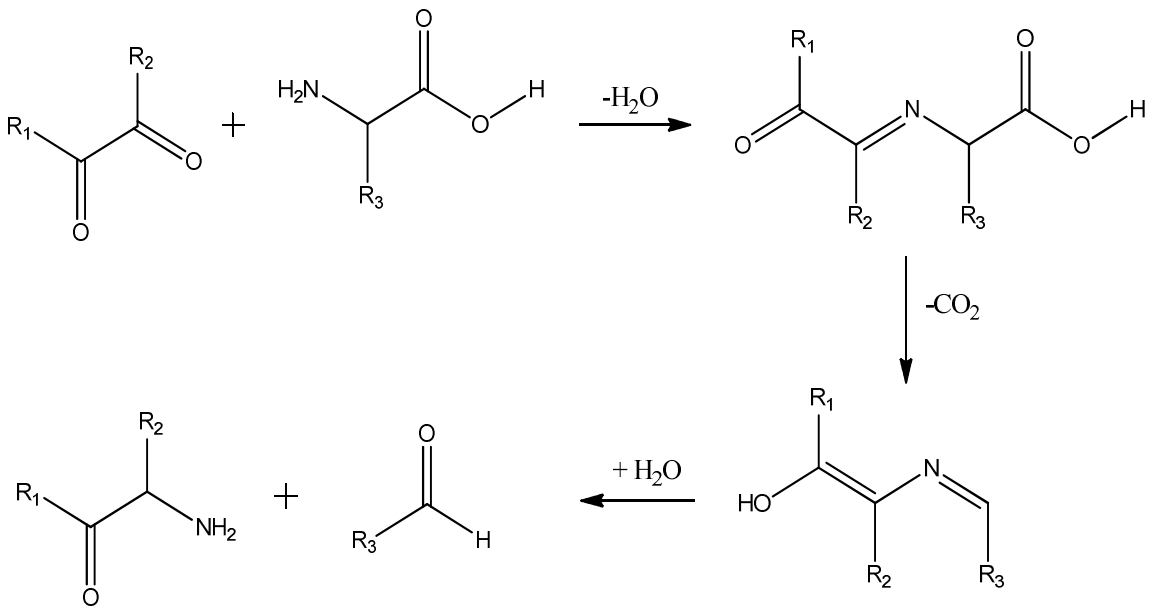
and proteins (Zamora and Hidalgo, 2005). This reaction usually requires temperatures above 100 °C (Gillatt, 2001). In foods, the starting materials include amino acids and/or free amino groups from proteins and reducing sugars/carbonyl compounds (Hidalgo and Zamora, 2000a). The reducing sugars include monosaccharides like glucose and fructose or disaccharides such as maltose and lactose whereas reducing pentoses from meat products can also be the precursors.

Scheme 8 shows the first step of the Maillard reaction where an N-substituted glycosylamine is formed. When aldose is involved, this reaction is called an Amadori rearrangement and in the case of ketose, Heynes rearrangement. In both cases, the primary amino group of an amino acid, peptide, or protein is involved (Hidalgo and Zamora, 2000a). The Amadori rearrangement in the **Scheme 8** example produces 1-amino-1-deoxy-2-ketose which then can undergo sugar dehydration, fragmentation or enolization or the Strecker degradation to form both amino and nonamino compounds. Carbohydrate oxidation can also create starting materials for the Strecker degradation of amino acids. For example, α-dicarbonyl compounds formed from sugars can react with the amino groups of amino acids through the Strecker degradation (Hidalgo and Zamora, 2005). Initially, a Schiff base is formed which then undergoes rearrangement, decarboxylation, and hydrolysis to form an α-amino carbonyl compound and a Strecker aldehyde (**Scheme 9**). Strecker aldehydes and pyrazines formed from dicarbonyl compounds are important in the final flavor formation of foods (Ho, 1996).

The amino and nonamino products produced by sugar dehydration, fragmentation, or enolization and the Strecker degradation, including imino Schiff bases and other volatile and non-volatile monomers can further congregate through aldol condensation or polymerization as described below (Zamora and Hidalgo, 2005). Extensive and repeated condensation produces brown, high molecular weight heterogeneous polymers often referred to as melanoidins.



Scheme 8: The Maillard reaction pathway. Adapted from Hidalgo and Zamora (2000a); Warner (2004)



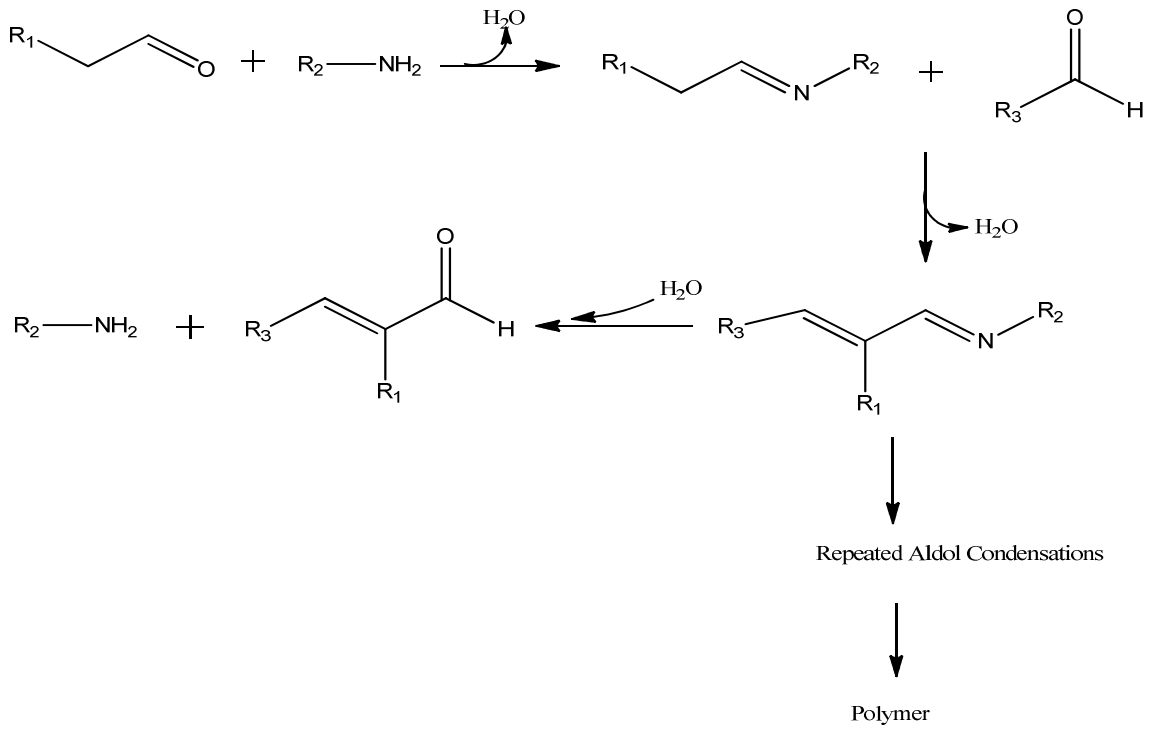
Scheme 9: The role of α-dicarbonyl compounds in the Strecker degradation of amino acids in the Maillard reaction pathway. Adapted from Hidalgo and Zamora (2005)

1.3.4.2 Aldol condensation

An aldol-type condensation has been suggested for the formation of melanoidins where sugar degradation products from early Maillard reactions polymerize to form the melanoidin backbone (Yaylayan and Kaminsky, 1998; Cämmerer et al., 2002). This backbone could possibly be linked through amino compounds when imino Schiff bases polymerize into dimers and high molecular weight brown macromolecules (**Scheme 10**). The instability of the polymeric brown materials leads to dehydration or scission to create new volatile and non-volatile compounds. The new volatiles can have a substantial effect on the flavor of the fried food products.

Regular repeating units in melanoidin structure have been proposed but more flexible structures allow the insertion of enamines of 1-amino-1-deoxyosuloses or decomposition products of diaminoketosyl and diketosylamino components (Kroh and Westphal, 1983; Cämmerer and Kroh, 1995). Intramolecular cyclization, furan body formation or N-

heterocyclic formation such as pyrrolaldehyde can also be formed due to reactive centers in the main and side chains (Kato and Tsuchida, 1981).

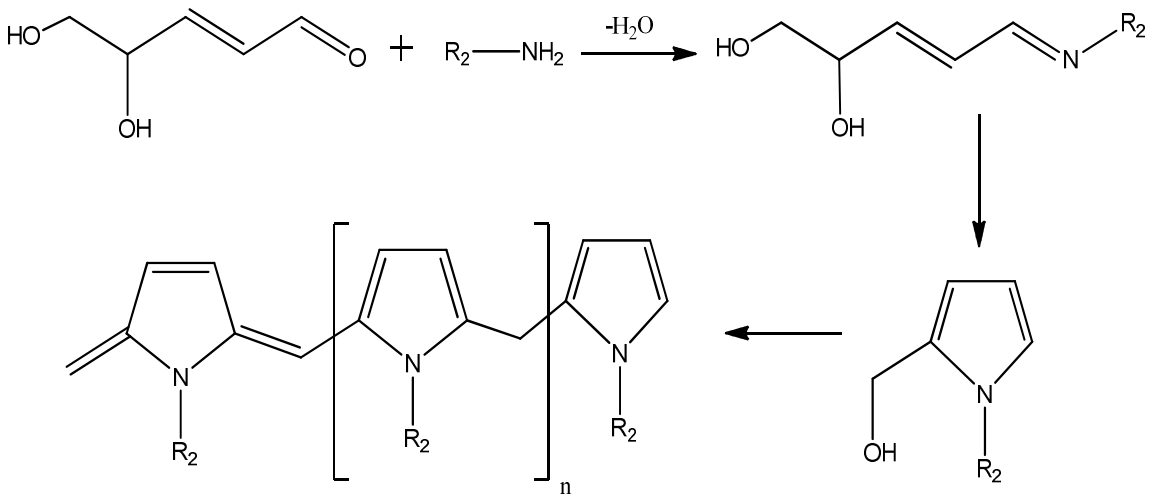


Scheme 10: Aldol condensation as a source of melanoidins formation. Adapted from Hidalgo and Zamora (2000a)

1.3.4.3 Pyrrole polymerization

The second proposed mechanism involves the polymerization of N-substituted hydroxymethylpyrroles into dimers, trimers, tetramers, and higher polymers (Hidalgo and Zamora, 2005). Maillard reactions produce an N-substituted hydroxymethylpyrrole intermediate, which has similar structure to a lipid oxidation intermediate described later. The reaction mechanism was elucidated following the isolation and characterization of N-

substituted 2-(hydroxymethyl) pyrrole derivative from the reaction of 2-deoxy-D-ribose and methyl 4-aminobutyrate (Wondrak et al., 1997). The first step involves a hexose derived 2-deoxy-pentose reacting with amino acids and/or proteins' amino group to produce an imine. Cyclization produces 2-hydroxymethylpyrrole which together polymerize into methylene-bridged polypyrroles formed through the repetitive electrophilic substitution and subsequent deformilation and dehydration/dehydrogenation (**Scheme 11)**. A 2, 3 dehydration of N-substituted 2-(hydroxymethyl) pyrrole followed by a vinylogous Amadori rearrangement and subsequent cyclization and polymerization leads to the pyrrole polymer. Up to 12 pyrrole units can form without cyclization of the tetramer which after dehydration forms a hexamer (Hidalgo and Zamora 1993; Tressl et al., 1998a).



Scheme 11: Formation of polymerized 2-hydroxymethylpyrroles during the Maillard reaction. Adapted from Hidalgo and Zamora (2005)

Applying size exclusion chromatography, fractions with molecular weights of 7000 Da or greater (> 95 % p. w.) were observed when sugars reacted with amines (Tressl et al., 1998b). Nonetheless, the isolation of homogeneous high molecular weight Maillard reaction

products was not possible and structures of these products are not known. ^{1}H and ^{13}C – NMR spectra of melanoidins separated by multiple research groups produced differing results. Some groups found signals resembling Amadori products (Kato and Tsuchida, 1981; Olsson et al., 1981) when others observed contrasting signals typical to olefin, aromatic, and carbonyl regions (Benzing-Purdie and Ratcliffe, 1986; Feather and Huang, 1986; Kato et al., 1986; Huang and Feather, 1988). The varieties of melanoidin structures and the difficulties in isolation of purified compounds can rationalize the opposing results (Hidalgo and Zamora, 2000a). In addition, pyrrole, indole, and amide signals have been found in ^{15}N-NMR spectra with labelled amino acids (Benzing-Purdie and Ratcliffe, 1986). These results coincide with proposed polypyrrolic melanoidin structures and those produced through oxidized lipid/protein reactions described below (Tressl et al., 1998a, 1998b).

The formation of polypyrrolic polymers can be assessed spectrophotometrically (Hidalgo et al., 1998; Zamora et al., 2000). The darkening of oil causes by changes in color and fluorescence explains the production of the polypyrrolic polymers as well as the short chain aldehydes and volatile heterocyclic compounds (Hidalgo and Zamora, 1993, 1995a, 1995b; Zamora and Hidalgo, 1995). Low nitrogen containing melanoidins cannot be assessed by the spectrophotometric methods. Possible explanations could be related to the production of low molecular weight monomers and/or unsaturated fatty acid or carbohydrate polymers or a non-homogeneous melanoidin polymer produced by different mechanisms but these have not been confirmed (Zamora and Hidalgo, 2005).

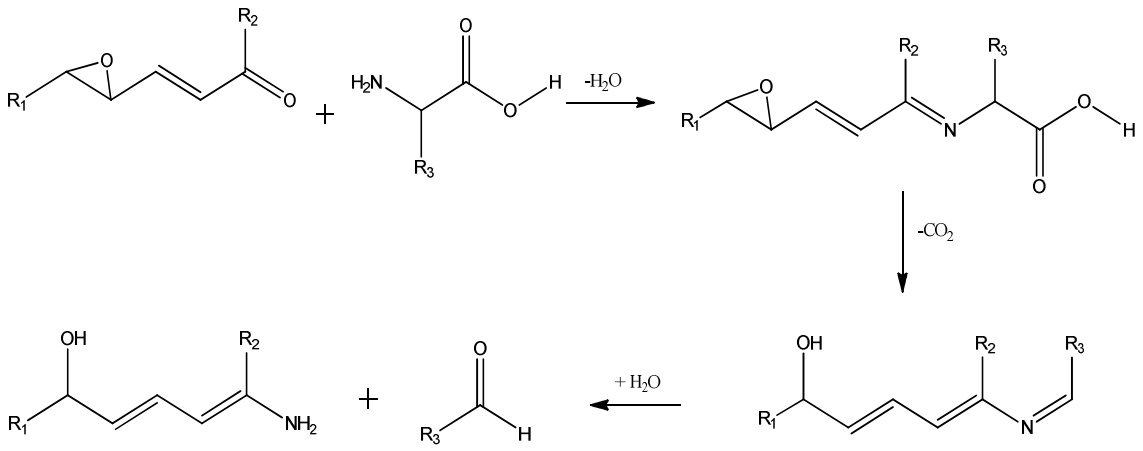
1.3.5 Role of lipids in nonenzymatic browning

Lipids are susceptible to oxidation that forms a variety of carbonyl compounds which are efficient precursors for nonenzymatic browning. Lipid oxidation products can polymerize into brown oxypolymers described above or can react with other compounds, particularly proteins to affect reaction rates, color, flavor, and texture of the fried foods

(Venolia and Tappel, 1958; Buttkus, 1975; Khayat and Schwall, 1983). Lipid oxidation products and their reaction with amines, amino acids and proteins have been implicated in many browning reactions happening in fatty foods (El-Zeany and Fattah, 1982; Pokorný, 1998; Hidalgo and Zamora, 2000b). Pokorný and Janicek (1975) reported that the carbonyl group of lipids reacted with the amino group of protein when a mixture of oxidized lipids and proteins were stored at 60 °C to form colored products.

Lipid oxidation and the Maillard reaction are two of the most important reactions affecting color of food products and have a similar mechanism of intermediate product formation (Zamora and Hidalgo, 2005). The intermediate products for lipids are lipid peroxides while for the Maillard reaction, Amadori compounds are intermediates. In both of these processes, the intermediate products undergo similar fragmentation, rearrangement, and degradation to produce volatile and non-volatile monomers which are the precursors forming highly colored polymers through aldol condensation and/or carbonyl-amine polymerization. Nonenzymatic browning is promoted through interaction of lipid oxidation products with Maillard reaction starting materials and intermediates to produce new compounds. Even though these reaction mechanisms are somewhat similar, they differ because different precursors are used.

The carbonyl group from carbohydrates or carbonyl compounds formed during lipid oxidation are involved in different Strecker degradation pathways (Hidalgo and Zamora, 2000a). Typical α-dicarbonyl derivatives generated from carbohydrates are not formed during lipid oxidation, however, compounds which follow the same mechanism in Strecker degradation are created. For example, 4,5-epoxyalkenals are involved in Strecker degradation of an amino acid where initially an imine is generated followed by rearrangement, decarboxylation, and hydrolysis leading to a Strecker aldehyde and a hydroxyl amino compound (Hidalgo and Zamora, 2004) (**Scheme 12**).

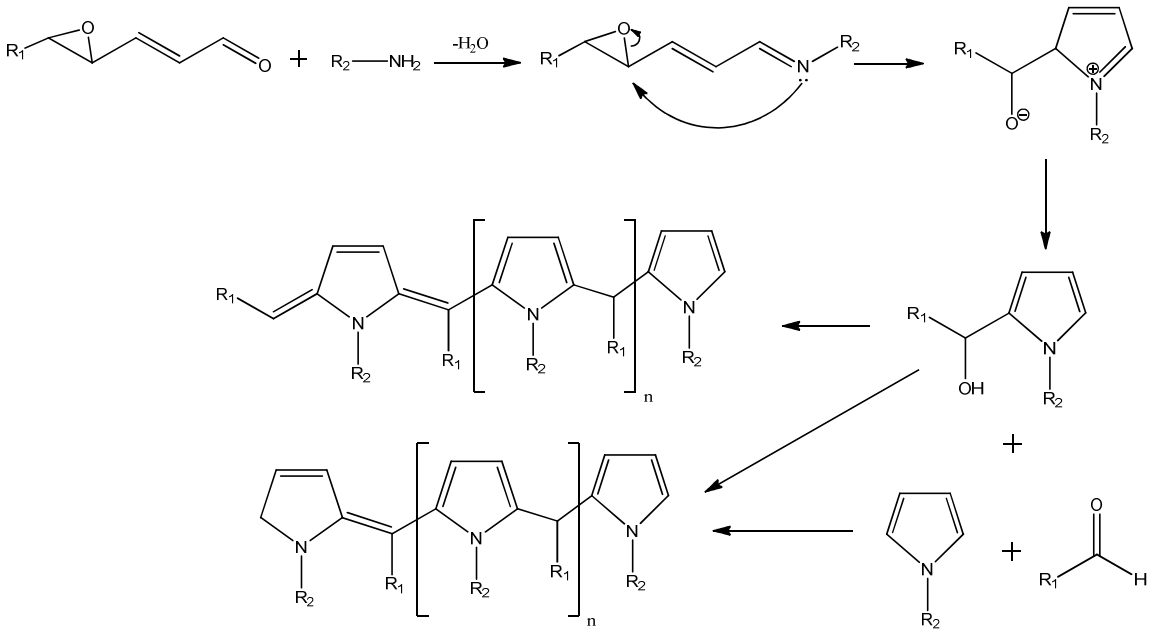


Scheme 12: 4,5-epoxy-2-alkenals as precursors in the Strecker degradation of amino acids. Adapted from Hidalgo and Zamora (2005)

Lipid oxidation intermediates undergo melanoidin formation reactions similar to aldol condensation and pyrrole polymerization. During aldol condensation, carbonyl compounds are bonded with amino groups forming imino Schiff bases that then polymerize into dimers and other high molecular weight brown pigments (Zamora and Hidalgo, 2005).

An important intermediate formed during lipid oxidation similar to the Maillard reaction are N-substituted hydroxyalkylpyrroles, which are the precursors for pyrrole polymerization. The N-substituted hydroxyalkylpyrroles produced through the lipid oxidation, transpire when epoxyalkenals and unsaturated epoxyketoacids react with amines, amino acids and proteins. The best known mechanism for pyrrole polymers formation is the reaction of 4,5-(*E*)-epoxy-2(*E*)-alkenals with butylamine and glycine methyl ester as proposed by Hidalgo and Zamora (1993). Similar to the Maillard reaction, the first step involves the carbonyl group of the aldehyde and the amino group reacting to produce an imine. The cyclic intermediate can be converted into two different pyrrole derivatives

depending upon reaction conditions. At carbon 2 of the pyrrole ring, a proton leaves and initiates a rearrangement to produce 2-(1-hydroxyalkyl)pyrroles. These types of products are unstable and readily undergo dehydration to produce a pyrrole polymer. Lipid oxidation products, 4,5-epoxy-2-alkenals, and lysine amino groups produce both N-substituted pyrroles and N-substituted hydroxyalkylpyrroles with the latter being able to polymerize spontaneously to produce melanoidin-like polymers (Zamora and Hidalgo, 1994; Zamora and Hidalgo, 1995). These polymers have been characterized by nuclear magnetic resonance and mass spectroscopies, and gel filtration chromatography (Hidalgo and Zamora, 1993). When the formations of short chain aldehydes are favored, stable N-substituted pyrroles are also created (**Scheme 13**).



Scheme 13: Lipid oxidation products as precursors for the formation of 2-(1-hydroxyalkyl)pyrroles and its polymers. Adapted from Hidalgo and Zamora (2005)

Low molecular weight monomers formed in reaction of oxidized lipids and proteins can contribute to coloration of oil during frying (Hidalgo and Zamora, 2000a). Malondialdehyde, a secondary lipid oxidation product, can generate conjugated Schiff bases, dihydropyridines and pyridinium salts among other compounds (Kikugawa and Ido, 1984; Nair et al., 1986, 1988; Gómez-Sánchez et al., 1990; Itakura et al., 1996). Nonenzymatically produced 4-hydroxynonenal during lipid oxidative degradation, is involved in colored and fluorescent products formation through Michael addition with reactive groups of amino acids and proteins (Esterbauer et al., 1990, 1991; Baker et al., 1998; Xu and Sayre, 1998). However, the amount produced is insignificant compared to that of high molecular weight products.

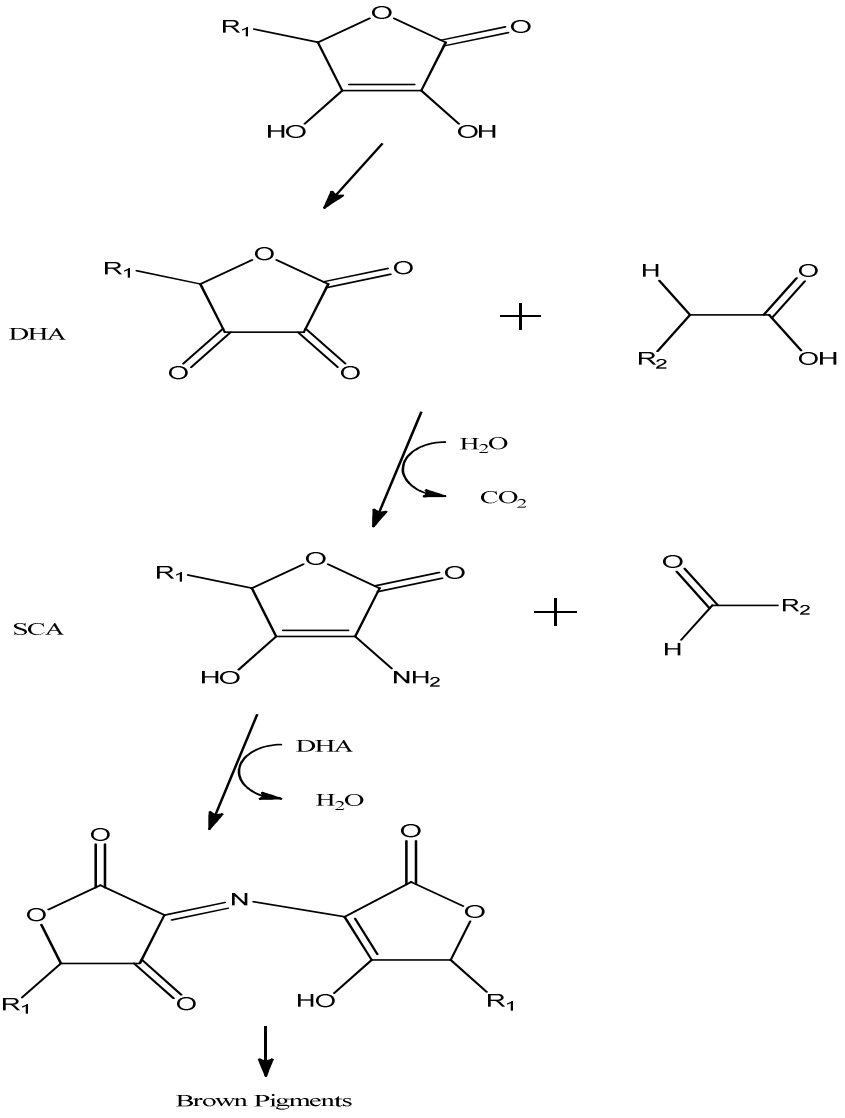
Lipid oxidation products have been found to affect the Maillard reaction pathway by promoting the reactions or reacting with intermediates and forming new compounds usually not formed in the absence of lipids. For example, lipids improve meat flavors when aldehydes from lipid oxidation react with hydrogen sulphide and the amount of sulphur containing heterocyclic volatiles are reduced (Gandemer, 1999; Zamora and Hidalgo, 2005). Lipid oxidation products also provide carbonyl or alcohol volatiles which add to the meat aroma. A deeper colored crust was observed in meatballs and Vienna cutlets fried in oxidized oil compared to fresh oil (Dagerskjog and Bengtsson, 1974). It is also worth noting that Maillard reaction products can promote lipid oxidation because Amadori products have been shown to increase phospholipid oxidation (Breitling-Utzmann et al., 2001). Nonenzymatically browned proteins have conversely exhibit antioxidant activities preventing lipid oxidation (Alaiz et al., 1997; Zamora et al., 1997; Ahmad et al., 1998; Alaiz et al., 1998; Hidalgo et al., 2001).

Both lipid oxidation and the Maillard reaction are complementary due to the differing conditions in which melanoidins form from oxidized lipids versus carbohydrate and protein reactions. Since both have common intermediates, polymerization mechanisms, and products, both reactions together influence highly colored polymer formation. The Maillard reaction and lipid oxidation mechanisms, kinetics, and products are so interrelated that when mixtures of carbohydrates, lipids, and proteins are involved, both reactions should be considered simultaneously (Hidalgo and Zamora, 2005).

1.3.6 Other nonenzymatic browning reactions

In addition to the high molecular weight nonenzymatic browning products described above, the structure of low molecular weight colored Maillard products have been found but only in model systems (Ledl and Severin, 1981; Hashiba, 1986; Arnoldi et al., 1997). In the absence of amino groups and at high temperatures, caramelization of carbohydrates occurs and produces high and low molecular weight brown colored compounds. Oxidation of ascorbic acid to form dehydroascorbic acid (DHA) followed by a series of reactions with amino compounds also forms pigments (Handwerk and Coleman, 1988; Namiki, 1988; Gregory, 1996). In this reaction, DHA reacts with an α amino acid to produce scorbamic acid (SCA) which converts to 2,2'-nitrilodi-2(2)-deoxy-L-ascorbic acid, ammonium salt and a red pigment (Hidalgo and Zamora, 2000a). Further polymerization of this compound produces melanoidin-like polymers shown in **Scheme 14**. In the presence of proteins and water, DHA also browned but the previously mentioned ammonium salt was not obtained. This reaction is rapid in an alkaline medium but is slower at a pH lower than 7 without metal catalysts. Peroxy free radicals formed during lipid oxidation have been found to polymerize

with proteins (Karel et al., 1975; Gardner, 1979; Kikugawa et al., 1990). Although these types of nonenzymatic browning reactions are important in food browning, their contribution to color change and oil degradation during frying is insignificant when compared to standard browning reactions.



Scheme 14: The nonenzymatic browning of ascorbic acid. Adapted from Hidalgo and Zamora (2000a)

1.3.7 Physical changes

As a result of the chemical breakdown of the frying oil, physical changes often occur. These changes include an increase in viscosity, color, and foaming and a decrease in the smoke point (Warner, 2004). Gertz and Matthäus (2008) described the changes in physical and chemical parameters during deep fat frying along with the main causes of these reactions and how well they correlate with oil deterioration. Color formation is one of the most important physical changes that occur in the frying oil. As discussed later, oil color is an important parameter in the decision to discard used frying oil and is often evaluated during the frying process.

Oil absorption is a physical change that is often dependent upon the type of food being fried. For example, starch based products such as potatoes were found to absorb oil more readily than non-breaded meat or protein products (Pokorný, 1999). Oil absorption is also dependent upon the extent of chemical breakdown. As the amount of polar compounds in the oil increases, an increase in surfactant content occurs and more fat is absorbed by the fried food (Blumenthal, 1991).

1.3.8 Health effects of oxidized oils and oligomer products

The degradation products arising from the aforementioned reactions affect the chemical and physical properties of oil and food products but also influence the nutritional quality. Most toxic and/or carcinogenic products are formed during oxidative degradation of oil and frying foods. Many degradation products are volatile and often evaporate to contaminate the frying facility. Non-volatile degradation products are important because they remain in the oil, are absorbed by the food, and affect digestion. Dangerous degradation

products often cause off-flavors for the fried food product. Fry operations would likely stop frying in oil containing hazardous oil degradation products due to flavor profile changes, especially when these products are in quantities that may cause health issues. In North America, the amount of degradation products is not regulated, in contrast to the European Union where levels are strictly enforced (Lawson, 1995).

For most nutritional trials or experimental diets involving abused fats and oils, the test subjects are fed large amounts of the degraded oils (Marquez-Ruiz and Dobarganes, 2006). These excessive amounts would most likely not ever be reached by normal culinary practices and consumed through fried foods. However, with the increasing reliance on the convenience and taste of fried products, the importance of the adverse effects of ingesting these detrimental food components becomes imperative (Ghidurus et al., 2010).

Some studies describing nutritional effects of used frying oils and fats have shown a decreased growth rate of experimental animals and caused liver enlargement as opposed to experiments where artificially abused oils and fats were used causing numerous biological and histopathological changes (Marquez-Ruiz and Dobarganes, 2006). In a 10 year study conducted by Lang and Lang et al. (1973; 1974; 1978), the effects of feeding three generations of rats with 175 °C heated soybean and hardened ground nut oils exhibited a reduction in size of benign and malignant tumours and the induction of microsomal enzymes. When the rat's diet contained polymers at 12-20 %, a depressed growth rate and higher mortality was observed (Marquez-Ruiz and Dobarganes, 2006). The oil polymers caused malabsorption of essential food nutrients and fat soluble vitamins so the rats were not able to properly utilize their ingested food (Ghidurus et al., 2011).

Elevated blood pressure and impaired vasorelaxation were observed in experimental Sprague-Dawley rats fed for 6 months with soybean oil used for extensive frying (Leong et al., 2010). Similar health effects were observed when rats were fed heated palm oil (Leong et al., 2008; Leong et al., 2009). In another study, rats fed thermally oxidized sunflower oil had increased intestinal oxidative stress (David et al*.,* 2010). This stress was attributed to the formation of non-volatile oxidative degradation products which interfered with normal liver and kidney function.

Maillard reaction products formed by the reaction of glucose with creatine or creatinine produced imidazoquinoline and imidazoquinoxaline mutagens (Pokorný, 1999). Creatine is a precursor to a number of heterocyclic carcinogenic compounds including compounds containing imidazol rings. These compound amounts increase with rising temperatures and frying time but are usually present in insignificant quantities in deep fat fried foods (Nielsen et al., 1988). Overall, the general consensus is that a moderate consumption of frying fats and oils produced under normal culinary procedures is safe (Marquez-Ruiz and Dobarganes, 2006).

1.4 Food ingredients in breading/batter mixtures

During frying, the major interactions occur between the atmosphere, frying oil, and food products and contribute significantly to oil deterioration. Decomposition compounds produced in the oil during frying have a direct effect on oil darkening which is the most apparent physical change in the oil during frying. Color is an important parameter of oil and is used as a guide to edible oil refining and also as an indicator of the condition of used oil in frying (Xu, 2003). Decomposition products not only affect color and oil quality but also the

flavor and nutritional value of the fried food. The types of food ingredients coming into contact with the frying oil play an important role in the oil deterioration (Jacobson, 1991). Understanding how ingredients affect oil degradation can help to optimize the frying process to produce good quality fried food and extend oil fry life (Warner, 2004). Most frying operations fry foods that contain a variety of ingredients and use an array of batters. Simple sugars and starches as well as proteins and amino acids are basic food ingredients that interact with the frying oil components. Minor endogenous food and oil components such as spices, antioxidants, phenolics and others also aid in or slow degradation of frying oil. Since a majority of fried foods are breaded or battered, it is important to look at the components that make up these mixtures.

Certain conditions and external factors also play a part in oil deterioration besides the types of food being fried, namely the type of oil used, oxygen absorption, oil temperature, frying time and duration, metal contaminants, and food materials remaining in the fryer during frying among other factors and conditions.

Breadings and batters are used in fried foods for a number of reasons but mostly to add flavor and texture, to control oil uptake, provide a thermal buffer between the hot oil and food, and to reduce water loss and the cost of the final food product (Sasiela, 2004). Batters are often wet coatings and are commonly used in deep frying as opposed to other types of frying because the coating would run off before it had the chance to cook and coalesce (Ivory, 1999). Dry coatings are commonly referred to as breadings because they often include the use of bread crumbs. These coatings can be used in pan frying as well as deep fat frying because of the thicker coating and typically use eggs as an adhesive (Sasiela, 2004).

Breadings involve dipping the food product in flour, followed by beaten eggs, and then bread crumbs to give a texture to the final crust of the food. The composition of these ingredients as well as whether or not all three steps are used is dictated by consumer preference. For example, typical wheat flours can be substituted for corn starch or even omitted completely. Eggs can be replaced with milk, buttermilk, honey or a starch-based water mixture. There is also an array of bread crumb types and substitutes such as crackers, crushed cereals or Panko (Japanese) bread crumbs. A blend of seasonings is usually added to the base ingredients including various herbs, spices, garlic, dried onion, or other aromatic vegetables (Lawson, 1995; Ivory, 1999; Sasiela, 2004).

Batters contain wet and dry components which are mixed together initially as base ingredients. The wet materials include milk, buttermilk, water or even beer and the dry ingredients can be flours and starches or similar to any previously mentioned dry breading ingredients. Alongside additional seasonings, batters can include products such as eggs, cheese, baking soda, or baking powder to add flavor and texture or to be used as a leavening agent.

During frying, each blend of food ingredients decomposes within the product and reacts with the oil or fat and other food components. These reactions lead to a number of changes in both the food product and the frying oil. Studies on the effects of commercial and laboratory made batter and breading ingredients during frying focus mainly on crust texture, porosity, fracturability, hardness, moisture content and crust color formation as well as frying oil uptake (Baixauli et al. 2002a, 2002b; Dogan et al., 2004; Salvador et al., 2005; Ngadi et al., 2007). The collective effect of multiple ingredients of batters or breadings on the physical and chemical changes in the frying oil has not been analyzed systematically. To

better understand the effect of the ingredients on oil deterioration, there is a need to assess the ingredients separately.

1.4.1 Simple and complex carbohydrates

Carbohydrates are considered to be either simple or complex, where the former, is found in fried food products including glucose, fructose, lactose, and maltose. Complex carbohydrates are mainly starches and fibers such as gums, pregelatinized starch, modified starch, high amylose starch, and dietary fibers (Chen et al., 2001).

The main reaction that carbohydrates undergo during frying is the Maillard reaction. As described previously, the Maillard reaction occurs between the "reducing" sugars group and amino group to form brown pigments in the oil (Sasiela, 2004). Reducing sugars as classically described are monosaccharides with a carbonyl group and occur naturally in or are added to the coating ingredients. Candidates for reducing sugars include glucose, fructose (in corn syrup or invert sugar), lactose (type of milk sugar), and maltose. Sucrose has been found to aid pigment formation upon hydrolysis during frying (Roe and Faulks, 1991). Caramelization of sugars occurs simultaneously with the Maillard reaction and may contribute to color development but is generally less important when compared to the contribution of Maillard reaction products to oil darkening.

In order for complex carbohydrates to contribute to oil degradation and color formation, they must first be broken down into simpler and smaller molecules. Most of the simpler molecules are simple sugars and thus contribute to browning and degradation reactions through similar mechanisms (Orthoefer and List, 2006b).

Glucose and fructose have both been shown to significantly contribute to color formation in oil during frying but only if other materials with higher color formation capabilities are not present during frying (Totani et al., 2006b). When compared to other food ingredients, starches usually do not significantly contribute to the color development in oil during frying. However, in this described study used concentrations of food ingredients and conditions not typically found in frying operations whereas the main objective for this study was to find the major contributor to oil color change. When comparing starch ingredients to proteins, oil color development was significantly faster for the latter (Jacobson, 1991). In the unpublished preparative work of another research group, starch did not significantly contribute to the oil color development (Totani et al., 2006a; 2006b). Among starch products, differences have been observed in their effect on oil deterioration. For example, bread crumbs have been found to darken frying oil at a faster rate when compared to cracker crumbs mainly due to differences in ingredients (Lawson, 1995).

1.4.2 Proteins

Proteins are always present in fried foods but vary in the quantities (Pokorný, 1999). Proteins provide structure, help flavor formation, contribute to browning reactions, improve water-holding capacity, and can serve as emulsifying and film-forming agents (Chen et al., 2001). A variety of proteins have been used in modern batters including soy and whey proteins, cheese powder, egg albumen, and gluten. Meat proteins can also contribute to the involvement of proteins in reactions taking place during frying.

The most reactive parts of proteins are the sulfhydryl and amino groups (Pokorný and Reblova, 1999). The sulfhydryl groups are present in sulphur-containing amino acids

such as cysteine, cystine or methionine whereas the amino groups are found in all amino acids with some such as lysine, arginine and asparagine having multiple amino groups. In food products, amino acids and peptides are present in smaller amounts but are more reactive than proteins mainly because more amino groups are available for reaction (Pokorný, 1999). Oligopeptides may contribute to color deterioration of the frying oil, especially when present in substantial quantities in the fried food (Totani et al, 2006a). No investigations have looked at these materials because of the difficulty in producing oligopeptides, their limited availability, and free amino acids would be expected to be more reactive with carbonyls when compared to oligopeptides.

As foods are heated, the tissues or food materials exude liquid and shrink due to the sudden increase in energy provided by the hot oil during frying. The water quickly evaporates and causes food components to remain in the oil. A majority of food materials are taken out of the oil once frying commences, however, when food materials are extruded by water as described above, the food components tend to stay in the frying oil. Amino acids have been found to leave food components in this fashion. For example, in soup prepared from beef, pork, or chicken, high levels of alanine, glutamine, methionine, and taurine, were found in the heated broth (Hukuie et al., 1994). Another study found extract from fresh bovine muscle was rich in alanine, taurine, serine, threonine, and asparagine (Omata, 1986). According to Totani et al. (2006a), juice exuded from foodstuffs such as beef, chicken, scallops, onion, and shrimp during frying contained enough amino acids to darken oil heated at 180 °C for 20 h, regardless of the presence of batters on the foods.

The main reactions that proteins participate in during frying are the Maillard reaction and thermo-degradation (Pokorný, 1999). All amino acids, peptides, and proteins have the

ability to participate in the Maillard reaction, however, the number and position of the amino groups in the amino acid molecule can determine its activity (Lento et al., 1958). In a sugar-amino acid reaction system, monoamino acids containing the amino group in the alpha position caused only a small increase in color compared to those containing the amino group in the terminal position or those containing two amino groups. Longer chained aliphatic alpha monoamino acids caused only a slightly higher color increase as compared to shorter chained acids in the same system.

A number of studies have looked at the effect of individual amino acids and browning reactions. Uematsu and Ishii (1983a; 1983b) found the most intensive browning in mixtures with methionine, glycine, proline, and serine after 10 days of storage at 50 °C with methyl linoleate on a cellulose support. The same intensive browning was observed in lysine stored for 18 days but development was significantly slower compared to the amino acids mentioned previously. Amino acids have been found to have different browning potentials based on the lipidic precursors present. When using common frying oils such as canola and soybean oils, browning reactions rate increased when cysteine, methionine, proline, and tryptophan were used with the fastest rate occurring when proline and tryptophan were applied (Koga et al., 1997). Similarly, glycine, alanine, and leucine, but not lysine, were found to vigorously increase oil coloration in a model system using canola oil at 180 °C frying temperature. On the contrary, glucose, sucrose, albumin, and sodium chloride showed no significant contribution. Thus based on the study performed by Koga et al. (1997), compared to starch-heavy fried foods, protein-based foods produced a more significant color change in the frying oil. Tynek et al. (2001) fried sliced potatoes and cod fillets and found 10 % less triacylglyceride (TAG) polymers and 40 % less TAG dimers when frying the latter.

The color formation in oil when cod fillets were fried was more intense when compared to frying potatoes and heating oil.

Though the effect of protein and amino acid browning of oil studied above is important, limitations to these experiments exist. Totani et al. (2006a) tested only a few amino acids and established that some have lower impact on darkening of oil during prolonged low temperature heating and not at frying temperatures. Studies performed by Uematsu and Ishii (1983a; 1983b) tested amino acid browning during prolonged storage and again not at frying temperatures. Koga et al. (1997) used a deep fat frying model system, however, the amounts of the frying materials were very small and glass-fiber filters were used as the bulk frying material. Totani et al. (2006a) utilized juice extracted from foods containing amino acids to test how amino acid browning occurs during frying. Considering the differences in results when different amino acids and the various precursors were used, it is evident that the effect of amino acids on browning is complex.

The present study investigated the effects of proteins, amino acids, and carbohydrates on pigment formation and thermo-oxidative deterioration. When food based ingredients containing amino acids and appropriate frying conditions will be applied, the role of compounds during actual deep fat frying can be better understood.

1.4.3 Fat exchange between food and frying oil

Some deep fat fried foods contain different types of lipids that are usually exchanged with the frying oil. Products such as potatoes or doughnuts have low amounts of lipids (Pokorný and Reblova, 1999). Most of these lipids are bound by membranes or lipoproteins and only contribute insignificant amounts to the frying oil. Lean meats such as chicken

breast or thighs and cod fillets do not release enough external fat into the frying oil to change it significantly (Henry, 1998). Pork, beef, lamb, and some poultry contain elevated amounts of fat and usually significantly affect changes in the frying oil (Dobarganes et al., 2000a). Frying fatty fish and other fatty products have the most significant effects on the frying oils and may speed degradation due to higher levels of unsaturation. These types of fish fat contain large amounts of polyunsaturated fatty acids such as eicosapentaenoic or docosahexaenoic acids and decrease the stability of the frying oil (Sanchez-Muniz et al., 1992; Toth-Markus and Sass-Kiss, 1993). In batters containing egg yolks, a darkening of oil and an increase in free fatty acids was observed due to diffusion of phospholipids from the egg yolk into the frying oil (Bennion et al., 1976).

More than 85 % of par-fried food lipids are exchanged during the final frying irrespective of the oil used and the type of food (Pérez-Camino et al., 1991). The degree of damage caused by the released par-fried oils is dependent upon the condition of the initial frying oil as well as its storage conditions after frying (Dobarganes et al., 2000a).

1.4.4 Water

When food products are fried, water inside the food product evaporates to form a protective steam blanket over the oil surface, limiting the amount of oxygen entering the fryer (Landers and Rathmann 1981; Peers and Swoboda, 1982; Dana et al., 2003; Kochhar and Gertz, 2004). Increasing the amounts of water in fried products usually causes an increased rate of hydrolysis of the oil and can also affect the oxidative degradation of the frying oil. The release of water as steam into the oil causes turbulent mixing of the frying oil, extending the surface area and more intensively oxygenating the oil by stimulating

unsaturated fatty acids of the frying oil. Color development during pan frying was only moderately influenced by water and fat content of fried meat patties at 120 – 220 °C (Dagerskog and Bengtsson, 1974).

1.4.5 Minor food ingredients

Minor components such as seasonings, spices, leavening agents or other ingredients found in breadings or batters can also affect the rate of oil deterioration and the ability of color formation. High molecular weight non-starch polysaccharides like dietary fibers decompose during frying into monomers which can stimulate the formation of melanoidins or other colored products (Augustin et al., 1989; Pokorný, 1999). Fresh doughnuts contain ammonium carbonate or ammonium bicarbonate as leavening agents and also have high egg content. Both components have been linked to excessive oil darkening during frying due to the phospholipids present in egg yolks (Bennion et al., 1976; Tomioka and Kaneda, 1976a, 1976b; Lawson, 1995).

Endogenous antioxidants and pigments present in products such as rosemary, sage, garlic and onion powders and others can be dissolved in the oil during frying. Utilization of rosemary as an antioxidant has been shown to decrease the oxidation rate as well as polymerization (Pokorný and Reblova, 1999). Spinach powder decreased the formation of polar components in soybean oil while red ginseng extract decreased free fatty acid, conjugated dienoic acid, and aldehyde formation in palm oil (Lee et al., 2002; Kim and Choe, 2003). Sodium pyrophosphate decreased free fatty acid formation and after-cooking darkening whereas edible films such as hydroxypropylmethylcellulose decreased the amount of free fatty acids in the frying oil (Mazza and Qi, 1992; Holownia et al., 2000). Ascorbyl

palmitate, sterols and their fatty acid esters, tocopherols and lignins improved the oxidative stability of the frying oils (Boskou and Morton 1976; Gordon and Magos 1984; Blekas and Boskou 1986; Gordon and Kourimska, 1995a; Choe and Lee, 1998; Kim and Choe, 2004). Other food ingredients such as carrot powder, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) also slowed oxidative degradation of frying lipids (Choe and Lee, 1998; Lee et al., 2003; Choe and Min, 2007).

Natural pigments such as chlorophylls and carotenoids in colored vegetables decompose at frying temperatures into light colored and/or colorless products which contribute to the oil darkening (Pokorný, 1999). Totani et al. (2006b) established that oils containing mineral components at 225 ppm rapidly change color after 10 hours of frying.

Anti-browning agents have been proposed by food ingredient producers to make light colored fried food products and these components also leech into the frying oil. Chemicals such as sulfites have been used to bleach fried products and frying oil, however the safety of sulfites is questioned and these compounds may initiate asthmatic reactions (Sullivan and Smith, 1985). Because of safety issues these products and anti-browning chemicals are no longer used (Taylor et al., 1986).

Breadings and batters often break apart during deep fat frying and their solid particles can stay in the oil increasing oil darkening and stimulate thermo-oxidative deterioration (Lawson, 1995). Fried food particles that stick to the surface of heated pans during pan frying have been shown to cause overheating and produce effectual coloring substances (Yuki et al., 1961). This phenomenon can be related to deep fat frying when food

particles and oil deterioration products can gum up on the top of the walls of fryers, usually at the upper level of the oil or above and similarly may affect oil darkening.

1.5 External factors and conditions

A number of other factors and conditions contribute to changes in the frying oil and include frying temperature and time, oil turnover, quality and type of frying oil, types of deep fryers, and food fragmentation.

1.5.1 Frying temperature and time

Since frying temperature and time are the most important factors affecting product quality and degradation of oil, many countries strictly regulate frying temperature (Chen et al., 2001). Most foods are fried at a temperature range of 163 - 191 °C (Lawson, 1995). In Germany, the maximum frying temperature is set at 165 °C, however, most frying operations in North America use temperatures between 195 - 215 °C (Gupta, 2004). As frying time increases so does the amounts of free fatty acids and polar compounds, the latter including oxidized triacylglycerides, dimers, and polymers (Mazza and Qi, 1992; Gordon and Kourimski, 1995b; Romero et al., 1998). The amount of polar compounds have been shown to rapidly increase during the first 20 rounds of frying in sunflower oil at 180 °C, thereafter no significant increase in degradation was observed due to exhaustion of precursors (Cuesta et al., 1993).

Increasing frying temperature accelerates thermo-oxidative degradation of oil and fried food ingredients including a higher rate of compound polymerization (Fedeli, 1988; Blumenthal, 1991). In a study comparing frying temperatures of 185 °C and 215 °C, the total

amounts of polar components formed after 7 days of frying were 19.8 % and 38 % respectively (Aladedunye and Przybylski, 2009). The amounts of polymers generated were 8 % at 185 °C and 15.6 % at 215 °C. The types of polymers also changed when higher temperatures were used. A decrease in the contribution of polymers with peroxide linkages whereas an increase in ether linked or carbon to carbon linked polymers was observed when elevated temperatures were applied (Kim et al., 1999).

During intermittent heating and cooling of frying oils, the solubility of oxygen increases when the oil cools down from the frying temperatures (Clark and Serbia, 1991). Because of this phenomenon, intermittent heating and cooling causes increased oil deterioration when compared to oils undergoing continuous heating. In sunflower oil, linoleic acid content decreased by 25 % during intermittent frying and only a 5 % decrease was observed during continuous heating (Peers and Swoboda, 1982).

Typically, most frying operations do not fry only one type of food product and often have issues with temperature regulation; therefore, an assessment of the frying performance of oil at high temperatures with different food ingredients becomes imperative.

1.5.2 Oil turnover

Better quality frying products were produced when a high ratio of fresh oil to the total frying oil was kept (Paul et al., 1997). The amount of polar components, diacylglycerides, and free fatty acids decreased when frying oil was frequently topped up with fresh oil and the fry life of oil increased providing better quality fried foods (Sanchez-Muniz et al., 1993b; Romero et al., 1998). More oxidative degradation was observed than

hydrolytic reactions when frying potatoes in frequently topped up frying oils (Cuesta et al., 1993).

1.5.3 Quality of oil

Both the type and the quality of the oil used affect deep fat frying performance and its susceptibility to deterioration. The fatty acid composition of the oil can have positive or negative effects on the oil fry-life. Canola oil is commonly used as a frying medium in Canada because of its nutritional properties such as low saturated fatty acid and high monounsaturated fatty acid content (Eskin et al., 1996). The frying stability of canola oil, however, is influenced by the thermally unstable polyunsaturated fatty acids. Frying oils containing high amounts of polyunsaturated fatty acids undergo the faster thermo-oxidative degradation. Specifically, the content of linolenic acid is vital to the frying performance of oil, its frying stability, and formation of proper flavor in fried foods. Oil containing 8.5 % of linolenic acid produced undesirable fishy and acrid odors when heated above 190 °C, having a higher odor intensity score when compared to hydrogenated oil (Frankel et al., 1985). Lower amounts of free fatty acids and polar components were found when low linolenic acid canola oil (2.5 %) was used for deep fat frying of potato chips at 190 °C (Xu et al., 1999). Eskin et al. (1989) found that heated low linolenic acid canola oil (1.6 %) produced lower amounts of free fatty acids and also these authors observed a reduced oxidation rate and a lower intensity of heated room odor when compared to standard canola oil (9 % linolenic acid). The amount of polar components in cottonseed oil increased proportionally to the content of linoleic acid during deep fat frying of potato chips (Warner et al., 1997). Increasing contribution of oleic acid decreased the rate of total polar component formation

and reduced the amount of total volatile compounds during storage of fried products (Warner et al., 1994). Fatty acid chain length has not been implicated in stimulation of thermo-oxidative degradation, however, increasing the level of free fatty acids significantly increased thermo-oxidative degradation (Choe and Min, 2007). By reducing linolenic acid and increasing oleic acid contents, canola oil becomes commercially suitable for deep fat frying operations (Eskin et al, 1989; Warner and Mounts, 1993; Warner et al., 1994).

1.5.4 Types of fryer

Fryer types can affect frying oil deterioration through efficient heat transfer, polymerized fat deposits on walls, surface to volume ratio, and catalytic metal contamination (Choe and Min, 2007). “Hot spots” on or in the deep fat fryer can cause scorching of the oil and degrade it faster. Polymerized fat usually stuck to the fryer interior walls at the oil level or slightly above it causing gumming of the oil as well as increases foaming, faster darkening, and stimulates frying oil deterioration. A small surface to volume ratio is necessary to minimize oil and air contact. Metals such as copper or iron can be released into the frying oil from the food, deep fat fryer, or its accessories at high temperatures and become catalysts increasing the rate of oxidation.

1.5.5 Antioxidants

Antioxidants naturally present or added to oils and foods can influence oil quality during frying. Antioxidants such as tocopherols, butylated hydroxyanisole (BHA), hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ) slow down oil oxidation at room temperature but become less effective at frying temperatures due to

losses through evaporation and/or decomposition (Choe and Lee, 1998, Choe and Min, 2007).

1.6 Assessing performance of oil during frying

Physical changes in the oil such as color darkening, odor formation, excessive foaming, and smoking have traditionally been used as indicators of oil frying quality. Visual indicators are often inadequate and unreliable because of the subjective nature of their assessment. Simple, objective, and reliable analytical methods have been developed to quantify a specific group of degradation products that are used to assess the quality and performance of the frying oil are described below (Paradis and Nawar, 1981; Melton et al., 1994).

Decomposition and oxidative degradation products formed during frying are both volatile and non-volatile. The majority of volatile products are continuously removed from the oil and their collection and analysis is difficult and laborious (Fritsch, 1981; Gertz and Matthäus, 2008). Non-volatile products remain in the frying oil and can also be absorbed by the fried food. The non-volatile products are more practical to study and most analyses assessing frying oil quality depend upon their formation (Melton et al., 1994; Gertz and Matthäus, 2008). Formation and accumulation of newly formed components as a result of physical and chemical changes happening in the oil are often used as indicators of oil quality and deterioration because their detection methods are easy, quick, inexpensive, and reproducible. Physical changes are usually measured by: color, density, and viscosity changes, refractive index, conductivity, specific heat, among others. Assessment of chemical changes rely on specific compounds formed in the oil such as polymers and free fatty acids,

total polar components or overall alteration of endogenous oil compounds such as fatty acids, tocopherols and others (Gonzalez-Quijano and Dobarganes, 1988).

1.6.1 Color analysis

During frying, the color of the oil darkens and is the first sensory indicator used to "assess" when oil will produce unacceptable flavor and odor of fried products (Paul et al., 1997). It is considered to be a major measurement used to assess acceptance of further used frying oil and is evaluated on a daily basis (Orthoefer, 1988). Oil color is used as a conclusive parameter in many countries as to whether or not to discard the frying oil (Bansal et al., 2010a). The U.S. Department of Agriculture published a document entitled the Manufacturing Process Inspection that states that oil darkening is evidence of an unsuitable frying oil and must be rejected (USDA, 1985).

As discussed previously, a number of conditions contribute to oil color change during frying. Many products from thermo-oxidative deterioration and nonenzymatic browning increase oil color intensity (Blumenthal, 1991). Pigments and Maillard reaction products from the fried food can leech into the oil and cause color to darken (Gutierrez et al., 1988; Lalas et al., 2006; Delgado-Andrade et al., 2010). Particles of food left in the oil after food frying can cause faster color formation as well as caramelization of the food product (Vijayan et al., 1996). Oil components such as tocotrienols and phenolics have also been shown to contribute to faster oil darkening through oligomerization and other chemical changes. Color formation has been associated with pigments having molecular weights of 300-551 Daltons and containing double bonds, carboxyl, ester, peroxide, or hydroxyl functional groups (Min et al., 1975).

Official methods for measurement of color in frying oils include American Oil Chemists' Society procedures using different colorimeters: Wesson (AOCS method Cc 13b-45), Lovibond (AOCS method Cc 13e-92), and spectrophotometric (AOCS Cc 13c-50) (Firestone, 2009). The first two procedures utilize colorimetric comparators while spectrophotometric method measures photometric color index (PCI) calculated according to the equation:

$$PCI = 1.29\ (A_{460}) + 69.7\ (A_{550}) + 41.2\ (A_{620}) - 56.4\ (A_{670})$$

where A_{460}, A_{550}, A_{620}, and A_{670} are absorbances measured at 460, 550, 620, and 670 nm, respectively.

Xu (2000) reported that results from the spectrophotometric method assessing color of frying oils are highly correlated with absorbance measured at 490 nm and the total polar components (TPC) value ($r = 0.953$). Bansal et al. (2010b) showed that good correlation exists for absorbance measured at 400 - 500 nm.

Rates of color intensity differ from oil to oil and depend upon the initial oil color, the type of food product fried in it, the composition of oil, and the results of color changes must be interpreted with caution because darkening of oil is not always directly correlated with the frying quality of oil (Gertz, 2000).

1.6.2 Total polar components (TPC)

As mentioned previously, polar components form in the mostly nonpolar, fresh oil as a result of chemical reactions occurring during frying with the amount steadily increasing

with frying time. Numerous types of polar components are formed in the oil during frying, including: oxidized and oligomerized triacylglycerides and sterols, free fatty acids, mono- and diacylglycerides, degraded antioxidant and phospholipids, and other degraded oil and food constituents.

Total polar component (TPC) analysis directly measures all of the degraded products in the oil and thus is the best indicator of frying oil degradation. TPC amount is often used as an indicator to regulate the level of oil abuse in commercial and institutional frying operations. This measurement is widely accepted in European Union countries, however, the upper limits of TPC values vary (Firestone, 2006). Gertz (2000) stated that the level of 24 - 25 % of TPC should be taken as borderline to discarding frying oil. The following limits of TPC are applied in: Germany 24 % maximum; 25 % in Spain, Belgium, and France; and 27 % in Austria with these values being strictly enforced (Firestone, 2006). Regulations controlling the amount of TPC levels in Canada or the United States are not in place yet.

A gravimetric procedure used as an official method to assess TPC involves chromatographic separation of both polar and nonpolar components using 5 % adjusted water content silica gel (AOAC 982.27; AOCS Cd 20-91; IUPAC 2.507). The elution of the nonpolar fraction from the silica column uses a combination of solvents and contains unaltered triacylglycerides. The TPC is then calculated by the difference and expressed as weight percent of the starting sample. IUPAC and AOCS conducted collaborative tests and proved the method to be exact and reproducible with a lower than 5 % coefficient of variation (Dobarganes et al., 2000b; Firestone, 2009). A small sample size and column variation has been proposed to minimize solvent and silica gel use and to reduce analysis time (Schulte, 2004; Marmesat et al., 2007). Because of the small sample size, accuracy and

precision both decrease when using this method especially in low TPC level oils. An automated and accelerated solvent extraction has been proposed to replace the manual chromatographic step to speed analysis and reduce experimental effort (Zainal and Isengard, 2010).

TPC determination through nuclear magnetic spectroscopy (NMR), near infrared spectroscopy (NIR) and differential scanning calorimetry (DSC) had been proposed to substitute the adsorption chromatography and to eliminate chemicals (Sun and Moreira, 1996; Hein et al., 1998; Tan and Che Man, 1999; Gerde et al., 2007). Although these methods have their advantages and require advanced and expensive analytical equipment, adsorption chromatography is simple and uses readily available and affordable materials and will most likely not be replaced soon.

2.6.3 Composition of polar materials (CPM)

In order to provide more information about the types and levels of reactions occurring in the frying oils and the levels of toxicity, separation and characterization of specific compounds within TPC is necessary (Paradis and Nawar, 1981). For example, hydrolytic changes create diacylglycerides and free fatty acids that contribute to the TPC level but are less important nutritionally when compared to thermo-oxidative and polymerization products (Dobarganes and Marquez-Ruiz, 2006).

The separation and quantification of polar components by their molecular weights is done using high performance size exclusion chromatography (HPSEC). Whether the method is applied directly to used frying oil or to isolated polar fractions, the polar materials are separated into dimers, polymers, oxidized monomeric triacylglycerides, diacylglycerides and

free fatty acids (Dobarganes and Marquez-Ruiz, 2006). The delegates at the 3rd International Symposium on Deep Fat Frying stated that TPC and CPM are the best indicators of oil degradation (DGF, 2000). Regulations in some European countries also included the total amount of dimers and polymers as indicators of frying oil quality indicating that its amount should be less than 10 % (Firestone, 2006).

1.6.4 Changes in tocopherols

Tocopherols are natural antioxidants found in vegetable oils that under frying conditions decrease in concentration due to direct antioxidant activity, evaporation, or through thermal oxidation and polymerization. A rate of tocopherols disappearance is also affected by the temperature and time and the performance of frying oil can be assessed by measurement of antioxidants degradation (Gordon and Kourimska, 1995b; Normand et al., 2001; Warner and Moser, 2009).

Analytical methods used for quantifying tocopherols have been extensively reviewed by Abidi (2000), Cert et al. (2000), Ruperez et al. (2001), Carrasco-Pancorbo et al. (2005), Ladislav et al. (2005), Bendini et al. (2007), Jensen and Laurisen (2007), Liu et al. (2008), and Tarascou et al. (2010). The most widely accepted method for their quantification is the direct normal-phase high performance liquid chromatography (HPLC) oil analysis. The most common and sensitive approach for these compounds uses fluorescence detection with an excitation wavelength of 290 nm and an emission wavelength at 330 nm (Chase et al., 1994; Cert et al., 2000). A UV detector set at 292 nm should be used only in the absence of a fluorescence detector according to the AOCS official method (Firestone, 2009). Reverse-

phase HPLC has a shorter equilibrium and analysis time with a higher reproducibility but β- and γ- isomers cannot be resolved (Cert et al., 2000; Ruperez et al., 2001).

1.6.5 Fatty acid composition

The change in fatty acid composition can be used as an indicator of thermo-oxidative degradation occurring during deep fat frying (Warner, 2004). The degree of changes occurring to the fatty acid composition increases with temperature and frying time. Polyunsaturated fatty acids such as linoleic and linolenic acid primarily decrease as frying ensues. Gas chromatography (GC) is often used alongside an official method to analyze fatty acid composition by first transforming fatty acids into methyl esters and then using GC to separate and quantify the methyl ester derivatives (AOCS methods Ce 1-62, Ce 1f-96, Ce 2-66, ISO method 5509, IUPAC method 2.301).

The gas chromatogram peak areas are used in routine analysis to calculate the fatty acids content (Dijkstra et al., 2007). By default, fatty acid content is set to 100 % in GC analysis and thus a decrease in polyunsaturated fatty acids is counterbalanced by increasing the more stable monounsaturated and saturated fatty acids. It also must be taken into account that chromatograms only account for unaltered fatty acids and exclude degraded oxidized, dimerized and polymerized fatty acids. An internal standard (e.g. C17:1) becomes useful by demonstrating that the amounts of the monounsaturated and saturated fatty acid remain unchanged, and that the sum of all fatty acids indeed falls below 100 % as a consequence of thermo-oxidative deterioration.

1.6.6 *p*- Anisidine value (AV)

Primary oxidation products can be quantified by standardized methods such as peroxide values and conjugated dienes but their thermal instability makes their measurements unreliable. Secondary oxidation products formed during frying are from the thermal decomposition of hydroperoxides. Carbonyls compounds such as aldehydes are the most dominant and some are volatile and lost through evaporation but non-volatiles stay in the oil and can be analyzed by AV (Chang et al., 1978; Perkins, 2006). The definition of *p*-anisidine value is 100 times the optical density of 1.00 g of oil in 100 mL of a mixture of solvent and reagent measured at 350 nm in a 1 cm cuvette (Firestone, 2009). The reaction between aldehydes, principally 2-alkenals and 2,4-dienals and the *p*- anisidine reagent in a glacial acetic acid solution forms Schiff bases. The official procedure utilizes the characteristic UV absorbance of the Schiff bases at 350 nm with the absorption increasing with the amount of non-volatile carbonyl compounds, the values being arbitrarily expressed as absorbance units per 1.00 g of fat (ISO 6885; AOCS Cd 18-90; IUPAC 2.502; White, 1995).

Fourier transformed infrared spectroscopy (FTIR) can be used in AV determination and provides a rapid, automated, and solvent free alternative to the current official chemical method (Dubois et al., 1996). A strong correlation (r^2= 0.998) has been found between the AV of the thermo-oxidized canola oil determined by FTIR and the official chemical method (Szabo et al., 2009).

1.6.7 4-Hydroxynonenal (HNE) formation

A number of toxic components form during deep frying due to lack of control over frying conditions or unstable frying oil. One of the most studied and reviewed due to its high reactivity is 4-hydroxynonenal with recent reviews by Niki (2009), Gueraud et al. (2010) and Spickett et al. (2010). HNE exhibits mutagenic, cytotoxic, and genotoxic properties, and can modify proteins, nucleic acids, and other biomolecules leading to several diseases and medical conditions including Alzheimer's disease and atherosclerosis.

In non-biological samples, HNE is analyzed as 5(1'-hydroxyhexyl)-1-methyl-2-pyrazoline adduct formed by reaction of HNE with N-methylhydrazine, quantified by gas chromatography with a nitrogen detector (Tamura and Shibamoto, 1991). Another study used water containing 0.1 % BHT to extract HNE from oil followed by chloroform extraction and transformation of HNE into diol analyzed by GC-MS (Surh and Kwon, 2003). The extraction and clean-up procedure is a lengthy and error-prone multistep process used to purify complex mixtures of carbonyl hydrazones. Aladedunye et al. (2011) developed a method that omits derivatization and purification of HNE by first isolating oil polar components using a 5 % water silica column and 15 % diisopropyl ether in hexane to elude the nonpolar fraction. Methanol was then used to collect the polar fraction which was concentrated, centrifuged and the supernatant analyzed for HNE by HPLC. With a coefficient of variation below 6 %, this method is precise, sensitive, simple and accurate at all HNE levels.

In summary, no single method has been proven to be absolutely reliable for measuring frying oil deterioration components and therefore employing the use of several tests can help to fully understand the extent of changes in the frying oil.

1.7 Focus of the Thesis

Discussed above published data showed clearly that all experiemnts were designed to assess how simple carbohydrates such as glucose and fructose and individual amino acids contributed to pigments formation. Additionally, these compounds were assessed individually and using simple model conditions. Frying foods is a relatively complex system where many factors are involved concurrently and usually interaction of a few components has to be taken into account. We could not find studies describing the effect of food ingredients on darkening of frying oil and how color changes affecting quality of frying oil and fried products. Following the lack of data in this very important method of food preparation, it is imperative to perform a systematic study to assess how typical food ingredients affect frying oil darkening and quality of fried foods. To evaluate pigment formation the following objectives are included in this work:

1. Performing all experiments using standard frying conditions
2. How individual and complex carbohydrates affect darkening of the frying oil
3. How selected amino acids and proteins affect color change in the frying oil
4. Effect of the carbohydrates with proteinous components combination on oil quality
5. How standard breading and battering products affect frying oil darkening
6. How the oxidation of oil and food components affect color of frying oil
7. How temperature of frying oil affects color changes
8. How the formation of pigments affects the development of toxic compounds such as HNE (Hydroxynonenal)
9. How darkening of frying oil affects degradation of antioxidants

10. How darkening of frying oil affects quality of fried foods and frying oil
11. To develop some recommendations for frying food industry to improve quality of fried foods

Chapter 2 - Materials and Methods

The materials and methods used in this study are presented in this chapter.

2.1 Materials

2.1.1 Oils and food ingredients

Two types of canola oil were selected for use in this study. Regular canola oil (RCO) and high oleic, low linolenic canola oil (HLCO) without antioxidants added, both were commercially refined, bleached and deodorized at Richardson Oilseed Processing (Lethbridge, AB, Canada).

Corn starch, glucose, whey and soy proteins were purchased from a local health food store. All of the commercial breading products (Corn Flake® and Shake and Bake®) and batter mixtures were purchased from a local food store. Amino acids glycine (Gly), lysine (Lys), and L-α-phosphatidylcholine (> 99 %) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.1.2 Chemicals

All of the solvents and chemicals used in this study unless otherwise stated were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Tripalmitin, a standard used for polar component analysis, and fatty acid methyl ester standards were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA). Gamma tocopherol standard was purchased from ChromaDex (Irvine, CA, USA) while α-tocopherol and δ-tocopherol standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Silica gel 60 (70 –

230 mesh), potassium iodide, potassium iodate, sodium thiosulfate, and potato starch were obtained from Sigma-Aldrich (St. Louis, MO).

2.1.3 Instruments

Spectrophotometric analysis for UV-Vis data was done using a Beckman DU-65 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA).

HPLC analyses were carried out on a Finnigan Surveyor LC system (Thermo Electron Corp., Waltham, MA, USA) equipped with a Finnigan Surveyor Autosampler Plus, FL Plus fluorescence detector, photo-diode-array detector, UV-Vis Plus detector and Sedex 75 evaporative light scattering detector (Sedere, Alfortville, France).

GC analyses were performed on a Trace GC Ultra (Thermo Electron Corporation, Rodano, Italy).

2.2 Methods

2.2.1 Frying procedures and oil samples

2.2.1.1 Standard frying conditions

Frying for all conditions was performed in 1-L capacity domestic deep fryers (Toastess International, Dollard-des-Ormeaux, Quebec, Canada). A starting volume of 800 mL was used for either type of canola oil. The frying oil was heated to a frying temperature of 185, 200, or 215 ± 5 °C and kept at this temperature for 8 hours each day for 4 days (32 hours total). At the beginning of each frying day, the oil was heated for 90 min prior to commencing frying of product. Each fryer was shut off at the end of each frying day and left to cool overnight. The oils were filtered to remove any solid debris and two 25 mL samples

of oil were taken each morning and kept at -20 °C until analyzed. Fresh oils (50 mL) were then added daily to replenish oil levels.

2.2.1.2 Preparation of oxidized oil and food products for frying

Pigment formation and frying performance of the oil were assessed during large scale frying of the following components:

1. Starch (St)
2. Starch + glucose (99:1, wt/wt; Glu 1 % or SGlu)
3. Starch + glucose (90:1, wt/wt; Glu 10 %)
4. Starch + glycine (99.9:0.1, wt/wt; SGly)
5. Starch + lysine (99.9:0.1, wt/wt; SLys)
6. Starch + glucose + glycine (98.9:1:0.1, wt/wt/wt; SGG)
7. Soy
8. Whey
9. Whey + glycine (99.9:0.1, wt/wt; WGly)
10. Whey + lysine (99.9:0.1, wt/wt; WLys)
11. Whey + glucose + glycine (98.9:1:0.1, wt/wt/wt; WGG)
12. Oxidized oil (0.1 % Ox, 1 % Ox, and 5 % Ox)
13. Oxidized oil (1 % Hydroperoxides reduced- 1 % RP)
14. Whey + glycine (99.9:0.1, wt/wt) + oxidized oil (OxWG)
15. Whey + starch + glucose + glycine (33:65.9:1:0.1, wt/wt/wt/wt; MH)
16. Whey + starch + glucose + glycine (33:65.9:1:0.1, wt/wt/wt/wt) + oxidized oil MO)

17. Commercial batter mixture (BM)
18. Corn flake® breading (CF)
19. Shake and bake® breading (S&B)
20. Phospholipids (425 µg/g Phos)
21. Polymer
22. No polymer

Laboratory prepared mixtures of food ingredients were chosen to mimic typical fried food composition. The ingredients of the commercial breadings and batters used in this study as itemized on the packaging are listed in **Table 1**.

For frying with oxidized oils involving food frying, OxWG and MO, 1 % of fresh oil was replaced with equal volume of oxidized oil at the beginning of frying only. When solely oxidized oil was used for frying, 0.1 %, 1 %, and 5 % of fresh oil was replaced with oxidized oil at the beginning of frying only. The oxidized oil was prepared by placing canola oil in an oven at 60 °C for 7 d with free air access and in a container providing a surface area to volume ratio of 1 and oxidized oil had a PV= 417 meq/kg whereas fresh oil had a PV= 1.3 meq/kg. The oxidized oil with high peroxide value was heated at a frying temperature of 185 °C for one hour to reduce the peroxide value to PV= 183 meq/kg. Oil with reduced peroxide value was used as source of precursors produced by thermal degradation of hydroperoxides.

Table 1: Ingredients of commercial battering and breading ingredients as listed on their respective packaging.

Type of Breading/ Batter	**Ingredients**
Commercial Batter Mixture	Wheat flour, corn meal, baking powder, buttermilk powder, sugar, spices, and mustard
Corn Flake® Breading	Flaked milled corn, sugar/glucose-fructose, malt flavoring, salt, natural color, thiamin hydrochloride, niacinamide, pyridoxine hydrochloride, folic acid, *d*-Calcium pantothenate, and iron
Shake and Bake® Breading	Bread crumbs (contains wheat flour), corn meal, corn dextrin, modified corn starch, salt, hydrogenated soybean and cottonseed oils, canola oil, spices, color and citric acid

Starch and starch based mixtures were prepared by mixing starch first in cold water followed by hot water at 4:1:5 wt/v/v ratio forming gel used in frying (Aladedunye and Przybylski, 2011). Whey and soy proteins and commercial breadings were combined with water at 40:60 wt/v ratio. Oils were heated only when, 0.1 %, 1 %, 5 % of oxidized oil, 425 μg/g phospholipids, 1 % oil with reduced peroxide value, polymer and without polymer were applied to these experiments.

During the large scale frying, a batch of 80 g of product was fried for 10 min, every hour and a half, for a total of 4 batches fried daily. For small scale frying, a batch of 2 g or 10 g of product was placed in the oil 4 times throughout the frying day. Oil was filtered as described above. In all trials and for all components, a frying temperature of 185 ± 5 °C was used as a standard temperature. Temperatures of 200 and 215 ± 5 °C were applied for frying products and during heating oil without frying. Two replications of each of the trials were performed.

2.2.3 Isolations

2.2.3.1 4-Hydroxy-2-nonenal

During polar components analysis, the nonpolar fractions (triacylglycerides) were removed. The polar fractions were recovered by eluting the column four times with 5 mL of methanol. The final volume of 5 mL was achieved through evaporation of the combined eluants under a stream of nitrogen. The cloudy solution was then centrifuged at 1000 g for 5 min, and the clear supernatant transferred into a clean vial and analyzed by HPLC according to the procedure described by Aladedunye et al. (2011).

2.2.4 Analysis by spectrophotometry

2.2.4.1 Color analysis

The AOCS Official method Cc 13c-50 was used to determine oil color (Firestone, 2009). Oil (1.00 g) was weighed and dissolved in a 2 mL volumetric flask and filled to the mark with isooctane. The absorbance was read against an isooctane blank at 490 nm.

2.2.4.1 Anisidine value

Anisidine values (AV) were assessed according to ISO Method 6885 (2004). The oil was weighed directly into a 25 mL volumetric flask. The oil was dissolved in isooctane. The oil solution (5 mL) and 1 mL of anisidine reagent (0.25 % solution of anisidine in glacial acetic acid) were pipetted into a clean screw-capped test tube. After the test tube was closed and vortexed, it was kept in a dark at room temperature for 8 min. Within the proceeding 2 min, the absorbance of the solution was read at 350 nm. When the measured absorbance was outside the range of 0.2 to 0.8 units, an adjusted amount of oil was weighed and the

procedure repeated. An unreacted test solution was prepared using glacial acetic acid (1 mL) instead of anisidine reagent. The blank consisted of isooctane (5 mL) instead of the oil solution.

The following formula was used to calculate anisidine value:

$$AV = 25/m\ [1.2 \times (A_1 - A_2 - A_0)]$$

Where: A_0 is the absorbance of the unreacted test solution, A_1 is the absorbance of the reacted solution, A_2 is the absorbance of the blank, and m is the mass of the oil in grams.

2.2.5 Analysis by GC

2.2.5.1 Fatty acid composition

Initially, fatty acid methyl esters (FAME) were prepared by weighing 25 mg of oil directly into a screw-capped tube and 0.5 mL of an internal standard (methyl heptadecenoate; 2 mg/mL) in isooctane was added. This was followed by 5 mL of 0.5 M solution of sodium methoxide in methanol, was mixed, and heated at 60 °C in a heating block for 1 h. The solution was then cooled to room temperature and 0.3 mL glacial acetic acid, 3 mL isooctane and 3 mL distilled water were added. The solution was mixed and centrifuged and the clear upper layer (FAME) transferred into a dry chromatographic vial and analyzed on a Trace GC Ultra gas chromatograph.

The GC was equipped with a Trace TR-FAME fused silica capillary column (100 m x 0.25 mm x 0.25 μm) using hydrogen as a carrier gas with a flow rate of 1.5 mL/min. The temperature of the column was programmed from 70 to 160 °C at 25 °C/min, held for 30

min, and further programmed to 210 °C at 3 °C/min. The starting and final temperatures were held for 5 and 30 min respectively. A PTV injector was used to make a splitless injection with a detector temperature set at 250 °C. A AS 3000 autosampler was used to inject 1 µL of FAME samples. The fatty acids were identified by comparison of retention times with authentic standards.

2.2.6 Analysis by HPLC

2.2.6.1 Composition of polar material

High-performance size-exclusion chromatography (HPSEC) was used to analyze the composition of polar components following the ISO Method 16931: 2007 procedure. Three size exclusion columns (Phenogel 500 Å, 100 Å, and 50 Å, 5 µm, 300 x 4.60 mm; Phenomenox, Torrance, CA) connected in series were used to separate the components. Tetrahydrofuran (THF) was used as the mobile phase with a flow rate of 0.3 mL/min, and column temperature of 30 °C. A Sedex 75 evaporative light scattering detector (Sedere, Alfortville, France) operated at 30 °C with purified air at a pressure of 2.5 bar was used to detect the components in a 10 µL injected sample.

2.2.6.2 Tocopherols

High-performance liquid chromatography (HPLC) was used to analyze the amount of tocopherols using the AOCS Official Method Ce 8-89 (Firestone, 2009). Oil samples (40 mg) were weighed directly into threaded 1.5 mL HPLC vials and dissolved in 1 mL of hexane. Analysis was conducted using a Finnigan Surveyor LC fitted with a Finnigan Surveyor FL Plus fluorescence detector with an excitation wavelength at 292 nm and

emission at 325 nm (Thermo Electron Corporation, Rodano, Italy). Each oil sample (10 μL) was injected by a Finnigan Surveyor Autosampler Plus into a normal-phase Diol column (5 μm; 250 x 4.6 mm; Monochrom, Varian, CA, USA). Using a 0.6 mL/min flow rate, the mobile phase comprised of 7 % methyl-*tert*-butyl-ether in hexane. All tocopherol levels were quantified using the external calibration method with separate calibration curves for α-, γ-, and δ- tocopherol.

2.2.6.3 Analysis of 4-hydroxynonenal

The analysis of 4-hydroxynonenal (HNE) used high-performance liquid chromatography (HPLC) equipped with a C18 column (4 μm; 300 x 3.9 mm; Novapak). A 20 μL sample was injected on the column; mobile phase was pumped at 0.75 mL/min consisting of acetonitrile and water (30:70 v/v). Components were detected by UV detector set at 223 nm (Lang et al., 1985). HNE identification was completed through comparison of retention data and by co-elution of HNE standard with selected samples. Quantification of HNE was carried out using external calibration.

2.2.7 Analysis by potentiometric titration

2.2.7.1 Peroxide Values (PV)

Peroxide values (PV) were analyzed by potentiometric endpoint determination according to ISO Method 27107 (2008). The oil (5.0 g) was weighed directly into a beaker and dissolved in 50 mL of glacial acetic acid/isooctane mixture (60:40 v/v). Saturated potassium iodide solution (0.5 mL) was added and mixed for 1 min then immediately 100 mL of distilled water was added and mixture titrated with a 0.01 M sodium thiosulfate

solution until an equivalence point at 25 mV was reached, measured by Pt electrode. The blank solution contained all reagents and was prepared following described procedure. The peroxide value was calculated using the following formula:

$$PV = \frac{(V-V_0) \cdot c \cdot F \cdot 1000}{m}$$

Where: V is the volume of sodium thiosulfate solution used for determination in mL; V_0 is the volume of sodium thiosulfate solution used in blank test; F is the factor converting concentration of thiosulfate solution used in assessment to 0.01 M and calculated according to the formula below; c is the concentration of sodium thiosulfate solution in mol/L; and m is the mass of the sample in grams. Results were reported as meq of active oxygen per kg (meq/kg).

Conversion factor (F) calculation:

$$F = \frac{E_{KIO3} \cdot V_1 \cdot 6 \cdot 1000 \cdot c_{KIO3}}{M_{KIO3} \cdot V_2 \cdot V_3 \cdot c_{thiosulfate} \cdot 100}$$

Where: E_{KIO3} is the mass of potassium iodate in grams; 6 is the equivalent mass for the titer (1 mol KIO_3 → 3 mol I_2); V_1 is the volume of potassium iodate solution used for titer determination (5 or 10 mL); V_2 is the total volume of potassium iodate solution in mL (250 or 500 mL); V_3 is the volume of 0.01 M sodium thiosulfate solution used for determination in mL; c_{KIO3} is the purity of potassium iodate in g/100 g; M_{KIO3} is the molecular mass of potassium iodate (214 g/mol); and $c_{thiosulfate}$ is the concentration of sodium thiosulfate solution used for titration in moles per liter.

2.2.8 Data Analysis

All frying experiments and analyses were performed in duplicate and results are presented as mean values for the two frying replicates (n = 4). Data was analyzed by single factor analysis of variance (ANOVA) and regression analysis using Minitab 2000 statistical software (Minitab Inc. PA, ver. 15). Statistically significant differences between means were determined by pairwise comparison of the means by Duncan's multiple range tests. Statistically significant differences were determined at the $P < 0.05$ level.

Chapter 3 - Results

3.1 Influence of carbohydrates and proteins on color formation and thermo-oxidative oil degradation

3.1.1 Color

Regulations in many countries stipulate that color must be one of the criteria used for discarding frying oils (Bansal et al., 2010a). Frying with whey protein as a base food ingredient showed the fastest increase in the color intensity ($p < 0.05$) during the whole frying period (**Figure 2**). Whey protein showed the fastest color change with a rate ten times faster compared to heated oil alone. The same protein stimulated one and a half times faster the formation of pigments that darkened oil when compared to soy protein, the latter being the second fastest color forming ingredient. After the first day of frying, all protein based food ingredients caused a significant change in oil color when compared to heated oil alone and continued this trend until the end of frying. On the second day of frying with proteins, the increase in the intensity of oil color was faster compared to frying carbohydrates. The rates of oil color changes were significantly higher when protein products were fried than heated oil. Frying with starch increased the rate of color formation almost two-fold when compared to heated oil. As the amount of glucose in the fried product increased, so did the rate of color changes but the rates were only significantly different for starch and starch with 10 % glucose. When free amino acids were added to whey protein, darkening of the oil was faster compared to soybean protein however, pigments were formed at the lower rate when whey protein was fried alone.

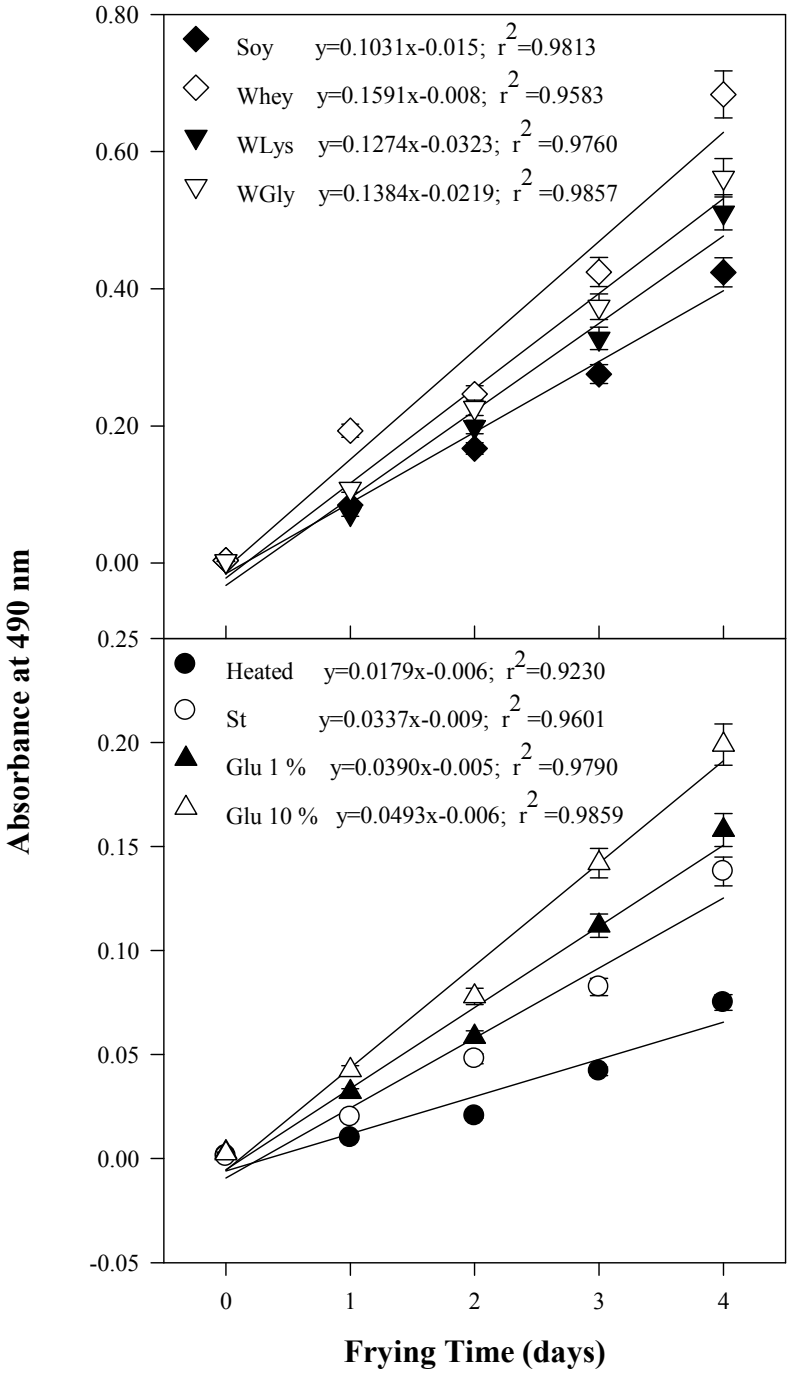


Figure 2: Effect of carbohydrate and protein products on color formation of frying oil. No products were fried in heated oil and it is used as a reference for both graphs above. Note the different Absorbance scales. Lines represent regressions. WLys - Whey + lysine; WGly - Whey + glycine; St – Starch; Glu - glucose + starch

3.1.2 Total polar compounds (TPC)

For all the frying protocols, the TPC amount increased after each day of frying and continued to do so as frying progressed (**Figure 3**). Measuring the TPC is a very reliable method used to measure oxidative degradation of the oil and is utilized as a predictor of fried food quality (Stier, 2001). Major oxidative degradation products formed during frying were polymerized triacylglycerides (Fritsch, 1981). Polymer formation in the oil is known to cause oil darkening and is one of the parameters which correlates well with change in TPC (Choe and Min, 2007). The pattern of TPC followed that of color changes during frying with protein products forming larger amounts of TPC than during frying with carbohydrates and the heated oil. Glycine enriched whey protein (WGly) formed TPC one and a half times faster than heated oil and 1 % glucose enriched starch. WGly produced a larger amount of TPC when compared to whey protein alone and less polar materials were observed during frying starch alone compared to 1 % glucose enriched starch although both of these differences were not statistically significant.

3.1.3 Residual tocopherols

Tocopherol concentration is highly dependent upon the extent of oil oxidation and can be used to measure the level of oil deterioration (Warner, 2004). Fresh canola oil used in this study had an average tocopherol profile of 216 μg/g of α-tocopherol and 355 μg/g of γ-tocopherol. All groups showed a significant decline in tocopherol amounts from fresh oil to the end of the frying period (Figure 4). At the end of frying, less than 10 % total tocopherols remained in the oil with the exception of heated oil (16 %) and in oil where lysine enriched whey protein (WLys) was fried (13 %). At the end of frying, for a system where WGly, WLys or whey protein was fried, complete depletion of tocopherols was observed. Heated oil exhibited the least tocopherol degradation and after frying for one day, 60 % of tocopherols remained still intact and depletion continued until the end of frying (16 %). After frying day one, the rate of tocopherol degradation in oils where whey and soy protein

were fried increased two fold compared to heated oil alone. An inverse relationship between TPC formation and tocopherol reduction was observed.

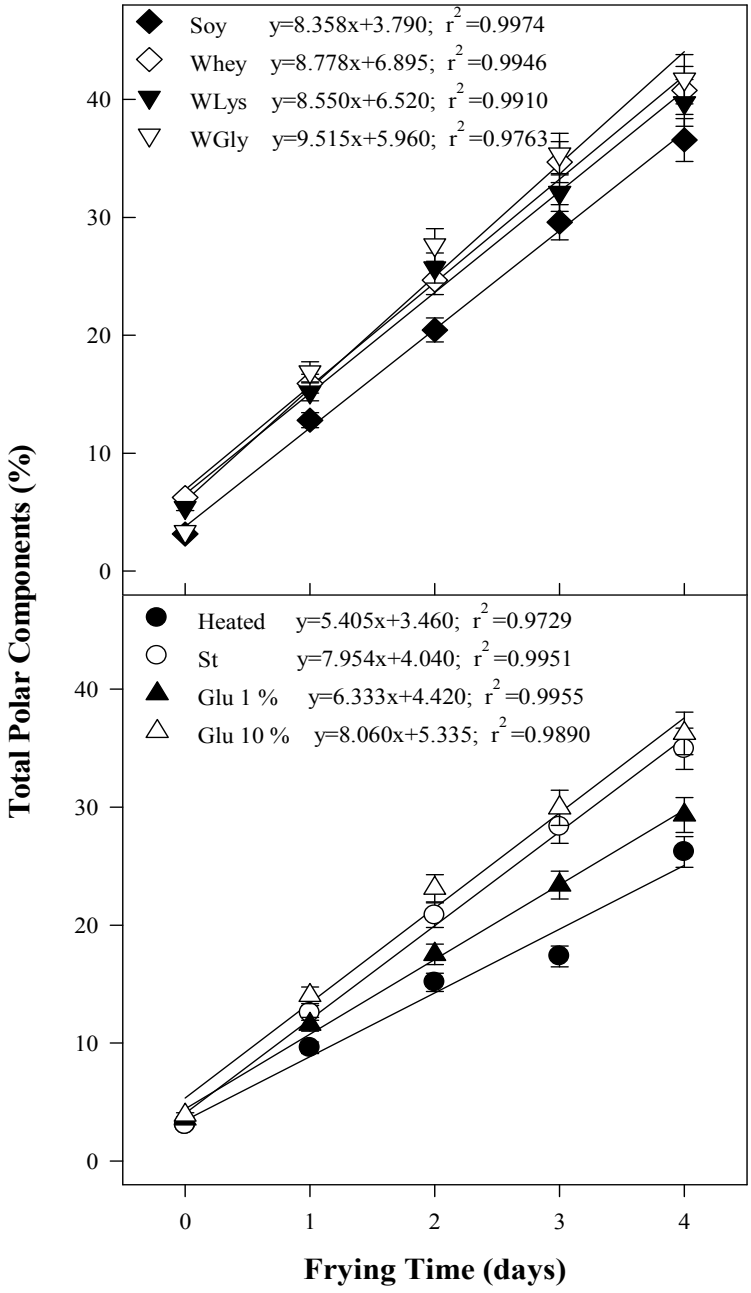


Figure 3: Changes in polar components during frying with carbohydrates and proteins. Heated oil is a reference for both graphs. Lines represent regressions. WLys - Whey + lysine; WGly - Whey + glycine; St – Starch; Glu - glucose + starch

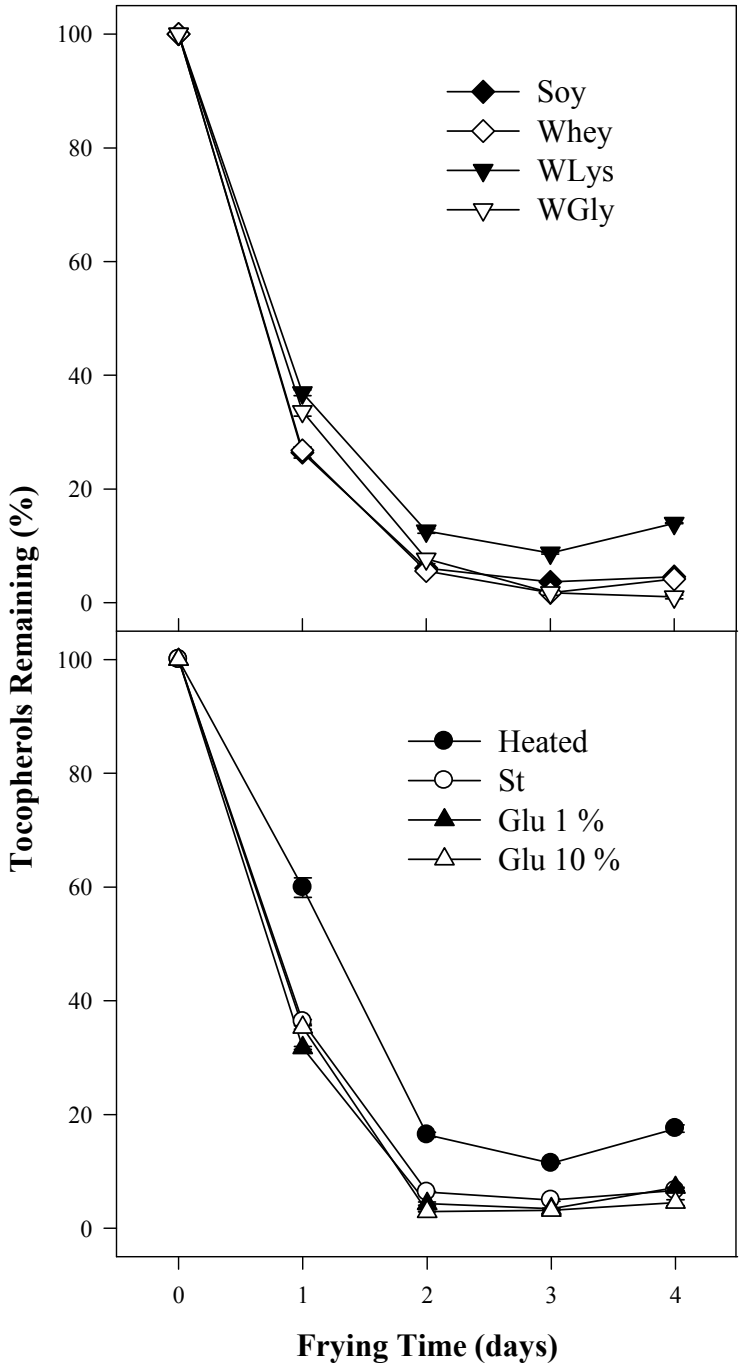


Figure 4: Total tocopherols remaining during frying with carbohydrates and proteins. Heated oil is a reference for both graphs. WLys - Whey + lysine; WGly - Whey + glycine; St – Starch; Glu - glucose + starch

3.1.4 Composition of polar components

Polar component composition was analyzed using HPSEC and separated into diacylglycerides (DG), oxidized triacylglycerides (OTG), dimers, oligomers, and free fatty acids. Their contribution was calculated using peak area with dimers and oligomers combined together shown as oligomers in **Figure 5**. The standard tripalmitin was used for proper identification of peaks with all oligomers eluting before this standard and oxidized triacylglycerides after. For all of the frying conditions, oligomers increased at fast rate and at the third day of frying, the contribution of these compounds plateaued. At the end of frying, oligomer formation rate was in heated oil and all oils where carbohydrates were fried significantly lower than in oils where whey protein, glycine and lysine enriched whey protein were fried.

The amounts of formed OTG decreased significantly in all runs after the first day of frying and continued to decrease until the end of frying period (**Figure 6**). A larger decrease in the amount of OTG was observed in oils where protein products were fried when compared to both carbohydrates fried and heated oils.

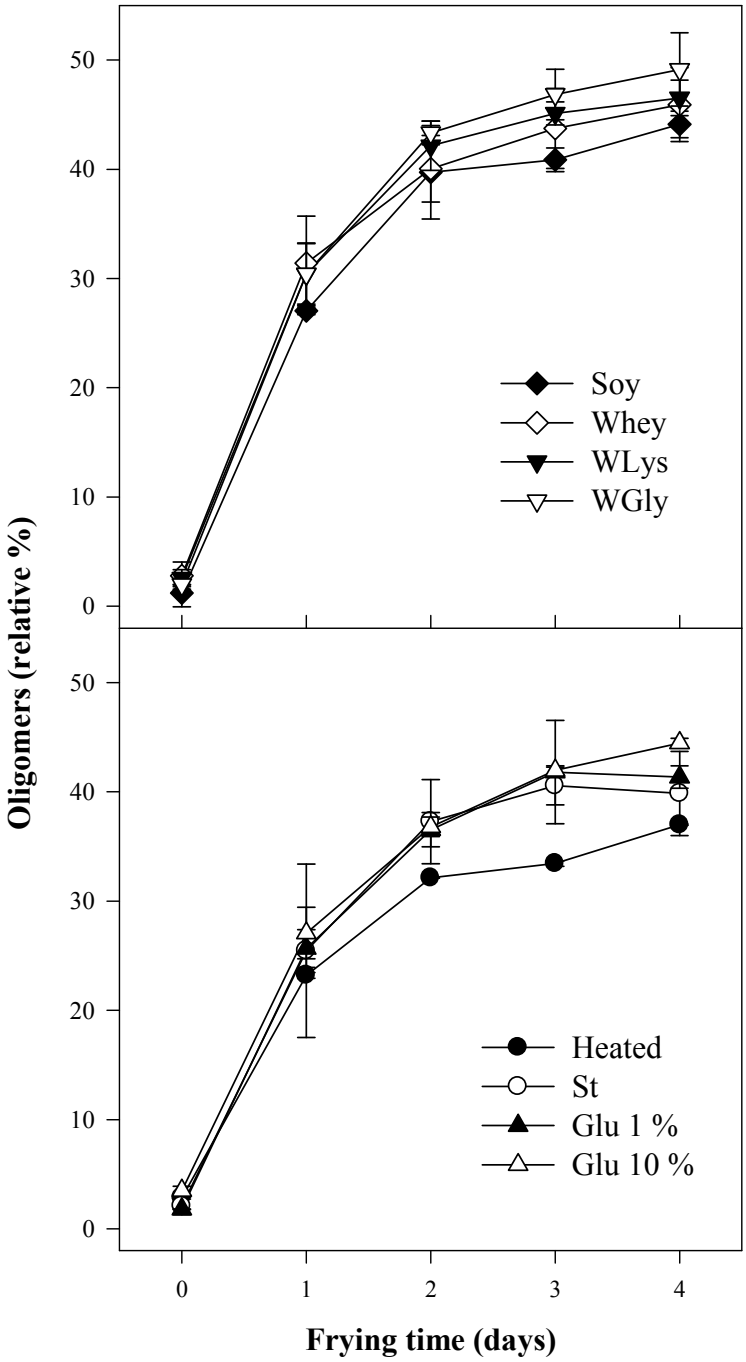


Figure 5: Changes in the amounts of oligomers during frying with carbohydrates and proteins. Heated oil is a reference for both graphs. WLys - Whey + lysine; WGly - Whey + glycine; St – Starch; Glu - glucose + starch

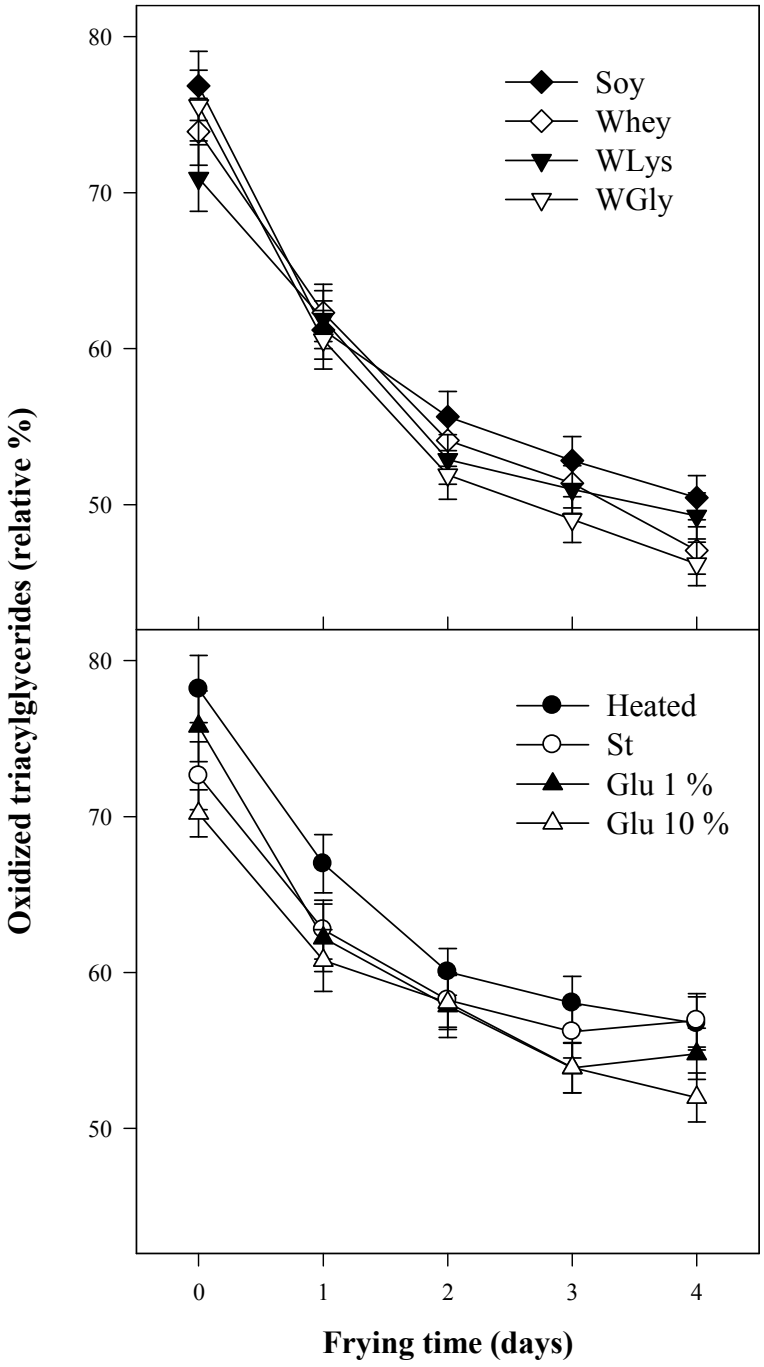


Figure 6: Changes in oxidized triacylglycerides during frying with carbohydrates and proteins. Heated oil is a reference for both graphs. WLys - Whey + lysine; WGly - Whey + glycine; St – Starch; Glu - glucose + starch

3.1.5 Anisidine value

Oxidative degradation of unsaturated fatty acids forms secondary oxidation products, such as carbonyls, and non-volatile compounds remain in the oil during frying (Chang et al., 1978; Perkins, 2006). Since secondary oxidation products are more stable than primary products, they become a good indicator for oil oxidation levels. In all conditions tested, the anisidine values increased significantly until the end of the first frying day as shown in **Figure 7**. From the second frying day until the end of frying, the anisidine values increased insignificantly for fried starch products and heated oil. In oils fried with proteins, the anisidine values from frying day two until the end of frying reached a plateau and only small fluctuations of values were observed. Heated oil had the slowest rate of anisidine value increase with the values increasing at a rate following a day behind the other oils. Adding 1 % glucose to fried starch caused anisidine values to increase faster compared to both starch and 10 % glucose in starch. Whey protein had the largest increase in anisidine values when compared to soy protein and both amino acid enriched whey proteins, however, the differences were not statistically significant until the last day of frying. Similar pattern of changes was observed for oils where carbohydrates and proteins were fried.

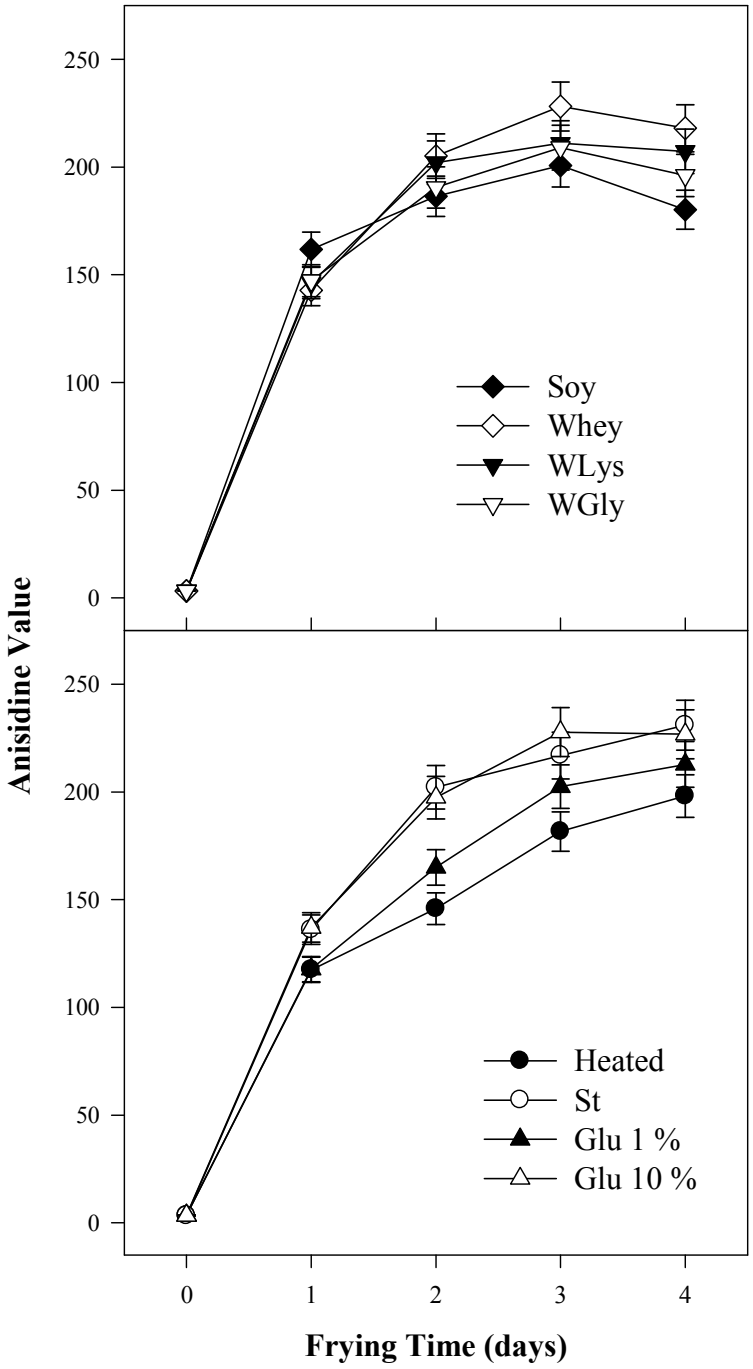


Figure 7: Effect of carbohydrates and proteins on anisidine value during frying. Heated oil is a reference for both graphs. WLys - Whey + lysine; WGly - Whey + glycine; St – Starch; Glu - glucose + starch

3.1.6 Fatty acid composition

Table 2 and **Table 3** show the changes to the major fatty acid compositions during frying with different food ingredients. The contribution of linoleic and linolenic acid for all tested conditions decreased significantly during frying. Frying with WLys caused the largest decrease in linoleic acid from 18.3 % to 13.5 % and a reduction of about twice as much of linolenic acid, 8.7 % to 4.6 %. The addition of amino acids to the food product decreased the polyunsaturated fatty acid content faster than any other conditions tested (**Table 2** and **Table 3**). In the heated oil used as a control, the slowest changes in the amount of linoleic and linolenic acids were observed when compared to all other conditions tested. The addition of glucose to starch only slightly improved linoleic and linolenic fatty acid integrity, however, as more glucose was added, faster degradation of these fatty acids occurred. When soy protein was fried in oil, the slowest changes of linoleic and linolenic acids were observed when compared to all other proteins fried in oils. These degradation/oxidation rates were comparable to changes when starch was fried in oils. The formation of *trans* fatty acids was slightly affected by the product being fried in the oil. The differences were within analytical error of assessment applied, indicating not statistically important changes.

Table 2: The changes to the major fatty acid compositions of canola oil that occur during frying with different carbohydrate components. Heated oil is a control for all assessments.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
Heated						
0	4.12 ± 0.18	2.40 ± 0.19	58.84 ± 1.59	18.19 ± 0.25	8.64 ± 0.08	1.45 ± 0.13
1	4.37 ± 0.22	2.48 ± 0.18	60.87 ± 1.39	17.10 ± 0.13	7.49 ± 0.13	1.41 ± 0.11
2	4.54 ± 0.20	2.79 ± 0.22	61.29 ± 1.57	16.28 ± 0.35	6.63 ± 0.36	1.72 ± 0.13
3	4.68 ± 0.18	2.64 ± 0.16	62.52 ± 1.54	15.54 ± 0.52	5.96 ± 0.32	1.68 ± 0.11
4	4.78 ± 0.19	2.65 ± 0.19	63.42 ± 1.51	14.88 ± 0.52	5.41 ± 0.12	1.68 ± 0.09
Starch						
0	4.10 ± 0.18	2.31 ± 0.18	59.11 ± 1.59	18.19 ± 0.25	8.65 ± 0.08	1.42 ± 0.13
1	4.33 ± 0.22	2.47 ± 0.18	60.57 ± 1.38	17.14 ± 0.13	7.47 ± 0.13	1.50 ± 0.12
2	4.50± 0.20	2.53 ± 0.20	61.79 ± 1.58	16.30 ± 0.35	6.60 ± 0.35	1.51 ± 0.11
3	4.66 ± 0.17	2.53 ± 0.15	62.96 ± 1.55	15.40 ± 0.52	5.84 ± 0.31	1.48 ± 0.09
4	4.83 ± 0.19	2.61 ± 0.19	63.98 ± 1.52	14.46 ± 0.51	5.16 ± 0.11	1.50 ± 0.08
Glucose 1 %						
0	4.03 ± 0.18	2.24 ± 0.18	58.99 ± 1.59	18.35 ± 0.26	8.75 ± 0.08	1.38 ± 0.13
1	4.25 ± 0.22	2.39 ± 0.18	60.41 ± 1.38	17.33 ± 0.13	7.64 ± 0.13	1.44 ± 0.11
2	4.48 ± 0.19	2.50 ± 0.19	61.82 ± 1.58	16.29 ± 0.35	6.68 ± 0.36	1.48 ± 0.11
3	4.60 ± 0.17	2.56 ± 0.15	62.82 ± 1.55	15.56 ± 0.52	5.94 ± 0.32	1.53 ± 0.10
4	4.79 ± 0.19	2.58 ± 0.19	63.91 ± 1.52	14.70 ± 0.52	5.29 ± 0.12	1.55 ± 0.08
Glucose 10 %						
0	4.09 ± 0.18	2.22 ± 0.18	59.05 ± 1.59	18.25 ± 0.26	8.70 ± 0.08	1.37 ± 0.13
1	4.23 ± 0.22	2.37 ± 0.18	60.44 ± 1.38	17.37 ± 0.13	7.62 ± 0.13	1.46 ± 0.11
2	4.43 ± 0.19	2.37 ± 0.18	61.89 ± 1.58	16.38 ± 0.35	6.65 ± 0.36	1.47 ± 0.11
3	4.60 ± 0.17	2.51 ± 0.15	62.84 ± 1.55	15.53 ± 0.52	5.85 ± 0.31	1.58 ± 0.10
4	4.78 ± 0.19	2.58 ± 0.19	63.80 ± 1.52	14.72 ± 0.52	5.24 ± 0.11	1.60 ± 0.08

[a] All values are averages of duplicate analysis

Table 3: The changes to the major fatty acid compositions of canola oil that occur during frying with different protein components. Heated oil is a control to all assessments.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
Soy						
0	3.94 ± 0.17	2.46 ± 0.19	61.28 ± 1.65	17.26 ± 0.24	7.55 ± 0.07	1.22 ± 0.11
1	4.08 ± 0.21	2.55 ± 0.19	62.40 ± 1.43	16.46 ± 0.12	6.51 ± 0.11	1.46 ± 0.11
2	4.29 ± 0.19	2.66 ± 0.21	63.82 ± 1.63	15.33 ± 0.33	5.66 ± 0.30	1.50 ± 0.11
3	4.45 ± 0.17	2.71 ± 0.16	64.75 ± 1.60	14.62 ± 0.49	5.01 ± 0.27	1.56 ± 0.10
4	4.58 ± 0.18	2.77 ± 0.20	65.62 ± 1.56	13.88 ± 0.49	4.47 ± 0.10	1.59 ± 0.08
Whey						
0	4.09 ± 0.18	2.29 ± 0.18	58.99 ± 1.59	18.21 ± 0.25	8.67 ± 0.08	1.41 ± 0.13
1	4.26 ± 0.22	2.36 ± 0.17	60.42 ± 1.38	17.29 ± 0.13	7.58 ± 0.13	1.47 ± 0.11
2	4.43 ± 0.19	2.51 ± 0.20	61.65 ± 1.58	16.41 ± 0.35	6.65 ± 0.36	1.57 ± 0.11
3	4.63 ± 0.17	2.58 ± 0.16	62.88 ± 1.55	15.47 ± 0.52	5.87 ± 0.31	1.55 ± 0.10
4	4.77 ± 0.19	2.58 ± 0.19	63.85 ± 1.52	14.73 ± 0.52	5.23 ± 0.11	1.55 ± 0.08
WLysine						
0	4.06 ± 0.18	2.23 ± 0.18	59.13 ± 1.59	18.25 ± 0.26	8.69 ± 0.08	1.39 ± 0.13
1	4.26 ± 0.22	2.38 ± 0.18	60.69 ± 1.39	17.18 ± 0.13	7.44 ± 0.13	1.50 ± 0.12
2	4.52 ± 0.20	2.45 ± 0.19	62.26 ± 1.59	15.95 ± 0.34	6.26 ± 0.34	1.55 ± 0.11
3	4.80 ± 0.18	2.61 ± 0.16	63.79 ± 1.57	14.69 ± 0.49	5.30 ± 0.28	1.63 ± 0.10
4	5.07 ± 0.20	2.71 ± 0.20	65.41 ± 1.56	13.53 ± 0.48	4.56 ± 0.10	1.61 ± 0.08
WGlycine						
0	4.12 ± 0.18	2.51 ± 0.20	58.83 ± 1.59	18.07 ± 0.25	8.55 ± 0.08	1.41 ± 0.13
1	4.39 ± 0.23	2.60 ± 0.19	61.11 ± 1.40	16.79 ± 0.13	7.07 ± 0.12	1.47 ± 0.11
2	4.55 ± 0.20	2.69 ± 0.21	62.27 ± 1.59	15.90 ± 0.34	6.17 ± 0.33	1.57 ± 0.11
3	4.73 ± 0.18	2.76 ± 0.17	63.19 ± 1.56	15.00 ± 0.50	5.43 ± 0.29	1.71 ± 0.11
4	4.89 ± 0.19	2.82 ± 0.20	64.40 ± 1.53	14.07 ± 0.50	4.81 ± 0.10	1.74 ± 0.09

[a] All values are averages of duplicate analysis

3.2 Influence of selected food ingredients on color formation and thermo-oxidative oil degradation in regular and high oleic, low linolenic canola oils

3.2.1 Color

A significant ($p < 0.05$) increase in pigment formation was observed for all conditions and food ingredients tested (**Figure 8**). Frying whey protein provided the fastest color change for both types of oils with a rate ten times faster that of the rate for heated oils without frying foods, the latter having the lowest rate of color change. By the end of the first frying day when proteins were fried in both oils, the darkening occurred at least two times faster compared to solely heating the oils. After the second day of whey protein frying, a faster rate of pigment formation was observed when compared to all other conditions and this trend was kept until the end of frying. When soybean protein was fried, lower changes in color of oil were observed when compared to oils where whey proteins were fried. Frying starch caused a lower rate of pigment formation than during frying proteins in both oils and significant differences were found after the first frying day for both oils (**Figure 8**). Color development in starch fried oils was not significantly different when compared to heated oils at any point during frying for both oils. When comparing the two oils, the differences between them in color development were only significant when proteins were fried. The rate of pigments formation doubled when whey protein was fried in HLCO compared to RCO. In the case of soy protein, RCO had the larger rate of color changes when compared to HLCO.

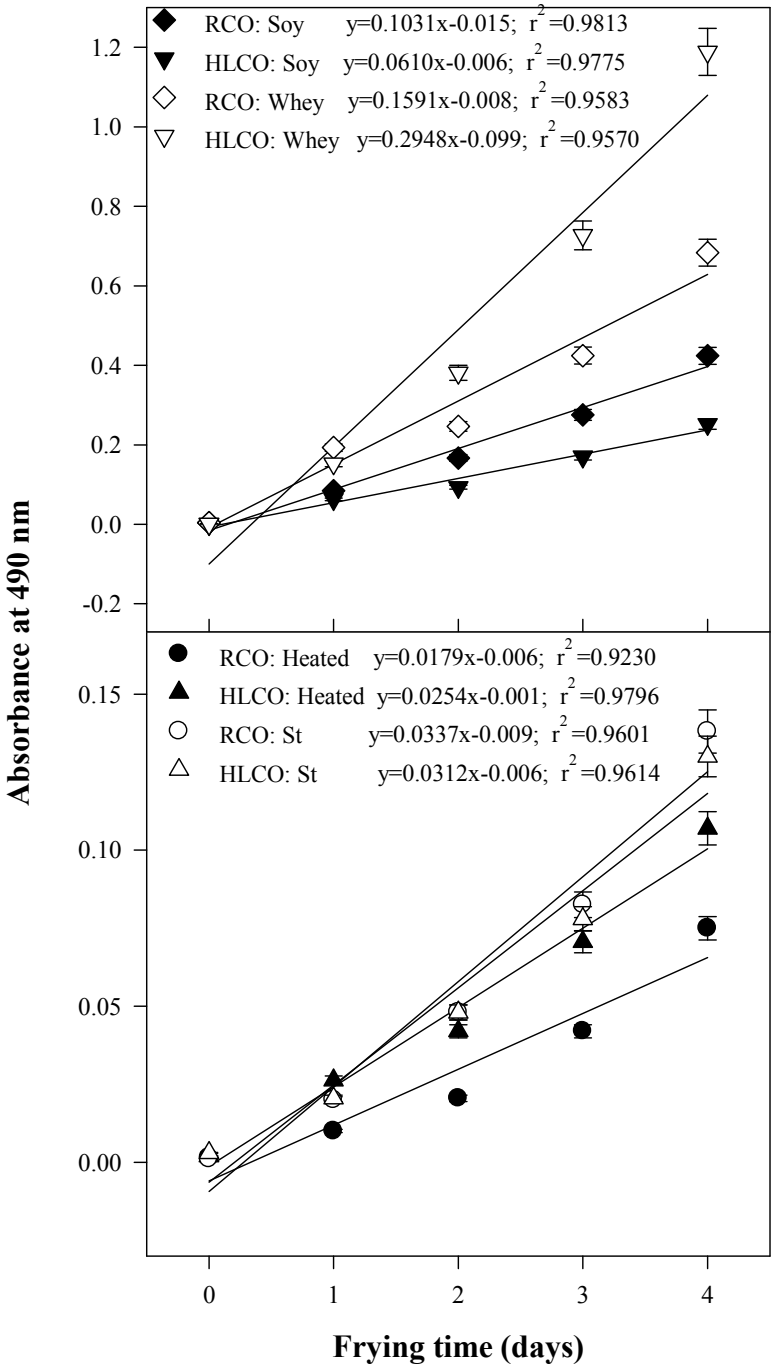


Figure 8: Effect of carbohydrates and proteins on color formation during frying in high oleic low linolenic (HLCO) and regular (RCO) canola oil. Heated oils are controls for all assessments. Lines represent regressions. St - Starch

3.2.2 Total polar components (TPC)

For all of the frying protocols, TPC increased significantly ($p < 0.05$) as frying time progressed (**Figure 9**). The TPC were formed at a lower rate in RCO when compared to HLCO in all conditions. After the first frying day, significant differences were found between RCO and the HLCO for all frying experiments, which continued up to the end of the frying period. After the second day of frying, RCO produced significantly less TPC when compared to all other oils and products fried. Soy and whey proteins fried in HLCO stimulated a faster increase in TPC formation when compared to heated HLCO and RCO and starch fried in RCO at the end of frying.

3.2.3 Residual tocopherols

The average tocopherol profile of fresh RCO and HLCO was 216 and 248 μg/g of α-tocopherol and 355 and 340 μg/g of γ-tocopherol, respectively. Tocopherol degradation occurred at a faster rate in HLCO when compared to regular canola oil for all conditions tested (Figure 10). The heating of RCO caused the slowest reduction in tocopherols with 60 % of the total amount remaining but during heating of HLCO tocopherols were completely depleted after the first frying day (Figure 10). Frying all food ingredients stimulated tocopherol degradation, indicating a pro-oxidative effect of these compounds.

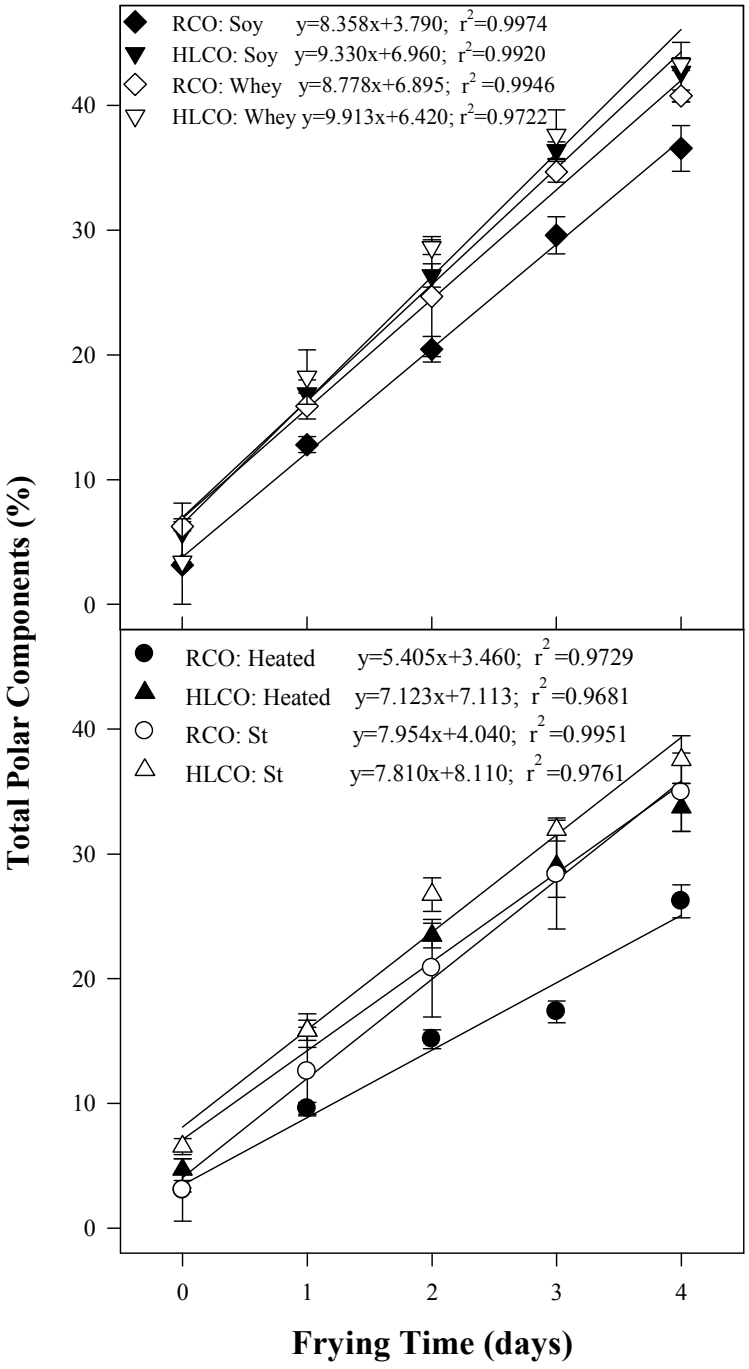


Figure 9: Changes in polar components during frying with carbohydrates and proteins in high oleic, low linolenic (HLCO) and regular (RCO) canola frying oils. Heated oils are a reference for both graphs. Lines represent regressions. St -Starch

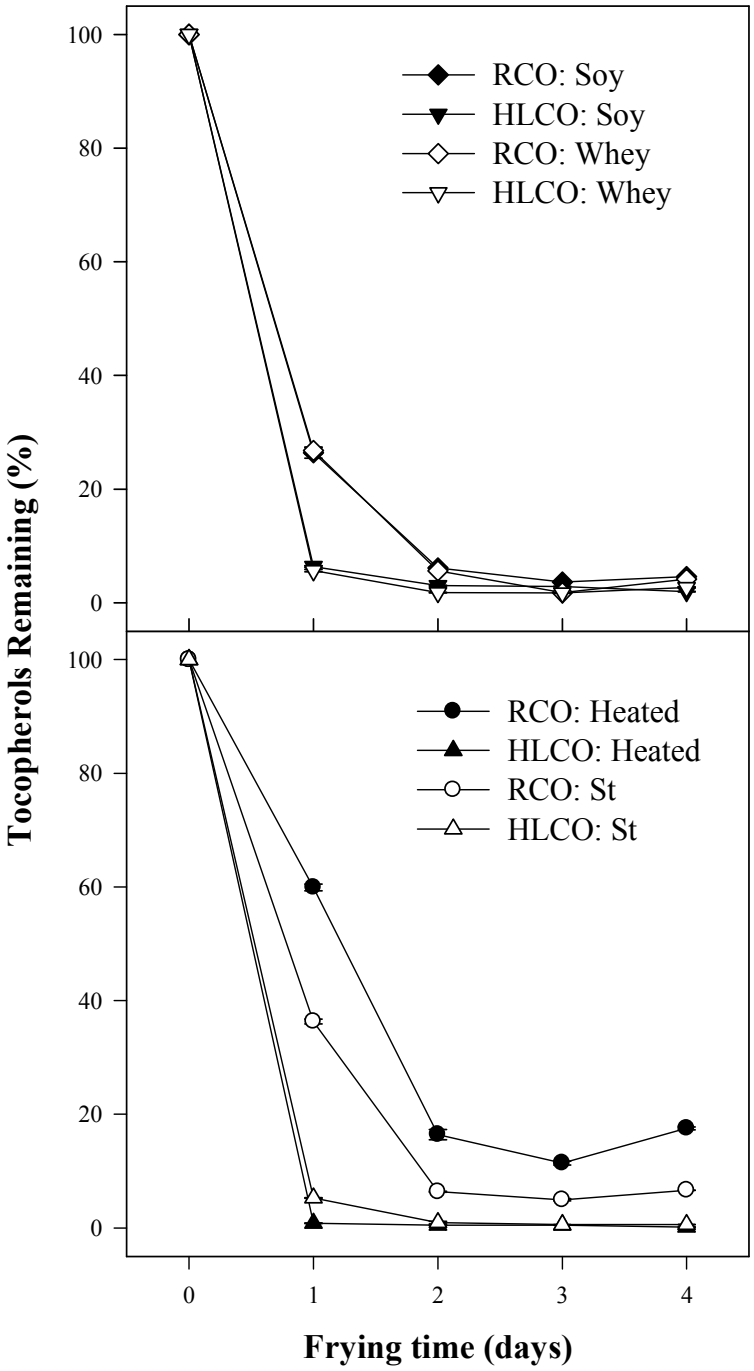


Figure 10: Total tocopherols remaining over frying time with carbohydrates and proteins fried in high oleic, low linolenic (HLCO) and regular (RCO) canola oils. Heated oils are a reference for both graphs. St - Starch

3.2.4 Composition of polar components

The contribution of oligomers increased with frying time for all experiments (**Figure 11**). At the end of frying, the highest oligomer content was in RCO when whey protein was fried in it and achieved 46 % of the polar components. Similar levels of these compounds were observed under the same conditions in HLCO. Heating RCO caused a significant increase in the oligomer contribution; however, it had a lower amount of oligomers by 9 % compared to when whey was fried in RCO. All of the proteins fried in both oils produced a significantly higher amount of oligomers when compared to frying starch and heated oils alone. When proteins were fried in HLCO, a faster rate of oligomer formation was noticed compared to RCO until the final fry day. All food ingredients fried in both oils shared a similar pattern of oligomers and color formation with exception of fried whey protein in HLCO where lower than expected amounts of oligomers were formed at the end of frying.

The amount of oxidized triacylglycerides (OTG) decreased significantly after the first day of frying in all conditions with this trend continuing in RCO until the end of frying (**Figure 12**). Whey protein fried HLCO experienced the greatest drop of 23 % in OTG after the first day of frying but stabilized afterwards. Starch fried in RCO, the heated oils, and all fried food ingredients in HLCO exhibited the levelling of OTG amount from the second day until the end of frying. Heated oils and starch fried oils had significantly higher amounts of OTG when compared to protein fried oils for the same period of frying.

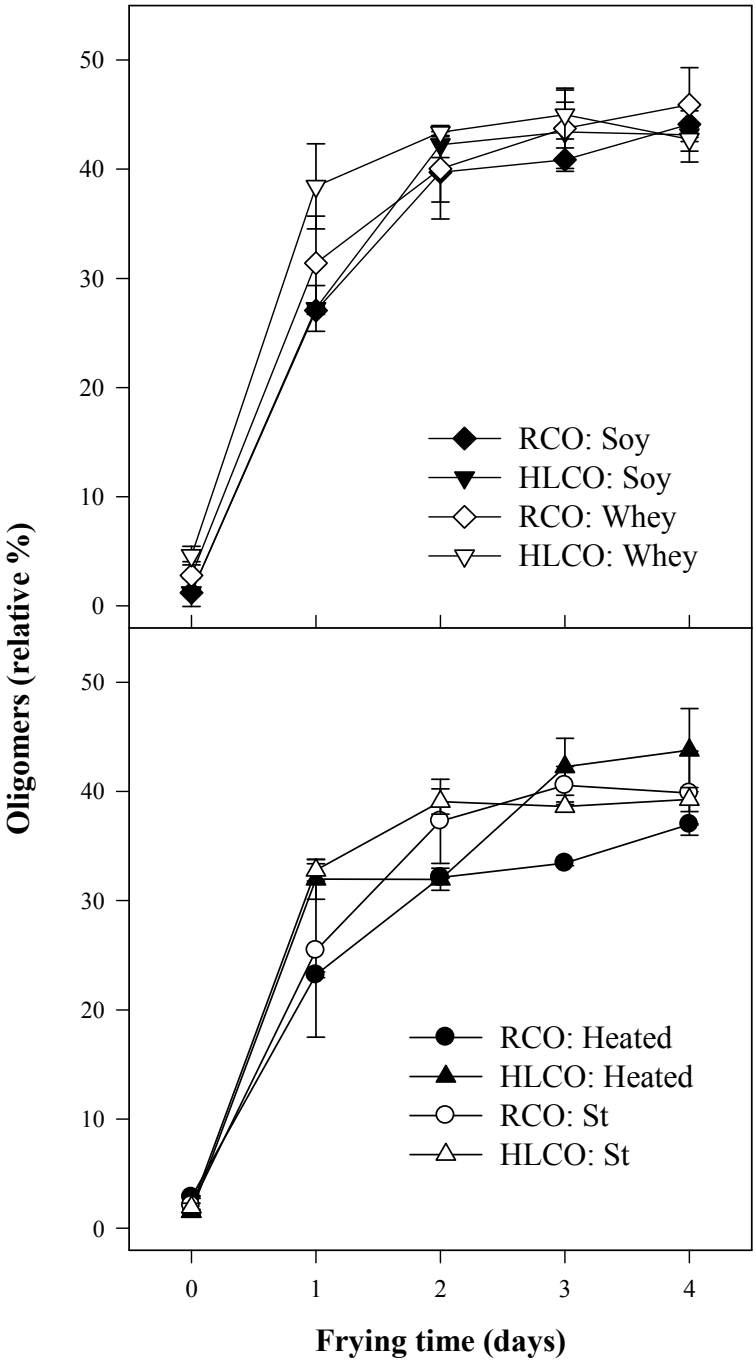


Figure 11: Change in amount of oligomers during carbohydrate and protein frying in high oleic, low linolenic (HLCO) and regular (RCO) canola oils. Heated oils are controls for both graphs. St - Starch

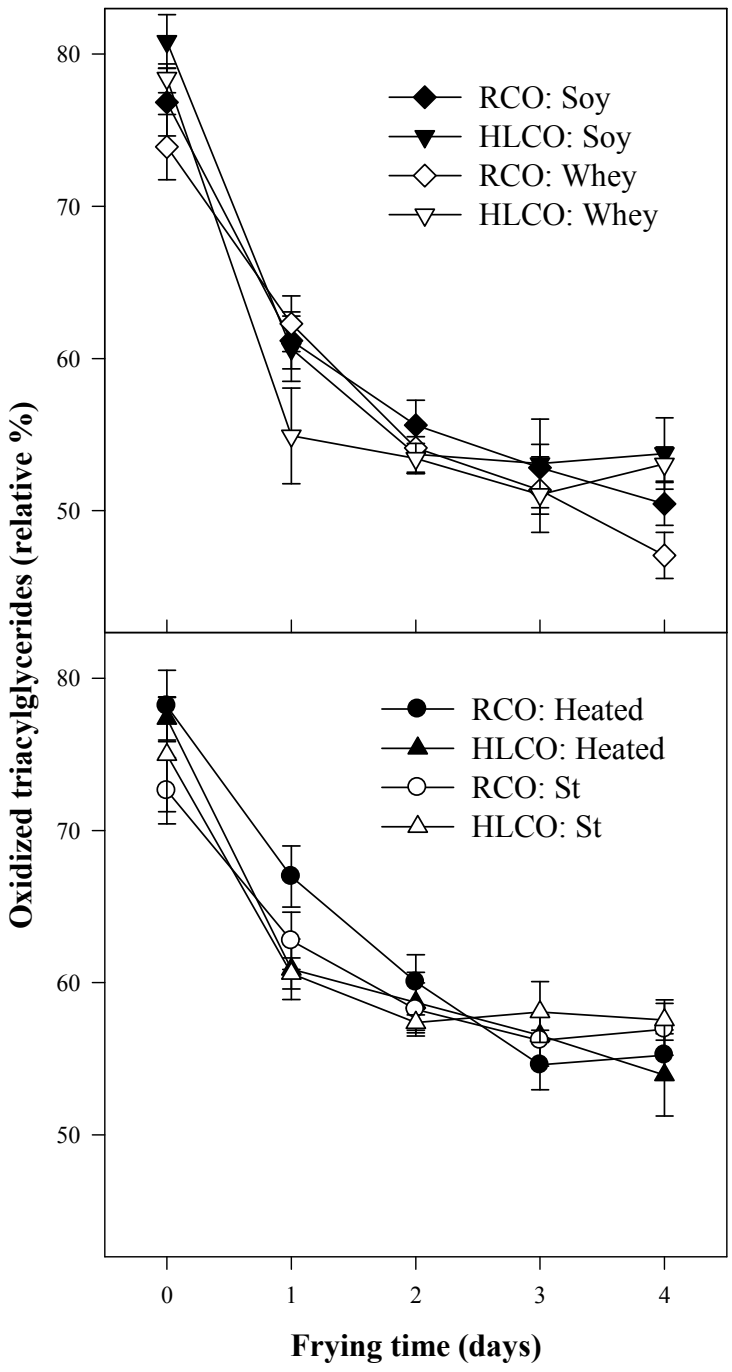


Figure 12: Changes in oxidized triacylglycerides during carbohydrate and protein frying in high oleic, low linolenic (HLCO) and regular (RCO) canola oils. Heated oils are controls for both graphs. St - Starch

3.2.5 Anisidine value

The anisidine values for HLCO were significantly less than RCO values when comparing between the same fried products (**Figure 13**). At the end of frying, the anisidine values of HLCO were comparable to the values at the end of first day of frying in RCO for the same ingredients fried. During heating, RCO achieved lower anisidine values until the end of second day of frying and after that time it surpassed HLCO keeping significantly larger values until the end of frying. For all frying conditions tested, there was no significant increase in the anisidine values after the second day of frying except when starch was fried in HLCO. In heated RCO, significant reduction in anisidine values was found when compared to oils in which food ingredients were fried, particularly at the end of frying. Heated HLCO produced significantly lower anisidine values from oils where products were fried. When comparing the anisidine values to other results describing thermo-oxidative degradation, the oils in which starch was fried produced higher than expected values for this parameter. When comparing values for this indicator for oils used for frying starch and protein, significant differences were observed from frying day three until the end of frying (**Figure 13**).

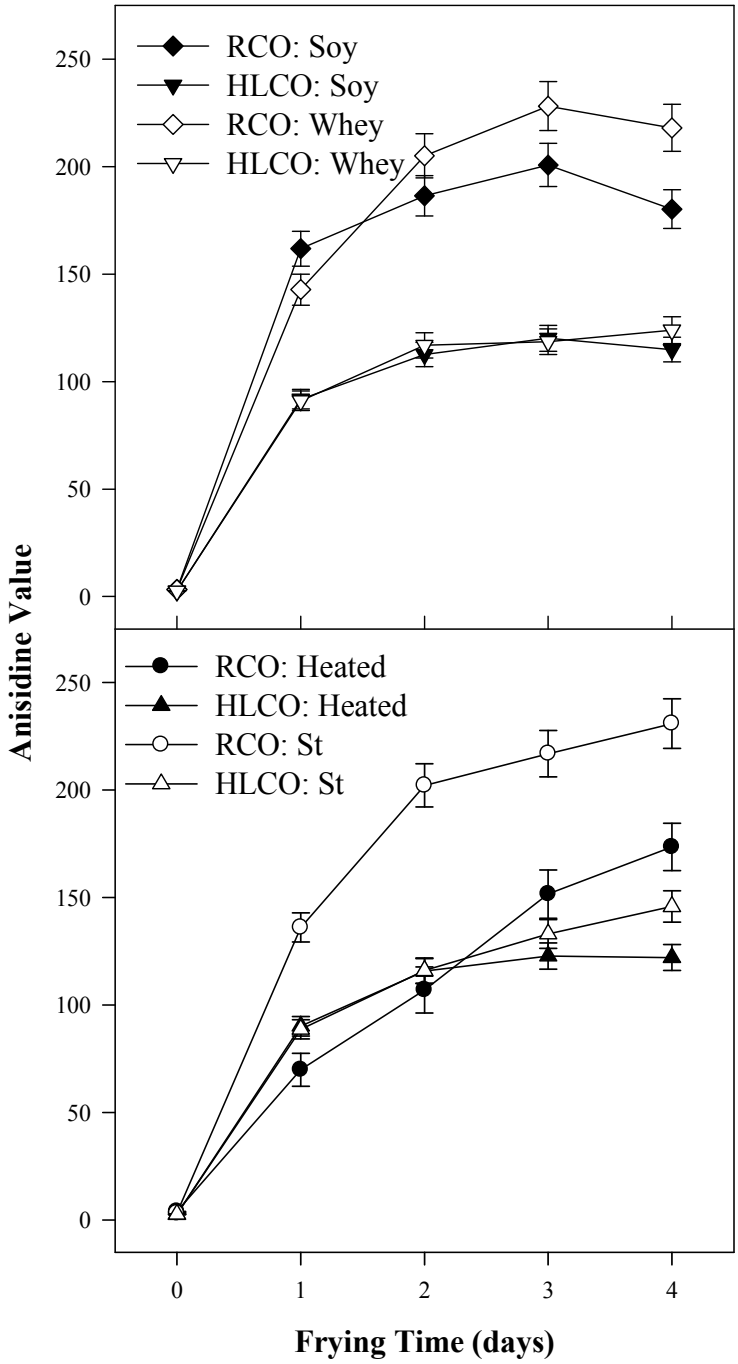


Figure 13: Effect of carbohydrates and proteins on anisidine value during frying in high oleic low linolenic (HLCO) and regular (RCO) canola oils. Heated oils are controls for all experiments. St - Starch

3.2.6 Fatty acid composition

Looking at the fatty acid profiles of the fresh oils, HLCO had a slightly lowered (12 % decrease) palmitic acid (C16:0) when compared to RCO. The contribution of stearic acid (C18:0) was the same in both oils (**Table 4**). The main differences were in the content of oleic acid (C18:1) with HLCO containing on average 12.0 % more than RCO. This was compensated by 5 % lower contribution of linoleic acid (C18:2) and lower by 6.2 % in the amount of linolenic acid (C18:3) in HLCO. During frying, linoleic and linolenic acids were the most affected and in all experiments and in both oils, the amount of these fatty acids significantly decreased over frying time. When comparing the two oils, linolenic acid content decreased at a faster rate in RCO in all conditions tested. When the oils were heated or fried with starch, linoleic acid decreased significantly faster in RCO as compared to HLCO. Linoleic acid decreased faster in HLCO when the two proteins were fried in it. When whey protein was fried in HLCO, the fastest decrease in unsaturated fatty acids content was observed, followed by soy protein, whereas when starch was fried, the slowest degradation rate to these acids was found. In heated RCO, the slowest degradation of unsaturated fatty acid was noticed whereas when starch was fried, the fastest rate of oxidative degradation was observed. The formation of *trans* fatty acids was significant in all experiments when comparing to both fresh oils and within the same frying experiments. The rates of *trans* fatty acid formation during frying whey and soy proteins in HLCO were five and four times faster, respectively, than in a heated oil. However, rates of formation of *trans* fatty acids were three and four times slower during frying starch.

Table 4: The changes to the major fatty acids of regular (RCO) and high oleic, low linolenic (HLCO) canola oils that occurred during frying with different food ingredients. Heated oils are controls to all experiments.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
RCO: Heated						
0	4.12 ± 0.18	2.40 ± 0.19	58.84 ± 1.59	18.19 ± 0.25	8.64 ± 0.08	1.45 ± 0.13
1	4.37 ± 0.22	2.48 ± 0.18	60.87 ± 1.39	17.10 ± 0.13	7.49 ± 0.13	1.41 ± 0.11
2	4.54 ± 0.20	2.79 ± 0.22	61.29 ± 1.57	16.28 ± 0.35	6.63 ± 0.36	1.72 ± 0.13
3	4.68 ± 0.18	2.64 ± 0.16	62.52 ± 1.54	15.54 ± 0.52	5.96 ± 0.32	1.68 ± 0.11
4	4.78 ± 0.19	2.65 ± 0.19	63.42 ± 1.51	14.88 ± 0.52	5.41 ± 0.12	1.68 ± 0.09
RCO: Starch						
0	4.10 ± 0.18	2.31 ± 0.18	59.11 ± 1.59	18.19 ± 0.25	8.65 ± 0.08	1.42 ± 0.13
1	4.33 ± 0.22	2.47 ± 0.18	60.57 ± 1.38	17.14 ± 0.13	7.47 ± 0.13	1.50 ± 0.12
2	4.50 ± 0.20	2.53 ± 0.20	61.79 ± 1.58	16.30 ± 0.35	6.60 ± 0.35	1.51 ± 0.11
3	4.66 ± 0.17	2.53 ± 0.15	62.96 ± 1.55	15.40 ± 0.52	5.84 ± 0.31	1.48 ± 0.09
4	4.83 ± 0.19	2.61 ± 0.19	63.98 ± 1.52	14.46 ± 0.51	5.16 ± 0.11	1.50 ± 0.08
RCO: Soy						
0	3.94 ± 0.17	2.46 ± 0.19	61.28 ± 1.65	17.26 ± 0.24	7.55 ± 0.07	1.22 ± 0.11
1	4.08 ± 0.21	2.55 ± 0.19	62.40 ± 1.43	16.46 ± 0.12	6.51 ± 0.11	1.46 ± 0.11
2	4.29 ± 0.19	2.66 ± 0.21	63.82 ± 1.63	15.33 ± 0.33	5.66 ± 0.30	1.50 ± 0.11
3	4.45 ± 0.17	2.71 ± 0.16	64.75 ± 1.60	14.62 ± 0.49	5.01 ± 0.27	1.56 ± 0.10
4	4.58 ± 0.18	2.77 ± 0.20	65.62 ± 1.56	13.88 ± 0.49	4.47 ± 0.10	1.59 ± 0.08
RCO: Whey						
0	4.09 ± 0.18	2.29 ± 0.18	58.99 ± 1.59	18.21 ± 0.25	8.67 ± 0.08	1.41 ± 0.13
1	4.26 ± 0.22	2.36 ± 0.17	60.42 ± 1.38	17.29 ± 0.13	7.58 ± 0.13	1.47 ± 0.11
2	4.43 ± 0.19	2.51 ± 0.20	61.65 ± 1.58	16.41 ± 0.35	6.65 ± 0.36	1.57 ± 0.11
3	4.63 ± 0.17	2.58 ± 0.16	62.88 ± 1.55	15.47 ± 0.52	5.87 ± 0.31	1.55 ± 0.10
4	4.77 ± 0.19	2.58 ± 0.19	63.85 ± 1.52	14.73 ± 0.52	5.23 ± 0.11	1.55 ± 0.08
HLCO: Heated						
0	3.49 ± 0.15	2.39 ± 0.19	71.96 ± 1.94	13.07 ± 0.18	1.81 ± 0.02	1.05 ± 0.10
1	3.62 ± 0.19	2.50 ± 0.18	72.86 ± 1.67	11.98 ± 0.09	1.52 ± 0.03	1.16 ± 0.09
2	3.74 ± 0.16	2.53 ± 0.20	73.51 ± 1.88	11.15 ± 0.24	1.30 ± 0.07	1.20 ± 0.09
3	3.83 ± 0.14	2.64 ± 0.16	73.86 ± 1.82	10.45 ± 0.35	1.13 ± 0.06	1.31 ± 0.08
4	3.90 ± 0.15	2.64 ± 0.19	74.28 ± 1.77	9.98 ± 0.35	1.02 ± 0.02	1.38 ± 0.07
HLCO: Starch						
0	3.51 ± 0.15	2.46 ± 0.19	71.78 ± 1.93	12.97 ± 0.18	1.80 ± 0.02	1.08 ± 0.10
1	3.64 ± 0.19	2.54 ± 0.19	72.77 ± 1.66	11.91 ± 0.09	1.49 ± 0.03	1.21 ± 0.09
2	3.77 ± 0.16	2.59 ± 0.20	73.40 ± 1.88	11.00 ± 0.24	1.26 ± 0.07	1.29 ± 0.09
3	3.90 ± 0.15	2.69 ± 0.16	73.97 ± 1.83	10.15 ± 0.34	1.05 ± 0.06	1.44 ± 0.09
4	3.99 ± 0.16	2.70 ± 0.19	74.35 ± 1.77	9.53 ± 0.34	0.94 ± 0.02	1.51 ± 0.08
HLCO: Soy						
0	3.51 ± 0.15	2.43 ± 0.19	71.98 ± 1.94	13.00 ± 0.18	1.80 ± 0.02	1.09 ± 0.10
1	3.63 ± 0.19	2.50 ± 0.18	72.78 ± 1.66	11.96 ± 0.09	1.51 ± 0.03	1.18 ± 0.09
2	3.81 ± 0.17	2.58 ± 0.20	73.61 ± 1.88	10.70 ± 0.23	1.19 ± 0.06	1.39 ± 0.10
3	4.00 ± 0.15	2.61 ± 0.16	74.30 ± 1.83	9.62 ± 0.32	0.94 ± 0.05	1.49 ± 0.09
4	4.20 ± 0.16	2.71 ± 0.20	74.63 ± 1.78	8.57 ± 0.30	1.31 ± 0.03	2.29 ± 0.12
HLCO: Whey						
0	3.48 ± 0.15	2.16 ± 0.17	73.05 ± 1.97	13.24 ± 0.19	1.75 ± 0.02	0.43 ± 0.04
1	3.68 ± 0.19	2.31 ± 0.17	73.14 ± 1.67	11.83 ± 0.09	1.46 ± 0.03	0.97 ± 0.07
2	3.85 ± 0.17	2.59 ± 0.20	73.60 ± 1.88	10.52 ± 0.23	1.14 ± 0.06	1.52 ± 0.11
3	4.02 ± 0.15	2.68 ± 0.16	73.84 ± 1.82	9.47 ± 0.32	0.94 ± 0.05	1.91 ± 0.12
4	4.19 ± 0.16	2.71 ± 0.20	74.40 ± 1.77	8.65 ± 0.31	0.76 ± 0.02	2.05 ± 0.11

[a] All values are averages of duplicate analysis

3.3 Influence of breading and battering ingredients on color formation and thermo-oxidative oil degradation

3.3.1 Color

The highest amounts of pigment formation was observed for oil where glycine enriched whey protein was fried in canola oil with addition of 1 % of oxidized oil (OxWG), with a rate ten times higher than in control heated oil (**Figure 14**). There were no significant differences in the rate of color formation between oil supplemented with 1 % oxidized oil and oil heated alone. The effect of the oxidized oil on color development was observed only when protein based food ingredients were fried. For instance, the rate of pigment formation in sample containing whey and glycine (WGly) was almost doubled in the presence of 1 % oxidized oil (OxWG).

When starch was enriched in both glycine and glucose (SGG), the largest color change was found when compared to other starch based ingredients. The rate doubled when starch was fried and tripled for SGG compared to heated oil. When starch was enriched with either glycine (SGly) or glucose (SGlu) it produced greater amounts of pigments when compared to starch alone, however, for these two groups of ingredients these differences were statistically not significant from starch fried alone.

The commercial batter mixture (BM) fried in oil formed pigments at the slowest rate similar to heated oil. Shake and Bake® (S&B) breading produced the largest amount of pigments, darkening oil faster compared to all other commercially produced batters, however, differences were statistically significant only compared to BM. The laboratory made mixture of food ingredients (MH) caused color formation at a slightly lower rate than Corn Flakes® (CF) and S&B® commercial breadings but initially adding oxidized oil to the frying oil stimulated color formation faster when compared to the two previously mentioned breadings.

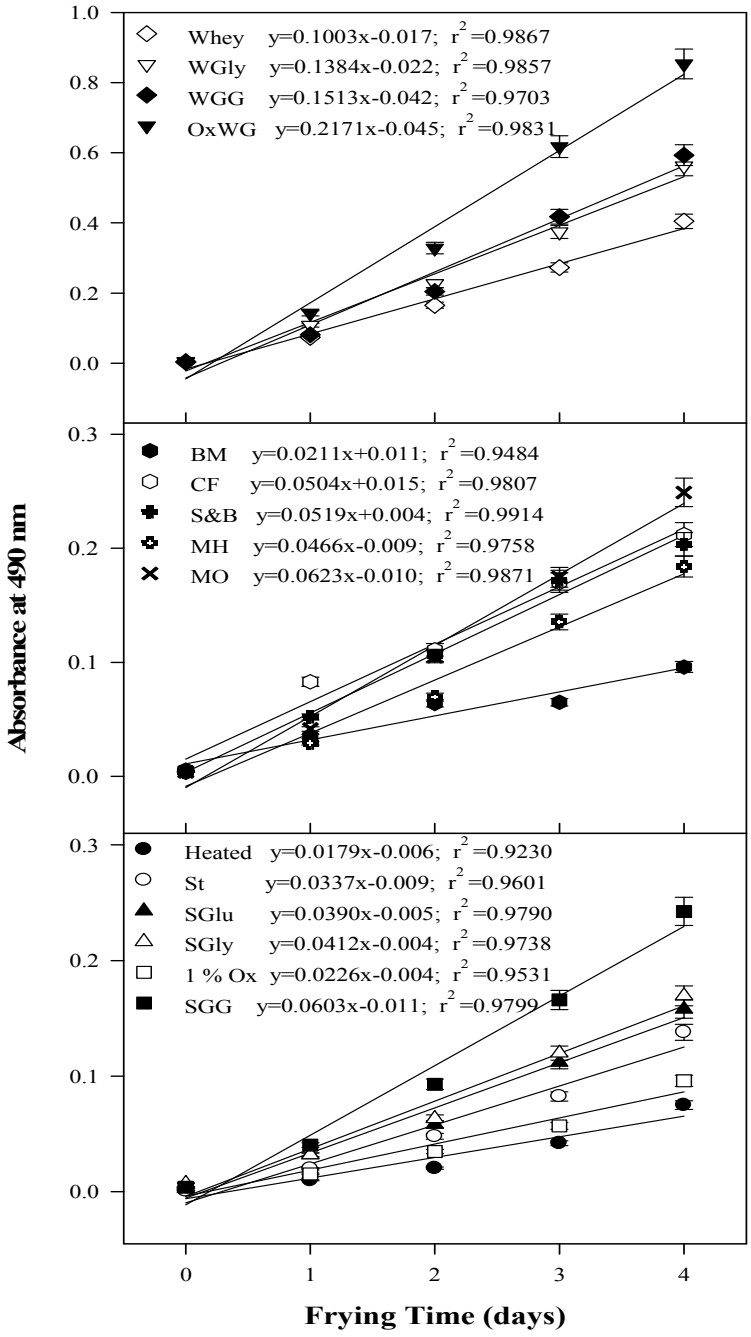


Figure 14: Effect of breadings and batterings on color formation during frying in canola oil. Heated or oxidized oils are references for graphs. For abbreviations see materials and methods. Note the different absorbances scale. Lines represent regressions.

3.3.2 Total polar components (TPC)

In all conditions tested, a significant amount of TPC was produced (**Figure 15**). The largest amount of TPC was formed when glycine enriched whey protein was fried in canola oil with the addition of 1 % oxidized oil (OxWG), consistent with results for color formation above. All of the food ingredients containing whey protein produced significantly higher amounts of TPC at the end of frying but statistically significant differences were found only between fried whey protein ingredients and heated oil with and without added 1 % oxidized oil. Glucose and glycine enriched starch (SGG) consistently produced the largest amount of TPC among starch based food ingredients, with an amount twice higher than in the heated oil control. For starch based products the trend was similar to that observed for color formation, however, during frying glucose enriched starch (SGlu) a lower TPC amount was observed. For the breadings and batters, S&B® and CF® produced the largest amount of TPC with the batter, BM producing the least amount of TPC. There were no statistically significant differences observed among any of the protein based products and commercial breadings or the laboratory made batters.

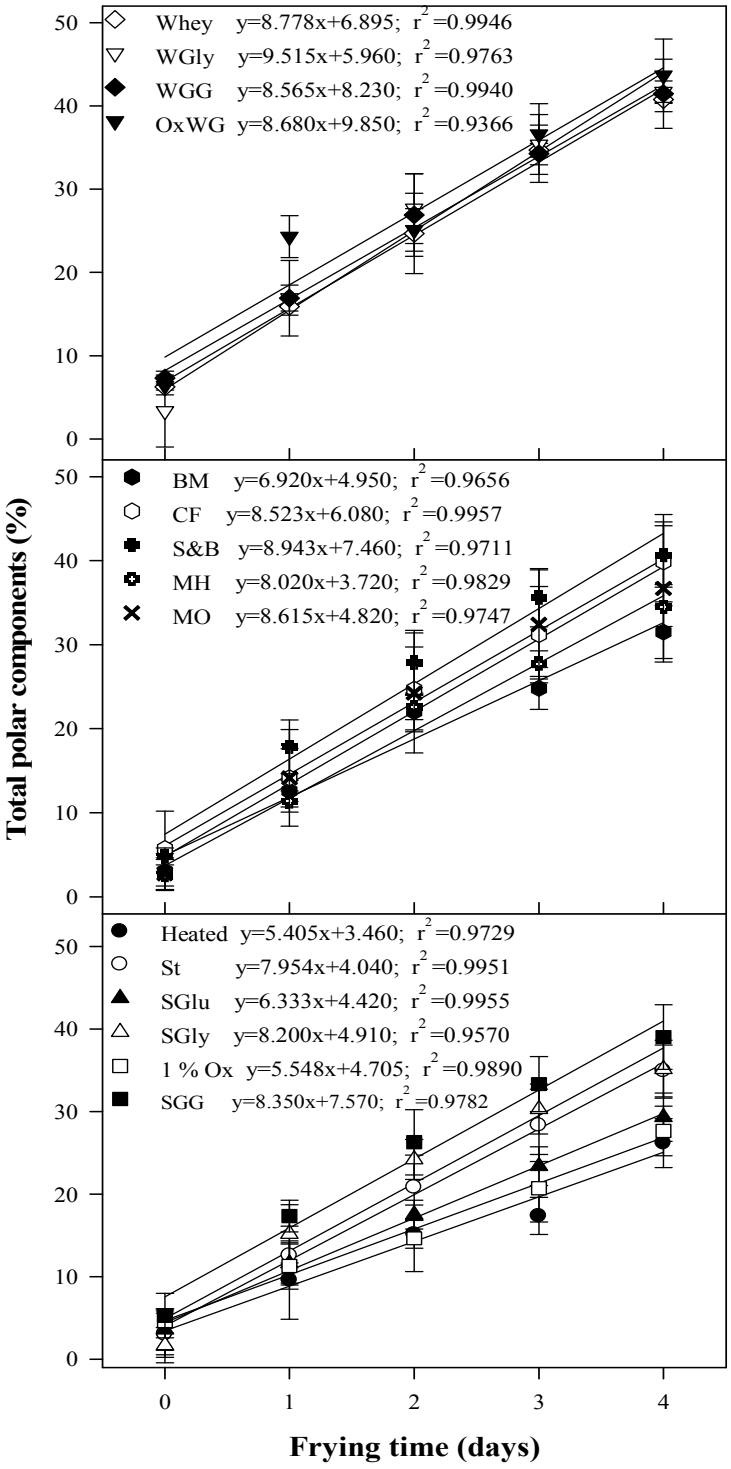


Figure 15: Changes in polar components during frying with batters and breadings. Heated oil and oxidized oil are used as references for all graphs. For abbreviations see the materials and methods. Lines represent regressions.

3.3.3 Residual tocopherols

For fresh canola oil, the average content of tocopherols was described above, however, when 1 % oxidized oil was added the content was lowered slightly to 213 μg/g of α-tocopherol and 354 μg/g of γ-tocopherol.

During frying all products and under all conditions, there was a significant decrease in tocopherol concentration until the end of the 3rd day of frying, when 10 % or less of initial value was detected (**Figure 16**). Heated oil sustained tocopherols at the best with a decrease by only 40 % after the first day of frying and an average residual tocopherol concentration remained at 10 % at the end of the frying period. Oil supplemented with 1 % oxidized oil had a significantly faster rate of tocopherol degradation when compared to the heated oil. The addition of glycine and glucose or both products to starch found a significant decrease in the tocopherol amounts when compared to heated oil and starch alone. Following the trend of both TPC and color, the commercial BM retained the highest tocopherol concentration when compared to the commercial breading products and the laboratory mixture. However, these differences were not statistically significant after the second day of frying mainly due to the small amounts of tocopherols left in the oils. For protein fried oils, whey alone had the slowest tocopherol degradation after the first day of frying with 27 % left. Glucose and glycine added to whey protein individually did not affect the tocopherol concentration after second day of frying but when combined the tocopherol concentration was lowered faster.

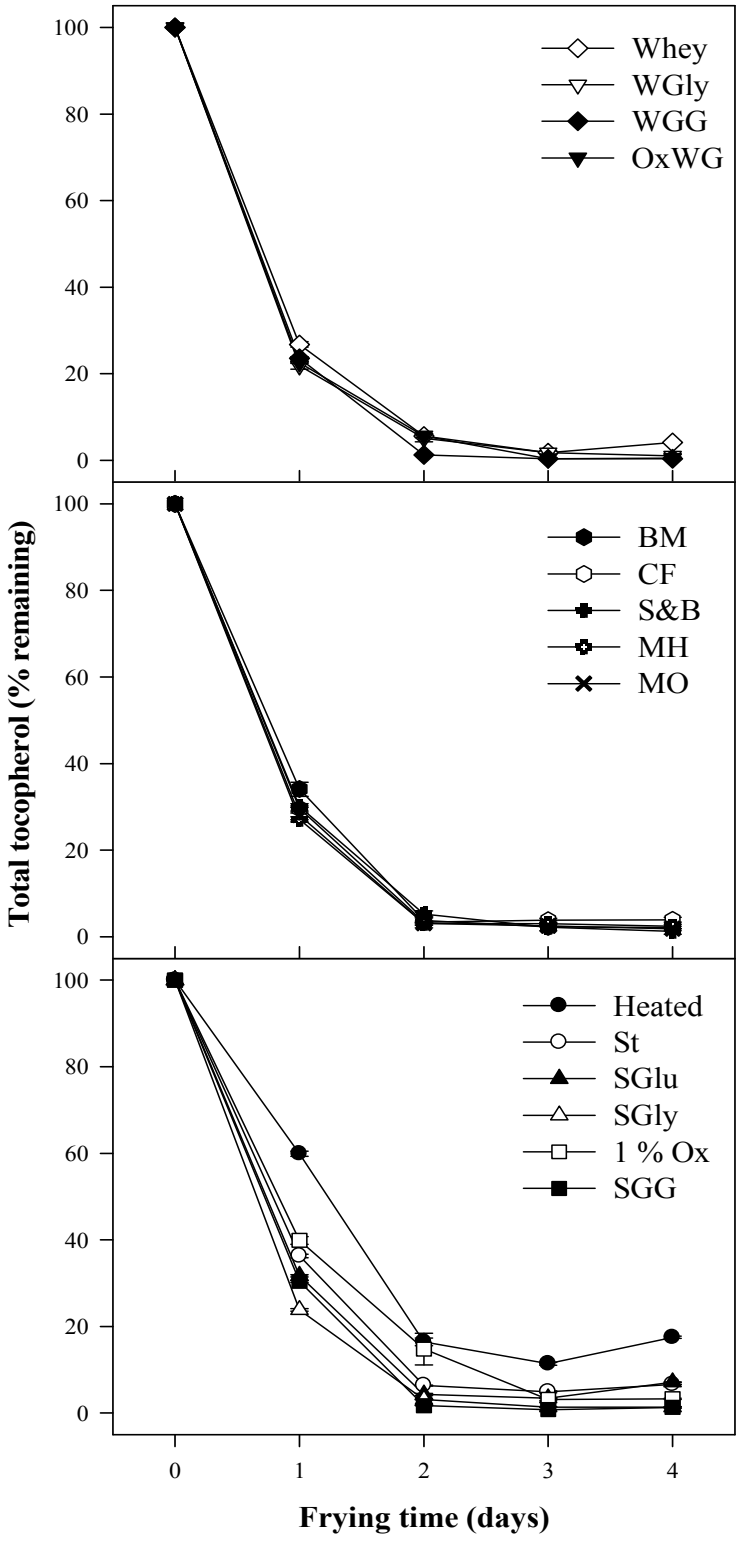


Figure 16: Total tocopherols remaining over frying batters and breadings. Heated oil and oxidized oils are a reference for all graphs. For abbreviations see the materials and methods.

3.3.4 Composition of polar components

In all protocols, oligomer production significantly increased until the second day of frying when the rate plateaued (**Figure 17**). Amongst starch based products, SGly caused the fastest accumulation of oligomers (33 % of polar components) after the first day of frying. At the end of frying, SGG also produced significantly higher amounts of oligomers (43 %) when compared to other starch products and heated oil. Heating the oil alone produced the least amount of oligomers even at the end of frying (37 %). The largest amount of oligomer production occurred when whey protein enriched with glucose and glycine (WGG) was fried which was 14 % higher than in heated oil. All whey protein based products were not significantly different from each other or any of the batters and breadings, but had higher amounts of oligomers when compared to heated oil, added oxidized oil, starch and SGlu. Following the pattern of the results, S&B® and CF® produced the most oligomers and at a faster rate than both the commercial BM and the laboratory mixture with and without oxidized oil added.

All trials had a significantly lower amount of oxidized triacylglycerides (OTG) in the oil until the end of the second day of frying where the OTG levels either slightly decreased or stayed stable (**Figure 18**). In glycine enriched starch, a decrease in the amount of OTG was found and the decreasing rate was greater when compared to heated oil, starch, and SGlu. Glycine and glucose enriched starch also had a large OTG reduction but the difference was not statistically significant from the starch products and the heated oil control. S&B® and CF® breadings formed significantly lower amounts of OTG at the end of the frying when compared to other batters, heated and oxidized added oil and most of the starch based products. Whey protein based products significantly decreased the amount of

OTG throughout the whole frying period similarly to the starch and batter mixtures. Again, all protein products were not significantly different from each other but had produced significantly smaller amounts of OTG when compared to all other conditions, except in S&B® and CF® breadings.

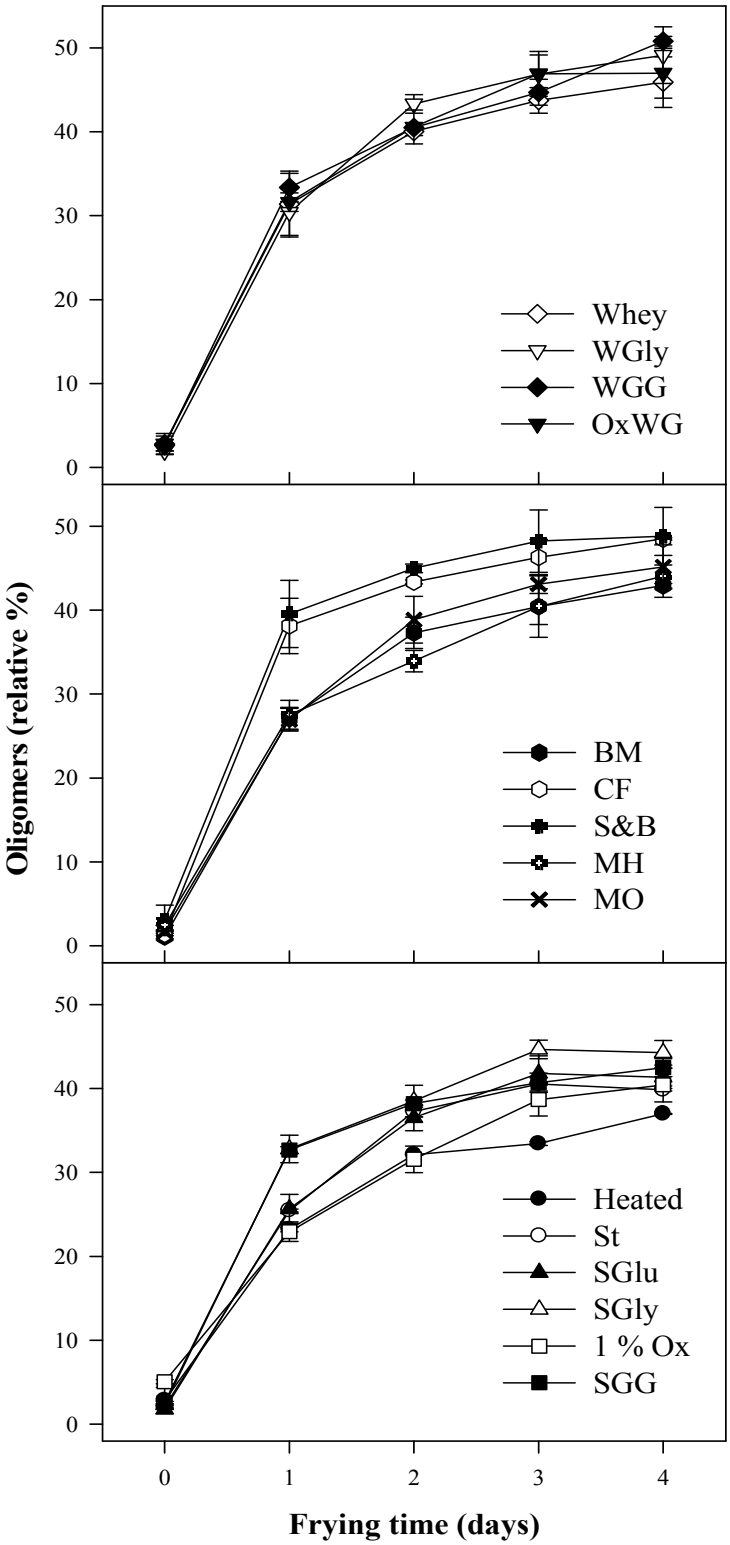


Figure 17: Changes in the amount of oligomers during frying batters and breadings in canola oil. Heated oil is reference for all graphs. For abbreviations see the materials and methods.

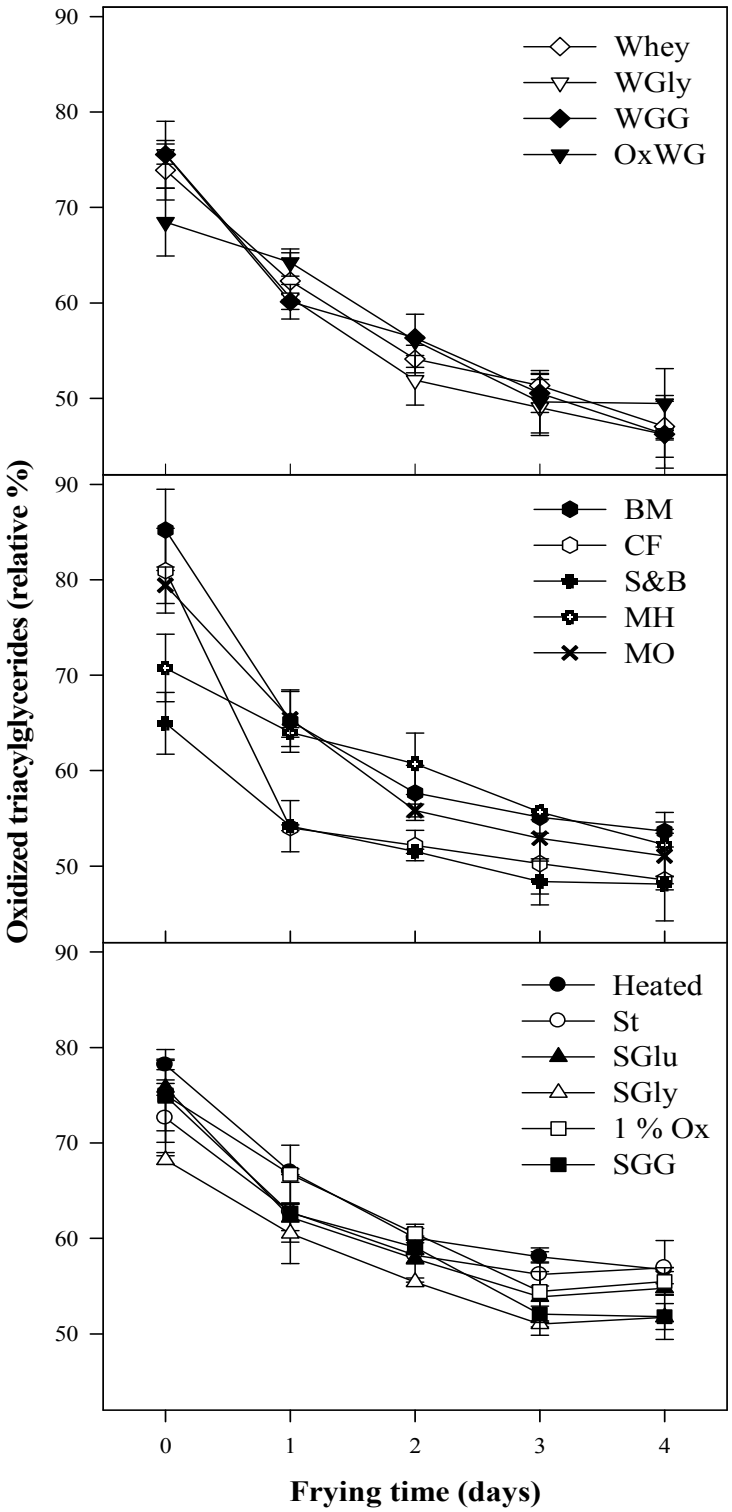


Figure 18: Changes in oxidized triacylglycerides during frying batters and breadings in canola oil. Heated and oxidized oils are references for all graphs. For abbreviations see the materials and methods.

3.3.5 Anisidine value

For all conditions tested, the anisidine values increased significantly until the end of the first day of frying and continued but at the slower rate until the end of frying. Proteins and amino acids fried in oils showed a decrease in anisidine value after the third day of frying, however, these changes were statistically not significant (**Figure 19**). At the end of frying, the anisidine values of heated oil and all of the commercial batters and breadings fried in oils were comparable to the end of the second day of frying for proteins, starches, except when 1 % glucose was added, and the laboratory made batter mixtures. The addition of glucose or glycine or both did not affect anisidine values when added to either starch or whey protein. The addition of oxidized oil to heated oil had slower effect on anidisine value changes when compared to heated oil. However, when the laboratory formulated batter mixture was fried in oil containing oxidized oil (MO), a significant increase in anisidine value was observed, particularly when compared to oil without added oxidized oil (MH).

3.3.6 Fatty acid composition

Table 5 shows the changes to canola oil major fatty acids during frying with starch based breading ingredients, **Table 6** shows for commercial and the laboratory made breadings and batters, and **Table 7** shows for protein based food ingredients. Linoleic and linolenic acid amounts for all conditions tested decreased significantly during frying. The addition of 1 % oxidized oil had the slowest rate of both linoleic and linolenic acid degradation. Starch had the smallest effect on fatty acid isomerization that increased faster when glycine was added to the fried product. The addition of both glucose and glycine together to starch increased isomerization even further. For all of the starch based ingredients, the addition of glycine (with and without glucose) caused the most significant decrease in the amount of both linoleic and linolenic acids.

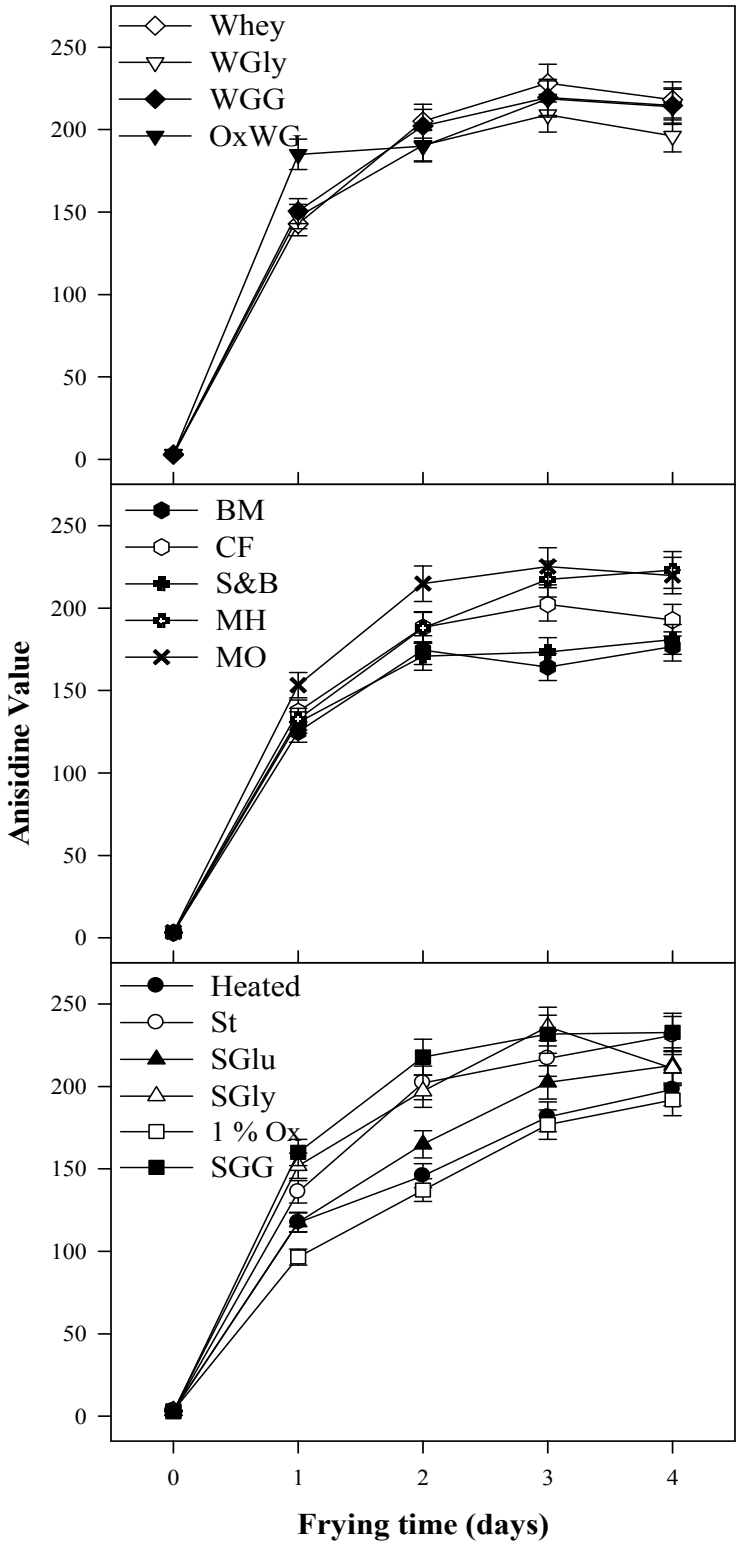


Figure 19: Effect of breadings and batterings on anisidine value during frying canola oil. Heated or oxidized oil is a reference for all graphs above. For abbreviations see the materials and methods.

Table 5: The changes to canola oil major fatty acids that occurred during frying with different carbohydrates. Heated oil or added 1 % oxidized oils are controls for all samples. For abbreviations see the materials and methods.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
Heated						
0	4.12 ± 0.18	2.40 ± 0.19	58.84 ± 1.59	18.19 ± 0.25	8.64 ± 0.08	1.45 ± 0.13
1	4.37 ± 0.22	2.48 ± 0.18	60.87 ± 1.39	17.10 ± 0.13	7.49 ± 0.13	1.41 ± 0.11
2	4.54 ± 0.20	2.79 ± 0.22	61.29 ± 1.57	16.28 ± 0.35	6.63 ± 0.36	1.72 ± 0.13
3	4.68 ± 0.18	2.64 ± 0.16	62.52 ± 1.54	15.54 ± 0.52	5.96 ± 0.32	1.68 ± 0.11
4	4.78 ± 0.19	2.65 ± 0.19	63.42 ± 1.51	14.88 ± 0.52	5.41 ± 0.12	1.68 ± 0.09
Starch						
0	4.10 ± 0.18	2.31 ± 0.18	59.11 ± 1.59	18.19 ± 0.25	8.65 ± 0.08	1.42 ± 0.13
1	4.33 ± 0.22	2.47 ± 0.18	60.57 ± 1.38	17.14 ± 0.13	7.47 ± 0.13	1.50 ± 0.12
2	4.50± 0.20	2.53 ± 0.20	61.79 ± 1.58	16.30 ± 0.35	6.60 ± 0.35	1.51 ± 0.11
3	4.66 ± 0.17	2.53 ± 0.15	62.96 ± 1.55	15.40 ± 0.52	5.84 ± 0.31	1.48 ± 0.09
4	4.83 ± 0.19	2.61 ± 0.19	63.98 ± 1.52	14.46 ± 0.51	5.16 ± 0.11	1.50 ± 0.08
Glucose 1 %						
0	4.03 ± 0.18	2.24 ± 0.18	58.99 ± 1.59	18.35 ± 0.26	8.75 ± 0.08	1.38 ± 0.13
1	4.25 ± 0.22	2.39 ± 0.18	60.41 ± 1.38	17.33 ± 0.13	7.64 ± 0.13	1.44 ± 0.11
2	4.48 ± 0.19	2.50 ± 0.19	61.82 ± 1.58	16.29 ± 0.35	6.68 ± 0.36	1.48 ± 0.11
3	4.60 ± 0.17	2.56 ± 0.15	62.82 ± 1.55	15.56 ± 0.52	5.94 ± 0.32	1.53 ± 0.10
4	4.79 ± 0.19	2.58 ± 0.19	63.91 ± 1.52	14.70 ± 0.52	5.29 ± 0.12	1.55 ± 0.08
SGly						
0	4.05 ± 0.18	2.22 ± 0.18	59.08 ± 1.59	18.25 ± 0.26	8.68 ± 0.08	1.41 ± 0.13
1	4.29 ± 0.22	2.37 ± 0.18	60.64 ± 1.39	17.13 ± 0.13	7.44 ± 0.13	1.52 ± 0.12
2	4.52 ±0.20	2.44 ± 0.19	62.05 ± 1.59	16.12 ± 0.35	6.43 ± 0.34	1.56 ± 0.11
3	4.69 ± 0.18	2.59 ± 0.16	63.58 ± 1.57	14.97 ± 0.50	5.55 ± 0.30	1.66 ± 0.11
4	4.83 ± 0.19	2.58 ± 0.19	64.26 ± 1.53	14.41 ± 0.51	5.07 ± 0.11	1.65 ± 0.09
1 % Ox						
0	4.07 ± 0.18	2.19 ± 0.17	59.26 ± 1.60	18.29 ± 0.26	8.59 ± 0.08	1.38 ± 0.13
1	4.23 ± 0.22	2.31 ± 0.17	60.43 ± 1.38	17.52 ± 0.13	7.69 ± 0.13	1.43 ± 0.11
2	4.36 ± 0.19	2.40 ± 0.19	61.43 ± 1.57	16.72 ± 0.36	6.94 ± 0.37	1.54 ± 0.11
3	4.45 ± 0.17	2.38 ± 0.14	61.91 ± 1.53	16.33 ± 0.55	6.56 ± 0.35	1.58 ± 0.10
4	4.59 ± 0.18	2.54 ± 0.18	62.70 ± 1.49	15.63 ± 0.55	5.99 ± 0.13	1.65 ± 0.09
SGG						
0	4.11 ± 0.18	2.57 ± 0.20	58.94 ± 1.59	18.18 ± 0.25	8.67 ± 0.08	1.29 ± 0.12
1	4.40 ± 0.23	2.73 ± 0.20	61.06 ± 1.40	16.75 ± 0.13	7.16 ± 0.12	1.40 ± 0.11
2	4.54 ± 0.20	2.80 ± 0.22	62.12 ± 1.59	15.97 ± 0.34	6.25 ± 0.33	1.52 ± 0.11
3	4.90 ± 0.18	2.84 ± 0.17	63.13 ± 1.56	14.98 ± 0.50	5.46 ± 0.29	1.59 ± 0.10
4	4.84 ± 0.19	2.83 ± 0.20	63.95 ± 1.52	14.43 ± 0.51	4.90 ± 0.11	1.68 ± 0.09

[a] All values are averages of duplicate analysis

Table 6: The changes to the canola oil major fatty acids during frying with different commercial and laboratory made breadings and batterings. For abbreviations see the materials and methods.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
BM						
0	3.86 ± 0.17	1.72 ± 0.14	61.41 ± 1.65	17.97 ± 0.25	7.90 ± 0.07	0.78 ± 0.07
1	4.00 ± 0.21	1.83 ± 0.14	62.00 ± 1.42	17.54 ± 0.13	7.35 ± 0.13	0.88 ± 0.07
2	4.12 ± 0.18	1.88 ± 0.15	62.95 ± 1.61	16.88 ± 0.36	6.64 ± 0.36	0.97 ± 0.07
3	4.22 ± 0.16	1.90 ± 0.11	63.86 ± 1.58	16.26 ± 0.54	6.00 ± 0.32	1.02 ± 0.06
4	4.40 ± 0.17	2.00 ± 0.14	64.75 ± 1.54	15.44 ± 0.54	5.44 ± 0.12	1.07 ± 0.06
CF						
0	4.12 ± 0.18	2.31 ± 0.18	59.16 ± 1.59	18.06 ± 0.25	8.56 ± 0.08	1.49 ± 0.14
1	4.32 ± 0.22	2.43 ± 0.18	60.89 ± 1.39	16.95 ± 0.13	7.28 ± 0.13	1.56 ± 0.12
2	4.52 ± 0.20	2.54 ± 0.20	62.38 ± 1.60	15.73 ± 0.34	6.22 ± 0.34	1.65 ± 0.12
3	4.77 ± 0.18	2.66 ± 0.16	63.52 ± 1.57	14.75 ± 0.49	5.37 ± 0.49	1.72 ± 0.11
4	4.94 ± 0.19	2.70 ± 0.19	64.46 ± 1.53	14.04 ± 0.50	4.76 ± 0.50	1.77 ± 0.09
S&B						
0	4.06 ± 0.18	2.32 ± 0.18	59.05 ± 1.59	18.17 ± 0.25	8.60 ± 0.08	1.54 ± 0.14
1	4.39 ± 0.23	2.61 ± 0.19	60.50 ± 1.38	16.90 ± 0.13	7.23 ± 0.13	1.72 ± 0.13
2	4.62 ± 0.20	2.77 ± 0.22	62.28 ± 1.59	15.56 ± 0.33	6.03 ± 0.32	1.84 ± 0.13
3	4.95 ± 0.19	3.07 ± 0.18	63.10 ± 1.56	14.42 ± 0.48	5.17 ± 0.28	1.99 ± 0.13
4	5.00 ± 0.20	3.14 ± 0.23	63.34 ± 1.51	14.15 ± 0.50	4.80 ± 0.10	2.13 ± 0.11
MH						
0	4.07 ± 0.18	2.31 ± 0.18	59.06 ± 1.59	18.21 ± 0.25	8.62 ± 0.08	1.46 ± 0.13
1	4.25 ± 0.22	2.41 ± 0.18	60.46 ± 1.38	17.27 ± 0.13	7.58 ± 0.13	1.51 ± 0.12
2	4.47 ± 0.19	2.50 ± 0.19	61.78 ± 1.58	16.28 ± 0.35	6.63 ± 0.36	1.51 ± 0.11
3	4.58 ± 0.17	2.51 ± 0.15	62.80 ± 1.55	15.66 ± 0.52	5.97 ± 0.32	1.57 ± 0.10
4	4.73 ± 0.19	2.50 ± 0.18	63.74 ± 1.52	14.84 ± 0.52	5.34 ± 0.12	1.63 ± 0.09
MO						
0	4.04 ± 0.18	2.18 ± 0.17	59.10 ± 1.59	18.26 ± 0.26	8.66 ± 0.08	1.50 ± 0.14
1	4.36 ± 0.22	2.39 ± 0.18	61.05 ± 1.40	16.83 ± 0.13	7.17 ± 0.12	1.59 ± 0.12
2	4.54 ± 0.20	2.48 ± 0.19	62.43 ± 1.60	15.96 ± 0.34	6.22 ± 0.33	1.67 ± 0.12
3	4.73 ± 0.18	2.52 ± 0.15	63.78 ± 1.57	14.89 ± 0.50	5.39 ± 0.29	1.71 ± 0.11
4	4.84 ± 0.19	2.59 ± 0.19	64.39 ± 1.53	14.32 ± 0.51	4.89 ± 0.11	1.75 ± 0.09

[a] All values are averages of duplicate analysis

Table 7: The changes to canola oil major fatty acids during frying different protein components. For abbreviations see the materials and methods.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
Whey						
0	4.09 ± 0.18	2.29 ± 0.18	58.99 ± 1.59	18.21 ± 0.25	8.67 ± 0.08	1.41 ± 0.13
1	4.26 ± 0.22	2.36 ± 0.17	60.42 ± 1.38	17.29 ± 0.13	7.58 ± 0.13	1.47 ± 0.11
2	4.43 ± 0.19	2.51 ± 0.20	61.65 ± 1.58	16.41 ± 0.35	6.65 ± 0.36	1.57 ± 0.11
3	4.63 ± 0.17	2.58 ± 0.16	62.88 ± 1.55	15.47 ± 0.52	5.87 ± 0.31	1.55 ± 0.10
4	4.77 ± 0.19	2.58 ± 0.19	63.85 ± 1.52	14.73 ± 0.52	5.23 ± 0.11	1.55 ± 0.08
WGlycine						
0	4.12 ± 0.18	2.51 ± 0.20	58.83 ± 1.59	18.07 ± 0.25	8.55 ± 0.08	1.41 ± 0.13
1	4.39 ± 0.23	2.60 ± 0.19	61.11 ± 1.40	16.79 ± 0.13	7.07 ± 0.12	1.47 ± 0.11
2	4.55 ± 0.20	2.69 ± 0.21	62.27 ± 1.59	15.90 ± 0.34	6.17 ± 0.33	1.57 ± 0.11
3	4.73 ± 0.18	2.76 ± 0.17	63.19 ± 1.56	15.00 ± 0.50	5.43 ± 0.29	1.71 ± 0.11
4	4.89 ± 0.19	2.82 ± 0.20	64.40 ± 1.53	14.07 ± 0.50	4.81 ± 0.10	1.74 ± 0.09
OXWG						
0	4.11 ± 0.18	2.66 ± 0.21	59.04 ± 1.59	18.09 ± 0.25	8.55 ± 0.08	1.35 ± 0.12
1	4.62 ± 0.24	2.96 ± 0.22	62.16 ± 1.42	15.62 ± 0.12	5.99 ± 0.10	1.66 ± 0.13
2	4.39 ± 0.19	2.79 ± 0.22	60.90 ± 1.56	16.74 ± 0.36	7.07 ± 0.38	1.50 ± 0.11
3	4.88 ± 0.18	3.04 ± 0.18	63.52 ± 1.57	14.42 ± 0.48	5.02 ± 0.27	1.77 ± 0.11
4	5.04 ± 0.20	2.99 ± 0.22	64.33 ± 1.53	13.83 ± 0.49	4.53 ± 0.10	1.84 ± 0.10
WGG						
0	4.11 ± 0.18	2.49 ± 0.20	59.04 ± 1.59	18.21 ± 0.25	8.66 ± 0.08	1.27 ± 0.12
1	4.39 ± 0.23	2.72 ± 0.20	60.53 ± 1.38	16.96 ± 0.13	7.44 ± 0.13	1.39 ± 0.11
2	4.63 ± 0.20	2.78 ± 0.22	62.37 ± 1.60	15.66 ± 0.34	6.23 ± 0.33	1.48 ± 0.11
3	4.85 ± 0.18	2.83 ± 0.17	63.63 ± 1.57	14.60 ± 0.49	5.31 ± 0.28	1.60 ± 0.10
4	5.08 ± 0.20	2.84 ± 0.20	64.95 ± 1.55	13.37 ± 0.47	4.55 ± 0.10	1.64 ± 0.09

[a] All values are averages of duplicate analysis

Frying the two commercial breadings oxidized linoleic and linolenic acids faster as well as produced *trans* fatty acids faster when compared to frying the commercial batters as well as the laboratory prepared mixture (**Table 6**). However, frying the laboratory prepared mixture in fresh oil supplemented with oxidized oil (MO) resulted in comparable rates of linoleic and linolenic acid deterioration, and *trans* fatty acid formation to the commercial breadings. The oil fried with the commercial batter and the laboratory made mixture had comparable rates of linoleic and linolenic acids deterioration and *trans* fatty acid formation to heated oil. When the S&B® commercial breadings was fried the highest overall *trans* fatty acid formation rate was found which was twice faster than in the case of all other products fried as well as heated oil with and without oxidized oil added.

Whey protein fried in oil lowered linoleic and linolenic acids deterioration faster when compared to all of the starch based products (**Table 7**). Adding glycine or glucose or both to the whey protein or oxidized oil caused faster deterioration of both linoleic and linolenic acids. The *trans* fatty acids were also formed at significantly higher rate in oils where whey protein based products were fried when compared to starch based products. An exception was found when whey proteins were alone (**Table 7**).

3.4 Influence of frying temperature on color formation and thermo-oxidative oil degradation when using basic food ingredients

3.4.1 Color

In all conditions tested there was a significant increase in color formation throughout the entire frying period (**Figure 20**). As oil temperatures were elevated, the amount of pigments formation increased for all conditions with the largest increases occurring when temperatures surpassed 200 °C. At the end of frying for all temperatures, all heated oils and starch fried in oils had significantly lower rate of color formation when compared to proteins fried in oils of the same temperature. Glycine enriched whey protein (WGly) fried at 215 °C caused the fastest darkening of oil with a rate 70 times faster than heating at 185 °C, 12.5 times faster than heating at 215 °C and 1.5 times faster when whey protein was fried at 215 °C. When starch was fried at 185 °C and 200 °C, higher changes in color of oil were observed when compared to heated oil at the same temperature. Soy protein consistently caused lower rate of oil color formation when compared to whey protein and WGly at the same temperatures. The addition of glycine to whey protein caused much faster oil darkening only at 215 °C.

3.4.2 Total Polar Compounds

Total polar compounds (TPC) formation was significant in all conditions tested (Figure 21). The rate of TPC formation was the lowest in 185 °C heated oil at a rate half of all 200 °C and 215 °C protein fried oils. TPC formation was higher when starch was fried at 185 °C and oil was heated alone at 200 °C and 215 °C as compared to heated at 185 °C. The differences in TPC formation between 200 °C and 215 °C were not significant when starch was fried. At the end of frying, starch produced more TPC than heated oils at equivalent temperatures although the differences were not significant between 200 °C and 215 °C. Protein products caused formation of larger amounts of TPC when compared to starch and heated oils at the same temperatures. Comparing proteins, soy protein at both 185 °C and

215 °C produced the lowest amounts of TPC while WGly produced the most at these temperatures. At 200 °C the order of TPC amounts reversed with soy protein producing significantly higher amounts of TPC compared to the whey protein and WGly. At 185 °C, heated oil, starch, and soy protein produced significantly lower amounts of TPC than at the 200 °C and 215 °C counterparts for all testing ingredients. Temperature increase affected TPC production at all conditions with products fried between each set of temperatures but the differences were only statistically significant for most between 185 °C and 215 °C.

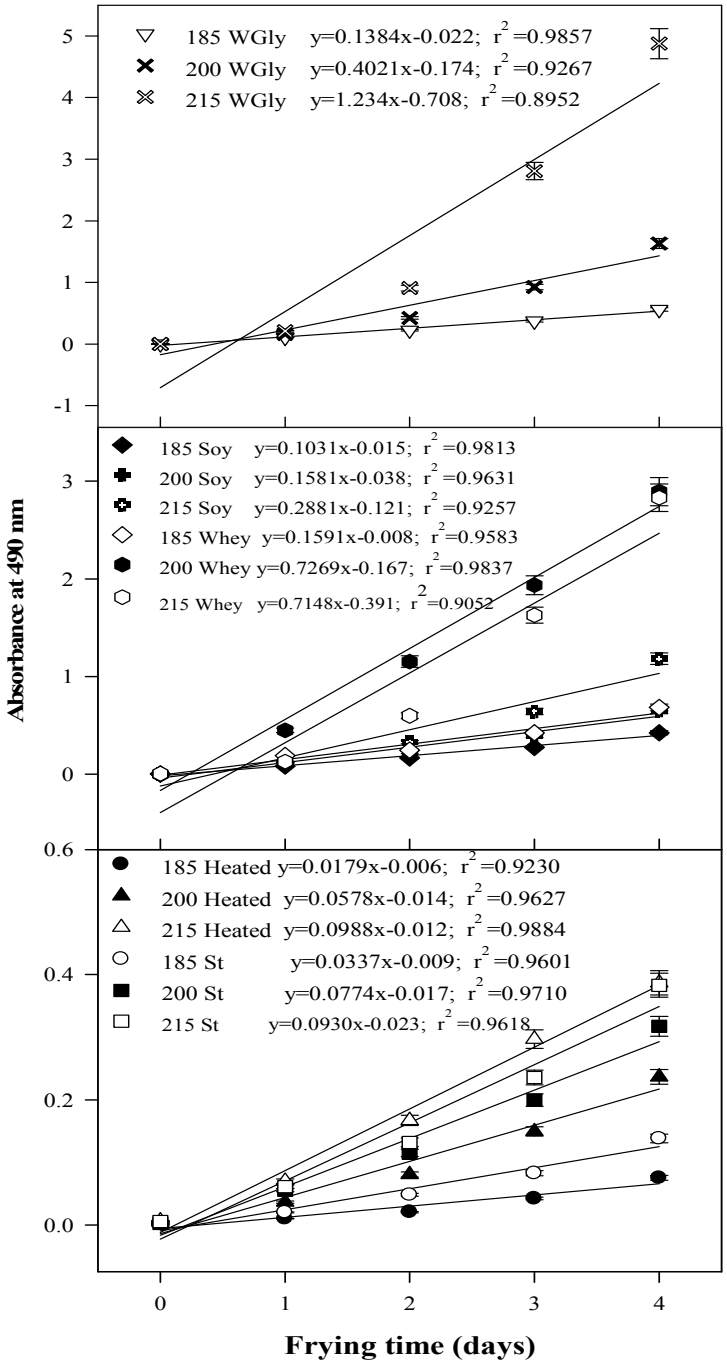


Figure 20: Effect of frying carbohydrates and proteins in canola oil at 185, 200, and 215 °C on oil color changes. Heated oil is a reference for graphs. Note the differences in absorbance scaling. Lines represent regressions. St – starch; WGly - whey + glycine

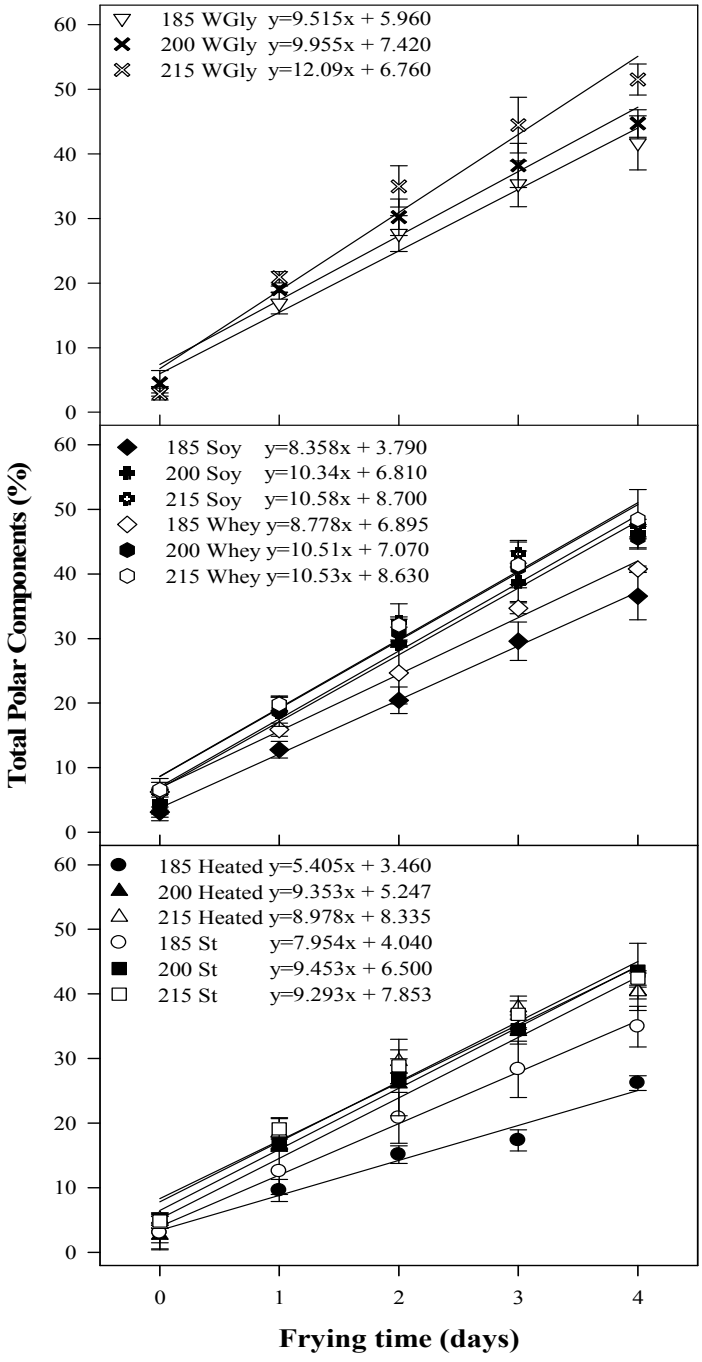


Figure 21: Changes in polar components during frying carbohydrates and proteins in canola oil at 185, 200 and 215 °C. Heated oil is a reference for all graphs. r^2 > 0.9479 for all regression lines. St – starch; WGly - whey + glycine

3.4.3 Residual tocopherols

A significant decrease in tocopherol retention was observed during frying until the third day of frying (**Figure 22**). The most significant losses of tocopherols were seen up to the end of the first day of frying or after primary 8 h of frying for all tested conditions. Heated oil without food at 185 °C retained 59 % tocopherols after the first day of frying and continued this trend of best retention until the end of frying with the final 11 % tocopherols remaining. Significantly higher amounts of tocopherols were retained when frying at 185 °C independently of products fried or oil heated. The amount of tocopherols remaining was at 8.5 % or lower after the second day of frying for all ingredients fried and conditions. Fried starch and heated oils consistently retained more tocopherols than in oils where proteins were fried at the same temperatures. Heated oil retained more tocopherols when compared to starch at all temperatures. At both 185 °C and 200 °C soy protein initially had a lower amount of tocopherols after the first day of frying when compared to the other two proteins, however, the tocopherol levels equalized after the second frying day.

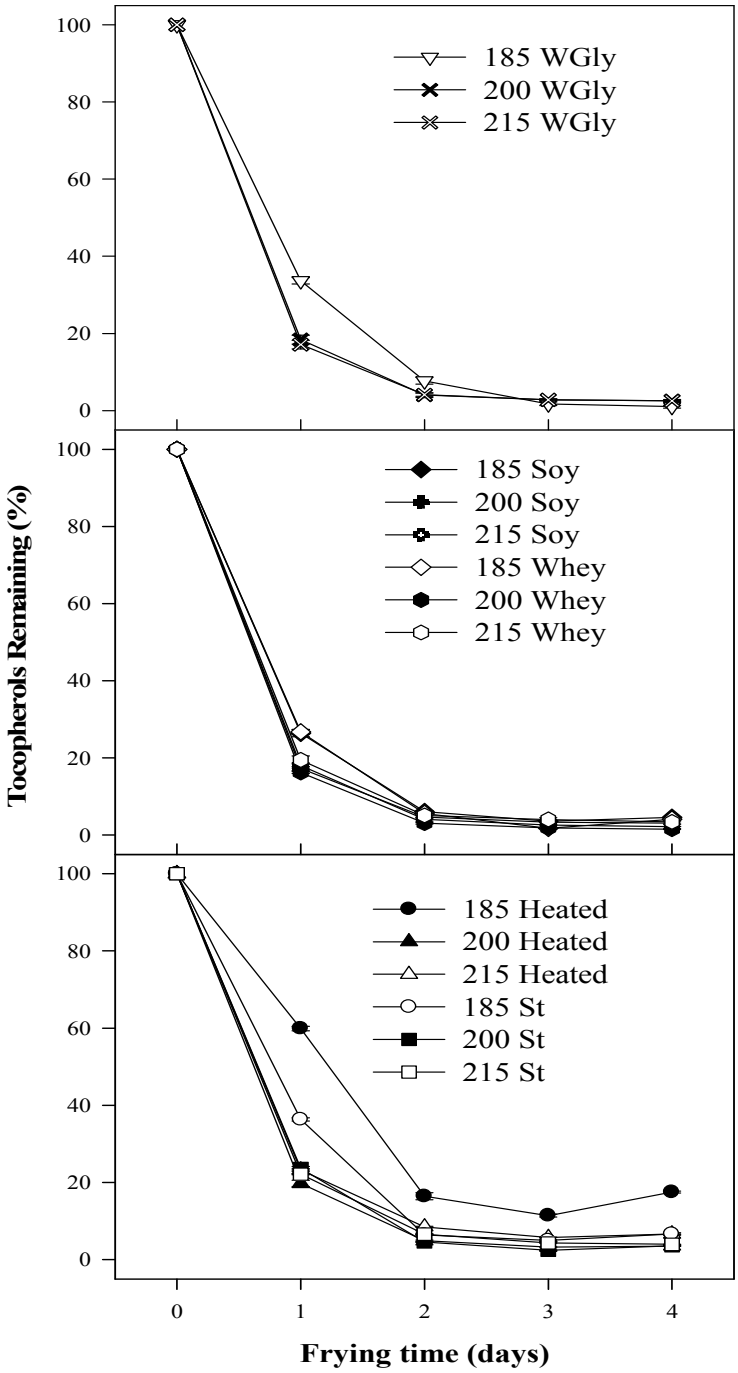


Figure 22: Total tocopherols remaining over frying time in canola oil with carbohydrates and proteins fried at 185, 200 and 215 °C. Heated oil is a reference for all graphs. St – starch; WGly - whey + glycine

3.4.4 Composition of polar components

Significant amounts of oligomers were formed at all temperatures until the end of the second day of frying (**Figure 23**). As temperature increased, so did the amount of oligomers for each of the conditions tested. At 185 °C oligomer formation was largest when WGly was fried. Whereas at 200 °C, heated oil produced the highest amounts of oligomers when compared to all other products fried except when whey protein was fried in oil when the amounts were comparable. Whey protein fried at 215 °C produced the highest amount of oligomers, reaching 57 % at the end of frying. For heated oil and starch fried oil, oligomer production at the end of frying at 185 °C was comparable to oligomer production at the end of the first day of frying at 215 °C. During proteins frying at 185 °C, the amount of formed oligomers at the end of frying was comparable to the amount made at the second day of frying at 215 °C. The 16 % limit of oligomers proposed by Firestone et al. (1991) was doubled or tripled when frying at both higher temperatures. The limit assigned by European countries at 25 % was exceeded at the end of the second frying day (Husain et al., 1991). For all of the other conditions tested, these limits were surpassed after the first day of frying.

The levels of oxidized triacylglycerides (OTG) decreased significantly after the first day of frying and continued for one more frying day (**Figure 24**). Heated oil and starch fried oils at 185 °C had significantly more OTG when compared to all other conditions after the first day of frying. At 215 °C, heated oil and starch fried oils had significantly lower amounts of OTG when compared to its protein counterparts. Increasing the temperature had a significantly negative effect on OTG retention for all conditions.

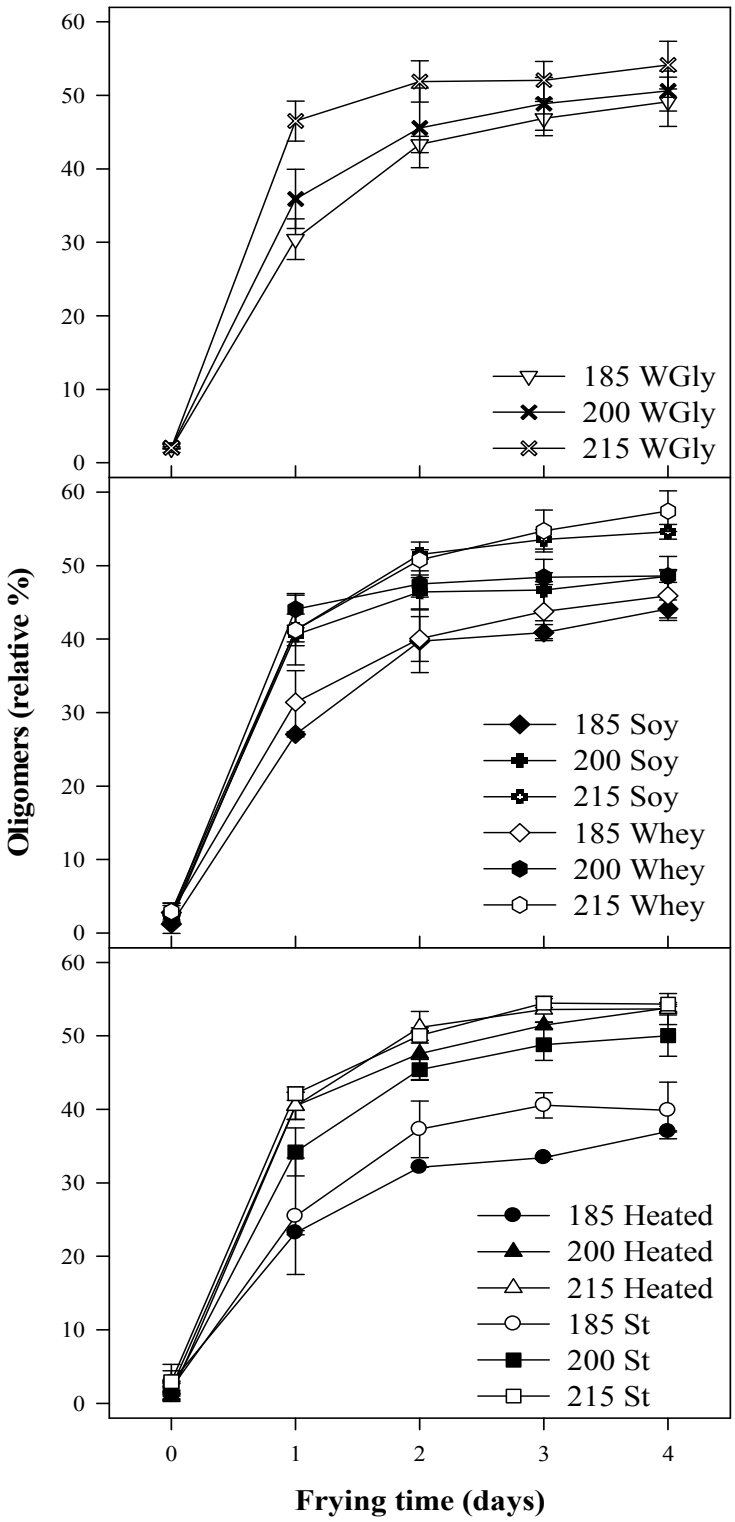


Figure 23: Changes in the amount of oligomers during frying carbohydrates and proteins in canola oil at 185, 200 and 215 °C. Heated oil is a reference for all graphs. St – starch; WGly - whey + glycine.

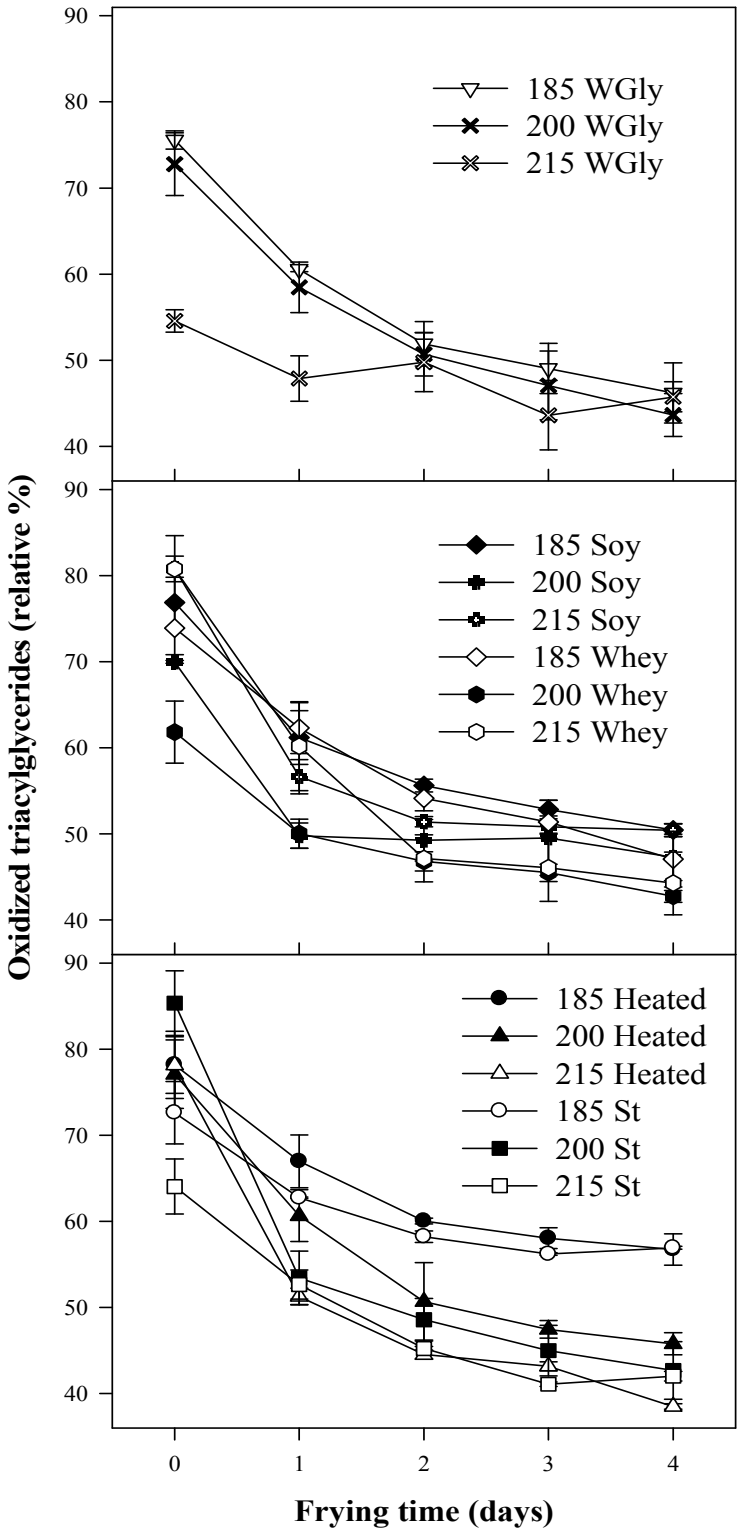


Figure 24: Change in oxidized triacylglycerides during frying carbohydrates and proteins in canola oil at 185, 200 and 215 °C. Heated oil is a reference for all graphs. St – starch; WGly - whey + glycine

3.4.5 Anisidine value

As frying temperatures increased, the anisidine values decreased (**Figure 25**). At 185 °C, there was a significant increase in the anisidine values after the first day of frying for all products fried, however, no significant changes were observed thereafter. A similar pattern was observed for frying at 200 °C, where an increase in anisidine values was observed up until the second day of frying and again, the values stabilized afterwards. At all temperatures, starch fried oils contained more carbonyl components than heated oil at the same temperatures. Soy protein fried oils produced a lower amount of carbonyl components when compared to both whey protein and WGly at the same temperatures. The addition of glycine to whey protein lowered the anisidine values when compared to whey protein alone at the same temperatures.

3.4.6 Fatty acid composition

Tables 8-10 show the changes in canola major fatty acids during frying basic food ingredients at 185, 200, and 215 °C, respectively. The initial contribution of linoleic and linolenic acid for all conditions decreased significantly with increasing frying time and temperature. The rate of *trans* fatty acid formation increased significantly for all conditions when time and temperature increased. The largest drop in linoleic and linolenic acid was observed at 215 °C when whey protein was fried in oil. At 200 °C and 215 °C, protein fried oils had a more significant drop in linoleic and linolenic acids content and a larger increase in *trans* fatty acid content when compared to both starch fried and heated oils. For all of the protein fried oils at 215 °C, linoleic acid content decreased by 30 % while linolenic acid by 60 % and the *trans* fatty acids amount increased by 57, 63, and 62 % when soy protein, whey protein and WGly were fried respectively. At 185 °C in oil where starch was fried, significant decrease in linoleic and linolenic acids amounts were observed when compared to soy protein and WGly fried oils as well to heated oil.

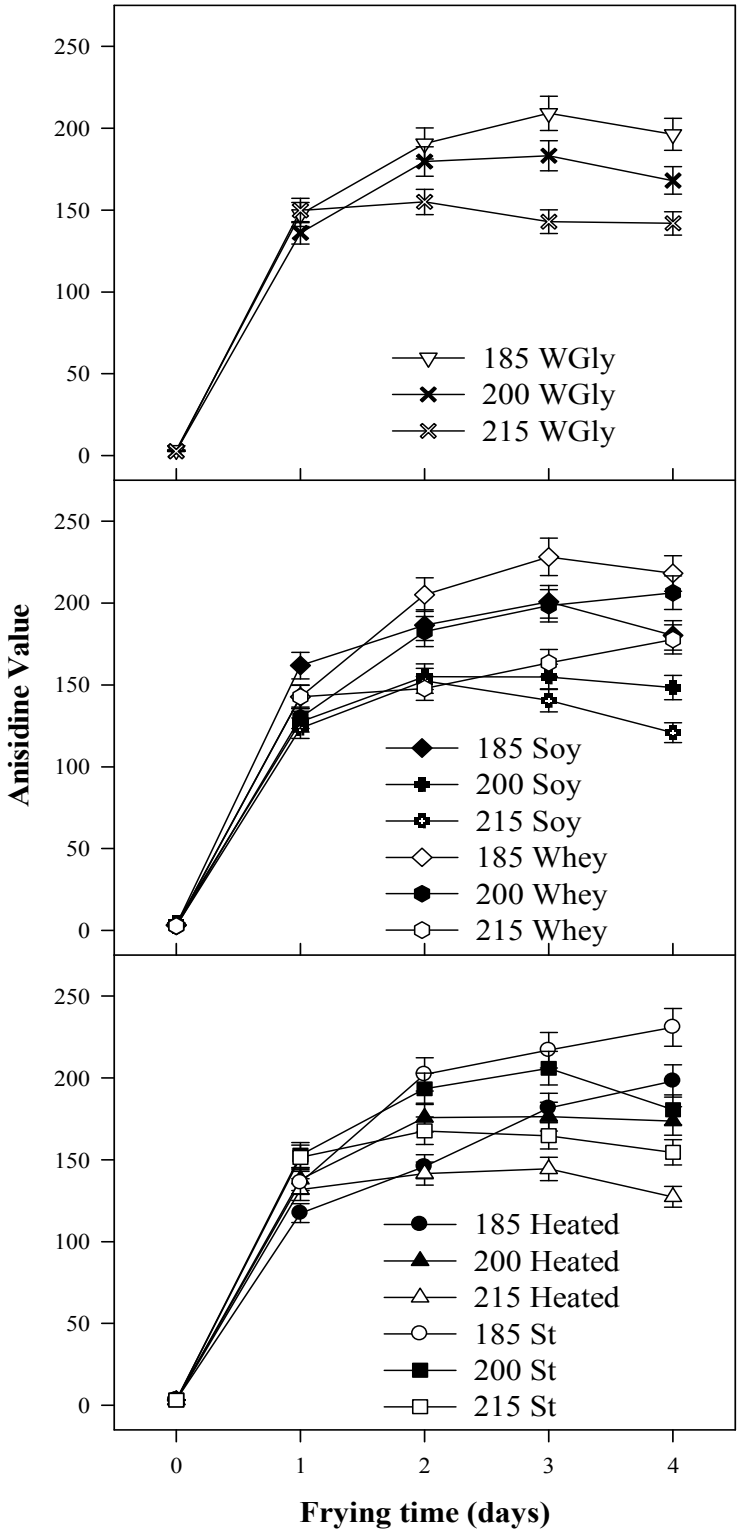


Figure 25: Effect of carbohydrates and proteins on anisidine value during frying in canola oil at 185, 200, and 215 °C. Heated oil is a reference for all graphs. St – starch; WGly - whey + glycine

Table 8: The changes to canola oil major fatty acids during frying at 185 °C with different food ingredients. No food products were fried in heated oil. St – starch; WGly - whey + glycine

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
185 Heated						
0	4.12 ± 0.18	2.40 ± 0.19	58.84 ± 1.59	18.19 ± 0.25	8.64 ± 0.08	1.45 ± 0.13
1	4.37 ± 0.22	2.48 ± 0.18	60.87 ± 1.39	17.10 ± 0.13	7.49 ± 0.13	1.41 ± 0.11
2	4.54 ± 0.20	2.79 ± 0.22	61.29 ± 1.57	16.28 ± 0.35	6.63 ± 0.36	1.72 ± 0.13
3	4.68 ± 0.18	2.64 ± 0.16	62.52 ± 1.54	15.54 ± 0.52	5.96 ± 0.32	1.68 ± 0.11
4	4.78 ± 0.19	2.65 ± 0.19	63.42 ± 1.51	14.88 ± 0.52	5.41 ± 0.12	1.68 ± 0.09
185 St						
0	4.10 ± 0.18	2.31 ± 0.18	59.11 ± 1.59	18.19 ± 0.25	8.65 ± 0.08	1.42 ± 0.13
1	4.33 ± 0.22	2.47 ± 0.18	60.57 ± 1.38	17.14 ± 0.13	7.47 ± 0.13	1.50 ± 0.12
2	4.50± 0.20	2.53 ± 0.20	61.79 ± 1.58	16.30 ± 0.35	6.60 ± 0.35	1.51 ± 0.11
3	4.66 ± 0.17	2.53 ± 0.15	62.96 ± 1.55	15.40 ± 0.52	5.84 ± 0.31	1.48 ± 0.09
4	4.83 ± 0.19	2.61 ± 0.19	63.98 ± 1.52	14.46 ± 0.51	5.16 ± 0.11	1.50 ± 0.08
185 Soy						
0	3.94 ± 0.17	2.46 ± 0.19	61.28 ± 1.65	17.26 ± 0.24	7.55 ± 0.07	1.22 ± 0.11
1	4.08 ± 0.21	2.55 ± 0.19	62.40 ± 1.43	16.46 ± 0.12	6.51 ± 0.11	1.46 ± 0.11
2	4.29 ± 0.19	2.66 ± 0.21	63.82 ± 1.63	15.33 ± 0.33	5.66 ± 0.30	1.50 ± 0.11
3	4.45 ± 0.17	2.71 ± 0.16	64.75 ± 1.60	14.62 ± 0.49	5.01 ± 0.27	1.56 ± 0.10
4	4.58 ± 0.18	2.77 ± 0.20	65.62 ± 1.56	13.88 ± 0.49	4.47 ± 0.10	1.59 ± 0.08
185 Whey						
0	4.09 ± 0.18	2.29 ± 0.18	58.99 ± 1.59	18.21 ± 0.25	8.67 ± 0.08	1.41 ± 0.13
1	4.26 ± 0.22	2.36 ± 0.17	60.42 ± 1.38	17.29 ± 0.13	7.58 ± 0.13	1.47 ± 0.11
2	4.43 ± 0.19	2.51 ± 0.20	61.65 ± 1.58	16.41 ± 0.35	6.65 ± 0.36	1.57 ± 0.11
3	4.63 ± 0.17	2.58 ± 0.16	62.88 ± 1.55	15.47 ± 0.52	5.87 ± 0.31	1.55 ± 0.10
4	4.77 ± 0.19	2.58 ± 0.19	63.85 ± 1.52	14.73 ± 0.52	5.23 ± 0.11	1.55 ± 0.08
185 WGly						
0	4.12 ± 0.18	2.51 ± 0.20	58.83 ± 1.59	18.07 ± 0.25	8.55 ± 0.08	1.41 ± 0.13
1	4.39 ± 0.23	2.60 ± 0.19	61.11 ± 1.40	16.79 ± 0.13	7.07 ± 0.12	1.47 ± 0.11
2	4.55 ± 0.20	2.69 ± 0.21	62.27 ± 1.59	15.90 ± 0.34	6.17 ± 0.33	1.57 ± 0.11
3	4.73 ± 0.18	2.76 ± 0.17	63.19 ± 1.56	15.00 ± 0.50	5.43 ± 0.29	1.71 ± 0.11
4	4.89 ± 0.19	2.82 ± 0.20	64.40 ± 1.53	14.07 ± 0.50	4.81 ± 0.10	1.74 ± 0.09

[a] All values are averages of duplicate analysis

Table 9: The changes to canola oil fatty acids during frying at 200 °C with different food ingredients. No food products were fried in heated oil. St – starch; WGly - whey + glycine

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
200 Heated						
0	4.06 ± 0.18	2.44 ± 0.19	58.93 ± 1.59	18.28 ± 0.26	8.69 ± 0.08	1.44 ± 0.13
1	4.30 ± 0.22	2.52 ± 0.19	60.63 ± 1.39	17.07 ± 0.13	7.31 ± 0.13	1.62 ± 0.12
2	4.54 ± 0.20	2.39 ± 0.19	62.17 ± 1.59	16.22 ± 0.35	6.37 ± 0.34	1.52 ± 0.11
3	4.64 ± 0.17	2.99 ± 0.18	62.81 ± 1.55	15.05 ± 0.50	5.27 ± 0.28	2.19 ± 0.14
4	4.85 ± 0.19	3.02 ± 0.22	63.43 ± 1.51	14.44 ± 0.51	4.73 ± 0.10	2.28 ± 0.12
200 St						
0	4.14 ± 0.18	2.66 ± 0.21	58.99 ± 1.59	17.98 ± 0.25	8.48 ± 0.08	1.49 ± 0.14
1	4.32 ± 0.22	2.70 ± 0.20	60.55 ± 1.38	17.03 ± 0.13	7.24 ± 0.13	1.68 ± 0.13
2	4.60 ± 0.20	2.82 ± 0.22	62.02 ± 1.59	15.87 ± 0.34	6.19 ± 0.33	1.78 ± 0.13
3	4.65 ± 0.17	2.73 ± 0.16	62.96 ± 1.55	15.31 ± 0.51	5.55 ± 0.30	1.86 ± 0.12
4	4.78 ± 0.19	2.73 ± 0.20	63.93 ± 1.52	14.60 ± 0.51	4.92 ± 0.11	1.97 ± 0.10
200 Soy						
0	4.16 ± 0.18	2.47 ± 0.20	59.22 ± 1.60	18.01 ± 0.25	8.55 ± 0.08	1.34 ± 0.12
1	4.31 ± 0.22	2.50 ± 0.18	60.65 ± 1.39	17.10 ± 0.13	7.33 ± 0.13	1.55 ± 0.12
2	4.57 ± 0.20	2.68 ± 0.21	62.37 ± 1.60	15.62 ± 0.33	6.01 ± 0.32	1.78 ± 0.13
3	4.88 ± 0.18	2.94 ± 0.18	63.14 ± 1.56	14.71 ± 0.49	5.21 ± 0.28	1.99 ± 0.13
4	5.05 ± 0.20	2.86 ± 0.21	64.48 ± 1.54	13.71 ± 0.48	4.49 ± 0.10	2.05 ± 0.11
200 Whey						
0	4.13 ± 0.18	2.42 ± 0.19	59.19 ± 1.59	18.12 ± 0.25	8.62 ± 0.08	1.34 ± 0.12
1	4.45 ± 0.23	2.64 ± 0.19	61.03 ± 1.40	16.60 ± 0.13	7.13 ± 0.12	1.54 ± 0.12
2	4.53 ± 0.20	2.53 ± 0.20	62.08 ± 1.59	16.10 ± 0.35	6.25 ± 0.33	1.71 ± 0.13
3	4.73 ± 0.18	2.64 ± 0.16	63.33 ± 1.56	15.06 ± 0.50	5.32 ± 0.28	1.88 ± 0.12
4	4.94 ± 0.19	2.76 ± 0.20	64.15 ± 1.53	14.02 ± 0.49	4.58 ± 0.10	2.05 ± 0.11
200 WGly						
0	4.07 ± 0.18	2.34 ± 0.18	59.20 ± 1.60	18.27 ± 0.26	8.64 ± 0.08	1.32 ± 0.12
1	4.39 ± 0.23	2.54 ± 0.19	61.21 ± 1.40	16.79 ± 0.13	6.88 ± 0.12	1.77 ± 0.14
2	4.64 ± 0.20	2.56 ± 0.20	62.54 ± 1.60	15.62 ± 0.33	5.71 ± 0.31	2.04 ± 0.15
3	4.80 ± 0.18	2.64 ± 0.16	63.26 ± 1.56	14.63 ± 0.49	4.99 ± 0.27	2.19 ± 0.14
4	5.00 ± 0.20	2.83 ± 0.20	64.30 ± 1.53	13.58 ± 0.48	4.25 ± 0.09	2.37 ± 0.12

[a] All values are averages of duplicate analysis

Table 10: The changes to canola oil fatty acids during frying at 215 °C with different food ingredients. No food products were fried in heated oil. St – starch; WGly - whey + glycine

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
215 Heated						
0	4.08 ± 0.18	2.30 ± 0.18	59.14 ± 1.59	18.21 ± 0.25	8.62 ± 0.08	1.34 ± 0.12
1	4.47 ± 0.23	2.52 ± 0.19	61.66 ± 1.41	16.40 ± 0.12	6.61 ± 0.11	1.71 ± 0.13
2	4.66 ± 0.20	2.59 ± 0.20	62.92 ± 1.61	15.44 ± 0.33	5.41 ± 0.29	2.12 ± 0.16
3	4.88 ± 0.18	2.69 ± 0.16	64.02 ± 1.58	14.28 ± 0.48	4.46 ± 0.24	2.45 ± 0.16
4	4.96 ± 0.19	2.69 ± 0.19	64.90 ± 1.55	13.67 ± 0.48	3.77 ± 0.08	2.72 ± 0.14
215 St						
0	4.07 ± 0.18	2.23 ± 0.18	59.33 ± 1.60	18.26 ± 0.26	8.65 ± 0.08	1.26 ± 0.12
1	4.41 ± 0.23	2.43 ± 0.18	61.41 ± 1.40	16.72 ± 0.13	6.92 ± 0.12	1.62 ± 0.12
2	4.56 ± 0.20	2.48 ± 0.19	62.53 ± 1.60	15.76 ± 0.34	5.79 ± 0.31	1.94 ± 0.14
3	4.78 ± 0.18	2.55 ± 0.15	63.85 ± 1.58	14.73 ± 0.49	4.69 ± 0.25	2.20 ± 0.14
4	5.02 ± 0.20	2.73 ± 0.20	64.96 ± 1.55	13.67 ± 0.48	3.97 ± 0.09	2.37 ± 0.12
215 Soy						
0	4.12 ± 0.18	2.28 ± 0.18	59.23 ± 1.60	18.23 ± 0.25	8.65 ± 0.08	1.22 ± 0.11
1	4.40 ± 0.23	2.47 ± 0.18	61.37 ± 1.40	16.61 ± 0.13	6.85 ± 0.12	1.63 ± 0.13
2	4.69 ± 0.20	2.66 ± 0.21	62.74 ± 1.61	15.08 ± 0.32	5.25 ± 0.28	2.17 ± 0.16
3	4.91 ± 0.18	2.76 ± 0.17	63.57 ± 1.57	13.87 ± 0.46	4.08 ± 0.22	2.58 ± 0.16
4	5.15 ± 0.20	2.77 ± 0.20	64.93 ± 1.55	12.96 ± 0.46	3.44 ± 0.07	2.87 ± 0.15
215 Whey						
0	4.08 ± 0.18	2.26 ± 0.18	59.19 ± 1.59	18.29 ± 0.26	8.68 ± 0.08	1.24 ± 0.11
1	4.37 ± 0.22	2.44 ± 0.18	61.03 ± 1.40	16.91 ± 0.13	6.94 ± 0.12	1.74 ± 0.13
2	4.74 ± 0.21	2.56 ± 0.20	62.99 ± 1.61	15.22 ± 0.33	5.19 ± 0.28	2.34 ± 0.17
3	5.01 ± 0.19	2.75 ± 0.17	64.29 ± 1.59	13.65 ± 0.46	3.93 ± 0.21	2.96 ± 0.19
4	5.19 ± 0.20	2.79 ± 0.20	64.89 ± 1.54	12.60 ± 0.44	3.33 ± 0.07	3.24 ± 0.17
215 WGly						
0	4.09 ± 0.18	2.27 ± 0.18	59.16 ± 1.59	18.26 ± 0.26	8.67 ± 0.08	1.22 ± 0.11
1	4.37 ± 0.22	2.40 ± 0.18	61.32 ± 1.40	16.85 ± 0.13	6.88 ± 0.12	1.71 ± 0.13
2	4.73 ± 0.21	2.58 ± 0.20	62.92 ± 1.61	15.11 ± 0.32	5.23 ± 0.28	2.37 ± 0.17
3	5.03 ± 0.19	2.69 ± 0.16	64.06 ± 1.58	13.62 ± 0.46	4.01 ± 0.21	3.07 ± 0.19
4	5.17 ± 0.20	2.71 ± 0.20	64.68 ± 1.54	12.82 ± 0.45	3.57 ± 0.08	3.30 ± 0.17

[a] All values are averages of duplicate analysis

3.5 Influence of solid food particles (SFP), polymers build up, and lipid hydroperoxides on color formation and thermo-oxidative oil degradation

3.5.1 Color

Frying with soy and whey proteins caused faster oil darkening when compared to all other minor components fried in the same oil. For both amounts of soy protein solid food particles (SFP) added to the frying oil, color development happened at the fastest rate (**Figure 26 and 27**). By the end of frying with 2.5 % SFP, soy protein caused a larger oil color change compared to whey protein but this difference was not significant at 12.5 % SFP. Adding 12.5 % of SFP into oil during frying caused faster darkening of the oil, indicating faster rate of the pigments formation when compared to 2.5 %. When compared to laboratory made mixture (MH), pigment formation occurred at slower rate in commercial batters and breadings, starch based ingredients and heated oil. The laboratory made mixture of SFP added at 2.5 % to oil during frying as well as commercial batters and breadings and starch based ingredients in both amounts added did not significantly affect color formation in the frying oil when compared to the heated oil.

Adding oxidized oil or oil with reduced peroxides in amounts up to 5 % of the frying oil did not significantly affect the oil color development in heated fresh oil or during frying for 4 consecutive days when compared to fresh unsupplemented canola oil (**Figure 28**). When 5 % of oxidized oil was added to frying oil a significant change in oil color was observed particularly when compared to heated fresh oil (**Figure 28**). The addition of phospholipids to the frying oil caused a significant color change during frying when compared to all oxidized oil additions, heating the oil, as well as frying with starch (**Figure 2**) and glycine and lysine enriched starch (**Figure 14**) but colored the oil slower than protein fried oils (**Figure 2**).

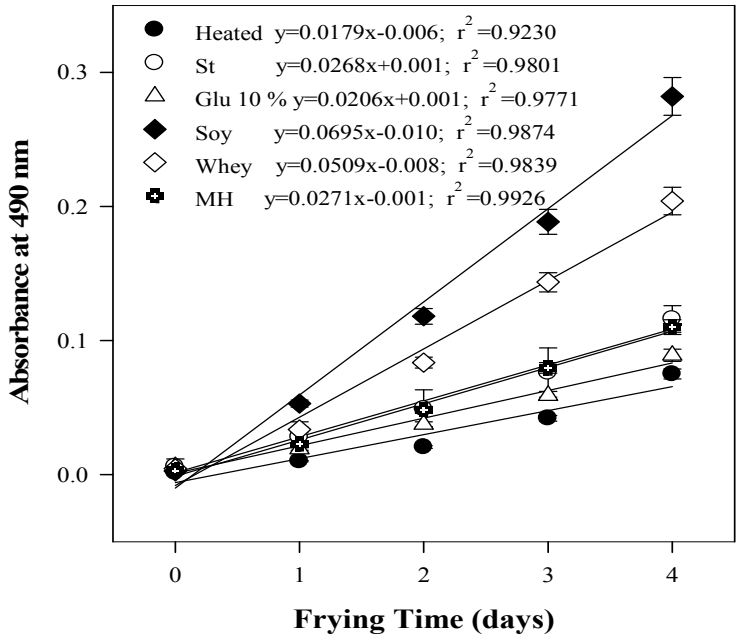


Figure 26: Effect of solid food particles (2.5 %) on color formation of frying oil. Heated oil is a reference to all experiments. For abbreviations see the materials and methods. Lines represent regressions.

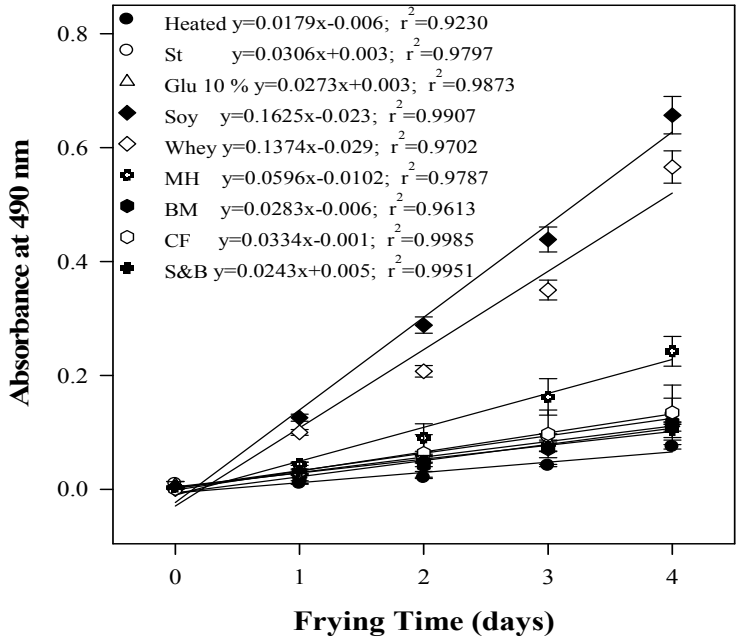


Figure 27: Effect of solid food particles (12.5 %) on color formation of frying oil. Heated oil is a reference to all experiments. For abbreviations see the materials and methods. Lines represent regressions.

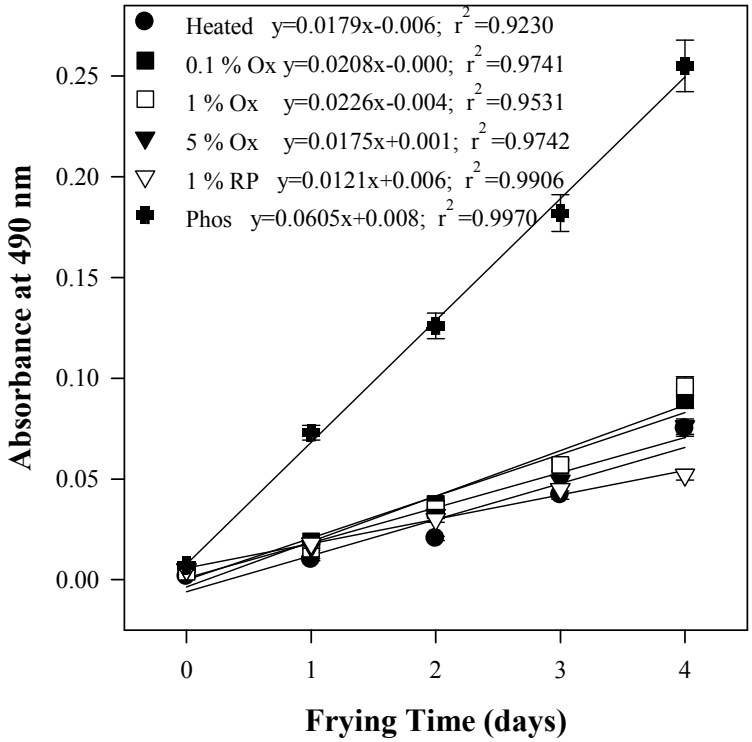


Figure 28: Effect of added oxidized oil, oil with reduced hydroperoxides content and phospholipids on color formation during frying. Heated oil is a reference to all experiments. Lines represent regressions. Ox - Oxidized oil; RP - Reduced peroxides; Phos - Phospholipids

Heating the oil with a brand new fryer caused a slower oil color formation when compared to both a slightly used and heavily oil polymer coated deep fryer (**Figure 29**). A heavy oil polymer coating on the walls of deep fryer exhibited faster trend in frying oil color development. When oil was heated without frying products, the build up of polymers on the fryer walls was very slow.

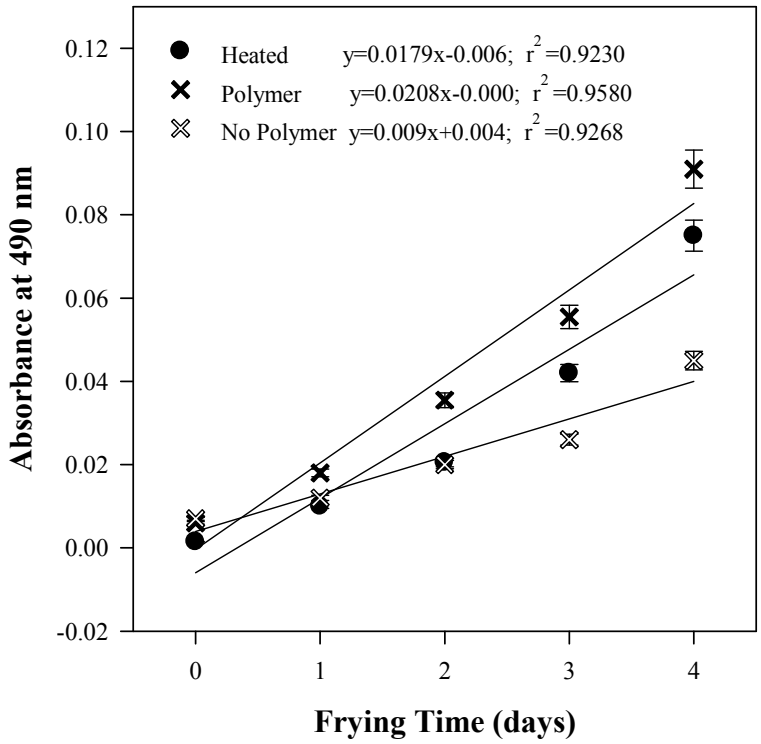


Figure 29: Effect of polymer build up on the sides of the deep fryer wall on color formation during frying. Heated oil is a reference for all experiments. Lines represent regressions.

3.5.2 Total polar components (TPC)

TPC formation was significant in all conditions tested for both amounts of SPF fried in oil from fresh oil and the end of frying. For both 2.5 % (**Figure 30**) and 12.5 % (**Figure 31**) SPF, there were no significant differences between any of the products fried and the heated oil control with the largest differences between any two conditions fried on the same day being less than 5 %.

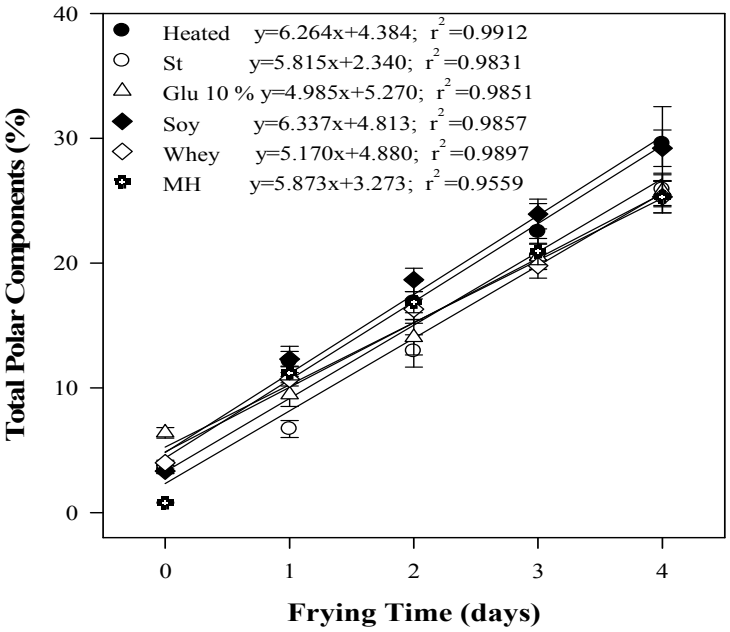


Figure 30: Changes in polar components during frying with food solid particles added at 2.5 % to the oil volume. Heated oil is a reference for these experiments. For abbreviations see the materials and methods. Lines represent regressions.

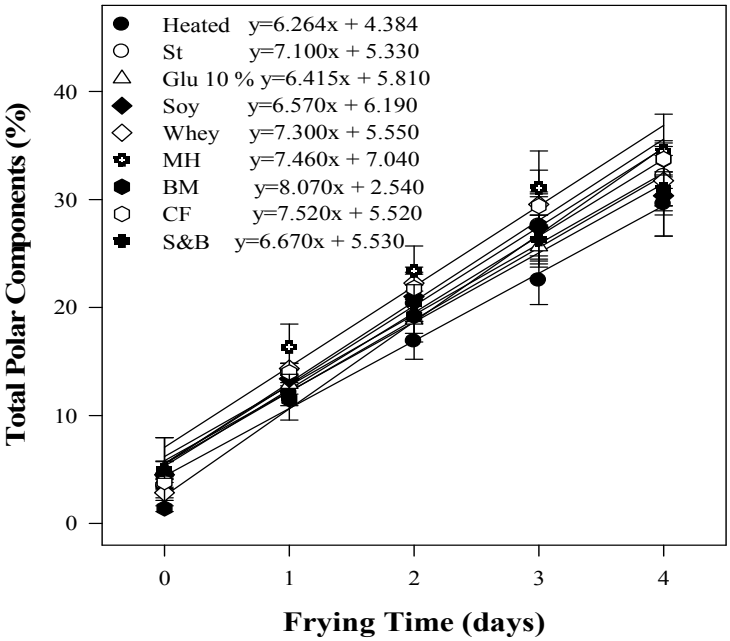


Figure 31: Changes in polar components during frying with food solid particles added at 12.5 % of oil volume. Heated oil is a reference for these experiments. For abbreviations see the materials and methods. $r^2 > 0.9512$ for all regression lines.

In all conditions when oxidized oil was added, the amount of TPC was significantly increased (**Figure 32**). However differences in TPC among oils with different amount of oxidized oils were statistically insignificant (**Figure 32**). When oil with reduced hydroperoxides (RP) was added, an insignificant decrease in TPC was observed at the end of frying as compared to 1 % oxidized oil without RP. Compared to the heated oil control, TPC development was slower during the first day of frying with phospholipids, however, this difference became insignificant by the end of frying day two.

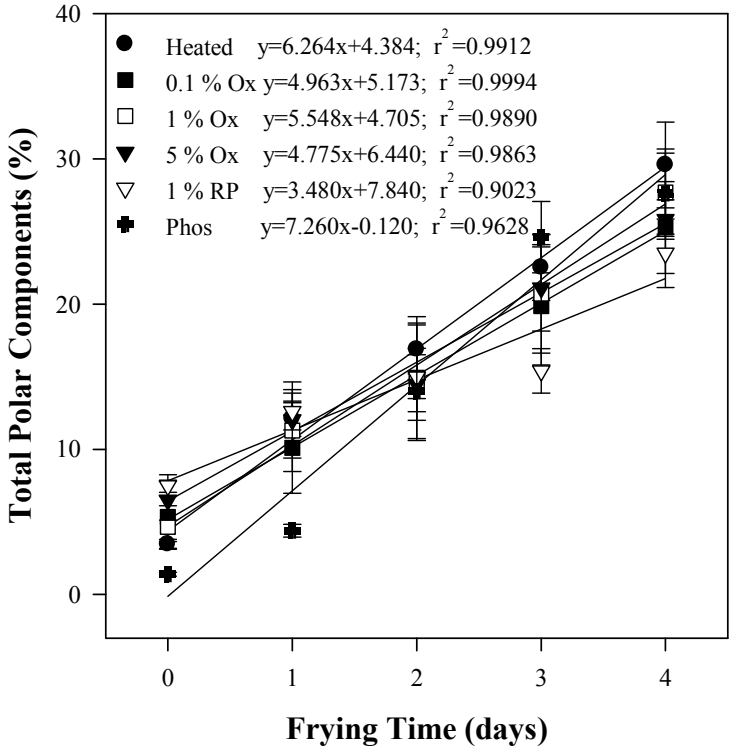


Figure 32: Changes in polar components during heating oil supplemented with oxidized oil, hydroperoxide reduced oil and phospholipids. Heated oil is a reference. Lines represent regressions. Ox - Oxidized oil; RP - Reduced peroxides; Phos - Phospholipids

TPC formation consistently increased as frying progressed regardless of whether or not there was polymer build up on the fryer (**Figure 33**). Observation of any polymers build up on the fryer walls always stimulated the rate of TPC formation.

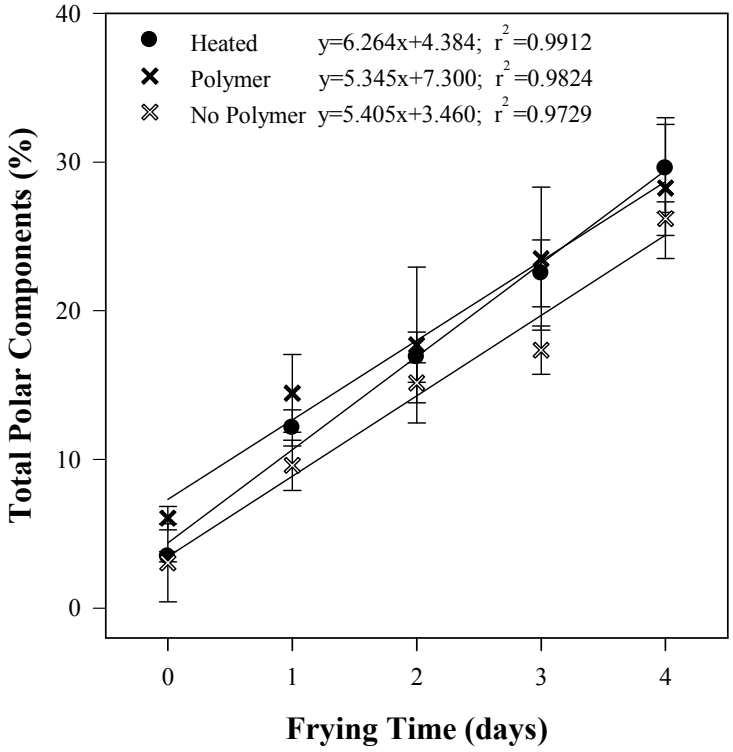


Figure 33: Changes in polar components during heating of oil with or without polymers build up on the deep fryer walls. Heated oil shows an average polymer build up and is a reference. Lines represent regressions.

3.5.3 Residual tocopherols

For solid food particles (SFP) added at both amounts to the frying oil the total amount of tocopherols decreased fast until at least the end of frying day three. The most significant drop in the tocopherol concentration was during the first day of frying where at

least 70 % of these compounds disappeared. When 2.5 % of SFP were fried with the laboratory made mixture, soy and whey protein, a further reduction of tocopherol concentration was observed after the first day of frying when compared to starch, glucose enriched starch, and the heated oil (**Figure 34**). After the second day of frying the tocopherols were depleted completely. The larger amount of SFP made of soy proteins caused a slower degradation of the tocopherols during frying than all other products fried including heated oil (**Figure 35**).

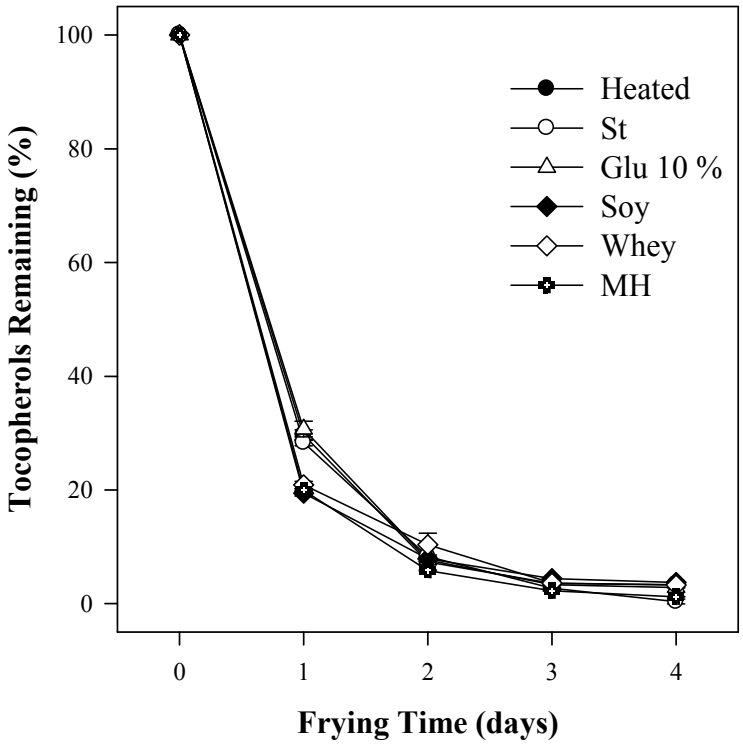


Figure 34: Total tocopherols remaining over frying time with 2.5 % of solid food particles in the frying oil. Heated oil is a reference. For abbreviations see the materials and methods.

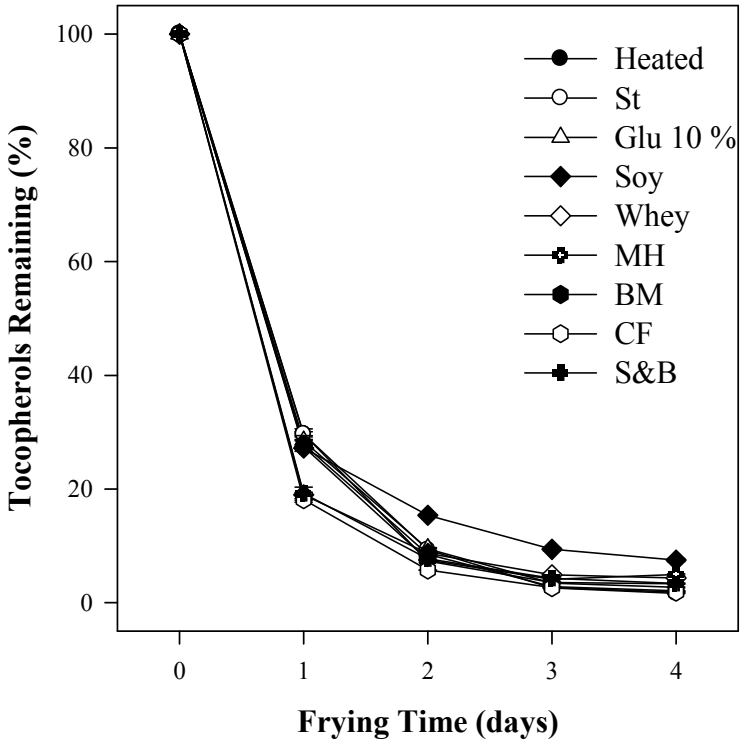


Figure 35: Total tocopherols remaining over frying time with 12.5 % of solid food particles in the frying oil. Heated oil is a reference. For abbreviations see the materials and methods.

Phospholipids added to the frying oil slowed degradation of tocopherols significantly, showing antioxidative properties (**Figure 36**). The addition of the 0.1 % of oxidized oil caused the fastest tocopherols degradation among all analyzed systems. Reducing peroxides in the oxidized oil before adding to the frying oil did not improve significantly the stability of tocopherols.

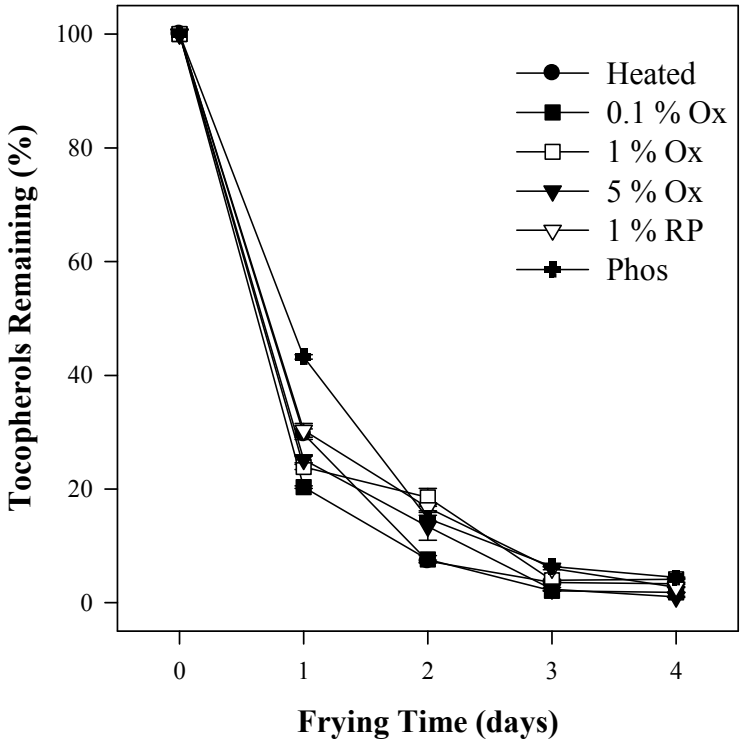


Figure 36: Total tocopherols remaining during frying in oil supplemented with oxidized oil, oil with reduced hydroperoxides and phospholipids. Heated oil is a control. Ox - Oxidized oil; RP - Reduced peroxides; Phos - Phospholipids

Polymers build up on the fryer walls caused a significantly faster rate of tocopherol degradation when compared to a fryer without polymers build up. For this system, tocopherols were completely depleted during the third day of frying (**Figure 37**). After the first day of frying, 52 % of tocopherols were observed in oil fried in the fryer without polymers build up and 29 % for the fryer with polymers build up. After the second day of frying only 16 % more tocopherols were observed in oil fried in clean fryer compared to fryer with polymers build up.

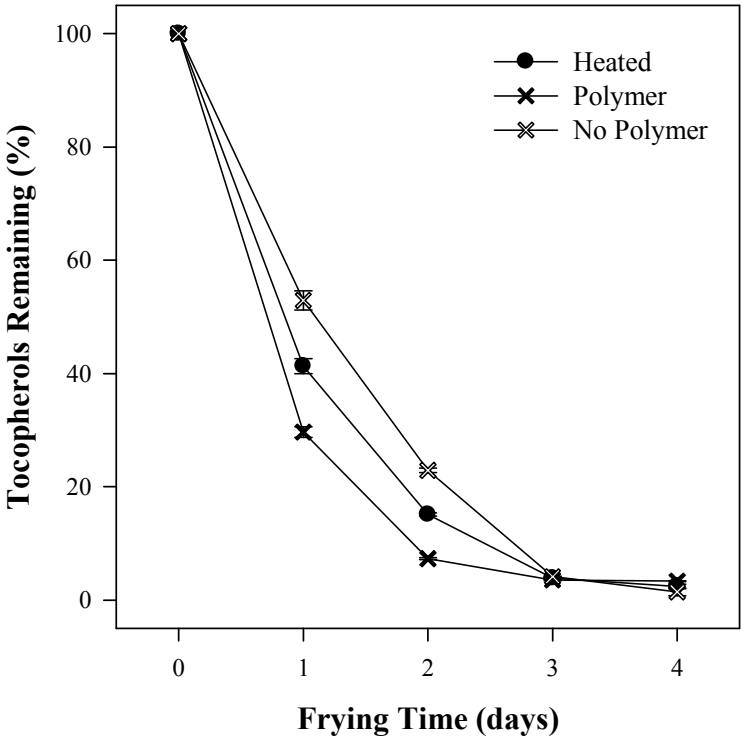


Figure 37: Total tocopherols remaining over frying time in fryers with or without oil polymers build up on the walls of the fryer.

3.5.4 Composition of polar components

When both amounts of SFP were added to the frying oil, oligomer formation was significantly higher in all systems fried. At the end of frying when 12.5 % of SFP were fried, a significantly lower amount of oligomers were observed during frying glucose enriched starch, starch, and in heated oil when compared to other 12.5 % SFP systems (**Figure 39**). During frying of whey protein using SFP, the largest amounts of oligomers were formed (**Figure 38** and **Figure 39**).

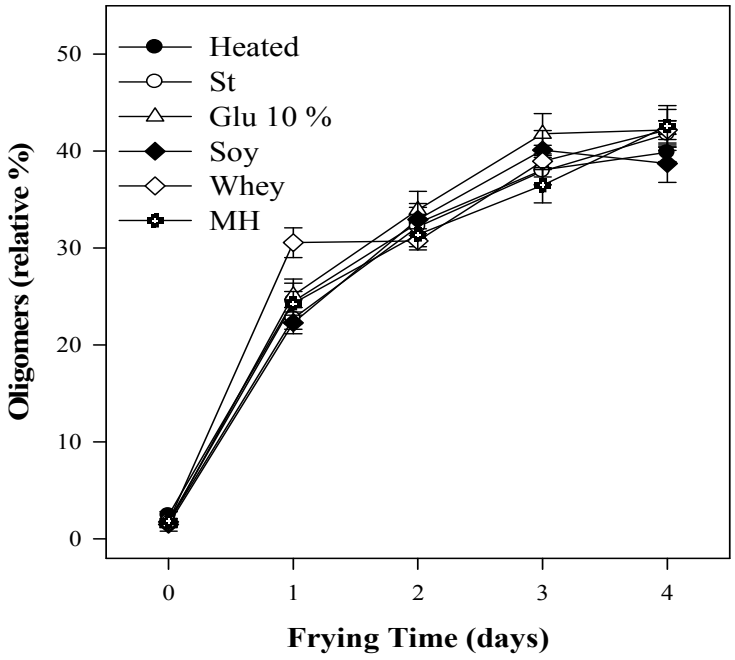


Figure 38: Change in the amount of oligomers during frying in the presence of 2.5 % of solid food particles in the frying oil. Heated oil is a control. For abbreviations see the materials and methods.

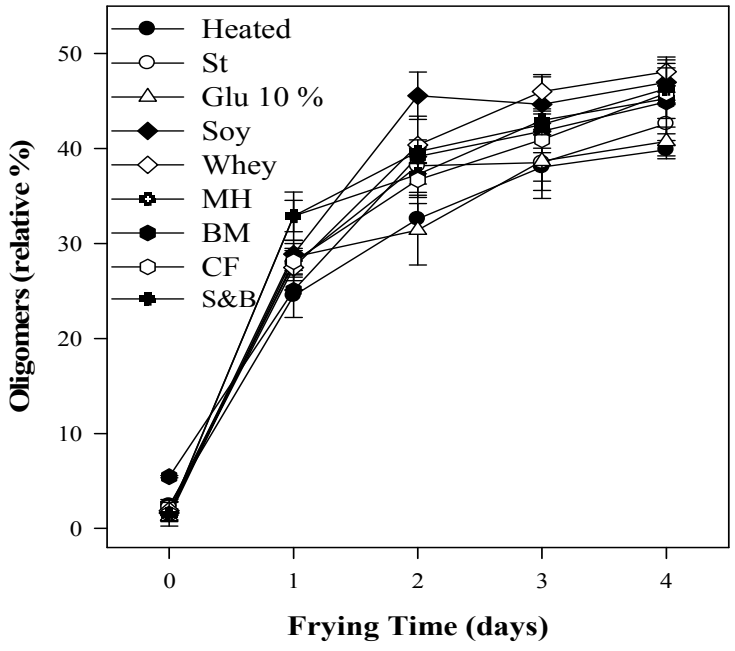


Figure 39: Change in the amount of oligomers during frying in the presence of 12.5 % of solid food particles in the frying oil. Heated oil is a control. For abbreviations see the materials and methods.

The amount of oxidized triacylglycerides (OTG) decreased at least up to the third day of frying. The most significant decrease in the amount of OTG was seen after the first day of frying. During frying whey protein at 2.5 % SFP, the amount of OTG reached the lowest level after the first day of frying as compared to other SFP added, followed by constant decrease up to the end of frying (**Figure 40**). When starch and soy protein were fried in oil as SFP, the largest amount of OTG was observed, however, by the end of frying, heated oil and soy protein surpassed to have the largest percentage of OTG. Statistically significant differences were ascertained between any system used for frying and heated oil for the whole frying period using 12.5 % SFP (**Figure 41**). At the end of frying using 12.5 % SFP, starch, glucose enriched starch, and heated oil had the highest amount of OTG when compared to other systems tested.

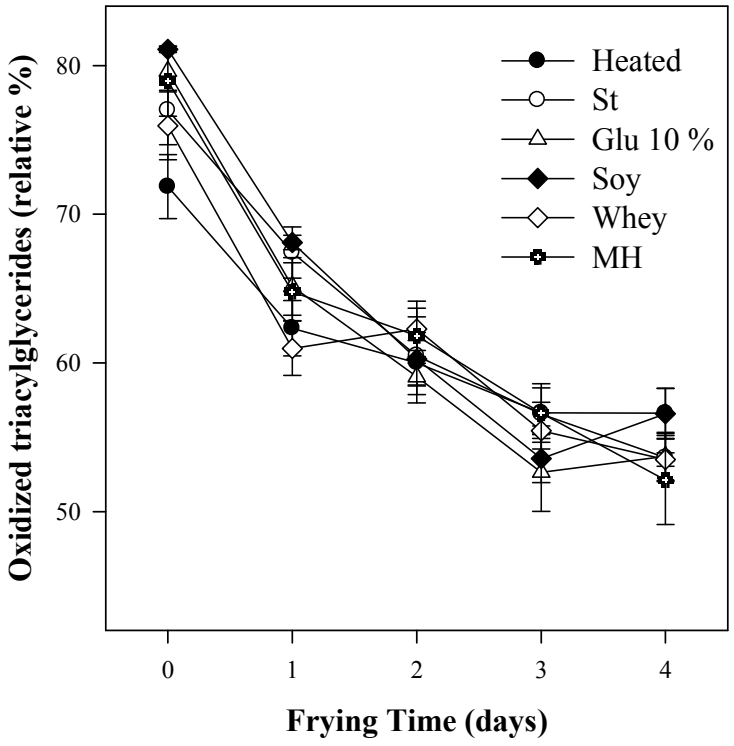


Figure 40: Changes in oxidized triacylglycerides during frying in the presence of 2.5 % solid food particles in frying oil. Heated oil is a control. For abbreviations see the materials and methods.

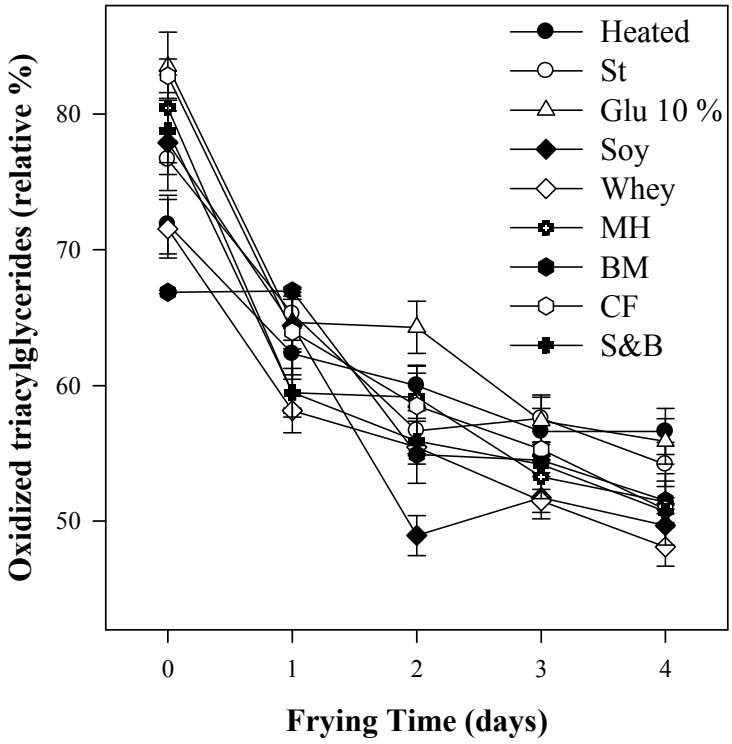


Figure 41: Changes in oxidized triacylglycerides during frying in the presence of 12.5 % solid food particles. Heated oil is a control. For abbreviations see the materials and methods.

Any addition of oxidized oil, oxidized oil with reduced peroxides and phospholipids significantly increased oligomers production for whole frying period (**Figure 42**). After the first day of frying in the presence of phospholipids, the amount of oligomers increased significantly compared to all other systems tested. The rate of oligomers formation when phospholipids were added to oil was lower after the second frying day, whereas samples supplemented with 5 % of oxidized oil and oxidized oil with reduced peroxides experienced significant increase in oligomers compared to other samples.

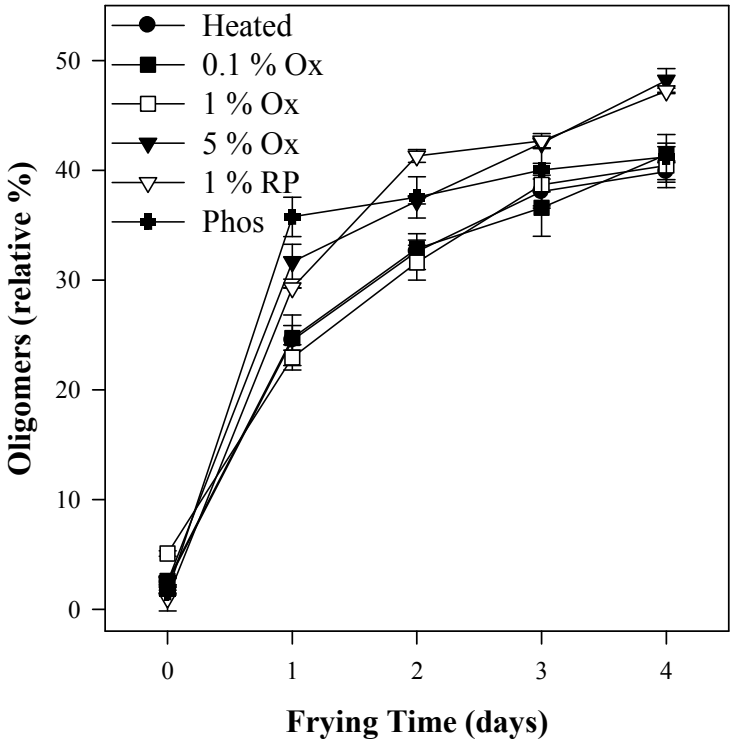


Figure 42: Changes in the amounts of oligomers in oils supplemented with oxidized oil, hydroperoxide reduced oil and phospholipids. Heated oil is a reference. Ox - Oxidized oil; RP - Reduced peroxides; Phos - Phospholipids

The addition of phospholipids to the frying oil decreased the amount of OTG in the oils by 25 % after the first day of frying and continued to have the lowest amount of these compounds in the frying oil until the end of frying (**Figure 43**). The addition of 0.1 % and 1 % of oxidized oil to the fresh oil did not significantly affect OTG amounts when compared to the heated oil. However, when the amount of added oxidized oil reached 5 %, the OTG levels during frying decreased significantly faster when compared to the heated oil and with 0.1 % and 1 % oxidized oil additions. No significant difference was observed between samples supplemented with 5 % oxidized oil and 1 % reduced peroxide oxidized oil, with regard to the amount of OTG (**Figure 43**).

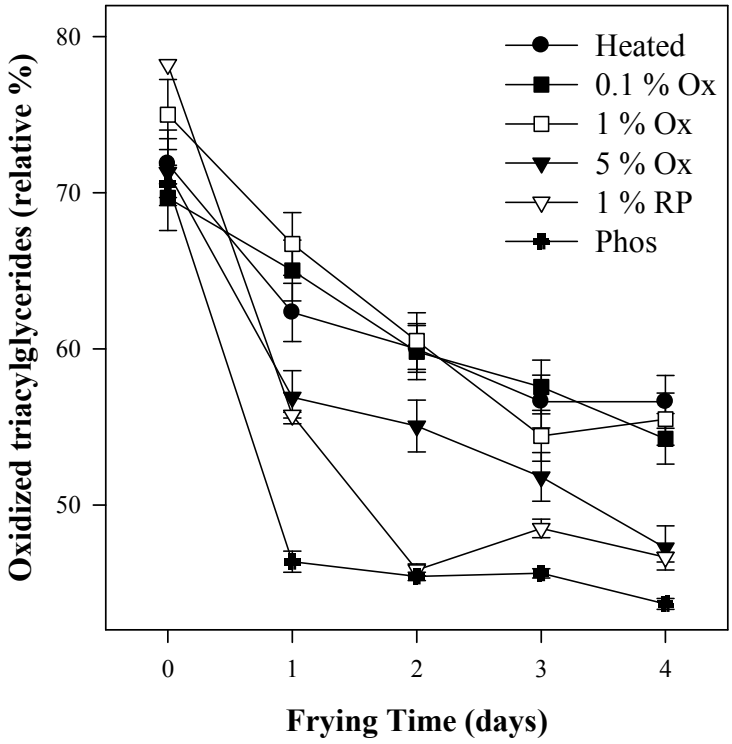


Figure 43: Changes in oxidized triacylglycerides during frying in oils supplemented with oxidized oil, hydroperoxide reduced oil and phospholipids. Heated oil is a reference. Ox - Oxidized oil; RP - Reduced peroxides; Phos – Phospholipids

Oligomer production in oils used for frying in fryers with and without polymer build up exhibited the same pattern of changes as for the heated oil and lacked any differences (**Figure 44**). No significant differences were found in OTG between fryers with polymers build up and without (**Figure 45**).

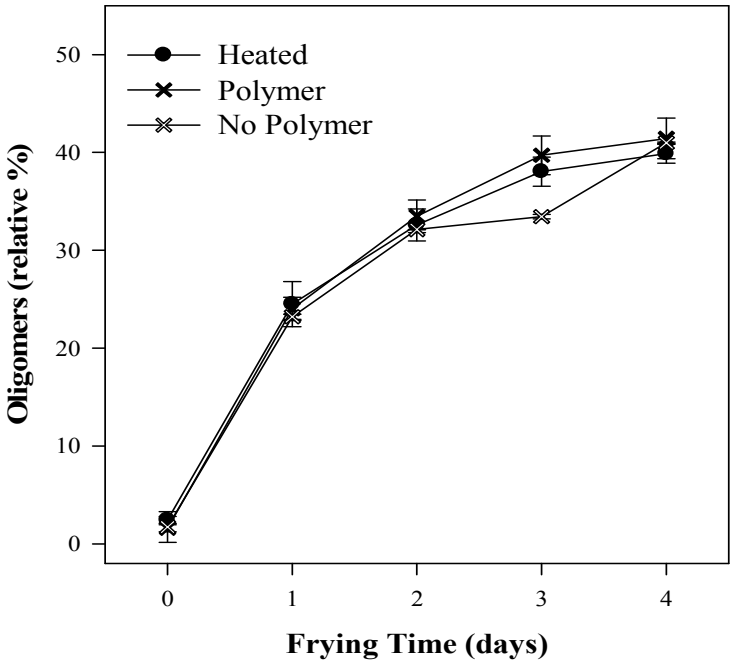


Figure 44: Changes in the amount of oligomers during frying with or without polymer build up on the deep fryer walls. Heated oil shows an average polymer build up and is a control.

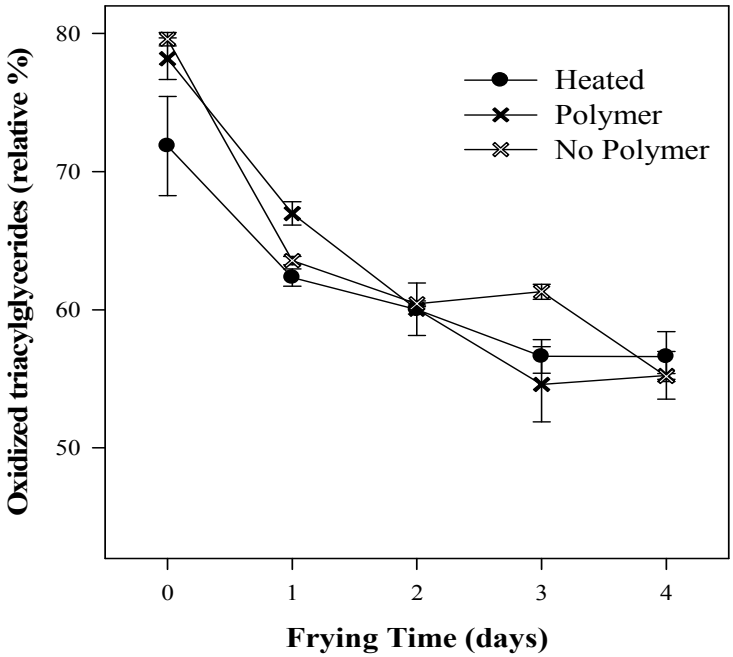


Figure 45: Changes in oxidized triacylglycerides during frying with or without polymer build up on the deep fryer walls. Heated oil shows an average oil polymer build up and is a control.

3.5.5 Anisidine value

When frying was done in the presence of SFP, continuous increase in the anisidine value was observed up to the end of frying (**Figure 46** and **Figure 47**). Addition of 12.5 % of SFP to frying oil caused the fastest increase in the anisidine value during the first day of frying (**Figure 47**). For 12.5 % SFP added, significant differences were not observed when different products were fried when compared to heated oil (**Figure 47**). For both amounts of SFP, oils with protein product particles had the lowest carbonyl formation as shown by low anisidine values at the end of frying (**Figure 46** and **Figure 47**).

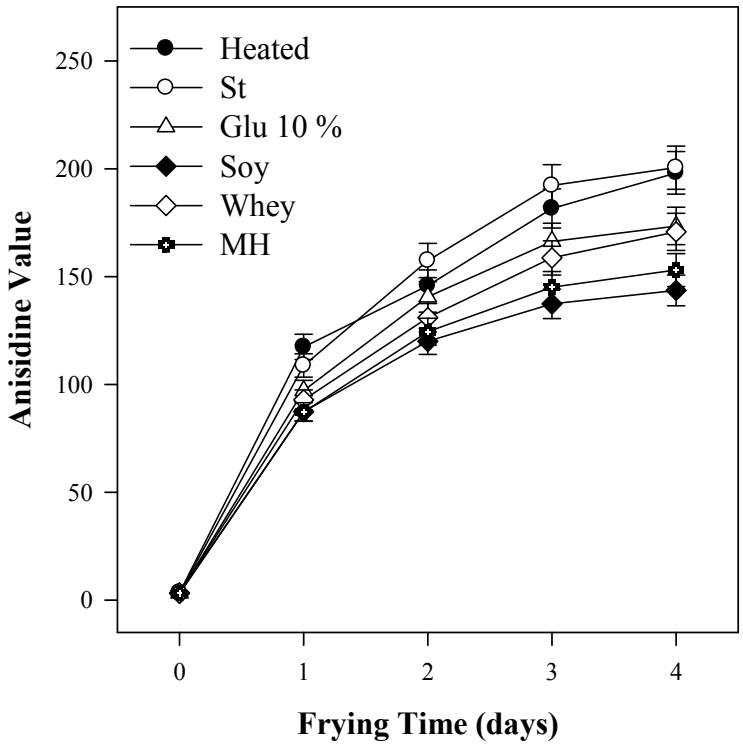


Figure 46: Effect of solid food particle present at 2.5 % in frying oil during frying on anisidine value. Heated oil is a control. For abbreviations see the materials and methods.

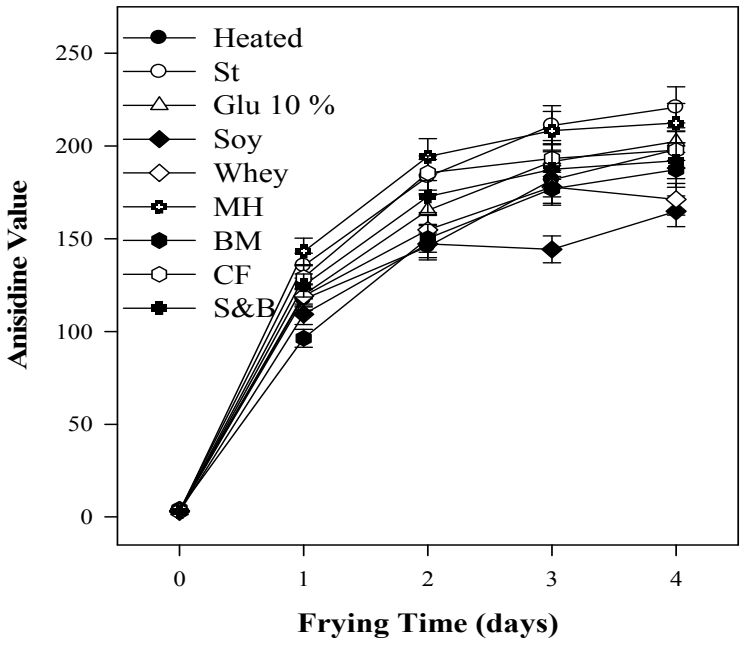


Figure 47: Effect of presence of solid food particles at 12.5 % on changes in anisidine value during frying. Heated oil is a reference. For abbreviations see the materials and methods.

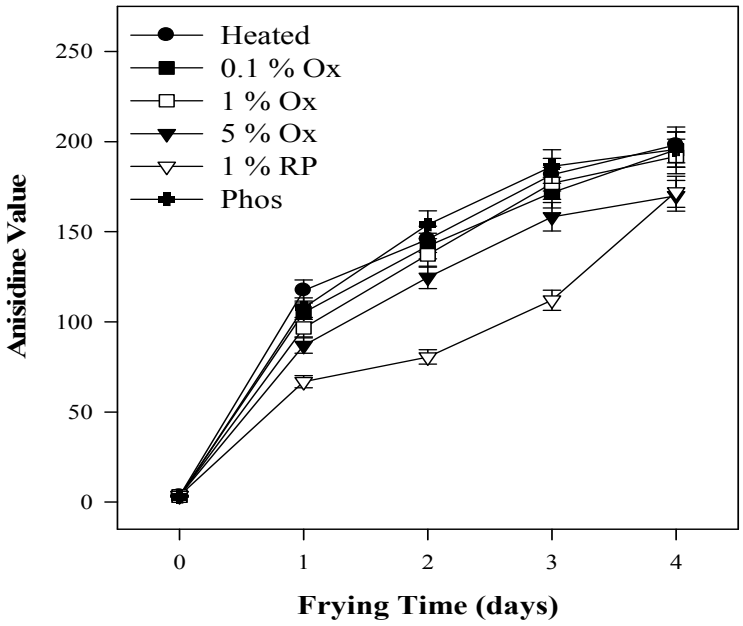


Figure 48: Effect of oil supplementation with oxidized oil, hydroperoxide reduced oil and phospholipids on anisidine value during frying. Heated oil is a control. Ox - Oxidized oil; RP - Reduced peroxides; Phos – Phospholipids

Adding oxidized oils and phospholipids to the frying oils stimulated formation of carbonyls as measured by anisidine value (**Figure 48**). When oil with reduced amount of hydroperoxides was implemented, significantly lower rate of anisidine value was observed compared to all other conditions.

Build up of polymers on the walls of the fryer stimulated carbonyl formation and an increase in the anisidine values were measured (**Figure 49**).

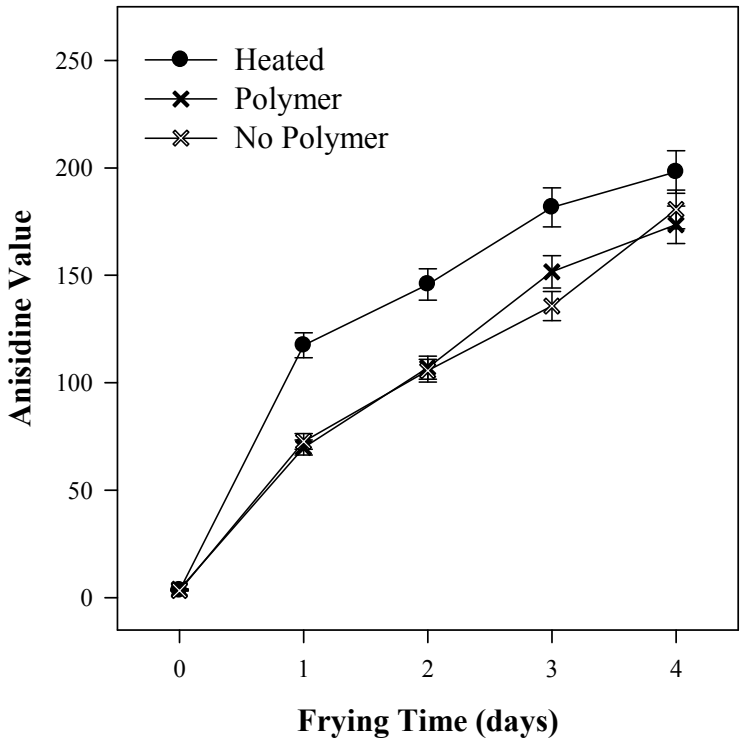


Figure 49: Effect of oil polymer build up on the deep fryer walls on anisidine value. No products were fried in any of the oils. Heated oil is a reference.

3.5.6 Fatty acid composition

For both amounts of SFP added to frying oils (**Table 11** and **Table 12**), a significant decrease in contribution of linoleic and linolenic acids were observed for all systems used for frying. Heating the oil alone caused a larger decrease in both linoleic and linolenic acid

concentrations when compared to any material fried in the presence of the SPF, however, the difference was not significant. When frying was done in the presence of 2.5 % SFP (**Table 11**), fried soy protein caused the largest drop in both linoleic and linolenic acids concentrations as well as the largest rise in *trans* fatty acid amounts as compared to other SFP added. In the case of 12.5 % SFP (**Table 12**), the commercial batters and breadings created the largest decrease in linoleic and linolenic acid concentrations as well as formed the largest rise in the *trans* fatty acid concentration. Also for both SFP amounts, proteins caused a larger decline in linoleic and linolenic acids concentrations when compared to fried starch and glucose enriched starch.

The addition of phospholipids to the frying oil initially caused the largest decrease in linoleic and linolenic acids concentrations when compared to oxidized oils added, however, the decrease was not statistically significant and a lower reduction was observed during heating oil alone (**Table 13**). Reducing the peroxides in the oxidized oil did not significantly affect the relative contributions of linolenic, linoleic or *trans* fatty acids in the oils. Increasing the amount of oxidized oil did not significantly affect either acids degradation nor *trans* fatty acid formation.

Table 11: The changes to canola oil major fatty acids during frying in the presence of 2.5 % of solid food particles. Heated oil is a control. For abbreviations see the materials and methods.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
Heated						
0	4.12 ± 0.18	2.40 ± 0.19	58.84 ± 1.59	18.19 ± 0.25	8.64 ± 0.08	1.45 ± 0.13
1	4.37 ± 0.22	2.48 ± 0.18	60.87 ± 1.39	17.10 ± 0.13	7.49 ± 0.13	1.41 ± 0.11
2	4.54 ± 0.20	2.79 ± 0.22	61.29 ± 1.57	16.28 ± 0.35	6.63 ± 0.36	1.72 ± 0.13
3	4.68 ± 0.18	2.64 ± 0.16	62.52 ± 1.54	15.54 ± 0.52	5.96 ± 0.32	1.68 ± 0.11
4	4.78 ± 0.19	2.65 ± 0.19	63.42 ± 1.51	14.88 ± 0.52	5.41 ± 0.12	1.68 ± 0.09
St						
0	4.10 ± 0.18	2.34 ± 0.18	59.08 ± 1.59	18.08 ± 0.25	8.57 ± 0.08	1.54 ± 0.14
1	4.21 ± 0.22	2.39 ± 0.18	60.23 ± 1.38	17.40 ± 0.13	7.74 ± 0.13	1.61 ± 0.12
2	4.29 ± 0.19	2.44 ± 0.19	60.85 ± 1.56	16.92 ± 0.36	7.21 ± 0.39	1.66 ± 0.12
3	4.43 ± 0.17	2.48 ± 0.15	61.62 ± 1.52	16.32 ± 0.55	6.67 ± 0.36	1.69 ± 0.11
4	4.54 ± 0.18	2.55 ± 0.18	62.49 ± 1.49	15.72 ± 0.55	6.18 ± 0.13	1.72 ± 0.09
Glu 10 %						
0	4.07 ± 0.18	2.36 ± 0.19	59.02 ± 1.59	18.11 ± 0.25	8.59 ± 0.08	1.57 ± 0.14
1	4.23 ± 0.22	2.44 ± 0.18	60.39 ± 1.38	17.27 ± 0.13	7.62 ± 0.13	1.65 ± 0.13
2	4.36 ± 0.19	2.50 ± 0.19	61.08 ± 1.56	16.71 ± 0.36	6.98 ± 0.37	1.73 ± 0.13
3	4.42 ± 0.17	2.48 ± 0.15	61.79 ± 1.53	16.30 ± 0.55	6.47 ± 0.34	1.82 ± 0.12
4	4.58 ± 0.18	2.52 ± 0.18	62.95 ± 1.50	15.40 ± 0.54	5.78 ± 0.13	1.84 ± 0.10
Soy						
0	4.04 ± 0.18	2.26 ± 0.18	59.16 ± 1.59	18.23 ± 0.25	8.66 ± 0.08	1.51 ± 0.14
1	4.20 ± 0.22	2.44 ± 0.18	60.04 ± 1.37	17.36 ± 0.13	7.66 ± 0.13	1.70 ± 0.13
2	4.38 ± 0.19	2.46 ± 0.19	61.07 ± 1.56	16.71 ± 0.36	6.95 ± 0.37	1.69 ± 0.12
3	4.45 ± 0.17	2.41 ± 0.15	62.31 ± 1.54	16.00 ± 0.54	6.32 ± 0.34	1.74 ± 0.11
4	4.58 ± 0.18	2.49 ± 0.18	62.81 ± 1.50	15.38 ± 0.54	5.74 ± 0.12	1.84 ± 0.10
Whey						
0	4.05 ± 0.18	2.23 ± 0.18	59.05 ± 1.59	18.19 ± 0.25	8.66 ± 0.08	1.49 ± 0.14
1	4.22 ± 0.22	2.34 ± 0.17	60.10 ± 1.37	17.50 ± 0.13	7.87 ± 0.14	1.56 ± 0.12
2	4.27 ± 0.19	2.35 ± 0.18	60.81 ± 1.56	17.08 ± 0.37	7.34 ± 0.39	1.60 ± 0.12
3	4.39 ± 0.16	2.41 ± 0.15	61.43 ± 1.52	16.59 ± 0.56	6.82 ± 0.36	1.65 ± 0.10
4	4.51 ± 0.18	2.55 ± 0.18	62.31 ± 1.48	15.85 ± 0.56	6.27 ± 0.14	1.72 ± 0.09
MH						
0	4.06 ± 0.18	2.36 ± 0.19	59.04 ± 1.59	18.16 ± 0.25	8.61 ± 0.08	1.56 ± 0.14
1	4.23 ± 0.22	2.49 ± 0.18	60.03 ± 1.37	17.42 ± 0.13	7.77 ± 0.13	1.66 ± 0.13
2	4.30 ± 0.19	2.48 ± 0.19	60.99 ± 1.56	16.91 ± 0.36	7.18 ± 0.38	1.71 ± 0.13
3	4.42 ± 0.17	2.53 ± 0.15	61.62 ± 1.52	16.38 ± 0.55	6.64 ± 0.35	1.75 ± 0.11
4	4.52 ± 0.18	2.52 ± 0.18	62.34 ± 1.48	15.88 ± 0.56	6.19 ± 0.13	1.77 ± 0.09

[a] All values are averages of duplicate analysis

Table 12: Changes to canola oil major fatty acids during frying with 12.5 % of solid food particles. Heated oil is a reference. Abbreviations see materials and methods.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
Heated						
0	4.12 ± 0.18	2.40 ± 0.19	58.84 ± 1.59	18.19 ± 0.25	8.64 ± 0.08	1.45 ± 0.13
1	4.37 ± 0.22	2.48 ± 0.18	60.87 ± 1.39	17.10 ± 0.13	7.49 ± 0.13	1.41 ± 0.11
2	4.54 ± 0.20	2.79 ± 0.22	61.29 ± 1.57	16.28 ± 0.35	6.63 ± 0.36	1.72 ± 0.13
3	4.68 ± 0.18	2.64 ± 0.16	62.52 ± 1.54	15.54 ± 0.52	5.96 ± 0.32	1.68 ± 0.11
4	4.78 ± 0.19	2.65 ± 0.19	63.42 ± 1.51	14.88 ± 0.52	5.41 ± 0.12	1.68 ± 0.09
St						
0	4.10 ± 0.18	2.33 ± 0.18	59.18 ± 1.59	18.22 ± 0.25	8.64 ± 0.08	1.29 ± 0.12
1	4.36 ± 0.22	2.42 ± 0.18	60.34 ± 1.38	17.35 ± 0.13	7.69 ± 0.13	1.35 ± 0.10
2	4.34 ± 0.19	2.42 ± 0.19	61.15 ± 1.56	16.97 ± 0.36	7.17 ± 0.38	1.39 ± 0.10
3	4.47 ± 0.17	2.50 ± 0.15	61.79 ± 1.53	16.35 ± 0.55	6.58 ± 0.35	1.51 ± 0.10
4	4.56 ± 0.18	2.49 ± 0.18	62.33 ± 1.48	16.00 ± 0.56	6.20 ± 0.13	1.53 ± 0.08
Glu 10 %						
0	4.09 ± 0.18	2.31 ± 0.18	59.12 ± 1.59	18.27 ± 0.26	8.67 ± 0.08	1.25 ± 0.11
1	4.28 ± 0.22	2.43 ± 0.18	60.40 ± 1.38	17.38 ± 0.13	7.69 ± 0.13	1.37 ± 0.11
2	4.32 ± 0.19	2.36 ± 0.18	61.01 ± 1.56	17.07 ± 0.37	7.20 ± 0.39	1.42 ± 0.10
3	4.45 ± 0.17	2.43 ± 0.15	61.78 ± 1.53	16.50 ± 0.55	6.61 ± 0.35	1.54 ± 0.10
4	4.52 ± 0.18	2.46 ± 0.18	62.26 ± 1.48	16.10 ± 0.57	6.20 ± 0.13	1.58 ± 0.08
Soy						
0	4.11 ± 0.18	2.32 ± 0.18	59.18 ± 1.59	18.19 ± 0.25	8.66 ± 0.08	1.30 ± 0.12
1	4.24 ± 0.22	2.43 ± 0.18	60.09 ± 1.37	17.55 ± 0.13	7.93 ± 0.14	1.33 ± 0.10
2	4.35 ± 0.19	2.46 ± 0.19	60.67 ± 1.55	17.16 ± 0.37	7.43 ± 0.40	1.39 ± 0.10
3	4.51 ± 0.17	2.51 ± 0.15	61.81 ± 1.53	16.27 ± 0.54	6.79 ± 0.36	1.42 ± 0.09
4	4.54 ± 0.18	2.58 ± 0.19	61.95 ± 1.47	16.16 ± 0.57	6.47 ± 0.14	1.51 ± 0.08
Whey						
0	4.17 ± 0.18	3.27 ± 0.26	58.52 ± 1.58	17.82 ± 0.25	8.32 ± 0.08	1.61 ± 0.15
1	4.28 ± 0.22	3.34 ± 0.25	59.64 ± 1.36	17.13 ± 0.13	7.51 ± 0.13	1.66 ± 0.13
2	4.38 ± 0.19	3.10 ± 0.24	60.52 ± 1.55	16.77 ± 0.36	7.03 ± 0.38	1.63 ± 0.12
3	4.52 ± 0.17	3.19 ± 0.19	61.30 ± 1.51	16.09 ± 0.54	6.43 ± 0.34	1.68 ± 0.11
4	4.67 ± 0.18	3.11 ± 0.22	62.40 ± 1.49	15.37 ± 0.54	5.91 ± 0.13	1.67 ± 0.09
MH						
0	4.13 ± 0.18	2.93 ± 0.23	58.73 ± 1.58	17.99 ± 0.25	8.48 ± 0.08	1.50 ± 0.14
1	4.35 ± 0.22	3.04 ± 0.22	60.29 ± 1.38	16.95 ± 0.13	7.41 ± 0.13	1.51 ± 0.12
2	4.43 ± 0.19	3.03 ± 0.24	61.05 ± 1.56	16.44 ± 0.35	6.77 ± 0.36	1.61 ± 0.12
3	4.59 ± 0.17	3.02 ± 0.18	61.94 ± 1.53	15.81 ± 0.53	6.16 ± 0.33	1.64 ± 0.10
4	4.64 ± 0.18	2.93 ± 0.21	62.76 ± 1.49	15.32 ± 0.54	5.71 ± 0.12	1.68 ± 0.09
BM						
0	3.89 ± 0.17	2.52 ± 0.20	61.04 ± 1.64	17.50 ± 0.24	7.67 ± 0.07	1.29 ± 0.12
1	4.04 ± 0.21	2.65 ± 0.20	61.90 ± 1.42	16.74 ± 0.13	6.95 ± 0.12	1.37 ± 0.11
2	4.22 ± 0.18	2.72 ± 0.21	63.01 ± 1.61	15.91 ± 0.34	6.21 ± 0.33	1.41 ± 0.10
3	4.36 ± 0.16	2.80 ± 0.17	64.19 ± 1.58	15.02 ± 0.50	5.51 ± 0.29	1.50 ± 0.09
4	4.45 ± 0.17	2.78 ± 0.20	64.76 ± 1.54	14.56 ± 0.51	5.04 ± 0.11	1.56 ± 0.08
CF						
0	4.13 ± 0.18	2.29 ± 0.18	59.52 ± 1.60	18.33 ± 0.26	8.71 ± 0.08	1.19 ± 0.11
1	4.28 ± 0.22	2.34 ± 0.17	60.58 ± 1.38	17.47 ± 0.13	7.71 ± 0.13	1.27 ± 0.10
2	4.39 ± 0.19	2.40 ± 0.19	61.70 ± 1.58	16.66 ± 0.36	6.95 ± 0.37	1.36 ± 0.10
3	4.58 ± 0.17	2.50 ± 0.15	62.53 ± 1.54	15.93 ± 0.53	6.24 ± 0.33	1.46 ± 0.09
4	4.68 ± 0.18	2.52 ± 0.18	63.27 ± 1.51	15.32 ± 0.54	5.72 ± 0.12	1.53 ± 0.08
S&B						
0	4.07 ± 0.18	2.26 ± 0.18	59.25 ± 1.60	18.31 ± 0.26	8.69 ± 0.08	1.26 ± 0.12
1	4.27 ± 0.22	2.41 ± 0.18	60.68 ± 1.39	17.29 ± 0.13	7.65 ± 0.13	1.36 ± 0.10
2	4.45 ± 0.19	2.51 ± 0.20	61.43 ± 1.57	16.61 ± 0.36	6.94 ± 0.37	1.44 ± 0.11
3	4.54 ± 0.17	2.52 ± 0.15	62.02 ± 1.53	16.23 ± 0.54	6.49 ± 0.35	1.49 ± 0.09
4	4.66 ± 0.18	2.58 ± 0.19	62.46 ± 1.49	15.72 ± 0.55	5.95 ± 0.13	1.61 ± 0.08

[a] All values are averages of duplicate analysis

Table 13: The changes to the major fatty acids composition during heating canola oil supplemented with oxidized oil, hydroperoxide reduced oil and phospholipids. Ox - Oxidized oil; RP - Reduced peroxides; Phos - Phospholipids

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
Heated						
0	4.12 ± 0.18	2.40 ± 0.19	58.84 ± 1.59	18.19 ± 0.25	8.64 ± 0.08	1.45 ± 0.13
1	4.37 ± 0.22	2.48 ± 0.18	60.87 ± 1.39	17.10 ± 0.13	7.49 ± 0.13	1.41 ± 0.11
2	4.54 ± 0.20	2.79 ± 0.22	61.29 ± 1.57	16.28 ± 0.35	6.63 ± 0.36	1.72 ± 0.13
3	4.68 ± 0.18	2.64 ± 0.16	62.52 ± 1.54	15.54 ± 0.52	5.96 ± 0.32	1.68 ± 0.11
4	4.78 ± 0.19	2.65 ± 0.19	63.42 ± 1.51	14.88 ± 0.52	5.41 ± 0.12	1.68 ± 0.09
0.1 % Ox						
0	4.08 ± 0.18	2.42 ± 0.19	59.06 ± 1.59	18.16 ± 0.25	8.58 ± 0.08	1.52 ± 0.14
1	4.21 ± 0.22	2.48 ± 0.18	59.98 ± 1.37	17.49 ± 0.13	7.78 ± 0.13	1.48 ± 0.11
2	4.35 ± 0.19	2.52 ± 0.20	61.09 ± 1.56	16.80 ± 0.36	7.11 ± 0.38	1.48 ± 0.11
3	4.52 ± 0.17	2.60 ± 0.16	61.84 ± 1.53	16.21 ± 0.54	6.59 ± 0.35	1.53 ± 0.10
4	4.52 ± 0.18	2.59 ± 0.19	62.42 ± 1.49	15.81 ± 0.56	6.09 ± 0.13	1.61 ± 0.08
1 % Ox						
0	4.07 ± 0.18	2.19 ± 0.17	59.26 ± 1.60	18.29 ± 0.26	8.59 ± 0.08	1.38 ± 0.13
1	4.23 ± 0.22	2.31 ± 0.17	60.43 ± 1.38	17.52 ± 0.13	7.69 ± 0.13	1.43 ± 0.11
2	4.36 ± 0.19	2.40 ± 0.19	61.43 ± 1.57	16.72 ± 0.36	6.94 ± 0.37	1.54 ± 0.11
3	4.45 ± 0.17	2.38 ± 0.14	61.91 ± 1.53	16.33 ± 0.55	6.56 ± 0.35	1.58 ± 0.10
4	4.59 ± 0.18	2.54 ± 0.18	62.70 ± 1.49	15.63 ± 0.55	5.99 ± 0.13	1.65 ± 0.09
5 % Ox						
0	4.09 ± 0.18	2.28 ± 0.18	59.11 ± 1.59	18.14 ± 0.25	8.58 ± 0.08	1.44 ± 0.13
1	4.19 ± 0.22	2.30 ± 0.17	60.08 ± 1.37	17.65 ± 0.13	7.98 ± 0.14	1.49 ± 0.11
2	4.27 ± 0.19	2.38 ± 0.19	60.62 ± 1.55	17.22 ± 0.37	7.42 ± 0.40	1.59 ± 0.12
3	4.38 ± 0.16	2.43 ± 0.15	61.39 ± 1.52	16.66 ± 0.56	6.81 ± 0.36	1.66 ± 0.11
4	4.54 ± 0.18	2.51 ± 0.18	62.03 ± 1.48	16.06 ± 0.57	6.27 ± 0.14	1.72 ± 0.09
1 % RP						
0	3.93 ± 0.17	1.85 ± 0.15	60.89 ± 1.64	17.91 ± 0.25	7.75 ± 0.07	0.96 ± 0.09
1	4.07 ± 0.21	1.94 ± 0.14	61.64 ± 1.41	17.28 ± 0.13	7.16 ± 0.12	1.09 ± 0.08
2	4.03 ± 0.17	1.90 ± 0.15	61.91 ± 1.58	17.29 ± 0.37	6.86 ± 0.37	1.14 ± 0.08
3	4.09 ± 0.15	1.88 ± 0.11	62.41 ± 1.54	17.03 ± 0.57	6.56 ± 0.35	1.16 ± 0.07
4	4.36 ± 0.17	2.09 ± 0.15	63.61 ± 1.51	16.03 ± 0.57	5.95 ± 0.13	1.31 ± 0.07
Phos						
0	4.16 ± 0.18	2.44 ± 0.19	59.25 ± 1.60	18.10 ± 0.25	8.61 ± 0.08	1.25 ± 0.11
1	4.25 ± 0.22	2.45 ± 0.18	60.19 ± 1.38	17.56 ± 0.13	7.84 ± 0.14	1.35 ± 0.10
2	4.43 ± 0.19	2.58 ± 0.20	61.19 ± 1.57	16.65 ± 0.36	6.96 ± 0.37	1.45 ± 0.11
3	4.57 ± 0.17	2.62 ± 0.16	62.11 ± 1.53	15.98 ± 0.53	6.28 ± 0.33	1.49 ± 0.09
4	4.71 ± 0.18	2.67 ± 0.19	62.83 ± 1.50	15.34 ± 0.54	5.73 ± 0.12	1.59 ± 0.08

[a] All values are averages of duplicate analysis

Polymer build up on the fryer walls did not affect linoleic and linolenic acids degradation nor *trans* fatty acid formation (**Table 14**).

Table 14: Changes to canola oil major fatty acids during heating in a deep fryer with and without polymer build up on the walls. Heated oil shows an average polymer build up and is a control.

Frying time (days)	Contribution[a] (relative percentage)					
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Total C_{trans}
Heated						
0	4.12 ± 0.18	2.40 ± 0.19	58.84 ± 1.59	18.19 ± 0.25	8.64 ± 0.08	1.45 ± 0.13
1	4.37 ± 0.22	2.48 ± 0.18	60.87 ± 1.39	17.10 ± 0.13	7.49 ± 0.13	1.41 ± 0.11
2	4.54 ± 0.20	2.79 ± 0.22	61.29 ± 1.57	16.28 ± 0.35	6.63 ± 0.36	1.72 ± 0.13
3	4.68 ± 0.18	2.64 ± 0.16	62.52 ± 1.54	15.54 ± 0.52	5.96 ± 0.32	1.68 ± 0.11
4	4.78 ± 0.19	2.65 ± 0.19	63.42 ± 1.51	14.88 ± 0.52	5.41 ± 0.12	1.68 ± 0.09
Polymer						
0	3.93 ± 0.17	1.84 ± 0.15	60.72 ± 1.64	17.89 ± 0.25	7.86 ± 0.07	0.95 ± 0.09
1	3.98 ± 0.20	1.86 ± 0.14	61.26 ± 1.40	17.58 ± 0.13	7.41 ± 0.13	1.03 ± 0.08
2	4.03 ± 0.17	1.88 ± 0.15	61.83 ± 1.58	17.26 ± 0.37	7.01 ± 0.38	1.11 ± 0.08
3	4.15 ± 0.16	1.95 ± 0.12	62.60 ± 1.55	16.71 ± 0.56	6.38 ± 0.34	1.15 ± 0.07
4	4.33 ± 0.17	2.03 ± 0.15	63.88 ± 1.52	15.97 ± 0.56	5.83 ± 0.13	1.33 ± 0.07
No Polymer						
0	3.92 ± 0.17	1.81 ± 0.14	61.17 ± 1.65	18.02 ± 0.25	7.93 ± 0.07	0.38 ± 0.03
1	3.97 ± 0.20	1.82 ± 0.13	61.85 ± 1.41	17.64 ± 0.13	7.41 ± 0.13	0.46 ± 0.04
2	4.03 ± 0.17	1.85 ± 0.14	62.36 ± 1.60	17.25 ± 0.37	6.96 ± 0.37	0.51 ± 0.04
3	4.16 ± 0.16	1.90 ± 0.11	63.15 ± 1.56	16.65 ± 0.56	6.44 ± 0.34	0.60 ± 0.04
4	4.26 ± 0.17	1.94 ± 0.14	63.97 ± 1.52	16.01 ± 0.56	5.82 ± 0.13	0.73 ± 0.04

[a] All values are averages of duplicate analysis

3.6 4-Hydroxy-2-nonenal (HNE) formation

After frying for the first day at all conditions, the amount of HNE increased continuously (**Figure 50)**. The frying oil with the addition of 1 % of oxidized oil produced the largest amount of HNE during the first day of frying and continued this trend until the end of frying. Heating the frying oil alone produced the largest amounts of HNE when compared to oils where starch based materials and protein based materials were fried, however, frying with soy protein and glycine enriched whey protein in oil supplemented with 1 % oxidized oil (OxWG) cause lower rate of HNE formation. The addition of lysine or glucose to starch reduced HNE formation for the first two days of frying but after the third day of frying, the differences were not significant. Fried soy protein produced significantly lower amounts of HNE compared to other proteins with exception of glycine enriched whey protein (WGly) fried at 215 °C. Increasing the oil temperature from 185 °C to 215 °C lowered HNE production when WGly was fried but these differences were only significant after the first day and at the end of frying. In the presence of oxidized oil and during WGly frying, an increased HNE production was observed after second day of frying.

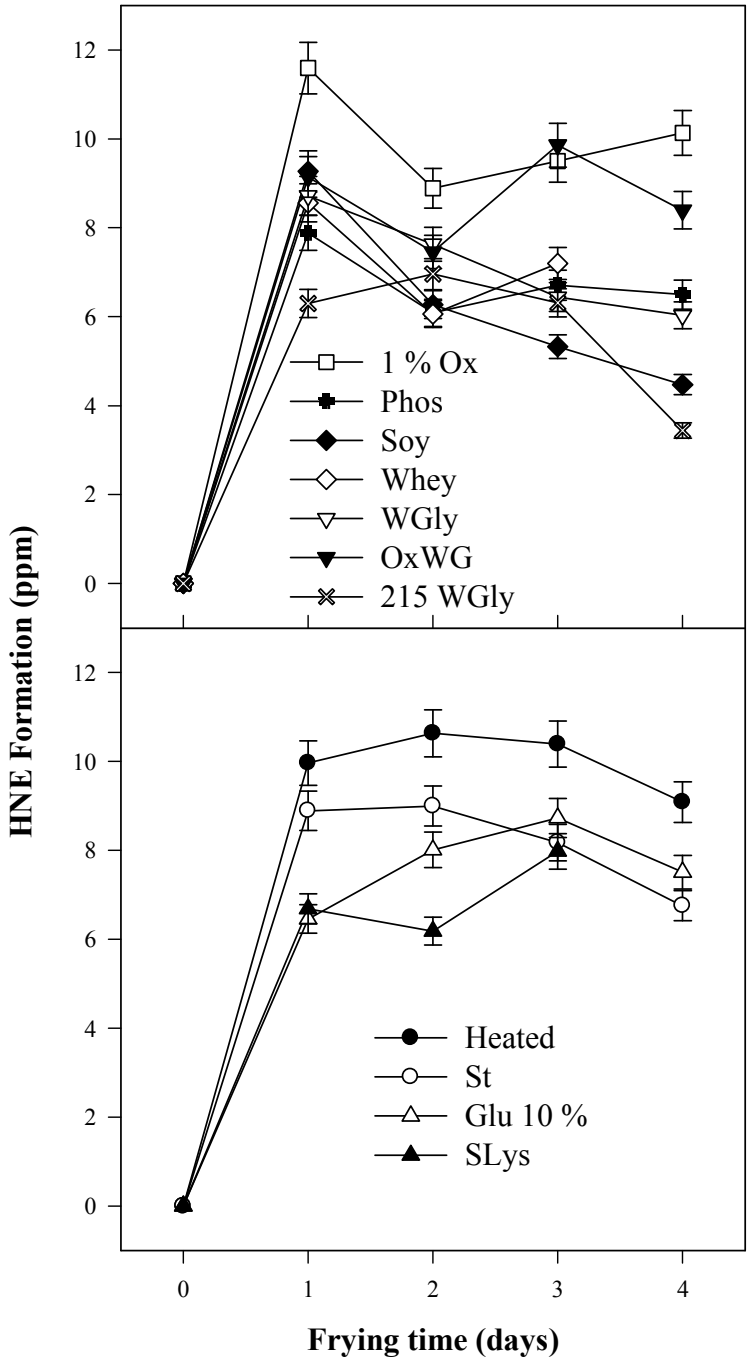


Figure 50: 4-Hydroxy-2-nonenal (HNE) formation during carbohydrates and proteins frying with the addition of oxidized oil and at frying temperature of 215 °C. No products were fried in heated, and oils with added 1 % Ox or Phospholipids. Heated oil is a control. For abbreviations see the materials and methods.

Chapter 4- Discussion

4.1 Food Ingredients

Deep fat frying provides the highest contact between the frying oil and the food being fried in it (Warner, 2004). Frying uses different amounts of products per unit volume of oil: (1) exhaustive industrial frying where tons of products are fried daily and extensive topping up of the oil occurs; (2) moderate restaurant frying where usually the product is fried occasionally during the day but oil is constantly heated and; (3) light or occasional frying at-home where the product is fried sporadically and the oil is heated for frying of food only and between frying sessions it is sitting at room temperature in the fryer. This versatility in deep fat frying techniques defines the extent of frying oil degradation and usually involves many factors. The fried food usually introduces solid particles into the oil which have the same ingredients as food products and these components stimulate typical reactions leading to oxidative degradation, particularly when these components are heated in oil at frying temperature for an extended period of time. One of the most prevalent changes in the frying oil during deep fat frying is formation of pigments, which cause darkening of oil (Paul et al., 1997). Changes in frying oil color are often used as an indicator of frying oil quality. Many operators believe that darkening of oil can be used to determine when the frying oil must be discarded (USDA, 1985; Bansal et al., 2010a). Because of this, the effect of food ingredients causing color changes during frying must be scrutinized.

4.1.1 Carbohydrates

All foods are comprised of the same basic ingredients that together greatly affect oil deterioration and color changes. Studying the behaviour of individual ingredients during the frying is the best way to establish these interactions. Carbohydrates are comprised of simple sugars, the most common being glucose, fructose, lactose and sucrose, and complex carbohydrates, including different types of starches and fibers.

We fried carbohydrate ingredients individually and observed faster oil color changes when compared to heating the frying oil solely at the same frying temperatures. Frying a corn starch product doubled the rate of pigment formation whereas when added to starch, glucose further increased the rate of color changes (**Figure 2**). Simple carbohydrates are the primary precursors in the nonenzymatic browning/Maillard reaction, one of the main processes leading to the pigment formation during frying. The addition of simple carbohydrates provides a higher number of carbonyl groups which are involved in the reactions leading to pigment formation (Tressl et al., 1998a; 1998b). Glucose molecules in starch are connected by glycosidic bonds that occupy the carbonyl groups and render it unavailable for the formation of pigments. The addition of glucose/carbonyl groups to the fried product caused amplification in oil darkening. Lawson (1995) compared carbohydrates contained in breadings and found that the presence of bread crumbs changed the color of frying oil faster than cracker crumbs. These two breadings contained different types of starches and simple sugars at different amounts and both can be directly related to the difference in brown pigment formation. Totani et al. (2006a) found that both wheat starch and bread crumbs contributed to oil color changes but their impact was trivial when compared to other food ingredients.

4.1.2 Proteins and amino acids

The other precursors necessary in nonenzymatic browning are compounds with an accessible amino group. The main sources of amino groups during frying are found in food proteins but they can also become accessible as amino acids. When the concentration of amino acids in the frying material was increased, higher amounts of pigments were also observed (Koga et al., 1997). It was presumed that amino acids with multiple amino groups, such as lysine, would stimulate pigment formation, however, this was not confirmed in our experiments (**Figure 2**). Koga et al. (1997) also found the coloring potential of lysine reduced when compared to other amino acids. Glycine, alanine, leucine, proline, methionine, tryptophan, and cysteine formed the brown pigments at the fastest rate (Koga et al., 1997; Hutapea et al., 2004).

Another important contributor to pigment formation in frying oils are whole protein food components which during frying, are solubilized in the frying oil and are direct precursors used in nonenzymatic browning. The brown pigments are also formed on or in the food product and are solubilized in the oil during frying (Fritsch, 1981). Both whey and soy proteins contributed the most significantly to the oil color compared to carbohydrate fried and heated oils in our study (**Figure 2**). Totani et al (2006a; 2006b) reported that proteins do not contribute to oil color formation. These authors used selected amino acids in small model systems to draw generalized conclusions regarding the effect of proteins on color changes during frying. The whey protein used in the present study caused the fastest and most extensive change in the color of the frying oil (**Figure 2**). One reason for whey protein color stimulating activity can be related to the substantial contribution of the color enhancing amino acids such as glycine, alanine, leucine, proline, methionine, tryptophan and

cysteine which were present at higher concentrations than in soy protein according to the manufacturer's nutrition label. The purity of proteins may also contribute to the difference in color change; whey protein contained 93 % of proteins whereas soy protein had 80 %. Another factor, probably most important, was the size of protein molecules and with it, accessibility and number of amino groups available for reactions. Soy protein contains mostly fractions with 65 and 55 kDa, representing mainly conglycinin (7S) and glycinin (11S) globulins, respectively (Liu, et al., 2008). Whey protein had proteins with 27 and 30 kDa fractions, representing α-lactalbumin and β-lactoglobulin, respectively (Qi and Onwulata, 2011). The size of the protein molecules affects the color of the frying oil in two different ways: (1) smaller protein molecules supply more free amino groups for nonenzymatic browning and pigment formation; (2) proteins with smaller molecular sizes have better solubility in oil, and are therefore able to provide precursors for pigment formation reactions.

Oil degradation indicators such as polar components, polymers, and oligomers have been correlated with the formation of pigments (Fritsch, 1981; Stier, 2001). Previous studies have shown that oil was no longer suitable for frying foods when high molecular weight components amounts exceeded 20 % (Husain et al., 1991) yet others consider oil deteriorated at 16 % (Firestone et al., 1991).

Higher amounts of TPC were formed with addition of free amino acids to the fried product namely glycine and lysine into whey protein (**Figure 3**), thus the addition of amino acids positively affected oil oxidative degradation in agreement with Totani (2006). A lower amount of TPC was formed when lysine was added compared to glycine and is consistent with the antioxidative products formed in the Maillard reaction which protected lipids from

oxidation (Manzocco et al., 2000). Soy protein contains endogenous antioxidants, including isoflavones and saponins, which usually dissolve in the oil and contribute to the reduction of TPC formation (Pokorný and Korczak, 2001).

Brown colored oligomers called melanoidins are produced in reactions between amino groups and carbonyl groups of reducing sugars or carbonyls formed during degradation of oxidized lipids (Choe and Min, 2007). An observed increase in the amount of polymers and colored components during frying proteins and glucose (**Figure 5)** is a direct indication of the formation of melanoidins through these reactions (Zamora and Hidalgo, 2005).

Condensation between the double bonds of oxidized triacylglyceride (OTG) compounds makes them precursors to oligomers and other large molecules (Quaglia and Bucarelli, 2001). The decline of OTG in all frying with proteins (**Figure 6**) can be mostly attributed to degradation and conversion into oligomeric and polymeric compounds (Arroyo et al., 1992).

The addition of glycine to whey protein produced a lower anisidine value in the oil during frying when compared to whey protein alone at the same temperatures (**Figure 7**). The decrease in carbonyl groups can be explained by probable reaction of the amino acid with carbonyl components occurring in the Maillard reaction (Gillatt, 2001).

4.1.3 Endogenous oil ingredients

Nonenzymatic browning is not specifically limited to carbohydrates as other sources of carbonyl groups, such as lipid oxidation products, are also important in pigment formation. In the case of deep fat frying, carbonyls are not the limiting precursor in

nonenzymatic browning because these compounds are continuously formed during oxidation of lipids, the main degradation process happening during frying. The results from this study revealed that the amount of oxidized oil added to the fresh frying oil affected the overall oil color change (**Figure 28**). Because lipid oxidation reactions are autocatalytic and require lipid radicals to continue, higher amounts of oxidized lipids added to frying oil sped up oxidation and precursor formation (Choe and Min, 2006). During frying in canola oil, the crucial importance of intensified oxidative degradation of lipids on the increase in color of frying oil has been shown (Aladedunye and Przybylski, 2009). When frying food in fresh oil supplemented with oxidized oil, the effect of the degraded oil on color changes was seen when only 1 % was added (**Figure 14**). This effect could be explained by the thermal degradation of hydroperoxides present in the oxidized oil to carbonyl compounds, secondary oxidation products, which are known precursors for Maillard reactions (Hidalgo and Zamora, 2000a). A number of frying operations add previously used oil in small percentages to fresh frying oil to achieve darker in color, and more flavorful, fried foods (Lawson, 1995).

Tocopherols are the major natural antioxidants present in oils that offer protection from oxidative degradation through donation of hydrogen atoms to lipid peroxy radicals to interfere with propagation or initiation reactions (Frankel, 2005; Seppanen et al., 2010). Frying oil supplemented with 1 % of oxidized oil exhibited faster degradation rate of tocopherols when compared to control heated oil (**Figure 36**). When oil is heated without frying, usually oxidative degradation increases and causes a faster disappearance of tocopherols (Mäkinen et al., 2001). The reduction of hydroperoxides in oxidized oil before using it as a supplement to frying provided better tocopherol retention as compared to not reducing hydroperoxides. This was probably due to fewer free radicals being present in the

frying oil and less tocopherols were required to quench them. Phospholipids usually act as catalysts to other antioxidants by enhancing access to the chain-initiating radicals and slowing the rate of oxidation and TPC production (**Figure 36)** (Koga and Terao, 1995; Lee and Choe, 2011).

4.1.4 Batter and breading ingredients

The cumulative effect of many food ingredients can result in a faster increase in pigment formation, changing frying oil color faster, and concomitantly more browning reactions are initiated because a more complex mixture of precursors is available at the same time. For example, both glycine enriched starch and glucose enriched starch change oil color faster than starch alone (**Figure 14**). The results indicate that Maillard reaction products formed in the food during frying and dissolved in the oil as precursors react concurrently forming brown pigments with the latter and transferring into frying oil during oil exchange to contribute to oil darkening (Koga et al. 1997; Totani et al., 2006a; 2006b). Fried glycine enriched whey protein in frying oil containing 1 % of oxidized canola oil exhibited higher rate of pigment formation compared to fried glycine and glucose enriched whey protein fried in unsupplemented frying oil. These results indicate that oxidized oil was a better source for carbonyl groups, the main precursor in the Maillard reaction leading to pigment formation, than glucose.

The influence of other food components listed in **Table 1** may also be taken into account when assessing pigment development of breadings and battering ingredients. More intense changes in color results were observed for the mixture of ingredients prepared in the laboratory compared to commercial batters (BM), clearly indicating that lower amounts of

precursors were available in the latter batter mixture (**Figure 14**). Higher amounts of reducing sugars and amino acids in laboratory mixtures contributed to more intense pigment formation and faster change in oil color.

An inverse relationship between TPC formation and tocopherol reduction (**Figure 4**) was observed and is consistent with other studies (Lopez-Varela et al., 1995). Food breading (wheat flour, egg and water) increased the tocopherol retention when compared to frying potatoes slices without breading (Miyagawa et al., 1991). The high content of reducing sugars in potatoes that usually leech into the oil and the lack of coating on the potato slices further stimulated sugar and amino acid amounts available for reaction and decreased the amount of tocopherols as a result of increased oil degradation (Miyagawa et al., 1991).

Amongst the commercial batters and breadings, BM, the only batter mixture tested, was the slowest in pigment formation (**Figure 14**) and was contaminating frying oil with solid food particles (SFP) less extensively than other two commercial breadings (**Figure 27**). The increased oil color intensity by these two breadings is probably caused by higher amounts of sugars available as ingredients and the intensive release of SFP contaminating the oil during frying. The SFP usually stayed in the frying oil for the daily duration of frying at an elevated temperature and as a result of these parameters, stimulated the rate of nonenzymatic browning (Lawson, 1995; Dogan et al., 2005).

4.1.5 Solid food particles (SFP)

In saying this, the addition of SFP in the oil did not significantly contribute to oil color formation unless the SFP were whey or soy protein (**Figure 26** and **Figure 27)**. In frying with breaded and batter ingredients, as little as 2.5 % of the batters or breadings can affect oil color formation if the concentration of proteins and/or amino acids exceeds one-third of the batters or breadings as shown by the laboratory batter mixture color forming ability (**Figure 26** and **Figure 27)**.

4.2 High oleic, low linolenic canola oil

When comparing regular and high oleic, low linolenic acid canola oils, the differences in pigment formation between the oils when frying the same product was dependent upon the product fried (**Figure 8**). For most foods fried, HLCO formed less pigments as compared to RCO with the exception of whey protein. This is an indication that smaller protein molecules are able to access oil easily due to better solubility and be a precursor to pigment formation. Reducing the linolenic acid content in frying oils has been shown to reduce color formation through reduced susceptibility to oxidation and formation of lower amounts of potential precursors (Xu et al., 1999). In this described study, the conclusion is based on frying potato chips, an ingredient containing starch, which confirms results of our study. Because oil darkening patterns are not similar amongst changing fatty acids profiles, the fatty acid differences slowed oxidation rates and thus reducing color formation under the conditions employed (Warner and Mounts, 1993).

Compared to regular canola oil, decreasing the polyunsaturated fatty acid (PUFA) concentration in HLCO should have resulted in formation of smaller amounts of oxidation

products but this expected result was not confirmed in this study (**Figure 9**). A similar distribution in the amounts of TPC were reported by Normand et al. (2001) when frying was done in high oleic, low linolenic and regular canola oils. The significantly slower tocopherol degradation rate in RCO, also observed in the present study, was adduced for the comparative thermo-oxidative stability despite the higher PUFA content compared to HLCO (**Figure 10**). Usually there is an inverse relationship between tocopherol degradation rate and the level of oil unsaturation (Barrera-Arellano, 1999; Verleyen et al., 2001).

Because of the decreased amounts of oxidation prone polyunsaturated fatty acids in the HLCO as compared to RCO, the amount of secondary oxidation products was reduced in HLCO (**Figure 7**). A similar trend was shown during frying using modified frying oils (Matthäus, 2006). This study also observed an increase in anisidine value up to the 30^{th} h of frying French fries which is comparable to the 24^{th} h seen in the present study (**Figure 7**).

The frying performance and chemical changes of different oils are mainly affected by the fatty acid composition of the oil. Cyclization, polymerization, pyrrolic, hydrolytic, oxidation and other chemical reactions during deep frying occur in the oil to change the fatty acid profile (Xu et al., 1999). The degradation of polyunsaturated fatty acids, linoleic and linolenic acids, shown in **Table 5** was consistent with the trends of results describing degradation of the oil and were observed by others (Xu et al., 1999).

Linolenic and linoleic acid content has been shown to affect frying performance, oil stability, and the flavor and overall quality of the fried food. When comparing the two oils, linolenic acid content decreased at a faster rate in RCO in all conditions tested because of the higher concentration of this fatty acid present in it (**Table 6**). Linolenic acid is also the fatty acid most susceptible to oxidation and other types of reactions and will decrease at a faster

rate when compared to other fatty acids (Xu et al., 1999). The observed significant decrease in linoleic acid contents of HLCO and the poor performance compared to RCO during frying of soy and whey proteins in the present study indicated that linoleic acid content is as important as linolenic acid to the overall stability of frying oils. Furthermore, whereas most studies and frying operations using modified oils primarily fry starch based ingredients such as potatoes or potato chips, the results from the present study did suggest that frying protein products in HLCO may negate its supposed superior frying stability.

4.3 Temperature

As oil temperatures were elevated, the intensity of color formation increased for all conditions with a larger increase occurring when temperatures surpassed 200 °C (**Figure 20**). This is consistent with results from other high temperature frying using French fries (Aladedunye and Przybylski, 2009). Glycine enriched whey protein caused the highest rate of color changes during frying at 215 °C (**Figure 20**). The higher frying temperatures accelerated the Maillard reaction rate, a well-known fact in chemical reactions (Benzing-Purdie et al, 1985; Laurendeau, 2005). As we have established, frying temperature is one of the main factors affecting oil color changes and previously was observed during frying in canola, soy and other oils (Pritchard and Adam, 1994; Tyagi and Vasishtha, 1996; Takeoka et al., 1997; Aladedunye and Przybylski, 2009).

Takeoka et al. (1997) reported the temperature dependant formation of TPC and oligomers, confirming findings of this study. Raising the temperature of the frying oil causes almost proportional increase in the amount of polar components (**Figure 21**). A similar pattern of TPC changes was also observed in other studies where elevated frying

temperature was applied (Houhoula et al., 2003; Aladedunye and Przybylski, 2009). Higher temperature equals more energy which speeds up different reactions including hydrolysis, oxidation, and oligomerization, thus increasing TPC formation (Laurendeau, 2005).

Increased amounts of TPC in the frying oil leads to higher surfactant content, lower surface tension, and higher fat absorption by the fried food (Blumenthal, 1991). Thus, an increase in the fat content in fried foods as well as shortened fry-life of oils can be attributed to higher frying temperatures. Since many countries struggle with diets involving high fat consumption and the diseases associated with high fat diets, controlling the frying oil temperature is imperative (Mehta and Swinburn, 2001).

Furthermore, higher temperatures can increase the rate of hydroperoxide decomposition which increases the amount of highly reactive alkoxyl and hydroxyl radicals (Verleyen et al., 2002; Frankel, 2005). As oil temperature increases, so does the rate of oxidation reactions and more free radicals are produced to further deplete the tocopherol contents. The need for more quenching capacity from tocopherols and other antioxidants present in the oil is necessary (**Figure 22**).

According to previous research, a significant increase in anisidine value as a function of temperature has been found during frying of potatoes in cottonseed and canola oils (Houhoula et al., 2002; Aladedunye and Przybylski, 2009). Previous studies have reported reduction in the labile and reactive carbonyl components when elevated temperatures were used for frying; however, oil replenishment will also have effects on anisidine values (Tyagi and Vasishtha, 1996). At higher temperatures, the thermal degradation of aldehydes as well as the carbonyl reactivity with other components in the oil mostly account for the decrease in

anisidine value, an effect more pronounced at the higher frying temperatures (Firestone et al., 1991; Achir et al, 2006).

At higher frying temperatures, the quantity of *trans* isomers formed increased as also found by Aladedunye and Przybylski (2009). These results (**Table 8**) further support the claims that an increase in energy is required for transfer of double bonds from *cis* to *trans* configuration. As the number of *cis* double bonds increases, the activation energy for isomerization decreases due to the increase in methylene interrupted carbons being present in the fatty acid chain. This increase in weaker carbon-hydrogen bonds effectively lowers the activation energy (Augustin et al., 1987).

The increased frying temperature and its effect on *trans* isomer formation can cause issues with nutritional claims regarding zero *trans* content in a serving portion of fried products. The *trans* level in fried food products can be significantly increased through fast exchange of fats between the frying oil and the food during frying due to elevated frying temperatures. According to Dobarganes et al. (2000a), there was a lack of significant differences in the composition between fried products and the frying oils, the amount of *trans* isomers in fried products will increase proportionally with the amount of *trans* isomers in the oil during frying. Claiming the product contains zero *trans* fat could become invalid because as temperature increases beyond regulated frying temperature, the amount of *trans* isomers in fried products may exceed the specified definition limit. A similar fatty acid composition was observed in frying oil and the oil in fried food, even when par-frying was done in different oil, indicating the importance of controlling frying temperature and the recommendation of keeping it below 190 °C (Aladedunye and Przybylski, 2009). After

accounting for analytical error, the differences were so small that the effect of frying materials on polyunsaturated fatty acids and *trans* fatty acids cannot be firmly established.

4.4 Oxidized oil polymer

Polymers adhered on the fryer walls slightly above the oil level, are colored and may also affect color of the oil; however, they are less effective in doing so (**Figure 29**). Oil polymers build up on fryer walls blocks efficient heat transfer throughout the fryer and create "hot spots" in the oil, and locally increased temperature (**Figure 37**) can speed up the oxidation and increase tocopherol usage (Lawson, 1995; Frankel, 2005). Proper cleaning and operating at optimum frying conditions such as temperatures and frying loads can prevent polymer build up on the fryer walls and eliminate additional factors stimulating oil coloration and thermo-oxidative deterioration.

Chapter 5- Conclusions and future research perspectives

Since many frying operations do not limit their fried food products to one type of food ingredient, the understanding of the effect of multiple food groups and minor food components on the frying oil is important. The present study showed that:

1. Proteins caused the largest change in color of the oil as well as the highest levels of oxidative degradation. Thus, protein products can cause an increase in polymerized substances which can lead to increased health issues in an area where deep fried foods are becoming highly popularized.The antioxidant effect of Maillard reaction products expected from frying with proteins was not apparent in this study.
2. When frying batters or breadings that include proteins in the mixture, soy protein would be beneficial to use over whey protein to help extend oil fry-life. Another option would be to include less protein in the mixture or replace it with another ingredient such as starch because it has a minor effect on the oil.
3. The addition of amino acids had a more significant effect on oil degradation than color formation in the frying oil but this was in small concentrations.
4. Maillard reaction precursors, such as carbonyl secondary oxidation products, glucose, and glycine proved to cause significant color change in the frying oil with the effect being greater when starch products were used as a base food material.
5. Breading materials caused the greatest color change and oil deterioration when compared to battering materials most likely due to particles falling into the oil during frying.
6. Since HLCO was only found to be significantly different from RCO in tocopherol degradation and in color at the end of frying in whey protein, not enough evidence

supports that food ingredients have a different effect on the frying stability of HLCO when compared to RCO.

7. As oil temperature increased, both oil color formation and oil deterioration increased, thus, controlling and maintaining appropriate frying temperatures becomes important when prolonging oil fry-life.
8. The Maillard reaction is an important factor affecting oil color. Proper concentrations and type of ingredients in breading and battering will prolong oil fry-life and improve quality of fried food.
9. Utilizing proteins with higher molecular weight will help to reduce darkening of the frying oil and improve quality of foods.
10. Limiting oxidative degradation of frying oil will slow frying oil darkening and at the same time limit the amount of detrimental components found in fried foods.
11. Using standard or lower frying temperature will limit oxidative degradation of oil and food ingredients also lowering the amount of unhealthy compounds.

References

Abidi, S. L. 2000. Chromatographic Analysis of Tocol-Derived Lipid Antioxidants. J. Chromatogr. A. 881, 197–216.

Abidi, S. L. and Mounts, T. L. 1996. Normal Phase High-Performance Liquid Chromatography of Tocopherols in Polar Phases. J. Liq. Chromatogr. Rel. Technol. 19, 509–520.

Achir, N., Kara, W., Chipeaux, C., Trezzani, I., and Cuvelier, M. E. 2006. Effect of energy transfer conditions on the chemical degradation of frying oil. Eur. J. Lipid Sci. Technol. 108, 999–1006.

Ahmad, L., Alaiz, M., Zamora, R., and Hidalgo, F. J. 1998. Effect of oxidized lipid/amino acid reaction products on the antioxidative activity of common antioxidants. J. Agric. Food Chem. 46, 3768-3771.

Alaiz, M., Hidalgo, F. J., and Zamora, R. 1997. Antioxidative activity of nonenzymatically browned proteins produced in oxidized lipid/protein reactions. J. Agric. Food Chem. 45, 1365-1369.

Alaiz, M., Hidalgo, F. J., and Zamora, R. 1998. Effect of initial slight oxidation on stability of polyunsaturated fatty acid/protein mixtures under controlled atmospheres. J. Am. Oil Chem. Soc. 75, 1127-1133.

Aladedunye, F. A., Matthäus, B., Przybylski, R., Zhao, Y. Y., and Curtis J. 2011. Carbon dioxide blanketing impedes the formation of 4-hydroxynonenal and acrylamide during frying. A novel procedure for HNE quantification. Eur. J. Lipid Sci. Technol. 113, 916–923.

Aladedunye, F. A. and Przybylski, R. 2009. Degradation and nutritional quality changes of oil during frying. J. Am. Oil Chem. Soc. 86, 149-156.

Aladedunye, F. A. and Przybylski, R. 2011. Rapid assessment of frying performance using small size samples of oils/fats. J. Am. Oil Chem. Soc. 88, 1867-1873.

American Oil Chemists' Society. 1998. Determination of Polar Compounds in Frying Fats. In: Firestone, D. Official Methods and Recommended Practices of the American Oil Chemists' Society (5th edn). AOCS. Champaign, IL, Method Cd 20–91.

Andia, A. G. and Street, J. C. 1975. Dietary induction of hepatic microsomal enzymes by thermally oxidized fats. J. Agric. Food Chem. 23, 173-177.

Association of Official Analytical Chemists. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists (15th edn). AOAC Inc. Arlington, VA, Method 982.27.

Arnoldi, A., Corain, E. A., Scaglioni, L., and Ames, J. M. 1997. New Colored Compounds from the Maillard Reaction Between Xylose and Lysine. J. Agric. Food Chem. 45, 650-655.

Arroyo, R., Cuesta, C., Garrido-Polonio, C., Lopez-Varela, S. and Sanchez-Muniz, F. S. 1992. High-Performance Size-Exclusion Chromatographic Studies on Polar Components Formed in Sunflower Oil Used for Frying. J. Am. Oil Chem. Soc. 69, 557 – 563.

Artman, N. R. 1969. The Chemical and Biological Properties of Heated and Oxidized Fats. Adv. Lipid Chem. 7, 245–330.

Augustin, E., Monroe, K., Augustin, J., and Tassinari, P. 1989. Total Dietary Fiber in Fried Cereal Products. Cereal Foods World. 34, 545-546.

Augustin, M. A., Asap, T., and Heng, L. K. 1987. Relationships between measurements of fat deterioration during heating and frying in RBD olein. J. Am. Oil Chem. Soc. 64, 1670–1675.

Baixauli, R., Salvador, A., Fiszman, S. M. and Calvo, C. 2002a. Effect of the addition of corn flour and colorants on the color of fried, battered squid rings. Euro. Food Res. Technol. 215, 457-461.

Baixauli, R., Salvador, A., Fiszman, S. M. and Calvo, C. 2002b. Effect of oil degradation during frying on the color of fried, battered squid rings. J. Am. Oil Chem. Soc. 79, 1127–1131.

Baker, A., Zidek, L., Wiesler, D., Chmelik, J., Pagel, M., and Novotny, M. V. 1998. Reaction of N-acetylglycyllysine methyl ester with 2-alkenals: An alternative model for covalent modification of proteins. Chem. Res. Toxicol. 11, 730-740.

Bansal, G., Zhou, W., Barlow, P. J., Joshi, P. S., Lo, H. L., and Chung, Y. K. 2010a. Review of Rapid Tests Available for Measuring the Quality Changes in Frying Oils and Comparison with Standard Methods. Crit. Rev. Food Sci. Nutr. 50, 503–514.

Bansal, G., Zhou, W., Barlow, P. J. Joshi, P. S., Neo, F. L. and Lo, H. L. 2010b. Evaluation of Commercially Available Rapid Test Kits for the Determination of Oil Quality in Deep-Frying Operations. Food Chem. 121, 621–626.

Barrera-Arellano, D., Ruiz-Méndez, V., Márquez Ruiz, G., and Dobarganes, C. Loss of tocopherols and formation of degradation compounds in triacylglycerol model systems heated at high temperature. J. Sci. Food Agr. 79, 1923-1928.

Bendini, A. Cerretani, L., Carrasco-Pancorbo, A., Gomez-Caravaca, A., Segura-Carretero, A., Fernandez-Gutierrez, A., and Lercker, G. 2007. Phenolic Molecules in Virgin

Olive Oils: A Survey of their Sensory Properties, Health Effects, Antioxidant Activity and Analytical Methods. An Overview of the Last Decade. Molecules. 12, 1679–1719.

Bennion, M., Stirk, K. S., and Ball, B. H. 1976. Changes in frying fats with batters containing egg. J. Am. Diet. Assoc. 68, 234-236.

Benzing-Purdie, L.M. and Ratcliffe, C. I. 1986. A study of the Maillard reaction by ^{13}C and ^{15}N CP-MAS NMR: Influence of time, temperature, and reactants on mayor products. Dev. Food Sci. 13, 193-205.

Benzing-Purdie, L. M., Ripmeester, J. A., and Ratcliffe, C. I. 1985. Effects of temperature on Maillard reaction products. J. Agric. Food Chem. 33, 31-33.

Blekas, G. and Boskou, G. 1986. Effect of esterified sterols on the stability of heated oils. In: Charalambous, G. (ed). The shelf life of food and beverages. Elsevier, Amsterdam, pp. 641–645.

Blumenthal, M. M. 1987. Understanding Frying. Monograph Series (2nd edn). Libra Laboratories, Inc., Piscataway, NJ.

Blumenthal, M. M. 1991. A new look at the chemistry and physics of deep-fat frying. Food Technol. 45, 68–94.

Boskou, D. and Morton, I. D. 1976. Effect of plant sterols on the rate of deterioration of heated oils. J. Sci. Food Agric. 27, 928–932.

Breitling-Utzmann, C. M., Unger, A., Friedl, D. A., and Lederer, M. O. 2001. Identification and quantification of phosphatidylethanolamine-derived glucosylamines and aminoketoses from human erythrocytes- Influence of glycation products on lipid peroxidation. Arch. Biochem. Biophys. 391, 245-254.

Brutting, R. and Spiteller, G. 1994. Produkte Der Dimerisierung Ungesattigter Fettsauren. XII: Die Dimerisierung Von Konjuenfettsauren. Fat. Sci. Technol. 96, 445–451.

Buttkus, H. A. 1975. Fluorescent lipid autoxidation products. J. Agric. Food Chem. 23, 823-825.

Cämmerer, B., Jalyschko, W., and Kroh, L. W. 2002. Intact carbohydrate structures as part of the melanoidin skeleton. J. Agric. Food Chem. 50, 2083-2087.

Cämmerer, B. and Kroh, L. W. 1995. Investigation on the influence of reaction conditions on the elementary composition of melanoidins. Food Chem. 53, 55-59.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carreterol, A., Gallina-Toschi, T. and Fernandez-Gutierrez, A. 2005. Analytical Determination of Polyphenols in Olive Oils. J. Separ. Sci. 28, 837–858.

Cert, A., Moreda, W. and Perez-Camino, M. C. 2000. Chromatographic Analysis of Minor Components in Vegetable Oils. J. Chromatogr A. 881, 131–148.

Chang, S. S., Peterson, R. J., and Ho, C. T. 1978. Chemical Reactions Involved in Deep-Fat Frying of Foods. J. Am. Oil Chem. Soc. 55, 718–727.

Chase, G. W., Akoh, C. C. and Eitenmiller, R. R. 1994. Analysis of Tocopherols in Vegetable Oils by High-Performance Liquid Chromatography: Comparison of Fluorescence and Evaporative Light-Scattering Detection. J. Am. Oil Chem. Soc. 71, 877–880.

Chen C. S., Chang, C. Y., and Hsieh, C. J. 2001. Improving the texture and color of fried products. In: Rossell, J. B. (ed). Frying: improving quality. Woodhead Publishing Ltd., Cambridge, UK, pp. 337-358.

Choe, E. and Lee, J. 1998. Thermooxidative stability of soybean oil, beef tallow, and palm oil during frying of steamed noodles. Korean J. Food Sci. Technol. 30, 288–292.

Choe, E. and Min, D. B. 2006. Mechanisms and Factors for Edible Oil Oxidation. Compr. Rev. Food Sci. Food Safety 5, 169–186.

Choe, E. and Min, D. B. 2007. Chemistry of Deep-Fat Frying Oils. J. Food Sci. 72, R77–R86.

Clark, W. L. and Serbia, G. W. 1991. Safety aspects of frying fats and oils. Food Technol. 45, 84–89.

Cuesta, C., Sanchez-Muniz, F. J., Garrido-Polonio, C., Lopez-Varela, S., and Arroyo, R. 1993. Thermooxidative and hydrolytic changes in sunflower oil used in frying with a fast turnover of fresh oil. J. Am. Oil Chem. Soc. 70, 1069–1073.

Dagerskog, M. and Bengtsson, N. E. 1974. Pan frying of meat patties-relationship among crust formation, yield, composition, and processing conditions. Lebensm. Wiss. Technol. 7, 202-207.

Dana, D., Blumenthal, M. M., and Saguy, I. S. 2003. The protective role of water injection on oil quality in deep fat frying conditions. Eur. Food Res. Technol. 217, 104–109.

David, R. O., Bastida, S., Schultz, A., Torre, L. G., Gonzalez-Munoz, F. J., Sanchez-Muniz, M. J. and Benedi, J. 2010. Fasting Status and Thermally Oxidized Sunflower Oil Ingestion Affect the Intestinal Antioxidant Enzyme Activity and Gene Expression of Male Wistar Rats. J. Agric. Food Chem. 58, 2498–2504.

Delgado-Andrade, C., Seiquer, I., Haro, A., Castellano, R., and Navarro, M. P. 2010. Development of the Maillard Reaction in Foods Cooked by Different Techniques. Intake of Maillard-Derived Compounds. Food Chem. 122, 145–153.

DGF (German Society for Fat Research). 2000. Proceedings of the 3rd International Symposium of Deep-Fat Frying – Final Recommendations. Eur. J. Lipid Sci. Technol. 102, 594–594.

Dijkstra, A. J., Christie, W. W., and Knothe, G. 2007. Analysis. In: Gunstone, F. D., Harwood, J. L., and Dijkstra, A. J. (eds). The Lipid Handbook (3rd edn). CRC press, Boca Raton, FL, pp. 415–469.

Dobarganes, M. C. and Marquez-Ruiz, G. 2006. Formation and Analysis of Oxidized Monomeric, Dimeric, and Higher Oligomeric Triglycerides. In: Erickson, M. D. (ed). Deep frying: Chemistry, Nutrition, and Practical Applications (2nd edn). AOCS press, Champaign, IL, pp. 87–110.

Dobarganes, M. C., Marquez-Ruiz, G. and Velasco, J. 2000a. Interaction Between Fat and Food During Deep-Frying. Eur. J. Lipid Sci. Technol. 102, 521–528.

Dobarganes, M. C., Velasco, J. and Dieffenbacher, A. 2000b. Determination of Polar Compounds, Polymerized and Oxidized Triacylglycerols, and Diacylglycerols in Oils and Fats. Pure Appl. Chem. 72, 1563–1575.

Dogan, S. F., Sahin, S., and Sumnu G. 2005. Effects of batters containing different protein types on the quality of deep-fat-fried chicken nuggets. Eur. Food Res. Technol. 220, 502-508.

Dubois, J., van de Voort, F. R., Sedman, J., Ismail, A. A., and Ramaswamy, H. R. 1996. Quantitative Infrared Analysis for Anisidine Value and Aldehydes in Thermally Stressed Oils. J. Am. Oil Chem. Soc. 73, 787–794.

El-Zeany, B. A. and Fattah. L. E. A. 1952. Oxidised lipids-proteins browning reaction. Part 6. Browning produced by the interaction of free fatty acids with proteins. Grasas Aceites. 33, 216-219.

Eskin, N. A. M., McDonald, B. E., Przybylski, R., Malcolmson, L. J., Scarth, R., Mag, T., Ward, K., and Adolph, D. 1996. Canola oil. In: Hui, Y. H. (ed). Bailey's Industrial Oil and Fat Products. Wiley-Interscience Publications, New York, NY, pp. 1–95.

Eskin, N. A. M., Vaisey-Genser, M., Durance-Todd, S., and Przybylski R. 1989. Stability of low linolenic acid canola oil to frying temperatures, J. Am. Oil Chem. Soc. 66, 1081–1084.

Esterbauer, H., Schaur, R. J., and Zollner, H. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biol. Med. 11, 81-128.

Esterbauer, H., Zollner, H., and Schaur, R. J. 1990. Aldehydes formed by lipid peroxidation: Mechanisms of formation, occurrence, and determination. In: Vigo-Pelfrey C. (ed). Membrane lipid oxidation. Vol. I. CRC Press, Boca Raton, FL, pp. 239-268.

Feather, M.S. and Huang, R.D. 1986. Some studies on a Maillard polymer derived from L-alanine and D-glucose. Dev. Food Sci. 13, 183-192.

Fedeli, E. 1988. The behaviour of olive oil during cooking and frying. In: Varela, G., Bender, A. E., and Morton, I. D. (eds). Frying of food: principles, changes, new approaches. VCH Publishers, New York, NY, pp. 52–81.

Firestone, D. 2006. Regulation of Frying Fat and Oil. In: Erickson, M. D. (ed). Deep Frying: Chemistry, Nutrition, and Practical Applications (2nd edn). AOCS press, Champaign, IL, pp. 373–385.

Firestone, D. 2009. Official Methods and Recommended Practices of the American oil chemists' society (6th edn). AOCS, Champaign, IL.

Firestone, D., Stier, R. F., and Blumenthal, M. M. 1991. Regulation of frying fats and oils. Food Technol. 45, 90-94.

Frankel, E. N. 2005. Lipid Oxidation, Oily Press Lipid Library, Vol. 18. Oily Press, Bridgewater, UK.

Frankel, E. N., Warner, K., and Moulton, K. J. 1985. Effects of hydrogenation and additives on cooking-oil performance of soybean oil. J. Am. Oil Chem. Soc. 62, 1354–1358.

Fritsch, C. W. 1981. Measurements of Frying Fat Deterioration: A Brief Review. J. Am. Oil Chem. Soc. 58, 272–274.

Gandemer, G. 1999. Lipid and meat quality: lipolysis, oxidation, Maillard reaction and flavour. Sci. Aliments. 19, 439–458.

Gardner, H. W. 1979. Lipid hydroperoxide reactivity with proteins and amino acids: A review. J. Agric. Food Chem. 27, 220-229.

Garriguet, D. 2006. Overview of Canadians' Eating Habits (Catalogue no. 82-620-MWE). Nutrition: Findings from the Canadian Community Healthy Survey. 2, 1-43.

Gerde, J. A., Hardy, C. L., Hurburgh Jr., C. R. and White, P. J. 2007. Rapid Determination of Degradation in Frying Oils with Near-Infrared Spectroscopy. J. Am. Oil Chem. Soc. 84, 519–522.

Gertz, C. 2000. Chemical and Physical Parameters as Quality Indicators of Used Frying Fats. Eur. J. Lipid Sci. Technol. 102, 566-572.

Gertz, C. 2001. Determination of Polymerized (Dimeric and Oligomeric) Triglycerides Content at Low Level. Eur. J. Lipid Sci. Technol. 103, 181–184.

Gertz, C. and Matthäus, B. 2008. Optimum Deep-Frying. Recommendations by the German Society for Fat Science (DGF, Deutsche gesellschaft für Fettwissenschaft e.V.). pp. 6.

Ghidurus, M., Turtoi, M., Boskou, G., Niculita, P., and Stan, S. 2010. Nutritional and health aspects related to frying (I). Rom. Biotechnol. Lett. 15, 5675–5682.

Ghidurus, M., Turtoi, M., Boskou, G., Niculita, P., and Stan, S. 2011. Nutritional and health aspects related to frying (II). Rom. Biotechnol. Lett. 16, 6467–6472.

Gillatt, P. 2001. Flavour and aroma development in frying and fried foods. In: Rossell, J. B. (ed). Frying: Improving quality. CRC Press, Boca Raton, FL, pp. 266-336.

Gómez-Sánchez, A., Hermosín, I., and Maya, I. 1990. Heterocycle formation from malondialdehyde and amino sugars. In: Finot, P. A., Aesbacher, H. U., Hurrell, R. F. and Liardon R. (eds). The Maillard reaction in food processing, human nutrition and physiology. Birkhäuser Verlag, Basel, Switzerland, pp. 139-144.

Gordon, M. H. and Kourimska, L. 1995a. The effects of antioxidants on changes in oils during heating and deep frying. J. Sci. Food Agric. 68, 347–353.

Gordon, M. H. and Kourimska, L. 1995b. Effect of Antioxidants on Losses of Tocopherols During Deep-Fat Frying. Food Chem. 52, 175–177.

Gordon, M. H. and Magos, P. 1984. The effect of sterols on the oxidation of edible oils. Food Chem. 10, 141–147.

Gregory, J. F. 1996. Vitamins. In: Fennema, O. R. (ed). Food Chemistry (3rd edn). Marcel Dekker, New York, NY, pp. 531-616.

Gueraud, F., Atalay, M., Bresgen, N., Cipak, A., Eckl, P. M., Huc, L., Jouanin, I., Siems, W. and Uchida, K. 2010. Chemistry and Biochemistry of Lipid Peroxidation Products. Free Rad. Res. 44, 1098–1124.

Gupta, M. J. 2004. The effect of oil processing on frying oil stability. In: Gupta, M.K., Warner, K., and White, P. J. (eds). Frying technology and practice. AOCS, Champaign, IL, pp. 76–90.

Gutierrez, R., Quijano, G., and Dobarganes, M. C. 1988. Analytical Procedures for the Evaluation of Used Frying Fats. In: Varela, G., Bender, A. E., and Morton, I. D (eds). Frying of Food: Principles, Changes, New Approaches. Ellis Harwood Ltd., Chicester, UK, pp. 141–154.

Hashiba, H. 1986. Oxidative browning of Amadori compounds. Color formation by iron with Maillard reaction products. Dev. Food Sci. 13, 155-164.

Handwerk, R. L. and Coleman, R. L. 1988. Approaches to the citrus browning problem. A review. J. Agric. Food Chem. 36, 231-236.

Hein, M., Henning, H. and Isengard, H. D. 1998. Determination of Total Polar Parts with New Methods for the Quality Survey of Frying Fats and Oils. Talanta. 47, 447–454.

Henry, C. J. K. 1998. Impact of fried foods on macronutrient intake, with special reference to fat and protein. Grasas Aceites 49, 336–339.

Hidalgo, F. J., Alaiz, M., and Zamora, R. 1998. A spectrophotometric method for the determination of proteins damaged by oxidized lipids. Anal. Biochem. 262, 129-136.

Hidalgo, F. J., Alaiz, M., and Zamora, R. 2001. Pyrrolization and antioxidant function of proteins following oxidative stress. Chem. Res. Toxicol. 14, 582-588.

Hidalgo, F. J. and Zamora, R. 1993. Fluorescent pyrrole products from carbonyl-amine reactions. J. Biol. Chem. 268, 16190-16197.

Hidalgo, F. J. and Zamora, R. 1995a. Characterization of the products formed during microwave irradiation of the nonenzymatic browning lysine/(*E*)-4,5-epoxy-(*E*)-2-heptenal model system. J. Agric. Food Chem. 43, 1023-l028.

Hidalgo, F. J. and Zamora, R. 1995b. In vitro production of long chain pyrrole fatty esters from carbonyl-amine reactions. J. Lipid Res. 36, 725-735.

Hidalgo, F. J. and Zamora, R. 2000a. The role of lipids in nonenzymatic browning. Grasas Aceites. 51, 37–51.

Hidalgo, F. J. and Zamora, R, 2000b. Modification of bovine serum albumin structure following reaction with 4,5(*E*)-epoxy-2(*E*)-heptenal. Chem. Res. Toxicol. 13, 501-508.

Hidalgo, F. J. and Zamora, R. 2004. Strecker-type degradation produced by the lipid oxidation products 4,5-epoxy-2-alkenals. J. Agric. Food Chem. 52, 7126–7131.

Hidalgo, F. J. and Zamora, R. 2005. Interplay between the Maillard Reaction and Lipid Peroxidation in Biochemical systems. Ann. N. Y. Acad. Sci. 1043, 319-326.

Ho, C. T. 1996. Thermal generation of Maillard aromas. In: Ikan, R. (ed). The Maillard reaction: Consequences for the chemical and life sciences. Wiley, Chichester, UK, pp. 27-53.

Holownia, K. I., Chinnin, M. S., Erickson, M. C., and Mallikarjunan, P. 2000. Quality evaluation of edible film-coated chicken strips and frying oils. J. Food Sci. 65, 1087–1090.

Houhoula, D. P., Oreopoulou, V., and Tzia, C. 2002. A kinetic study of oil deterioration during frying and a comparison with heating. J. Am. Oil Chem. Soc. 79, 133–137.

Houhoula, D. P., Oreopoulou, V. and Tzia, C. 2003. The Effect of Process Time and Temperature on the Accumulation of Polar Compounds in Cottonseed Oil During Deep-Fat Frying. J. Sci. Food Agric. 83, 314–319.

Huang, R. D, and Feather, M. S. 1988. ^{13}C NMR study of some Maillard reaction products arising from D-glucose-DL-alanine interactions. J. Agric Food Chem. 36, 673-676.

Hukuie, S., Yamano, Y., and Yamaguchi, S. 1994. Oishisa no kagaku. Asakura Shoten, Tokyo, JP, pp. 69-79.

Husain, S., Sastry, G. S. R. , and Prasada, R. N. 1991. Molecular weight averages as criteria for quality assessment of heated oils and fats. J. Am. Oil Chem. Soc. 68, 822-826.

Hutapea, E. B., Parkanyiova, L., Parkanyiova, J., Miyahara, M., Sakurai, H., and Pokorný, J. 2004. Browning reactions between oxidised vegetable oils and amino acids. Czech. J. Food Sci. 22, 99-107.

Institute of Medicine. 2005. Macronutrients and Healthful Diets. In: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. National Academy Press, Washington, D.C. pp. 769-879.

International Organization for Standardization. 2000. Animal and Vegetable Fats and Oils - Preparation of Methyl Esters of Fatty Acids, ISO, Geneva, Standard No. 5509.

International Organization for Standardization. 2004. Animal and Vegetable Fats and Oils - Determination of Anisidine Value, ISO, Geneva, Standard No. 6885.

International Organization for Standardization. 2007. Animal and Vegetable Fats and Oils - Determination of Polymerized Triglycerides Content by High-Performance Size-Exclusion Chromatography (HPSEC), ISO, Geneva, Standard No. 16931.

International Organization for Standardization. 2008. Animal and vegetable fats and oils -- Determination of peroxide value -- Potentiometric end-point determination, ISO, Geneva, Standard No. 27107.

International Union of Pure and Applied Chemistry. 1992. Standard Methods for the Analysis of Fats, Oils and Derivatives (7th edn). Blackwell Scientific Publications, Oxford, UK.

Itakura, K., Uchida, K., and Osawa, T. 1996. A novel fluorescent malondialdehyde-lysine adduct. Chem. Phys. Lipids. 84, 75-79.

Ivory, J. E. 1999. Typical Seasoning Formulations. In: Underriner, E. W. and Hume, I. R. (eds). Handbook of Industrial Seasonings. Aspen Publishers, Inc., Gaithersburg, ML, pp. 62-79.

Jacobson, G. A. 1991. Quality control in deep-fat frying operations. Food Technol. 45, 72-74.

Jensen, S. K. and Lauridsen, C. 2007. Alpha-Tocopherol Stereoisomers. Vitam. Horm. 76, 281–308.

Kamal-Eldin, A., Gorgen, S., Pettersson, J. and Lampi, A.M. 2000. Normal-Phase High-Performance Liquid Chromatography of Tocopherols and Tocotrienols. Comparison of Different Chromatographic Columns. J. Chromatogr A 881, 217–227.

Karel, M., Schaich, K., and Roy, R. B. 1975. Interaction of peroxidizing methyl linoleate with some proteins and amino acids. J. Agric. Food Chem. 23, 159-163.

Kato, H., Kim, S. B., and Hayase, F. 1986. Estimation of the partial chemical structure of melanoidins by oxidative degradation and ^{13}C CP-MAS NMR. Dev. Food Sci. 13, 215-223.

Kato, H. and Tsuchida, H. 1981. Estimation of melanoidin structure by pyrolysis and oxidation. Prog. Food Nutr. Sci. 5, 147-156.

Khayat, A, and Schwall, D. 1983. Lipid oxidation in seafood. Food Technol. 37, 130-140.

Kikugawa, K., and Ido, Y. 1984. Studies on peroxidized lipids. V. Formation and characterization of 1,4-dihydropyridine-3,5-dicarbaldehydes as model of fluorescent components in lipofuscin. Lipids. 19, 600-608.

Kikugawa, K., Kato, T., Beppu, M., and Hayasaka, A. 1990. Fluorescent and cross-linked proteins formed by free radical and aldehyde species generated during lipid oxidation. In: Porta, E. A. (ed). Lipofuscin and ceroid pigments. Plenum Press, New York, NY, pp. 345-357.

Kim, I. and Choe, E. 2003. Effects of red ginseng extract added to dough on the lipid oxidation of frying oil and fried dough during frying and storage. Food Sci. Biotechnol. 12, 67–71.

Kim, I. and Choe, E. 2004. Oxidative stability and antioxidant content changes in roasted and bleached sesame oil during heating. Food Sci. Biotechnol. 13, 762–767.

Kim, I.-H., Kim, C.-J., and Kim, D.-H. 1999. Physicochemical properties of methyl linoleate oxidized at various temperatures. Korean J. Food Sci. Technol. 31, 600–605.

Kochhar, S. P. 2001. The composition of frying oils. In: Rossell, J. B. (ed). Frying: Improving quality. CRC Press, Boca Raton, FL, pp. 87-114.

Kochhar, S. P. and Gertz, C. 2004. New theoretical and practical aspects of the frying process. Eur. J. Lipid Sci. Technol. 106, 722–727.

Koga, H., Tone, N., Muramoto, N., Sakurai, H., and Katayama, O. 1997. The relation between the coloration of frying oil and the deterioration using fried model-materials. J. Jpn. Soc. Food Sci. Technol. 44, 666-670.

Koga, T. and Terao, J. 1995. Phospholipids Increase Radical-Scavenging Activity of Vitamin E in a Bulk Oil Model System. J. Agric. Food Chem. 43, 1450–1454.

Kramer, J.K.G., Blais, L., Fouchard, R.C., Melnyk, R.A. and Kallury, M.R. 1997. A Rapid Method for the Determination of Vitamin E Forms in Tissues and Diets by High-Performance Liquid Chromatography Using Normal-Phase Diol Column. Lipids 32, 323–330.

Kroh, L. and Westphat, W. 1983. Gezieite Reaktionen von Amadori-Verbindungen. Z. Chem. 23, 293-294.

Ladislav, F., Vera, P., Karel, S. and Karel, V. 2005. Reliability of Carotenoid Analyses: A Review. Curr. Anal. Chem. 1, 93–102.

Lalas, S., Gortzi, O., and Tsaknis J. 2006. Frying Stability of Moringa Stenopetala Seed Oil. Plant Foods Hum. Nutr. 61, 99–108.

Landers, R. E. and Rathmann, D. M. 1981. Vegetable-oils—effects of processing, storage and use on nutritional values. J. Am. Oil Chem. Soc. 58, 255–259.

Lang, J., Celotto, C., and Esterbauer, H. 1985. Quantitative Determination of the Lipid Peroxidation Product 4-Hydroxynonenal by High Performance Liquid Chromatography. Anal. Biochem. 150, 369–378.

Lang, K. 1973. The physiological effects of heated fats, especially the cooking oils. Fett. Wiss. Technol. 75, 73–76.

Lang, K. 1974. Nutritional physiology of heated fats: Long-term animal studies. Fett. Wiss. Technol. 76, 145-151.

Lang, K., Billek, G., Fuhr, J., Henschel, J., Von Jan, E., Kracht, J., Scharmann, H., Strauss, H.J., Unbehend, M., and Waibel, J. 1978. Nutritional characteristics of frying fats. Z. Ernahrungswiss. 21, 1–61.

Laurendeau, N. M. 2005. Statistical thermodynamics: fundamentals and applications. Cambridge University Press. pp. 434.

Lawson, H. 1995. Deep Fat Frying. In: Food oils and fats: technology, utilization, and nutrition. Chapman & Hall, New York, NY, pp. 66-115.

Ledl, F., and Severin, T. H. 1981. Investigation of a xilose- and glucose-methylammonium acetate reaction mixture. Prog. Food Nutr. Sci. 5, 65-69.

Lee, J. and Choe, E. 2011. Effects of phospholipids on the antioxidant activity of α-tocopherol in the singlet oxygen oxidation of canola oil. New Biotechnol. 28, 691-697.

Lee, J., Kim, M., Park, K., and Choe, E. 2003. Lipid oxidation and carotenoids content in frying oil and fried dough containing carrot powder. J. Food Sci. 68, 1248–1253.

Lee, J., Lee, S., Lee, H., Park, K., and Choe, E. 2002. Spinach (*Spinacia oleracea*) powder as a natural food-grade antioxidant in deep-fat-fried products. J. Agric. Food Chem. 50, 5664–5669.

Lento, Jr., H. G. Underwood, J. C., and Willits, C. O. 1958. Browning of Sugar Solutions. II. Effect of the Position of Amino Group in the Acid Molecule in Dilute Glucose Solutions. J. Food Sci. 23, 68-71.

Leong, X. F., Aishah, A., Nor, A. U., Das, S. and Jaarin, K. 2008. Heated Palm Oil Causes Rise in Blood Pressure and Cardiac Changes in Heart Muscle in Experimental Rats. Arch. Med. Res. 39, 567–572.

Leong, X. F., Mohd Najib, M. N., Das, S., Mustafa, M. R. and Jaarin, K. 2009. Intake of Repeatedly Heated Palm Oil Causes Elevation in Blood Pressure with Impaired Vasorelaxation in Rats. Tohoku J. Exp. Med. 219, 71–78.

Leong, X. F., Mustafa, M. R., Das, S. and Jaarin, K. 2010. Association of Elevated Blood Pressure and Impaired Vasorelaxation in Experimental Sprague-Dawley Rats Fed with Heated Vegetable Oil. Lipids Health Dis. 9, 1–10.

Liu, C., Wang, X., Ma, H., Zhang, Z., Gao, W., and Xiao, L. 2008. Functional properties of protein isolates from soybeans stored under various conditions. Food Chem. 111, 29-37.

Liu, E. H., Qi, L. W., Ping Li, J. C., Li, C. Y., and Peng, Y. B. 2008. Advances of Modern Chromatographic and Electrophoretic Methods in Separation and Analysis of Flavonoids. Molecules. 13, 2521–2544.

Lopez-Varela, S., Sanchez-Muniz, F. J., Garrido-Polonio, C., Arroyo, R., and Cuesta, C. 1995. Relationship between chemical and physical indexes and column HPSE chromatography methods for evaluating frying oil. Z. Ernahrungswiss. 34, 308–313.

Mäkinen, M., Kamal-Eldin, A., Lampi, A. M., and Hopia A. 2001. α, γ, δ-tocopherols as inhibitors of isomerization and decomposition of *cis, trans* methyl linoleate hydroperoxides. Eur. J. Lipid Sci. Technol. 103, 286–291.

Malcolmson, L. J., Vaisey-Genser, M., Przybylski, R., Ryland, D., Eskin, N. A. M. and Armstrong, L. 1996. Characterization of Stored Regular and Low-Linolenic Canola Oils at Different Levels of Consumer Acceptance. J. Am. Oil Chem. Soc. 73, 1153–1160.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., and Lerici, C. R. 2000. Review of non-enzymatic browning and antioxidant capacity in processed foods. Trends Food Sci. Tech. 11, 340-346.

Marmesat, S., Velasco, J., Marquez-Ruiz, G., and Dobarganes, M. C. 2007. A Rapid Method for Determination of Polar Compounds in Used Frying Fats and Oils. Grasas Aceites. 58, 189–183.

Marquez-Ruiz, G., and Dobarganes, M. C. 2006. Nutritional and Physiological Effects of Used Frying Oils and Fats. In Erickson, M. D. (ed). Deep frying: Chemistry, Nutrition, and Practical Applications (2nd edn). AOCS press, Champaign, IL, pp. 173–204.

Matthäus, B. 2006. Utilization of high-oleic rapeseed oil for deep-fat frying of French fries compared to other commonly used edible oils. Eur. J. Lipid Sci. Technol. 108, 200–211.

Mazza, G. and Qi, H. 1992. Effect of after-cooking darkening inhibitors on stability of frying oil and quality of French fries. J. Am. Oil Chem. Soc. 69, 847–853.

Mehta, U. and Swinburn, B. 2001. A Review of Factors Affecting Fat Absorption in Hot Chips. Crit. Rev. Food Sci. Nutr. 41, 133–154.

Melton, S. L., Jafar, S., Sykes, D. and Trigiano, M. K. 1994. Review of Stability Measurements for Frying Oils and Fried Food Flavor. J. Am. Oil Chem. Soc. 71, 1301–1308.

Min, D. B. and Boff, J. M. 2002. Lipid Oxidation of Edible Oil. In: Akoh, C. C. and Min, D. B. (eds). Food Lipids: Chemistry, Nutrition and Biotechnology. Marcel Dekker, New York, NY, pp. 335–363.

Min, D. B, Lins, S. S., and Chang, S. S. 1975. Study of Color Stability of Tall Oil Fatty-Acids Through Isolation and Characterization of Minor Constituents. J. Am. Oil Chem. Soc. 52, 28–33.

Miyagawa, K., Hirai, K., and Takezoe, R. 1991. Tocopherol and fluorescence levels in deep-frying oil and their measurement for oil assessment. J. Am. Oil Chem. Soc. 68, 163-166.

Moreira, R. G., Castell-Perez, M. E., and Barrufet, M. A. 1999. Frying oil characteristics. In: Deep fat frying; fundamentals and applications, Chapman & Hall, Gaithersburg, MD, pp 33–74.

Nair, V., Offerman, R. J., and Turner, G. A. 1986. Novel fluorescent 1,4-dihydropyridines. J. Am. Oil Chem. Soc. 108, 8283-8285.

Nair, V., Offerman, R. J., Turner, G. A., Pryor, A. N., and Baenziger, N. C. 1988. Fluorescent 1,4-dihydropyridines: the malondialdehyde connection. Tetrahedron. 44, 2793-2803.

Namiki, M. 1988. Chemistry of Maillard reactions: Recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. Adv. Food Res. 32, 115-184.

Nawar, W. W. 1969. Thermal Degradation of Lipids. A Review. J. Agric. Food Chem. 17, 18–21.

Nielsen, P. A., Vahl, M., and Gry, J. 1988. HPLC Profiles of Mutagens in Lean Ground Pork Fried at Different Temperatures. Z. Lebensm. Unters. For. 187, 451-456.

Niki, E. 2009. Lipid Peroxidation: Physiological Levels and Dual Biological Effects. Free Radic. Biol. Med. 47, 469–484.

Normand, L., Eskin, N. A. M. and Przybylski, R. 2001. Effect of Tocopherols on the Frying Stability of Regular and Modified Canola Oils. J. Am. Oil Chem. Soc. 78, 369–373.

Olsson, K., Pememalm, F. A., and Theander, O. 1981. Reaction products and mechanism in some simple model systems. Progr. Food Nutr. Sci. 5, 47-55.

Omata, Y. 1986. Oishisa to Mikaku no Kagaku. In: Popular Science Books. Nihon Kogyo Shinbunsha, Tokyo, JP, pp. 230-249.

Orthoefer, F. T. 1988. Care of Food Service Frying Oils. J. Am. Oil Chem. Soc. 65, 1417–1419.

Orthoefer, F. T. and List, G. R. 2006a. Dynamics of Frying. In: Erickson, M. D. (ed). Deep Frying: Chemistry, Nutrition, and Practical Applications (2nd edn). AOCS press, Champaign, IL, pp. 253–275.

Orthoefer, F. T. and List, G.R. 2006b. Evaluation of Used Frying Oil. In: Erickson, M. D. (ed). Deep Frying: Chemistry, Nutrition, and Practical Applications (2nd edn). AOCS press, Champaign, IL, pp. 329–342.

Ottaviani, P., Graille, J., Perfetti, P., and Naudet, M. 1979. Products of Thermal and Oxidative Reactions of Heated Oil .2. Nonpolar or Slightly Polar Compounds. Chem. Phys. Lipids 24, 57–77.

Paradis, A. J., and Nawar, W. W. 1981. Evaluation of New Methods for the Assessment of Used Frying Oils. J. Food Sci. 46, 449–451.

Paul, S. Mittal, G. S., and Chinnan, M. S. 1997. Regulating the Use of Degraded Oil/Fat in Deep-Fat/Oil Food Frying. Crit. Rev. Food Sci. Nutr. 37, 635–662.

Peers, K. E. and Swoboda, P. A. T. 1982. Deterioration of sunflower seed oil under simulated frying conditions and during small-scale frying of potato chips. J. Sci. Food Agric. 33, 389–395.

Pérez-Camino, M. C., Márquez-Ruíz, G., Ruíz-Méndez, M. V. and Dobarganes, M. C. 1991. Lipid changes during the frying of frozen prefried foods. J. Food Sci. 56, 1644–1647.

Perkins, E. G. 2006. Volatile Odor and Flavor Components Formed in Deep Frying. In: Erickson, M. D. (ed). Deep Frying: Chemistry, Nutrition, and Practical Applications (2nd edn). AOCS press, Champaign, IL, pp. 51-56.

Piironen, V., Varo, P., Syvaoja, E.L., Salminen, K. and Koivistoinen, P. 1984. High-Performance Liquid Chromatography Determination of Tocopherols and Tocotrienols and its Application to Diets and Plasma of Finnish Men. Internat. J. Vit. Nutr. Res. 54, 35–40.

Pocklington, W.D. and Diefenbacker, A. 1988. Determination of Tocopherols and Tocotrienols in Vegetable Oils and Fats by High-Performance Liquid Chromatography. Results of a Collaborative Study and the Standardized Methods. Pure & Appl. Chem. 60, 877–892.

Pokorný, J. 1989. Flavor Chemistry of Deep Fat Frying in Oil. In: Min, D. B. and Smouse, T. H. (eds). Flavor Chemistry of Lipid Foods. AOCS, Champaign, IL, pp. 113–155.

Pokorný, J. 1998. Substrate influence on the frying process. Grasas Aceites. 49, 265-270.

Pokorný, J. 1999. Changes of Nutrients at Frying Temperatures. In: Boskou, D. and Elmadfa, I. (eds). Frying of Food: Oxidation, Nutrient, and Non-Nutrient Antioxidants, Biologically Active Compounds and High Temperatures. CRC Press, Boca Raton, FL, pp. 69–104.

Pokorný, J. and Janicek, G. 1975. Interactions between Proteins and Oxidized Lipids. Nahrung. 19, 911-920.

Pokorný J. and Korczak J. 2001. Preparation of natural antioxidants. In: Yanishlieva, N., Pokorný, J., and Gordon, M. (eds). Antioxidants in food: practical applications. Woodhead Publishing Limited, Cambridge, UK, pp. 311-330.

Pokorný, J. and Reblova, Z. 1999. Effect of Food Components on Changes in Frying Oil. Food Technol. Biotechnol. 37, 139-143.

Pritchard, M. K. and Adam, L. R. 1994. Relationship between fry color and sugar concentration in stored russet burbank and shepody potatoes. Am. Potato J. 71, 59–68.

Qi, P. X. and Onwulata, C. I. 2011. Physical properties, molecular structures, and protein quality of texturized whey protein isolate: Effect of extrusion moisture content. J. Dairy Sci. 94, 2231-2244.

Quaglia, G. B. and Bucarelli, F. M. 2001. Effective process control in frying. In: Rossell, J. B. (eds). Frying: improving quality. Woodhead Publishing Limited, Cambridge, UK, pp. 236-265.

Rammell, C.G. and Hoogenboom, J.J.L. 1985. Separation of Tocols by HPLC on an Amino-Cyano Polar Phase Column. J. Liquid Chromatogr. 8, 707–717.

Roe, M. A. and Faulks, R. M. 1991. Color development in a model system during frying: Role of individual amino acids and sugars. J. Food Sci. 56, 1711-1713.

Romero, A., Cuesta, C. and Sanchez-Muniz., F. J. 1998. Effect of Oil Replenishment During Deep-Fat Frying of Frozen Foods in Sunflower Oil and High-Oleic Acid Sunflower Oil. J. Am. Oil Chem. Soc. 75, 161–167.

Ruperez, F. J., Martin, D., Herrera, E. and Barbas, C. 2001. Chromatographic Analysis of α-Tocopherol and Related Compounds in Various Matrices. J. Chromatogr A. 935, 45–69.

Salvador, A., Sanz, T., and Fiszman, S. M. 2005. Effect of the addition of different ingredients on the characteristics of a batter coating for fried seafood prepared without a pre-frying step. Food Hydrocolloid. 70, 235–240

Sanchez-Muniz, F. J., Cuesta, C., and Garrido-Polonio, C. 1993a. Sunflower oil used for frying: combination of column, gas and high-performance size-exclusion chromatography for its evaluation. J. Am. Oil Chem. Soc. 70, 235–240.

Sanchez-Muniz, F. J., Cuesta, C., Lopez-Varela, M. C., Garrido-Polonio, M. C., and Arroyo, R. 1993b. Evaluation of the thermal oxidation rate of sunflower oil using various frying methods. In: Applewhite, T. H. (ed). Proceedings of World Conference on Oilseed and Technology and Utilization. AOCS, Champaign, IL, pp. 448–452.

Sanchez-Muniz, F. J., Viejo, J. M., and Medina, R. 1992. Deep-frying of sardines in different culinary fats. Changes in the fatty acid composition of sardines and frying fats. J. Agric. Food Chem. 40, 2252-2256.

Sasiela, R. J. 2004. Technology of Coating and Frying Food Products. In: Gupta, M. K., Warner, K. and White, P. J. (eds). Frying Technology and Practices. AOCS, Champaign, IL, pp. 125–155.

Schulte, E. 2004. Economical Micromethod for Determination of Polar Components in Frying Fats. Eur. J. Lipid Sci. Tech. 106, 772–776.

Seppanen, C. M., Song, Q. and Csallany, A. S. 2010. The Antioxidant Functions of Tocopherol and Tocotrienol Homologues in Oils, Fats, and Food System. J. Am. Oil Chem. Soc. 87, 469–481.

Spickett, C. M., Wiswedel, I., Siems, W., Zarkovic, K., and Zarkovic, N. 2010. Advances in Methods for the Determination of Biologically Relevant Lipid Peroxidation Products. Free Rad. Res. 44, 1172–1202.

St. Angelo, A. J. 1996. Lipid Oxidation in Foods. Crit. Rev. Food Sci. Nutr. 36, 175–224.

Stier, R. F. 2001. The measurement of frying oil quality and authenticity. In: Rossell, J. B. (ed). Frying: improving quality. Woodhead Publishing Limited, Cambridge, UK, pp. 165-193.

Sullivan, D. M. and Smith, R. L. 1985. Determination of sulfites in foods by ion chromatography. Food Technol. 39, 45-53.

Sun, X. and Moreira, R. G. 1996. Correlation Between NMR Proton Relaxation Time and Free Fatty Acids and Total Polar Materials of Degraded Soybean Oils. J. Food Process Pres. 20, 157–167.

Surh, J. and Kwon, H. 2002. Quantification of 4-Hydroxy-2-Alkenals in Oils Consumed in Korea. Korean J. Food Sci. Technol. 34, 905–910.

Szabo, A., Bazar, G., Andrassy-Baka, G., Locsmandi, L. and Romvari, R. 2009. A Near Infrared Spectroscopic (NIR) Approach to Estimate Quality Alterations During Prolonged Heating of Lard. Acta Aliment. 38, 97–106.

Takeoka, G. R., Full, G. H., and Dao, L.T. 1997. Effect of heating on the characteristics and chemical composition of selected frying oils and fats. J. Agric. Food Chem. 45, 3244-3249.

Tamura, H. and Shibamoto, T. 1991. Gas Chromatographic Analysis of Malonaldehyde and 4-Hydroxy-2-(E)-Nonenal Produced from Arachidonic Acid and Linoleic Acid in a Lipid Peroxidation Model System. Lipids. 26, 170–173.

Tan, C. P. and Che Man, Y. B. 1999. Quantitative Differential Scanning Calorimetric Analysis for Determining Total Polar Compounds in Heated Oils. J. Am. Oil Chem. Soc. 76, 1047–1057.

Tarascou, I., Souquet, J. M., Mazauric, J. P., Carrillo, S., Coq, S., Canon, F., Fulcrand, H. and Cheynier, V. 2010. The Hidden Face of Food Phenolic Composition. Arch. Biochem. Biophys. 501, 16–22.

Taylor, S. L., Higley, N. A., and Bush, R. K. 1986. Sulfites in foods: uses, analytical methods, residues, fate, exposure assessment metabolism, toxicology, and hyper sensitivity. Adv. Food Res. 30, 1-76.

Tomioka, F. and Kaneda, M. 1976a. Browning of Phospholipids by Heating. Kagaku to Seibutsu. 14, 509-511.

Tomioka, F. and Kaneda, M. 1976b. Studies on the Brown Discoloration of Heated Phospholipids II. Yukagaku. 23, 782-786.

Tompkins, C. and Perkins, E. G. 2000. Frying performance of low-linolenic acid soybean oil. J. Am. Oil Chem. Soc. 77, 223–229.

Totani, N. 2006. A Small Reduction in Atmospheric Oxygen Decreases Thermal Deterioration of Oil during Frying. J. Oleo Sci. 55, 135-141.

Totani, N., Kuzume, T., Yamaguchi, A., Takada, M., and Moriya, M. 2006a. Amino acids brown oil during frying. J. Oleo Sci. 55, 441-447.

Totani, N., Yamaguchi, A., Takada, M., and Moriya, M. 2006b. Color deterioration of oil during frying. J. Oleo Sci. 55, 51-57.

Toth-Markus, M. and Sass-Kiss, A. 1993. Effects of cooking on the fatty acid composition of silver carp *(Hypophtalmichfis molitrix*). Acta. Aliment. 22, 25–35.

Tressl, R., Wondrak, G. T., Garbe, L. A., Krüger, R. P., and Rewicki, D. 1998b. Pentoses and hexoses as sources of new melanoidin-like Maillard polymers. J. Agric. Food Chem. 46, 1765-1776.

Tressl, R,. Wondrak, G. T., Kruger, R. –P., and Rewicki, D. 1998a. New melanoidin-like Maillard polymers from 2-deoxypentoses. J. Agric. Food Chem. 46, 104-110.

Tseng, Y-C., Moreira, R. G., and Sun, X. 1996. Total frying—use time effects on soybean oil deterioration and on tortilla chip quality. Intl. J. Food Sci. Technol. 31, 287–294.

Tyagi, V. K. and Vasishtha, A. K. 1996. Changes in the characteristics and composition of oils during deep-fat frying. J. Am. Oil Chem. Soc. 73, 499-506.

Tynek, M., Hazuka, Z., Pawlowicz, R., and Dudek, M. 2001. Changes in the frying medium during deep-frying of food rich in proteins and carbohydrates. J. Food Lipids. 8, 251-261.

Uematsu, T. and Ishii K. 1983a. Browning reaction of oxidized methyl linoleate with amino acids on filter paper. J. Jpn. Soc. Food Sci. Technol. 30, 383–390.

Uematsu, T., and Ishii, K. 1983b. Browning reaction of oxidized peanut oil with amino acids on filter paper. J. Jpn. Soc. Food Sci. Technol. 30, 391–396.

USDA. 1985. Information for Code of Federal Regulations (Manufacturing Process Inspection Regulation), Part 18.40, U.S. Department of Agriculture, Washington, D.C.

Venolia, A. W. and Tappel. A. L. 1958. Brown-colored oxypolymers of unsaturated fats. J. Am. Oil Chem. Soc. 35, 135-138.

Verleyen, T., Kamal-Eldin, A., Mozuraityte, R., Verhe, R., Dewettnck, K., Huyghebaert, A., and Greyt, W. D. 2002. Oxidation at Elevated Temperatures: Competition between α-Tocopherol and Unsaturated Triacylglycerols. Eur. J. Lipid Sci. Tech. 104, 228–233.

Verleyen, T., Verhe, R., Huyghebaert, A., Dewettinck, K., and De Greyt, W. 2001. Identification of alpha-tocopherol oxidation products in triolein at elevated temperatures. J. Agr. Food Chem. 49, 1508-1511.

Vijayan, J. Slaughter, D. C. and Singh, R. P. 1996. Optical Properties of Corn Oil During Frying. Int. J. Food Sci. Technol. 31, 353–358.

Warner, K. 2004. Chemical and Physical Reactions in Oil During Frying. In: Gupta, M. K., Warner, K. and White, P. J. (eds). Frying Technology and Practices. AOCS, Champaign, IL, pp. 77–90.

Warner, K. and Moser, J. 2009. Frying Stability of Purified Mid-Oleic Sunflower Oil Triacylglycerols with Added Pure Tocopherols and Tocopherol Mixtures. J. Am. Oil Chem. Soc. 86, 1199–1207.

Warner, K. and Mounts, T. L. 1993. Frying stability of soybean and canola oils with modified fatty acid compositions J. Am. Oil Chem. Soc. 70, 983–988.

Warner, K., Orr, P., and Glynn, M. 1997. Effect of fatty acid composition of oils on flavor and stability of fried foods. J. Am. Oil Chem. Soc. 74, 347–356.

Warner, K., Orr, P., Parrott, L., and Glynn, M. 1994. Effect of frying oil composition on potato chip stability. J. Am. Oil Chem. Soc. 71, 983-988.

White, P. J. 1995. Conjugated Diene, Anisidine Value, and Carbonyl Value Analyses. In: Warner. K. and Eskin, N. A. M. (eds). Methods to Assess Quality Stability of Oils and Fat-Containing Foods. AOCS, Champaign, IL, pp. 107–133.

Wondrak. G. T., Tressl, R., and Rewicki, D. 1997. Maillard reaction of free and nucleic acid-bound 2-deoxy-D-ribose and D-ribose with ω-amino acids. J. Agric Food Chem. 45, 321-327.

Xu, G., and Sayre, L. M. 1998. Structural characterization of a 4-hydroxy-2-alkenal-derived fluorophore that contributes to lipoperoxidation-dependent protein cross-linking in aging and degenerative disease. Chem. Res. Toxicol. 11, 247-251.

Xu, X-Q. 2000. A New Spectrophotometric Method for the Rapid Assessment of Deep Frying Oil Quality. J. Am. Oil Chem. Soc. 77, 1087–1089.

Xu, X-Q. 2003. A chromametric method for the rapid assessment of deep frying oil quality. J. Sci. Food Agric. 83, 1293–1296.

Xu X-Q., Tran, V. H., Palmer, M., White, K., and Salisbury, P. 1999. Chemical and physical analyses and sensory evaluation of six deep-frying oils. J. Am. Oil Chem. Soc. 76, 1091–1099.

Yaylayan, V. A. and Kaminsky, E. 1998. Isolation and structural analysis of Maillard polymers: caramel and melanoidin formation in glycine/glucose model system. Food Chem. 63, 25-31.

Yuki, E., Onishi, R., and Hata, C. 1961. Studies on the Deterioration of Frying Oil IV. Yukagaku. 10, 464-467.

Zainal and Isengard, H. D. 2010. Determination of Total Polar Material in Frying Oil Using Accelerated Solvent Extraction. Lipid Technol. 22, 134–136.

Zamora, R., Alaiz, M., and Hidalgo, F. J. 1997. Feed-back inhibition of oxidative stress by oxidized lipid/amino acid reaction products. Biochemistry. 36, 15765-15771.

Zamora, R., Alaiz, M., and Hidalgo, F. J. 2000. Contribution of pyrrole formation and polymerization to the nonenzymatic browning produced by amino-carbonyl reactions. J. Agric. Food Chem. 48, 3152-3158.

Zamora, R. and Hidalgo, F. J. 1994. Modification of lysine amino groups by the lipid peroxidation product 4,5(*E*)-epoxy-2(*E*)-heptenal. Lipids. 29, 243–249.

Zamora, R. and Hidalgo, F. J. 1995. Linoleic acid oxidation in the presence of amino compounds produces pyrroles by carbonyl amine reactions. Biochim. Biophys. Acta. 1258, 319–327.

Zamora, R. and Hidalgo, F. J. 2005. Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. Crit. Rev. Food Sci. 45, 49-59.