STRATEGIES TO INHIBIT THE FORMATION OF 3-MONOCHLOROPROPANE DIOL

DURING DEEP-FAT FRYING

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Title

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The Supervisory Committee certifies that this *disquisition* complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

3-monochloropropane-1,2-diol or 3-chloropropane-1,2-diol (3-MCPD) and glycidol are the most commonly occurring group of thermal process contaminants which are considered as "possible human carcinogen" and "probably carcinogenic to humans", respectively. Potato strips prepared from three different potatoes cultivars (Russet Burbank, Ranger Russet, and Umatilla Russet) grown in North Dakota from the crop year 2018 were fried with vegetable oil at 190 ℃, respectively, for five consecutive days (8 h/day). The dynamic changes of 3-MCPD and glycidol equivalents were investigated during deep-fat frying. 3-MCPD equivalent in oil and potato strips decreased with increased frying time. Meanwhile, the content of glycidol equivalent increased with increased frying time. The major 3-MCPD and glycidol equivalents that were detected in the fried potato strips were those that migrated from the oils during frying. The application of absorbents, i.e., Magnesol and Celite, achieved the mitigation of 3-MCPD and glycidol in frying oil.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

2-MCPD..2-monochloropropane-1, 2-diol

3-MCPD..3-monochloropropane-1, 2-diol

3-MBPD..3-monobromopropanediol

HVP...Hydrolyzed Vegetable Protein

AOCS..American Oil Chemists' Society

BSTFA...Bis-trimethylsiyl Trifluoroacetamide

CAFR...Cyclic Acyloxonium Free Radical

DAG...Diacylglycerol

FFA..Free Fatty Acid

FLD..Fluorescence Detector

GC..Gas Chromatography

GE..Glycidyl Ester

HFBA...Heptafluorobutyric Anhydride

HFBI..Heptafluorobutyrylimidazole

HPLC...High-Performance Liquid Chromatography

IARC...International Agency for Research on Cancer

LOD...Limit of Detection

LOQ...Limit of Quantification

MAG..Monoacylglycerol

PBA..Phenylboronic Acid

PL..Phospholipid

PMTDI...Provisional Maximum Tolerable Daily Intake

PTFE..Polytetrafluoroethylene

SEM...Scanning Electron Microscopy

1. LITERATURE REVIEW

1.1. MCPD, Glycidol, and their Fatty Acid Esters

1.1.1. Physical and Chemical Properties

3-monochloropropane-1, 2-diol (3-MCPD) is a colorless or pale-yellow oily liquid at room temperature. Its relative molecular mass is 110.54 g/mol with a density of 1.32 g/cm₃ and is hygroscopic. Because of its moderate to a high polarity and it is highly soluble in the polar solvents such as methanol, ethyl acetate, ethanol, acetone, and chloroform (Lee & Khor, 2015). 2-monochloropropane-1, 2-diol (2-MCPD) is also a colorless or pale-yellow oily liquid at room temperature. It is also hygroscopic and has a similar appearance to 3-MCPD (Lee & Khor, 2015). Glycidol (Figure 1) is a colorless hygroscopic liquid and can dissolve in water and most other polar organic solvents. 2- and 3-MCPD esters and glycidyl esters (GEs) have similar physical properties (e.g., solubility and polarity) to their respective free forms with slightly lower melting points (Lee & Khor, 2015). However, more detailed measurements of these properties have not been made.

3-MCPD exists in two enantiomer forms derived from prochiral *L*-glycerol and expresses different biological activity because of its chiral nature. In the edible oil refining process, 2- and 3-MCPD and glycidol could form their respective fatty acids ester with under high temperatures. Particularly, 2- and 3-MCPD esters including monoester and diester could be formed with monoacylglycerolS (MAG) and diacylglycerols (DAG). The existence of diesters positional isomers is because the two hydroxyl groups are esterified by different acids (EFSA CONTAM Panel, 2016). 2- and 3- MCPD esters and GEs are soluble in non-polar solvents and poorly soluble in water. Previous studies have found that 2- and 3-MCPD monoesters have a higher solubility than their diester in polar solvents; the longer fatty acid chain of both monoesters and

diesters have a lower solubility in polar solvents, and higher solubility in non-polar solvents (EFSA CONTAM Panel, 2016). However, the individual solubility of 2- and 3-MCPD esters, and GEs have not been fully investigated.
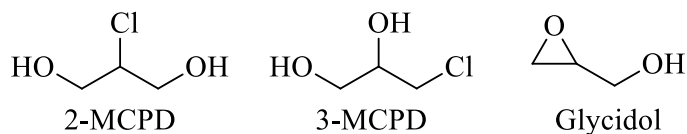
Figure 1. Structures of 2-MCPD, 3-MCPD, and glycidol

1.1.2. Analytical Methods

Presently, several quantification methods have been developed and validated for 3-MCPD. However, most of methods require derivatizing the 3-MCPD before analysis with high-performance liquid chromatography (HPLC), gas chromatography (GC), or gas chromatography-mass spectrometry (GC-MS) (Lee & Khor, 2015). Several analytical methods for 3-MCPD were published, and most of those methods used derivatization agents such as bis-trimethylsiyl trifluoroacetamide (BSTFA), phenylboronic acid (PBA), heptafluorobutyrylimidazole (HFBI), and heptafluorobutyric anhydride (HFBA) (Lee & Khor, 2015). Some methods do not need derivatization agents but have a high limit of detection (LOD) (Lee & Khor, 2015).

The difficulties of accurately quantifying 3-MCPD are due to its three main physical properties: high boiling point, low molecular weight, and the absence of a suitable chromophore (Hamlet et al. 2002). The lack of a suitable chromophore makes ultraviolet or fluorescence detection by HPLC unfavorable (Hamlet et al. 2002). Its high boiling point and low molecular weight makes GC analysis more complicate (Hamlet et al. 2002). For instance, 3-MCPD can cause unfavorable reactions with the column in the GC system because of its high polarity and low volatility, causing the low sensitivity and poor peak shape. Moreover, the low molecular weight of 3-MCPD makes the mass detection more difficult, because the diagnostic ions may not

be able to distinguish 3-MCPD from background chemicals (Hamlet et al. 2002). Consequently, the methods relying on the formation of the stable volatile derivatives of 3-MCPD are necessary. GC-MS is widely used to determine the derivative of 3-MCPD. However, new methods still need to be developed to produce repeatable results.

AOCS Official Method Cd 29a-13 is one of the quantification methods developed by the American Oil Chemists' Society (AOCS). GC-MS is used to determine the 2- and 3-MCPD esters, and GEs in edible oil by acid transesterification. The principle of this method is as follows:

First, GEs will be converted to 3-monobromopropanediol (3-MBPD) monoesters in an acid solution containing a bromide salt, as shown in Figure 2.
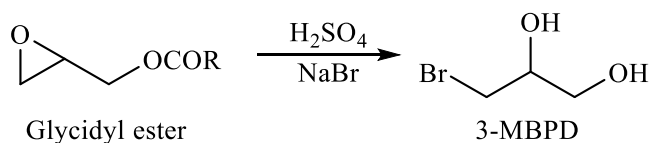
Figure 2. Principle of quantification methods (1/3) (AOCS, 2013)
Note - R represents the fatty acid group

Then, 2-MCPD esters, 3-MCPD esters, and 3-MBPD esters will be converted into the free (non-esterified) form in methanolic acid solution. Fatty acid methyl esters will be formed from the sample, as shown in Figure 3.
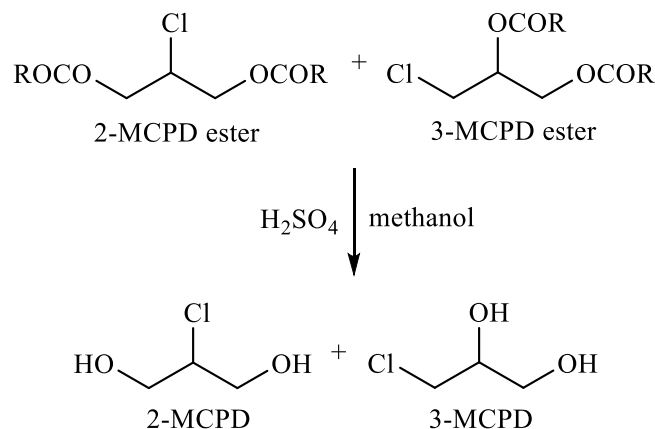
Figure 3. Principle of quantification methods (2/3) (AOCS, 2013)
Note - R represents the fatty acid

Finally, 2- MCPD, 3-MCPD, and 3-MBPD will be derivatized with PBA before GC-MS

analysis, as shown in Figure 4.
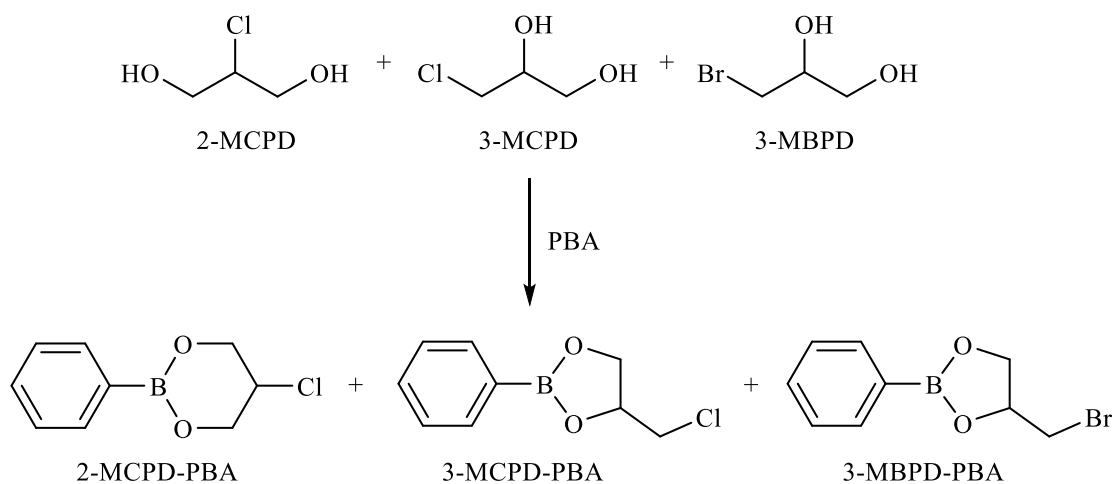
Figure 4. Principle of quantification methods (3/3) (AOCS, 2013)
Note - R represents the fatty acid

1.1.3. Toxicity of 2- and 3-MCPD and Glycidol

3-MCPD was identified as "possible human carcinogen", and GE was identified as

"probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC)

(Cheng, Liu, Wang, & Liu, 2017a). 3-MCPD showed carcinogenic effects in animal studies by

impacting kidneys, blood, and sperm. In addition, a variety of toxicological effects in acute studies were also reported (Cheng et al., 2017a).

Liu et al. (2016) synthesized and investigated five 3-MCPD esters for the toxicities in Swiss mice. Toxic effects were observed in the lung and thymus. The results also suggested that factors such as the relative substitution locations, chain length, number of substitutions, and degree of unsaturation of the 3-MCPD esters, altered the toxicity.

Before 3-MCPD was reported as a carcinogenic food contaminant, it was often used to treat rodents as an antifertility agent by blocking the glycolysis pathway. This pathway is a vital metabolic process for sperm mobility which makes up for the inadequacy of oxidative phosphorylation (Bakhiya, Abraham, Gürtler, Appel, & Lampen, 2011). 3-MCPD blocks the glycolysis by affecting tyrosine protein phosphorylation which impairs the 3'-5'-cyclic adenosine monophosphate/protein kinase A pathway of the sperm production (Lee & Khor, 2015). It was reported that 3-MCPD could reduce the production of progesterone in R2C rat Leydig cells. 3-MCPD also can induce morphological changes in Leydig cells and damage DNA (Lee & Khor, 2015).

Genotoxicity refers to chemicals that destroy genetic information within cells, leading to gene mutations that may cause cancer (Bakhiya et al., 2011). In 2016, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated the genotoxicity of 3-MCPD. They commented that no genotoxic potential had been found *in vivo* for 3-MCPD (Lee & Khor, 2015). However, long-term carcinogenicity studies identified that 3-MCPD is genotoxic *in vitro* (Lee & Khor, 2015).

The two enantiomers (Figure 5) of 3-MCPD possess different biological activities. For instance, (*R*)-isomer of 3-MCPD is detrimental to kidneys, whereas its (*S*)-isomer has the antifertility activity (Hamlet et al., 2002).

$$HO \overset{HO \quad H}{\diagup} Cl \qquad HO \overset{HO \quad H}{\diagup} Cl$$

(R)-stereoisomer   (S)-stereoisomer
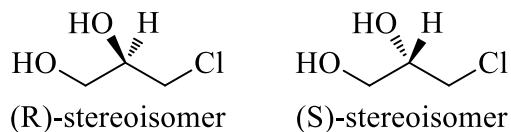
Figure 5. (*R*)- and (*S*)-stereoisomer of 3-MCPD

1.1.4. Public Health Significance and Legislation

In 2016, JECFA concluded that the significant levels of 2-MCPD esters, 3-MCPD esters, and GEs were found in refined vegetable oil. Since 3-MCPD is released by hydrolysis of its esterified forms in the gastrointestinal tract, 3-MCPD esters deliver the same toxicity as free 3-MCPD (EFSA CONTAM Panel, 2016). As no genotoxicity has been demonstrated for 3-MCPD, JECFA updated the Provisional Maximum Tolerable Daily Intake (PMTDI) at 4 µg/kg body weight per day for 3-MCPD. Its esters express as 3-MCPD equivalents that count into PMTDI as well. However, JECFA has not set the PMTDI for GEs which was substantially hydrolyzed in the GI tract to glycidol based on the evidence regarding the genotoxic and carcinogenic properties (EFSA CONTAM Panel, 2016).

In 2016, the European Food Safety Authority announced a relative conservative tolerable daily intake (TDI) for 3-MCPD, 0.8 µg/kg body weight. With this newly TDI, survey studies found that the mean exposure of multiple population groups (e.g., infants, toddlers) exceeded the TDI of 3-MCPD (EFSA CONTAM Panel, 2016). In consequence, both the United States and Canada has set the limit for the detectable levels of 3-MCPD in foodstuffs at 1.00 mg/kg (Lee & Khor, 2015). European Commission Regulation (EC) NO 290/2018 set the maximum standard at 1,000 µg/kg for GEs in vegetable oil and fat that are delivered to market or used as a food

6

ingredient for the general population; and 500 μg/kg for the vegetable oil and fat destining to process cereal-based infant and baby foods.

1.2. The Formation Mechanisms and Occurrence of MCPD and Glycidol

1.2.1. Formation Mechanisms of 2- and 3-MCPD and their Fatty Acid Esters

During the food manufacturing process, especially during treating cereal-derived foods containing proteinaceous oilseeds, 2- and 3-MCPD is usually formed in acid-hydrolyzed vegetable protein (HVP), which involves the presence of hydrochloric acid, high temperatures, and high pressures. Hydrochloric acid could react with lipids, including monoacylglycerols (MAGs), diacylglycerols (DAGs), triacylglycerols (TAGs), phospholipids (PLs), and glycerol which is formed from the acid hydrolysis of TAGs (Collier, Cromie, & Davies, 1991; Hamlet, Sadd, & Gray, 2004; Yao et al., 2019). In addition, there is a possible interconversion between 2- and 3-MCPD esters with glycidol as an intermediate (Hamlet et al., 2011). In food production, 2- and 3-MCPD are generally formed from the addition of chloride that reacts with the acylglycerols or glycerol present in raw food materials.

At present, the exact formation mechanisms of 2- and 3-MCPD and their corresponding fatty acid esters are still unknown. Rahn & Yaylayan (2011) proposed four mechanisms of 2- and 3-MCPD esters formation involving SN_2 reaction, which is the nucleophilic attack by chloride ions (Figure 6). Pathway a and b form 3-MCPD esters by an acyloxonium ion intermediate in the formation of glycidol before the nucleophilic attack by open the ring (pathway a) or the chloride ion (pathway b). Pathway c and d involved the direct nucleophilic substitution by the chloride ion in glycerol carbon carrying a protonated hydroxyl group (pathway c) or an ester group (pathway d). The summary of the four proposed pathways of 3-MCPD esters formation shows in Figure 6. However, the intermediate products produced are different depending on the chloride

ion binding sites in the pathway (similar to pathways a and b), which generates the acyloxynium ion.
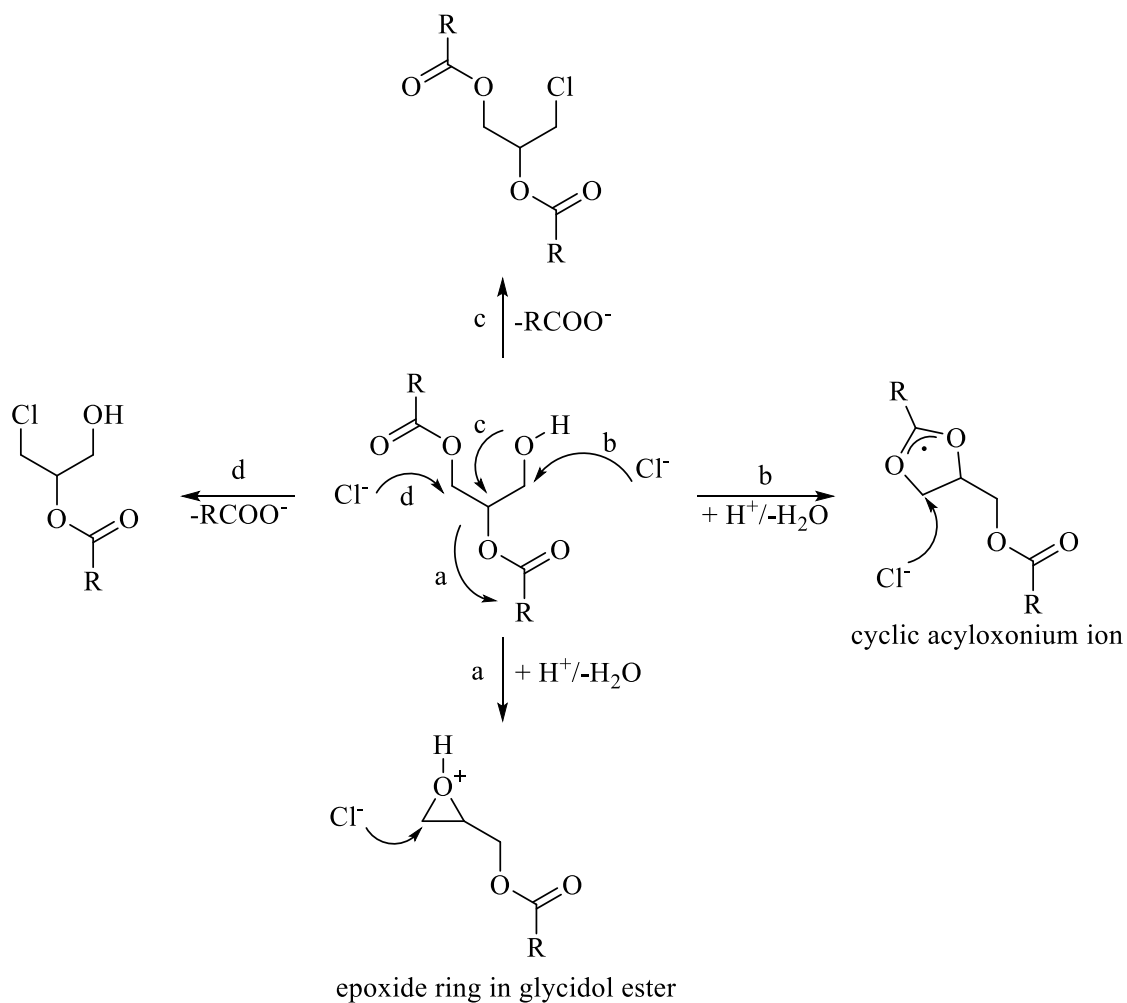
Figure 6. Summary of the proposed four pathways of the formation of 3-MCPD esters (adapted from Rahn & Yaylayan, 2011)
Note - R represents the fatty acid

In recent years, some researchers proposed the free radical mechanism (Figure 7, 8, & 9) to explain the formation mechanisms of 3-MCPD esters due to the discovery of the cyclic acyloxonium free radical (CAFR) intermediate from MAG (Zhao et al., 2016), DAG (Zhang et al., 2013), or TAG (Zhang et al., 2015).

8
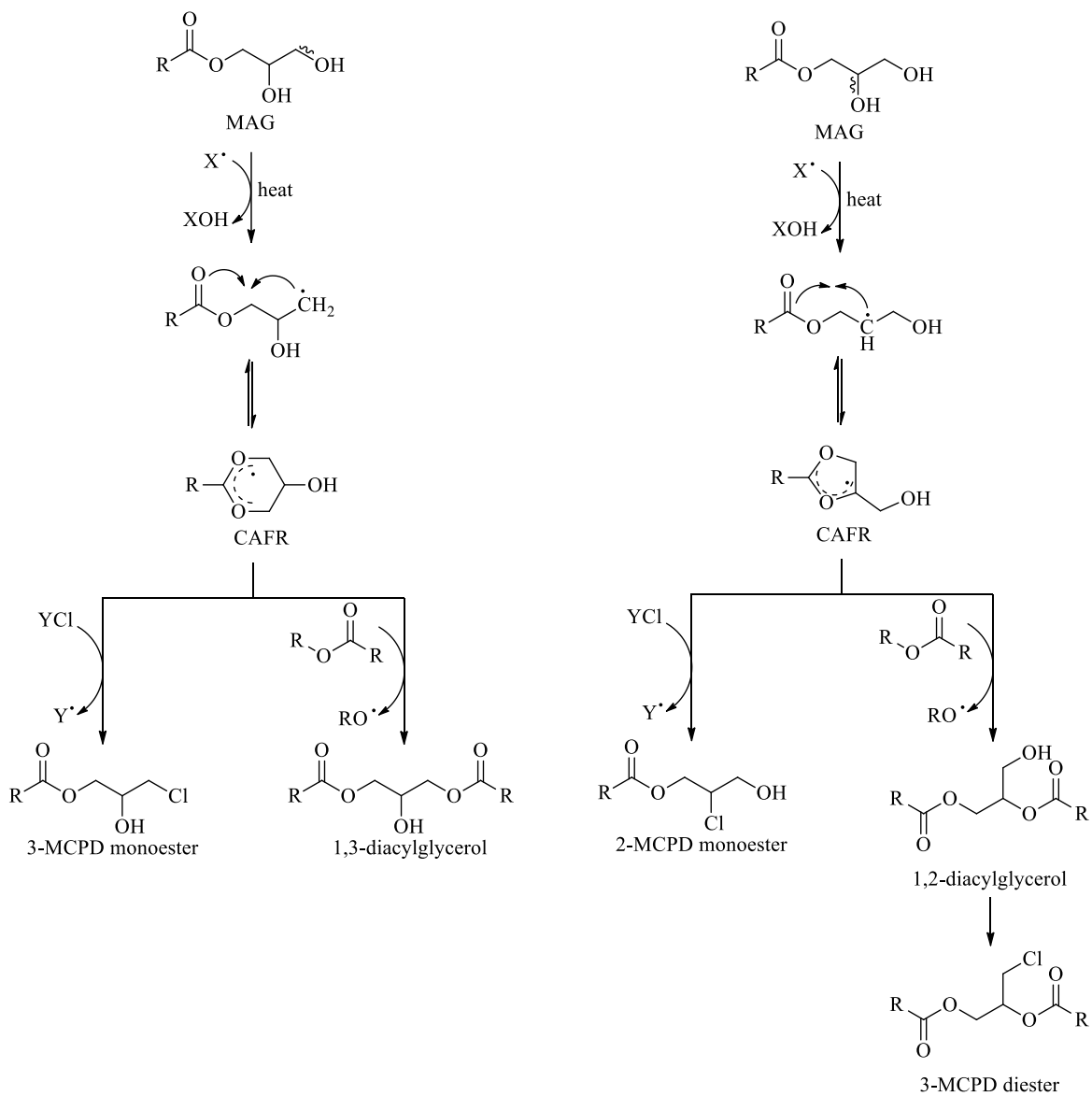
Figure 7. The proposed formation mechanism of 3-MCPD esters from MAGs (Zhao et al., 2016) Note - X represents lipid, Y represents $C_6H_6Cl_5$, MgCl, $AlCl_2$, CuCl, MnCl, SnCl, ZnCl and $FeCl_2$
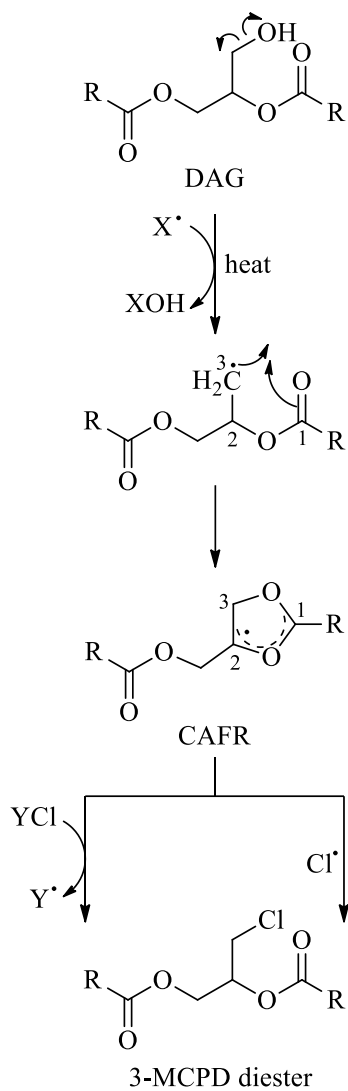
Figure 8. The proposed formation mechanism of 3-MCPD esters from DAGs (adapted from Zhang et al., 2013)
Note - X represents lipid, Y represents H, Cl, $C_6H_6Cl_5$, Na, K, FeCl, $FeCl_2$, CuCl, ZnCl
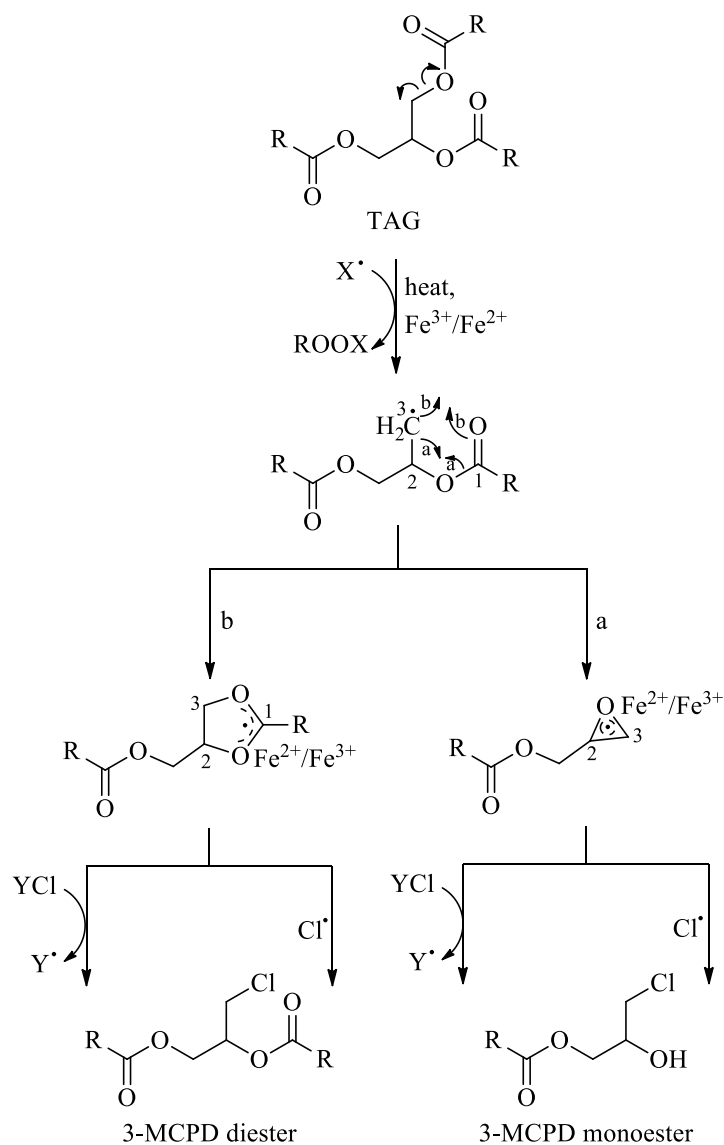
Figure 9. The proposed formation mechanism of 3-MCPD esters from TAGs (adapted from Zhang et al., 2015)

Note - X represents lipid, Y represents H, Cl, $C_6H_6Cl_5$, Na, K, FeCl, $FeCl_2$, CuCl, ZnCl

Furthermore, Zhang et al. (2013) also suggested that GEs could be the co-product from TAGs in the formation of 3-MCPD esters, and transition metals including Fe_{2+} and Fe_{3+} could be the catalyzers during the formation of 3-MCPD esters from TAGs. In the subsequent experiment of 3-MCPD esters formation from MAGs, they proposed the mechanism to explain the coexistence of 2- and 3-MCPD esters. They believe that the coexistence is because of the cyclic

acyloxonium structures of the free radical intermediates that are five or six-membered rings.

Thereinto, six-membered ring intermediate might be more stable than five-membered rings

intermediate, which may account for the quantity difference between 2-MCPD esters and 3-

MCPD esters (Zhao et al., 2016).

Although lots of studies have been made to elucidate the formation of 2- and 3-MCPD

esters, the mechanisms are still not clear (Collier et al., 1991; Yao et al., 2019; Zhang et al.,

2013; Zhao et al., 2016).

1.2.2. Formation Mechanisms of Glycidyl Fatty Acid Esters

The highest level of GE is found in refined palm oil that contains a high amount of DAG

(4-12%) (Destaillats et al., 2012). The proposed formation mechanisms are shown in Figure 10

& 11, where GEs are formed from MAGs and DAGs via elimination of water or free fatty acids

(FFAs) under high temperatures.

The scholars also demonstrated that GEs formation is independent of the 2- and 3-MCPD

esters formation that involving an acyloxonium ion or the intramolecular elimination of fatty

acids from DAGs (Destaillats et al., 2012).
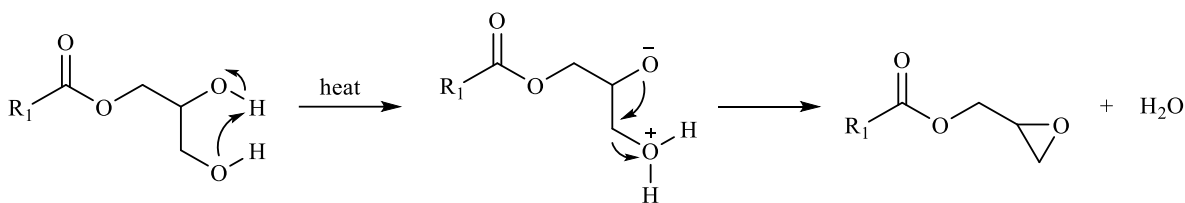
Figure 10. The proposed formation mechanism of GEs from MAGs under high temperatures
(adapted from Destaillats et al., 2012)
Note - R represents the fatty acid group
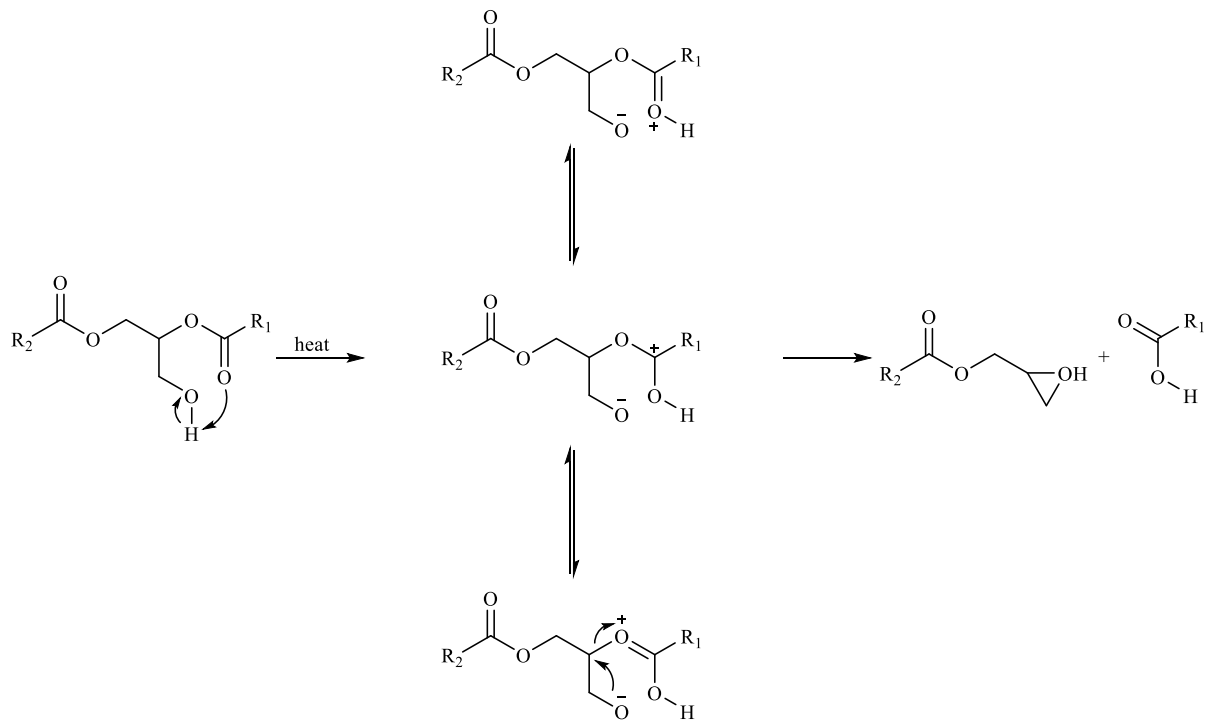
Figure 11. The proposed formation mechanism of GEs from DAGs under high temperatures (adapted Destaillats et al., 2012)
Note - R represents the fatty acid group

1.2.3. The Occurrence of MCPD, Glycidol and their Fatty Acid Esters in Foodstuffs

3-MCPD and its esters have been reported in various foodstuffs that have high salt content (sauce and soup), high fat content (meat and dairy), and are involved with thermal processing (cereal-derived products) (Arisseto et al., 2017). Some study also found fried food contains a significant level of 3-MCPD and its derivatives. One research group tested 85 samples of deep-fat fried foods and found 75% of positive samples in which concentrations of 3-MCPD were up to 0.99 mg/kg; potato strips were reported to contain 0.10-2.20 mg/kg of 3-MCPD (Arisseto et al., 2017).

3-MCPD esters were also found in refined edible oil. The process of oil refining includes degumming, neutralization, bleaching, and deodorization. 3-MCPD esters formed during the refining process of crude oil in the deodorization step. Deodorization is water-steam distillation

13

aiming to remove the odor and flavor (volatile compounds) in the oil at high temperatures (180–260 °C) and reduced pressures (3-20 mbar) (Šmidrkal et al., 2016). Before deodorization, the crude oil contains MAGs, DAGs, TAGs, FFAs, and some chloride compounds (Šmidrkal et al., 2016). Therefore, the oil contains many precursors of 3-MCPD esters. These precursors are possibly contributed to the development of 3-MCPD esters during the process of deep-fat frying of the foodstuffs. Moreover, the levels of 3-MCPD esters in oil after refining (deodorization) is dependent upon the fatty acid composition of the oil. Higher FFAs composition in oil resulted in higher 3-MCPD level during deodorization. In addition, the formation of 3-MCPD esters from DAGs is nearly 2–5 times quicker than that from MAGs (Šmidrkal et al., 2016). GEs are only identified in refined vegetable oil, which is formed during the deodorization of the oil refining by heating the partial glycerol (MAGs or DAGs) with the elimination of water or fatty acids under high temperatures (Destaillats, Craft, Dubois, & Nagy, 2012; Hrncirik & Duijn, 2011).

1.3. Deep-Fat Frying

Deep-fat frying causes several chemical reactions such as hydrolysis, oxidation, and polymerization of the frying oil.

1.3.1. Hydrolysis

For the hydrolysis of food oil, the water in the foodstuffs forms steam when the oil is heated and evaporated with bubbling and faded with frying (Naz, Siddiqi, Sheikh, & Sayeed, 2005). Water, steam, and oxygen trigger chemical reactions between the frying oil and the potato strips. Water is a weak nucleophile that attacks the ester linkage of TAG and produces FFA, DAG, MAG, and glycerol to accelerate further hydrolysis of oil. Glycerol will evaporate at 150 ºC, and the remaining glycerol promotes oil hydrolysis and FFAs production (Naz et al., 2005).

14

FFA is one of the indicators to evaluate oil quality. It forms during deep-fat frying from the hydrolysis of TAG or decomposition of hydroperoxides. Abundant research results have proven the content of FFA in frying oil increased with the increase of frying times (Chung, Lee, & Choe, 2004; Wong, Lai, et al., 2017; Wong, Muhamad, et al., 2017).

Hydrolysis occurs more readily in oils containing short and unsaturated fatty acids rather than oils containing long and saturated fatty acids (Choe & Min, 2007). This is because short and unsaturated fatty acids are relatively more soluble than long and saturated fatty acids in water. Water in foodstuffs hydrolyze the oil more rapid than steam (Choe & Min, 2007).

1.3.2. Oxidation

Oxidation of oil in deep-fat frying is a chemical reaction where oxygen is involved. Choe & Min (2006) summarized the factors affect the oxidation of oil, including oil processing, the type and concentration of oxygen, antioxidants, the fatty acid composition of the oil, peroxides, energy of heat, light exposure, pigments, FFAs, MAG, DAG, transition mental, and thermally oxidized compounds. Those factors interact closely to determine the degree of lipid oxidation. The principle of the oxidation of oil includes three steps, i.e., initiation, propagation, and termination (Figure 12).

$$
\begin{array}{lll}
\text{Initiation} & RH & \longrightarrow R\cdot + H\cdot \\
\text{Propagation} & R\cdot + O_2 & \longrightarrow ROO\cdot \\
 & ROO\cdot + RH & \longrightarrow ROOH + R\cdot \\
\text{Termination} & R\cdot + R\cdot & \longrightarrow RR \\
 & ROO\cdot + R\cdot & \longrightarrow ROOR \\
\end{array}
$$
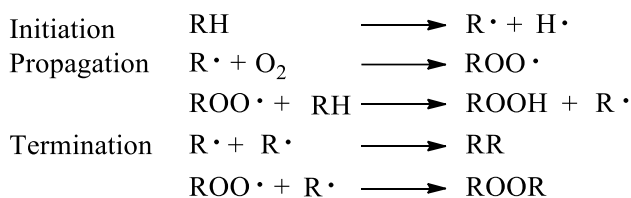
Figure 12. The principle of thermal oxidation of oil (Adapted from Choe & Min, 2006)
Note - R represents the fatty acid group

At the end of oxidation, non-radical volatile and non-volatile compounds are formed in the termination step (Choe & Min, 2007). During deep-fat frying, most of the volatiles were evaporated from the frying oil. The addition of water to the frying oil could decrease the content

15

of volatile compounds (Wu & Chen, 1992). However, the content of volatile compounds present in fried oil is dependent on the composition of the food, type of frying oil, frying time, and frying temperature. The loss of volatile compounds can be considered to evaporation, decomposition, or any reaction occurred between volatile compounds and other food components (Frankel, 1984). The remaining volatile compound in the oil can further undergo reactions such as dimerization, oxidation, and polymerization. The volatile compounds impact the oil quality and flavor of the fried food. The rate of oxidative degradation increases with the increase of the concentration of oxygen and free radicals (Choe & Min, 2007).

Mistry & Min (1987) reported that FFAs, MAG, and DAGs act as prooxidants which contain the hydrophilic and hydrophobic groups. Those prooxidants could decrease the interfacial tension of the oil, increase the diffusion rate of the oxygen from the headspace to the oil, thus accelerating the oil oxidation. In order to improve the oxidative stability of the edible oils, most FFA, MAG, and DAG are removed during the oil refining (Mistry & Min, 1988). During deep-fat frying, higher interfacial tension can destroy more steam bubbles and form a steam blanket on the oil surface (Choe & Min, 2007). The steam blanket could reduce the contact of oil and oxygen and reduce the rate of oil oxidative degradation (Choe & Min, 2007).

After the oil refining process, the initial content of MAG and DAG are minimal in refined oil. Generally, MAG and TAG present in soybean oil range from 0.07 % to 0.11 %, and 1.05 % to 1.20 %, respectively (Mistry & Min, 1987, 1988). According to the study by Wong, Muhamad et al. (2017), the initial percentage of MAG and FFA in palm olein was 0.12 %, DAG was 1.96 %, and TAG was 98.44 %. After five days of consecutive frying with potato chips at 160 ℃, the ratio of the MAG and DAG increased to 0.35 % and 3.00 %, respectively, while TAG decreased

to 96.65 %. The author suggested that the formation of partial glycerol (MAG and DAG) could further cause the formation of 3-MCPD esters and GEs.

1.3.3. Polymerization

Nonvolatile compounds, TAG dimers, and polymers are the primary decomposition product of oil during frying. Cyclic compounds are relatively lower in amount than nonvolatile compounds, TAG dimers, and polymers (Frankel, 1984). Christopoulou & Perkins (1989) isolated the dimers from partially hydrogenated soybean oil which were used for frying at 195 ºC. They found the evidence of the presence of the monohydrodimer, dehydroxydimer, ketodehydrodimer of linoleate, as well as dehydrodimer of oleate. The factors contributed to the formation of dimers and polymers include the type of oil, frying time, and frying temperature. In general, the amounts of polymers increase with the increase of the frying time and frying temperature. During deep-fat frying, the oil with more unsaturated fatty acids is more favorable to be polymerized; the oils with a higher amount of unsaturated fatty acids formed more polar compounds than those of saturated oils (Tompkins & Perkins, 2000). In addition to that, the formation of polymers increases during deep-fat frying if the frying system is rich in oxygen. Yoon, Jung, & Min (1988) reported that the thermally oxidized polymer compounds are prooxidants that accelerate lipid oxidation. During deep-fat frying, the polymers were formed which contribute to the further degradation of oil, the increase of oil viscosity, the reduction if heat transfer, the production of foam, and causing higher oil absorption of oil to the fried foods (Tseng, Moreira, & Sun, 1996).

2. THE CHANGE OF 3-MCPD AND GLYCIDOL DURING DEEP-FAT FRYING

2.1. Introduction

Potato (*Solanum tuberosum*) is one of the most important crops in the world. The chemical composition of potato mainly contains carbohydrates that become a major energy supplier in human diets (Bradshaw & Ramsay, 2009). In addition, potato provides a significant amount of nutrients such as protein, vitamins C, vitamins B1, vitamins B6, folate, and nutritional minerals (e.g. calcium, phosphorus, potassium, and magnesium). It is high in dietary fiber and contains abundant antioxidants such as tocopherols. As ungelatinized starch in potato causes low indigestibility for humans, it is oftentimes processed by deep-fat frying, baking, boiling, roasting, steaming, and microwaving.

North Dakota is one of the top potato producer in the United States. Russet Burbank, Ranger Russet, and Umatilla Russet are the top 3 grown potato cultivars in North Dakota (NASS, 2019). Russet potato has low moisture and high starch content that makes it an ideal choice for frying (Bradshaw & Ramsay, 2009).

The potato processers need to specify the characteristics of the potato varieties to satisfy different demands for potato products. The potato processing usually includes washing, peeling, and slicing or cutting. Then washing, blanching, and frying are carried to complete the chip by French fries manufacturer. Partial par-frying is applied in the manufacturing process of frozen French fries and other potato-derived foods (Bradshaw & Ramsay, 2009).

Deep-fat frying is a popular food processing around the world when the foodstuffs fried at high temperatures (150 °C to 190 °C). Lipid brings unique sensory taste to the consumers like flavor, texture, palatability, aroma, and color so that fried foods are highly preferred by the consumers (Wong, Lai, et al., 2017). During deep-fat frying, volatile and nonvolatile compounds

18

will be formed. Most of the volatile compounds in oil undergo further chemical reactions during frying, and the remaining volatile compounds will evaporate into the air (Choe & Min, 2007). The nonvolatile compounds in the oil will not only influence the physical and chemical properties of the frying oil and the fried foods, but also impact the flavor stability, quality, and texture of the fried foods during storage (Choe & Min, 2007).

During deep-fat frying, the oil is repeatedly heated at high temperatures, leading to the significant degradations of the oil (Wong, Lai, et al., 2017). The oil degradation could change the organoleptic properties and nutritive value of the fried foods as well as bringing food safety problems (Wong, Lai, et al., 2017). French fries are potato derived products followed by deep-fat frying and are a popular style for restaurant cooking due to its short preparation time (Millin et al., 2016a). During deep-fat frying, the fresh potato strips are submerged in hot oil, which causes rapid water removal and oil absorption, resulting in soft interior and crispy outer crust French fries, giving a loose and porous structure foods (Millin et al., 2016a). At the same time, there are structural and physicochemical changes in lipids, proteins, and carbohydrates, as well as microbiological changes, such as Maillard reaction, caramelization, protein denaturation, and starch gelatinization in deep-fried food (Zhang, Zhang, Fan, Li, & Fan, 2018). However, depending upon the potato cultivars, frying time, frying oils as well as frying temperatures, the final deep-fried potato products deliver dramatically different properties (Millin et al., 2016a).

Frying oil is a heat transfer medium that delivers desirable flavor, color, and texture to the potato strips (Choe & Min, 2007). Vegetable oil contains significant amount of polyunsaturated fatty acids that are susceptible to oxidation and polymerization (Banga & Varshney, 2010). However, the fate and formation of 3-MCPD and glycidol in vegetable oil during deep-fat frying is still unclear. The overarching goal of research is to understand the

dynamic changes of 3-MCPD and GE in frying oil and French fries over the course of 5 days frying.

2.2. Materials and Chemicals

Soybean oil was purchased locally, and fresh potatoes (Russet Burbank, Ranger Russet, and Umatilla Russet) from crop year 2018 were kindly provided by Potato breeding program at the North Dakota State University and stored at 6 ℃.

2-chloro-1,3-propanediol (2-MCPD), 2-chloro-1,3-propanediol-d5 (2-MCPD-d5), 3-Chloro-1,2-propanediol (3-MCPD), 3-chloro-1,2-propanediol-d5 (3-MCPD-d5), rac-1,2-bis-palmitoyl-3-chloropropanediol (PP-3-MCPDE), rac-1,2-bis-palmitoyl-3-chloropropanediol-d5 (PP-3-MCPDE-d5), 1,3-dipalmitoyl-2-chloropropanediol (PP-2-MCPDE), and 1,3-dipalmitoyl-2-chloropropanediol-d5 (PP-2-MCPDE-d5) were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Phenylboronic acid (PBA) and sodium bromide (NaBr) were purchased from Acros Organics (Morris Plains, NJ, USA). Hexane (HPLC grade) were purchased from EMD Millipore (Billerica, MA, USA). Tetrahydrofuran (THF) was purchased from Fisher Chemical (Fair Lawn, NJ, USA). Sodium bicarbonate (NaHCO3), sulfuric acid (H2SO4), sodium chloride (NaCl), methanol, ethyl acetate, anhydrous sodium sulfate, sodium phosphate dibasic, sodium phosphate monobasic, and acetone were purchased from VWR International (West Chester, PA, USA). Magnesol filter powder was purchased from The Dallas Group of America, Inc. (Whitehouse, NJ, USA). α-tocopherol, rac-β-tocopherol, δ-tocopherol, γ-tocopherol, and Celite were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

2.3. Methods

2.3.1. Sample Preparation

Fresh potatoes (Russet Burbank, Ranger Russet, and Umatilla Russet) were sorted, washed, hand-peeled, and then cut using commercial grade French fry cutter to strips (3/8 inch). The fresh potato strips were submerged in distilled water at room temperature for one hour, par-dried with tissues to remove surface moisture. The fresh potato strips were weighed into 100 g for a batch and blotted with tissues.

2.3.2. Deep-Fat Frying

Deep-fat frying was carried out by adding a 100 g batch of fresh-cut potato strips into a deep fryer that filled with 3 kg of fresh vegetable oil for 3.5 mins at a 30 mins interval at 190ºC for 8 h daily. The frying oil was fried continuously for five days without any replenishment. At the end of each day, three deep fryers were turned off, approximated 20 mL of fried oil was collected from each deep fryer after the temperatures were allowed to cool. All deep-fried potato strips were allowed to cool at room temperature, put into Ziplock bags, labeled and stored in a freezer at −20°C for further analyzing. A thermometer was used during deep-frying to monitor the temperature.

For the investigation on the impact of potato storage time on the formation of 3-MCPD and glycidol, Ranger Russet and Umatilla Russet were selected and stored at 6ºC for 6 months. The stored Ranger Russet and Umatilla Russet were deep-fried following the same deep-fat frying procedures as described above.

2.3.3. Quantification of 3-MCPD and Glycidol

2.3.3.1. Ester cleavage by acidic hydrolysis

Acidic hydrolysis was conducted by adding approximate 500 mg of oil sample, 100 µL of 20 mg/L PP-MCPDEs-d5, 0.5 mL of THF, and 1 mL of 1.8 % (v/v) H_2SO_4 in a screwed tube. The mixture was vortexed for 30 s and hydrolyzed for 10 h in a water bath at 40°C. The acidic hydrolysis was stopped the addition of 350 µL of saturated $NaHCO_3$.

2.3.3.2. Transformation of glycidol

The method of transformation of glycidol used was modified from Kuhlmann, (2011), Xu, Jin, Yang, Rao, & Chen, (2019) and Cheng, Liu, Wang, & Liu, (2017). After acidic hydrolysis, the experiment can be divided into assay A and assay B.

Assay A was conducted by adding 1 mL of 20% (w/v) NaBr solution into the above mixture after acidic hydrolysis to convert glycidol to 3-MCPD. Assay B was conducted by adding 1mL of 20% (w/v) NaCl solution to extract 2- and 3-MCPD. Then, the tube was vortexed for 30 s, and 5 mins were allowed to complete the reaction. Next, 2x2 mL hexane was added into the crewed tube. The supernatant was discarded by a glass pipette to remove the non-polar components and oils. The addition of 300 µL of 0.2 mol/L pH 6.5 phosphate buffer solution (PBS) solution was used to adjust the final pH to 6.8.

2.3.3.3. Derivatization for HS-SPME injection

For derivatization, the tube was directly added 2 mL of hexane and 100 µL of PBA solution and screwed tightly. The derivatization was completed in a water bath at 90 ºC for 30 mins. The supernatant was transferred into a 20 mL vial with an aluminum cap. The injection was performed by PAL RSI 120 autosampler automatically (CTC Analytics, Zwingen, Switzerland).

2.3.3.4. GC-MS analysis

The method of GC-MS analysis used was modified from Xu, Jin, Yang, Rao, & Chen, (2019). The analysis of each sample was made in duplicate.

2.3.4. Tocopherols Analysis

The concentration of tocopherols (α-tocopherol, β-tocopherol, δ-tocopherol, and γ-tocopherol) in vegetable oil during deep-frying was measured by normal-phase HPLC equipped with a fluorescence detector (FLD, G1321B). For HPLC analysis, approximated 0.1 g of the weighted oil sample was dissolved in 5 ml of hexane. The solution was then filtered by a 0.45 µm polytetrafluoroethylene (PTFE) filter after vortexing. The mobile phase of the HPLC system was consisted of hexane: isopropyl alcohol (98.5:1.5, v/v) using isocratic gradient at a flow rate of 0.5 mL/min. The separation of the solution was achieved by 1260 Infinity HPLC system with a Luna ® 3µm Silica (2) 100 A LC 150 mm x 4.6 mm column with 15 µL injection volume and column temperature of 40ºC. The detection was conducted by scanning fluorescence detector at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The analysis of each sample was made in triplicate.

The calibration curve was obtained by using 15 µL of serially diluted solutions at five different concentration of 0.1, 0.5, 1.0, 2.0 and 5.0 µL/mL. Linear regression analysis of the response area of the fluorescence versus the known concentration of tocopherol standards was used to create the calibration curve. All the samples were analyzed in triplicate.

2.3.5. Total Polar Compounds Measurement

Testo 270 is a digital cooking oil tester purchased from Testo, SE & Co. KGaA (West Chester, PA, USA). To measure total polar compound (TPC) in oil, the tester was inserted into

the deep fryer at the end of each day. The result of TPC in oil was displayed as a percentage (wt %). The measuring range of the tester is from 0.0 to 40 % TPC with (±) 2 % accuracy.

2.3.6. Oil Extraction

Accelerated Solvent Extractor (Dionex™ ASE™ 350) was used to extract the oils from potato strips and measured the oil uptake of the fried potato strips collected from the end of each day. Fried potato strips were thawed and weighed into 5 g, then ground with 3 g of Celite by a grinder. Extraction cell (SST, stainless steel cell) was inserted with filter and was then filled with the ground mixture of the fried potato strips and Celite. The rest space of the extraction cell was filled with extra Celite. The sample was heated to 100 ℃ in 5 mins, and three cycles of 20 mins static time was conducted. Hexane was used as the solvent of the system. Rinse volume was set at 50 % of the cell volume with 50 s purge. After the static step, the static valve opens and pump rinses 70 % of the cell volume of fresh solvent through the cell. The extraction ends with a 160 s nitrogen purge. The amount of oil collected from the extractor in glass tubes was weighed to determine the oil uptake of the fried potato. The oil uptake analysis was conducted in triplicate for each fried potato strips and expressed as g oil/100 dry sample.

2.3.7. Absorbent Application

Before the quantification of 3-MCPD and glycidol, appropriate 1 g of oil sample was weighed into a disposable glass tube and filled with 0.25 g of absorbent (Magnesol or Celite), respectively. The glass tube was vortexed for 5 mins and centrifuged at 4,000 rpm for 5 mins. Next, the supernatant was filtered by a 0.45 μm PTFE filter and weighed into 0.5 g prior to follow the procedures in Section 2.3.3.

2.3.8. Statistical Analysis

Seven replicates of pseudo blanks were performed for the determination of LOD and the limit of quantification (LOQ). Data were analyzed by one-way ANOVA. Tukey's test was used to determine significant differences (p<0.05) between means using Minitab (version® 18.1). Results were presented for mean ± SD. In figures and tables, different capital letters indicate significant differences between potato cultivars while different small letters indicate significant differences between frying days (p < 0.05) by Tukey's test.

2.4. Results and Discussion

2.4.1. The Impact of Frying Time on the Formation of 3-MCPD and Glycidol in Fried Oil

2.4.1.1. The change of 3-MCPD and glycidol during deep-fat frying

Figure 13 illustrates the change of 3-MCPD equivalent in oil fried with fresh potato strips. During five frying days, the levels of 3-MCPD equivalent detected in oil decreased from 1.37 μg/g to 0.23 μg/g (Russet Burbank), 0.23 μg/g (Ranger Russet), and 0.22 μg/g (Umatilla Russet). 3-MCPD equivalent decreased rapidly during the first two frying days and decreased slower during the rest of three frying days. Different cultivars did not show significant difference on the change of 3-MCPD equivalent.

Figure 14 illustrates the change of glycidol equivalent in oil fried with fresh potato strips. During five frying days, the levels of glycidol equivalent detected in oil increased from 0.02 μg/g to 0.70 μg/g (Russet Burbank), 0.59 μg/g (Ranger Russet), and 0.58 μg/g (Umatilla Russet). Glycidol equivalent increased rapidly during the first frying days and increased slower during the rest of three frying days. Different cultivars did not show significant difference on the change of glycidol equivalent.

Similar trend was also observed from Wong et al. (2017). The 3-MCPD equivalent decreased with the increased frying days indicated 3-MCPD was oxidized, decomposed, or migrated during the prolonged heat process. 3-MCPD esters are not stable during the long heat process, and their decomposition rate may be faster than their formation rate. Glycidol equivalent increased with the increased frying days suggested GEs are more stable than 3-MCPD esters in the prolonged heat process under a constant temperature.
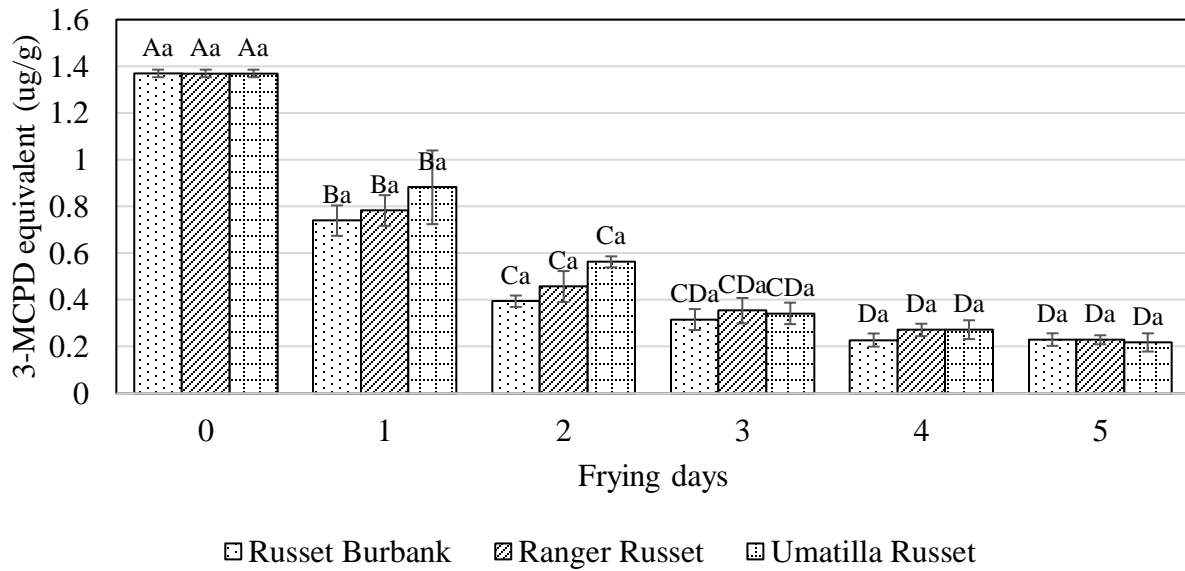
Figure 13. The change of 3-MCPD equivalent in oil fried with fresh potato strips
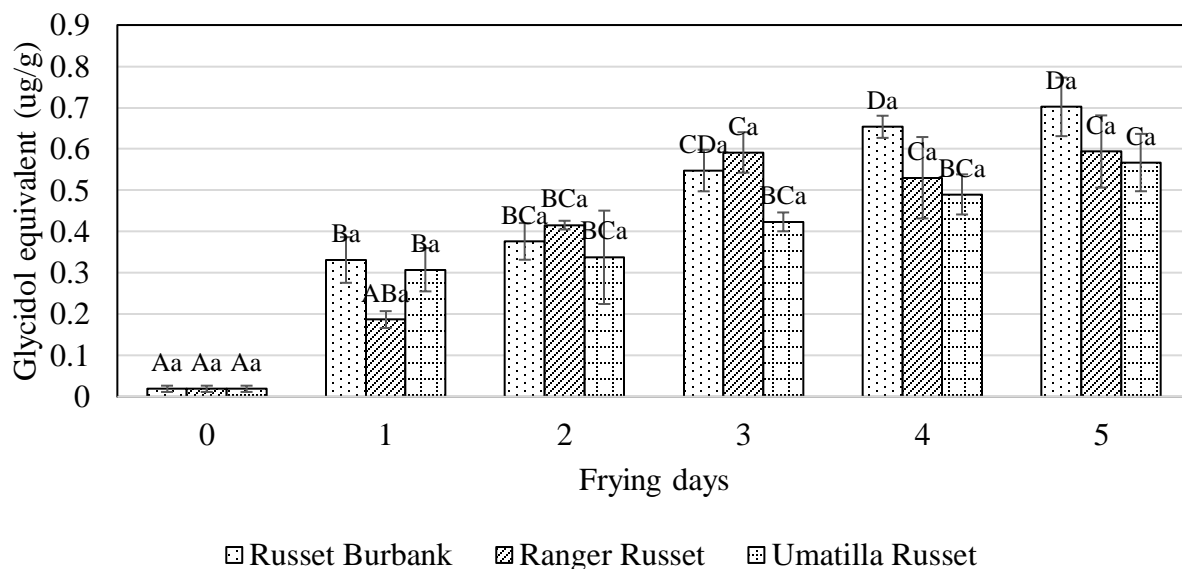
Figure 14. The change of glycidol equivalent in oil fried with fresh potato strips

2.4.1.2. Correlation between changes in tocopherols and 3-MCPD and glycidol during deep-fat frying

Tocopherols are antioxidants that naturally exist in vegetable oils and act to ensure oil quality. It is essential that sufficient tocopherols exits in the diet (Gordon & Kourimská, 1995). Therefore, extra tocopherols are usually added into the oil intentionally during refining to enhance oxidative stability (Choe & Min, 2006). The total amount of tocopherols content present in edible oils is influenced by cultivars, processing steps, the condition applied to convert the crude oils into edible oils, and the storage of the oils (Choe & Min, 2006). In general, vegetable oil contains four forms of tocopherol homologs: α-, β-, γ-, and δ-tocopherols. The concentration of those tocopherols and isomers plays a vital role in the tocopherol antioxidant activity in oils (Evans, Kodali, & Addis, 2002). δ-tocopherol has the strongest free radical scavenging activity, then followed by γ-, β-, and α-tocopherol (Choe & Min, 2006). In general, tocopherols exhibit the most significant antioxidant activity at lower concentrations and show decreased antioxidant activity, or become prooxidant at higher concentrations (Evans et al., 2002). For examples,

27

Evans et al. (2002) summarized the optimal concentrations of antioxidant activity of α-tocopherol (100–250 μg/g), γ-tocopherol (250–500 μg/g), δ-tocopherol (500–1000 μg/g), and the mixture of tocopherol homologs in soybean oil (500–750 μg/g).

Table 1 shows the change of tocopherols in oil during five frying days of Russet Burbank. As can be seen, the initial concentration of tocopherols in vegetable oil is DL-α-tocopherol (82.2 μg/g), rac-β-tocopherol (21.62 μg/g), γ-tocopherol (322.63 μg/g), and δ-tocopherol (817.22 μg/g). The results are in agreement with the data provided by Evans, Kodali, & Addis (2002).

Table 1. The change of tocopherols in oil (μg/g) during five frying days of Russet Burbank

Day	DL-α-Tocopherol	rac-β-Tocopherol	γ-Tocopherol	δ-Tocopherol
0	82.20±3.28a	21.62±1.58a	322.63±6.09a	817.22±40.83a
1	35.22±0.20b	13.01±0.62b	216.12±2.51b	322.65±3.41b
2	18.10±1.204c	9.72±1.29c	165.14±0.38c	131.90±0.76c
3	10.02±0.47d	4.81±0.71d	114.56±2.95d	36.91±4.06d
4	6.40±0.53de	2.92±0.21de	79.86±0.79e	11.32±0.76d
5	4.73±0.34e	1.78±0.41e	57.37±0.50f	5.19±0.42d

All four tocopherols decreased during frying process. This denotes the deep-fat frying process decomposed tocopherols and caused the antioxidant activity of frying oil to decrease with deep-fat frying. As antioxidants, tocopherols decrease the oxidation rate of oil at room temperature. Nonetheless, the antioxidant activity become less effective at high (deep-fat frying) temperatures because the tocopherols were lost by volatilization or decomposition (Eunok Choe & Lee, 1998).

Gordon & Kourimská (1995) reported the changes of tocopherol in oil that fried with potatoes. The decrease of α-tocopherol was faster than that of β-tocopherol, γ-tocopherol, and δ-tocopherol. The study also confirmed the loss of α-tocopherol is much more rapid than other tocopherols at frying temperature and showed the presence of more antioxidants might reduce

the loss of tocopherols as well. As a result, the addition of antioxidants (e.g., rosemary extract) can be considered when investigating the formation of 3-MCPD during deep-fat frying in the future.

Table 2 shows the results of Pearson correlation between change in tocopherols and change in 3-MCPD and glycidol during deep-fat frying. A positive correlation of the dynamic changes of 3-MCPD and a negative correlation of GE and the change of tocopherols was established. According to the results of the Pearson correlation, tocopherols might be an indicator to predict the dynamic changes of 3-MCPD and glycidol in oil samples during frying.

Table 2. The Pearson correlation between change in tocopherols and change in 3-MCPD and glycidol during deep-fat frying

	DL-α-Tocopherol	rac-β-Tocopherol	γ-Tocopherol	δ-Tocopherol
Fresh Russet Burbank				
3-MCPD in oil	0.9968**	0.9754*	0.9852*	0.9974**
Glycidol in oil	-0.9755*	-0.9938**	-0.9884*	-0.9772*
3-MCPD in strips	0.9901**	0.9841*	0.9981**	0.9919**
Fresh Ranger Russet				
3-MCPD in oil	0.9963**	0.9809*	0.9896*	0.9974**
Glycidol in oil	-0.9241	-0.9698*	-0.9720*	-0.9309
3-MCPD in strips	0.9998*	0.9554*	0.9833*	0.9999**
Stored Ranger Russet				
3-MCPD in oil	0.9948**	0.9905**	0.9962**	0.9964**
Glycidol in oil	-0.8981	-0.9591*	-0.9580*	-0.9059
3-MCPD in strips	0.9976**	0.9692*	0.9913**	0.9984**
Fresh Umatilla Russet				
3-MCPD in oil	0.981*	0.9941**	0.9982*	0.9847*
Glycidol in oil	-0.9887*	-0.9757*	-0.9749*	-0.9878*
3-MCPD in strips	0.9811*	0.9923**	0.9999**	0.9836
Stored Russet Umatilla				
3-MCPD in oil	0.9910**	0.9953**	0.9990**	0.9932**
Glycidol in oil	-0.9222	-0.9722*	-0.9726*	-0.9290
3-MCPD in strips	0.9999**	0.9493	0.9795	0.9999**

Note - *Correlation is significant at the 0.05 level (two-tailed)
**Correlation is significant at the 0.01 level (two-tailed)

2.4.1.3. Correlation between changes in total polar compounds and change in 3-MCPD and glycidol during deep-fat frying

As introduced, during the deep-fat frying process, the moisture of fresh potato strips, oxygen, and high temperatures affect the chemical reactions such as hydrolysis, oxidation, and

polymerization. The chemical composition of the oil changes during frying due to these reactions. All of these newly generated products (FFA, MAG, DAG, altered TAG, and some polar polymers) are considered as polar compounds. So the level of TPC in oil is a reliable measurement for oxidative degradation (Arafat, 2014).

Significant levels of polymers were formed during deep-fat frying because the medium contains abundant oxygen, then the oxidized polymer is proved to accelerate oil oxidation (Arafat, 2014). Those polymers can further accelerate oil degradation by decreasing oil viscosity, producing foam during deep-fat frying, reducing heat transfer, and delivering undesirable flavor to the fried food (Arafat, 2014). Besides, polymers lead to higher oil absorption to food. The FFA and their oxidized compounds delivered off-flavor that influences the frying oil quality and causes the frying oil unacceptable for further deep-fat frying (Arafat, 2014).

In this research, the initial TPC in vegetables was 8.5%, but after frying, the overall TPC (Table 3 & 4) increased drastically with increased frying days. Specifically, Umatilla Russet showed significant difference ($p < 0.05$) in TPC than the other two cultivars. Figure 15 shows the appearance of oil samples collected at the end of each day. The oil samples from Day 4 and Day 5 have already generated off-flavor and are greasy. The change in oil color intuitively showed the progress of oil deterioration for five days of deep-fat frying. Frying oil in the deep fryer was observed began to form the foam on the surface of the oil as well. More than that, the amount of foam increased and perceived a pungent off-flavor over frying time. The results proved deep-fat frying negatively impacted the flavor and color of the oil samples. If the end-point criteria of TPC set at the percentage of 30%, the oil is not able to use after two days (16 h) of frying.

Table 3. The percentages (%) of total polar compound (TPC) in oil fried with fresh potato strips

Cultivars	TPC (%)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Russet Burbank	8.5	19.0	25.0	36.0	>40	>40
Ranger Russet	8.5	18.0	25.5	36.5	>40	>40
Russet Umatilla	8.5	19.0	27.5	38.0	>40	>40

Note - The highest detectable percentage of the tester is 40.0%

Table 4. The percentages (%) of total polar compound (TPC) in oil fried with stored potato strips

Cultivars	TPC (%)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Stored Ranger Russet	8.5	18.4	27.6	37.3	>40	>40
Stored Russet Umatilla	8.5	18.6	28.1	39.0	>40	>40

Note - The highest detectable percentage of the tester is 40.0%

Figure 15. The change in color of the oil during five frying days
Note - Oils from left to right represent the oil samples collected at 0 (without frying), 1, 2, 3, 4, and 5 days, respectively.

By comparison of Table 3 & 4, it can be noticed that stored potato strips led to relatively higher TPC. For instance, TPC in fresh Ranger Russet were 8.5%, 18%, 25.5%, 36.5% at 0, 1, 2, and 3 days, respectively; TPC in stored Ranger Russet were 8.5%, 18.4%, 27.6%, 37.3% at 0, 1, 2, and 3 days, respectively. That indicated longer storage time of potato cultivars could negatively accelerate the oil degradation and impact the oil quality.

As shown in Table 5, the change in 3-MCPD showed a negative correlation, while glycidol showed a positive correlation with the change of tocopherols. According to the results of Pearson correlation and the observation during deep-fat frying, the TPC of the oil could be

used as a standard of oil quality for oil polymerization (FFA, MAG, DAG, and TAG), and be an indirect measurement of 3-MCPD and glycidol in oil samples during frying.

Table 5. The Pearson correlation between change in TPC and change in 3-MCPD and glycidol during deep-fat frying

	Fresh Russet Burbank	Fresh Ranger Russet	Stored Ranger Russet	Fresh Russet Umatilla	Stored Russet Umatilla
3-MCPD in oil	-0.9342	-0.9393	-0.9669*	-0.9848*	-0.9705*
Glycidol in oil	0.9723*	0.9926**	0.9949**	0.9228	0.9987**
3-MCPD in strips	-0.9732*	-0.9571*	-0.9894*	-0.9975**	-0.9703*

Note - *Correlation is significant at the 0.05 level (two-tailed)
**Correlation is significant at the 0.01 level (two-tailed)

2.4.1.4. The migration of 3-MCPD and glycidol in fried potato strips

Figure 16 illustrates the change of 3-MCPD equivalent in potato strips. During five frying days, the levels of 3-MCPD equivalent found in potato strips decreased from 1.14 to 0.03 μg/g (Russet Burbank), 1.09 to 0.06 μg/g (Ranger Russet), and 0.86 to 0.05 μg/g (Umatilla Russet). A similar result was found in fried oil, 3-MCPD equivalent in potato strips decreased rapidly during the first two frying days and decreased slower during the rest of three frying days.

Figure 17 illustrates the change of glycidol equivalent in potato strips. During five frying days, the levels of glycidol equivalent detected in potato strips increased from 0.13 to 0.3 μg/g (Russet Burbank), 0.06 to 0.32 μg/g (Ranger Russet), and 0.11 to 0.42 μg/g (Umatilla Russet).

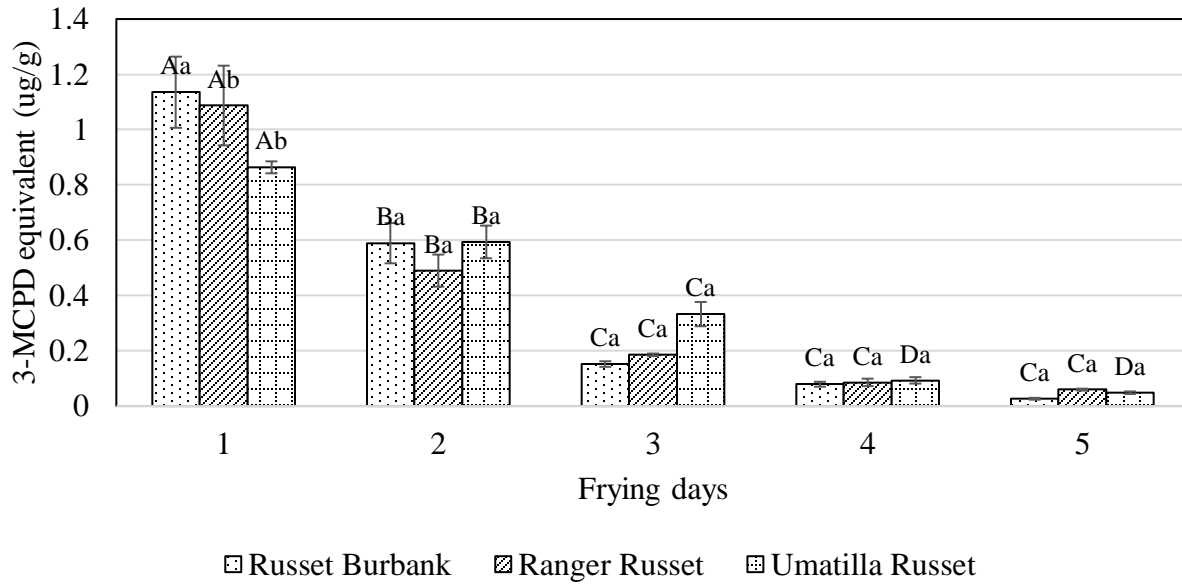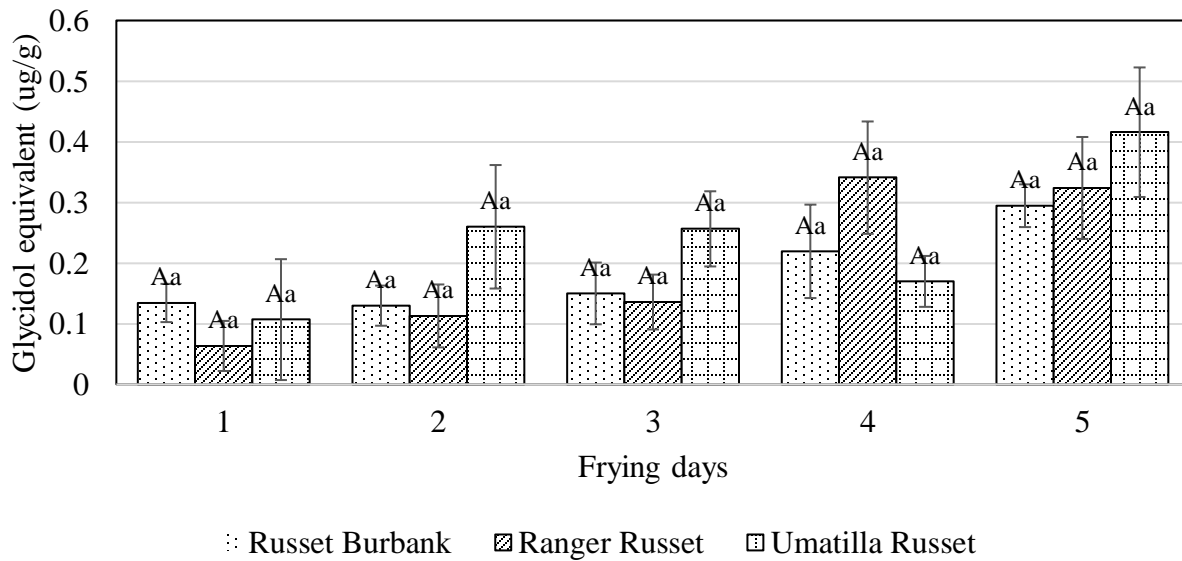Figure 16. The change of 3-MCPD equivalent in potato strips

Figure 17. The change of glycidol equivalent in potato strips

Zelinkova, Commission, & Velisek (2009) investigated the occurrence of 3-MCPD esters

in potato strips and found there was no significant differences ($p > 0.05$) regarding the potato

cultivars and growing location. Besides, 3-MCPD esters in palm oil (0.65 - 1.93 µg/kg) were

higher than in potato strips (0.23 - 1.00 µg/g). From that, they suggested that most of the 3-MCPD esters in potato strips were from the frying oil.

According to the comparison from Figure 16 & 17, it can be found that potato strips can absorb the oils which contained 3-MCPD and glycidol during the heating process. The major 3-MCPD and glycidol equivalent in potato strips were migrated from the oils. One of the reasons that caused 3-MCPD equivalent in fried oil decreased was because of the absorption of partial 3-MCPD and the removal of potato strips. Based on the results, it is speculated that oil uptakes by potato strips would increase with the increase of five frying days due to the oil degradation, which formed FFA, MAGs, DAGs and affected the interfacial tension of the frying oil. Therefore, the change of oil uptakes of the potato strips was further investigated.

The lipids in potato tubers are responsible for the membrane "fluidity". Galliard et al. (1973) stated that the lipid composition of the potato tubers indicated the biophysical properties of membrane structure. Lipoprotein is the essential lipids of the membrane structures of potato tubers that plays an effective role in osmotic characteristics and ion transport regarding these membranes' life function (Rastovski et al., 1981). As one of the precursors involved in the 3-MCPD formation, chorine ion can be affected by the lipoprotein structure and further influence the formation and migration of 3-MCPD and glycidol during deep-fat frying.

2.4.1.5. The change of oil uptake during deep-fat frying

Tables 6 & 7 show the oil uptake of potato strips prepared by fresh and stored potato. The highest oil uptake was 15.28 g oil/100g, and the lowest was 10.58 g oil/100g for potato strips made by fresh ones; For stored potato, the highest oil uptake in strips was 11.49 g oil/100g, and the lowest oil uptake was 8.37 g oil/100g. The ranges of oil uptake are relatively consistent with the study by Millin et al. (2016b) under a similar frying condition.

Table 6. The oil uptake (g oil/100 g) of strips made by fresh potato

	Day 1	Day 2	Day 3	Day 4	Day 5
Fresh Russet Burbank	13.60±0.52Aa	15.28±0.85Aa	13.03±0.30Aa	13.88±1.50Aa	13.90±0.89Aa
Fresh Ranger Russet	14.02±1.52Aa	15.18±0.27Aa	15.05±2.04Ab	14.88±0.81Aa	12.50±0.46Aa
Fresh Russet Umatilla	10.58±1.43Ab	16.00±1.05Ba	15.46±1.10Bb	15.03±1.97Ba	12.72±0.88ABa

Table 7. The oil uptake (g oil/100 g) of strips made by stored potato

	Day 1	Day 2	Day 3	Day 4	Day 5
Stored Ranger Russet	9.85±0.59ABa	11.54±1.38Aa	11.87±0.24Aa	8.63±0.52Ba	10.95±1.04Aa
Stored Russet Umatilla	10.47±1.89ABa	11.46±0.71Aa	11.49±0.49Aa	8.37±1.09Ba	9.01±0.60ABb

An increase in oil uptakes was anticipated in strips during deep-fat frying because of the oil degradation and the change of interfacial tension of the frying oil, as demonstrated in the previous discussion. Yet, the results obtained did not sufficiently support this assumption. With the increase of frying days, no simple trends were found regarding oil uptakes by both of the fresh and stored potato strips. Different cultivars did show significant differences ($p<0.05$) in some of frying days regarding the oil uptakes.

According to Table 6 & 7, the stored potato strips observed lower levels of oil uptakes than the fresh potato strips. For instance, the oil uptake of fresh Ranger Russet ranged from 12.5 to 15.18 g/100g; whereas the oil uptake of the stored Ranger Russet ranged from 8.63 to 11.87 g/100g. Arisseto et al. (2018) suggested that during frying of French fries (180ºC, 6 mins), the moisture in potato was lost and replaced by oil due to the heat and mass transfer. Consequently, the potatoes with higher moisture content tend to absorb more oil since more water was

evaporated. They also believed that 3-MCPD esters content in deep fat-fried potato strips was proportional to oil uptake. However, our findings cannot support such statements. The differences may attribute to the different frying conditions (frying time, type of oil, and frying temperature) used.

Zhang et al. (2018) found the total and structural oil absorbed by potato chips increased with the increased frying time; the penetrated surface oil increased during the first 2 mins, then decreased; the oil infiltration was consistent with the sequence from the outside to the inside of potato chips. Furthermore, they demonstrated the oil uptake was closely associated with the pore properties of the potato. The porosity was defined as the ratio of the volume of pores to the total volume of the potatoes (Millin et al., 2016b). Zhang et al. (2018) reported the pore volume and porosity have a proportional effect on the oil uptake. By applying X-ray micro-computed tomography (Micro-CT) scanning, a non-destructive technique to represents the cross-section of the internal structure of the potato strips, Millin et al. (2016b) analyzed the porosity and observed similar results that greater moisture loss resulted in more extensive crust development. Thus, oil is easier to permeate into the potato with thicker crust and larger pores.

Another factor that can be taken into consideration was the surface and interfacial tensions due to oil degradation, as discussed previously. The moisture evaporation during frying causes hydrolysis, which is the cleavage of the ester bonds between fatty acids and glycerol (Dana & Saguy, 2006). MAG and DAG are polar compounds that can increase the foam to entrap the steam bubbles that further accelerates the oil hydrolysis (Dana & Saguy, 2006). Dana & Saguy (2006) reviewed some data of related studies when frozen French fries was repeatedly fried by canola oil, a procedure similar with the current study. The surface tension of the canola oil had a slight change (30.1 to 29.3 mN/m); the interfacial tension only had marked decrease

36

within the first 10 h (from 24.4 to16.5 mN/m), followed by a slight decrease during the rest of 30 h (16.5 to 13 mN/m). Therefore, surface and interfacial tensions of the frying oil seemed not to be a major factor affecting the oil uptake at a certain frying temperature.

After combining with the analysis of the migration of 3-MCPD during deep-fat frying in Section 2.4.1.4, as well as the findings based on those studies, it can be postulated that more moisture content involved in the frying system caused higher moisture evaporation and oil uptakes during frying. Consequently, there is more opportunity to develop larger pores and resulting in higher 3-MCPD esters absorption.

2.4.2. The Impact of Storage Time of Potato on the Formation of 3-MCPD and Glycidol

To understand how storage time could potentially impact the formation of 3-MCPD and glycidol in fried strips, the fresh potato was stored at 6ºC in the dark and dry storage room for 6 months without any treatments. Figure 18, 19, 20 & 21 illustrate the change of 3-MCPD and glycidol equivalent in oil and potato strips, which fried with stored potato strips. In the fried oil, the levels of 3-MCPD equivalent decreased from 1.37 to 0.55 µg/g (stored Ranger Russet), and 0.50 µg/g (stored Umatilla Russet); the levels of glycidol equivalent in oil increased from 0.02 to 0.48 µg/g (stored Ranger Russet), and 0.56 µg/g (stored Umatilla Russet). In the potato strips, the levels of 3-MCPD equivalent decreased from 0.81 to 0.04 µg/g (stored Ranger Russet), and 0.81 to 0.04 µg/g (stored Umatilla Russet); the levels of glycidol equivalent increased from 0.52 to 0.61 µg/g (stored Ranger Russet), and 0.51 to 0.53 µg/g (stored Umatilla Russet).
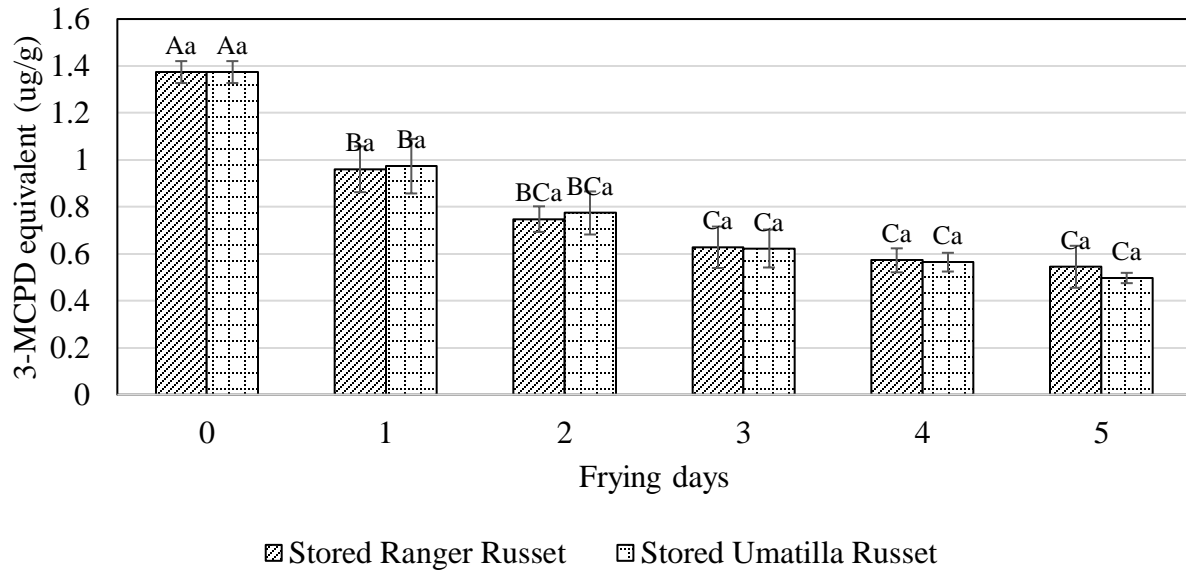
Figure 18. The change of 3-MCPD equivalent in oil fried with stored potato strips
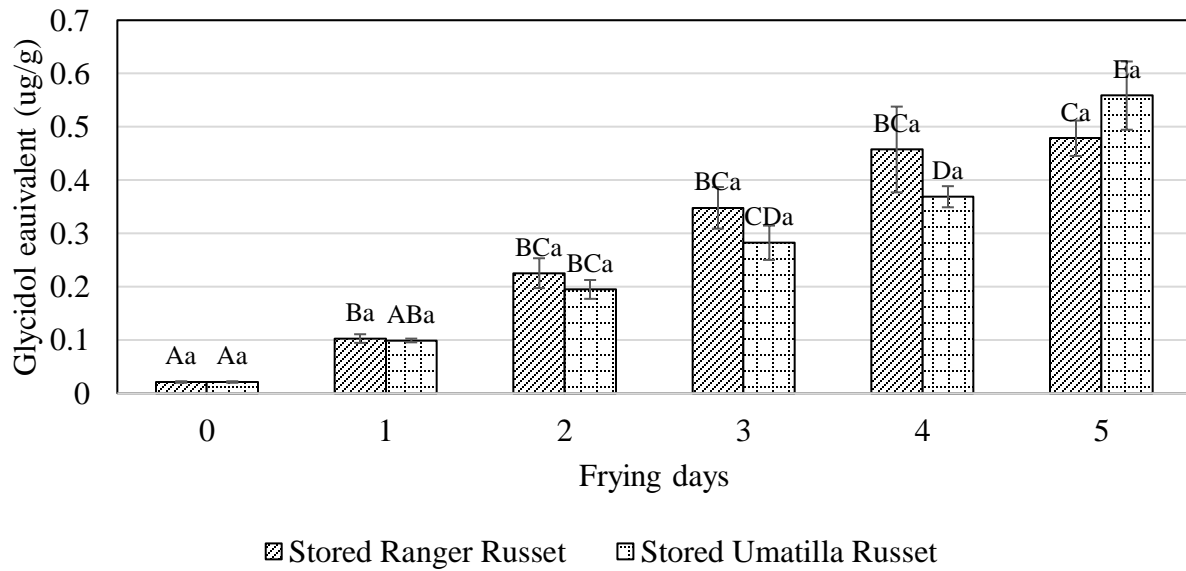

Figure 19. The change of glycidol equivalent in oil fried with stored potato strips
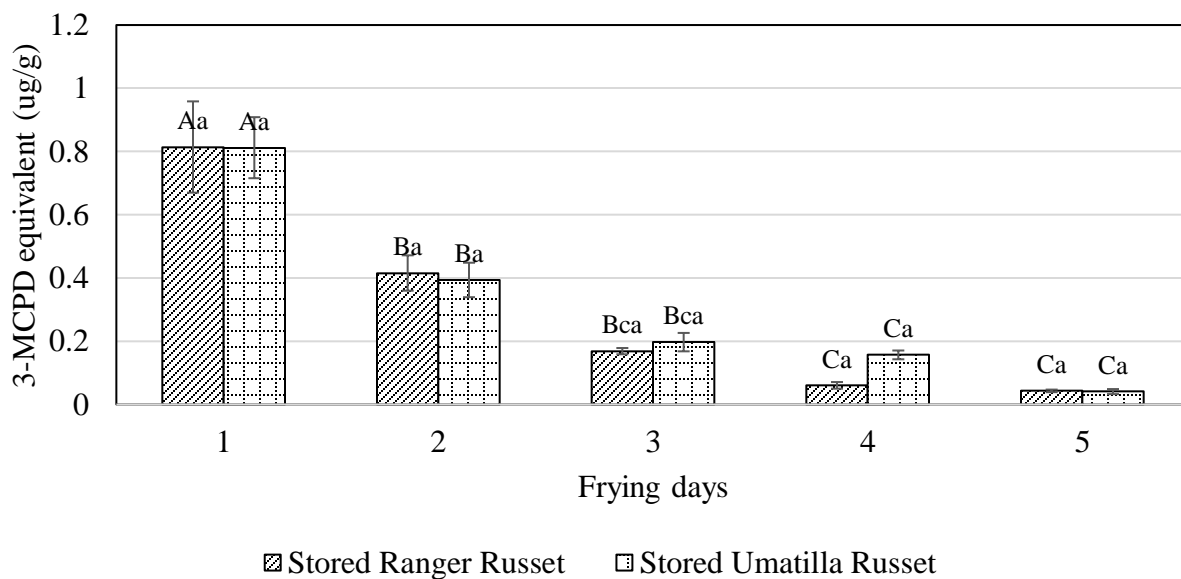
Figure 20. The change of 3-MCPD equivalent in stored potato strips
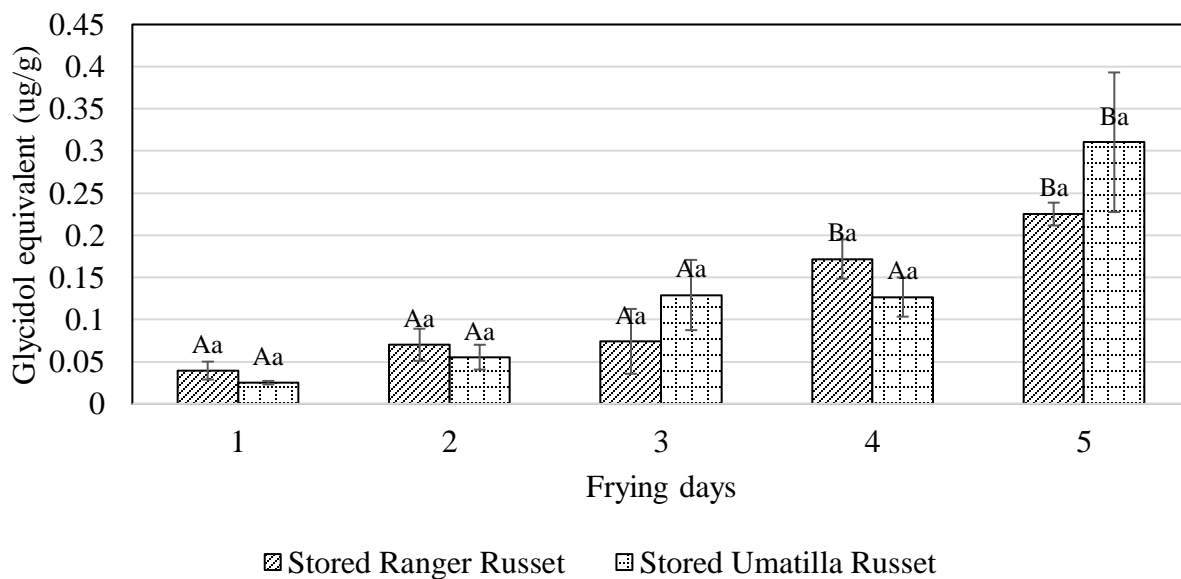
Figure 21. The change of glycidol equivalent in stored potato strips

After a comparison of those results of fresh potato strips, the stored potato strips had relatively higher levels of 3-MCPD equivalent and lower levels of glycidol equivalent than fresh potato strips in fried oil samples. Therefore, it speculated that longer storage time of potato cultivars slowed down the degradation of 3-MCPD. As previously discussed, water and oxygen

are important factors regarding oil degradation during deep-fat frying. During the long-term storage, the potato tubers constantly contacted with the storage atmosphere, which means the air composition (oxygen and water) surrounding the potatoes. After long-term storage, a phenomenon can be noticed that the stored potato tubers were much softer and moister than the fresh potato tubers. This allows us to believe there was moisture gain during the long-term storage due to the specific storage condition, resulting in a higher amount of water involved in the frying system.

Another speculation of these differences between fresh and stored potato tubers was there was a change of the lipid composition and polyunsaturated fatty acids during the long-term storage. The major lipids of the potato tubers are primarily related to the membrane structure, such as phospholipids and glycolipids. The degree of unsaturation of fatty acids is crucial for the functional properties of those membrane lipids. The activation of enzymes (lipoxygenase and lipolytic acyl hydrolase) in potato tubers during long term storage may accelerate lipid oxidation, thus producing off-flavor because the polyunsaturated fatty acids (e.g., linoleic acid and linolenic acids) will break down rapidly (Rastovski et al., 1981). Therefore, a lower level of polyunsaturated fatty acid present in potatoes is preferable to the stability of the processed potato products. Galliard et al. (1973) reported that the storage of potato was found a significant reduction ($p<0.05$) in polyunsaturated acid, which may enhance the oxidative stability of the lipids in potato products consequently.

Galliard, Berkeley, & Matthew (1975) analyzed the lipid composition of two potato cultivars stored at different storage temperatures (5 and 20ºC). They found an increase of TAG for both cultivars during both storage temperatures. TAG is one of the crucial precursors involving in the formation of 3-MCPD. They summarized that potato cultivar and storage

40

temperatures are not likely the main factors significantly ($p<0.05$) influencing the oxidative degradation of lipids and lead to off-flavor and rancidity to the potatoes. Therefore, lipid composition does not likely play a vital role in the storage of the potato tubers.

According to the results of TPC from Section 2.4.1.3, the strips made by stored potato had relatively higher TPC than the ones made by fresh potato. This manifests that the long-term storage of potato tubers without any treatments may promote the degradation of lipids. Lots of changes have occurred during the long-term storage of which cannot be easily analyzed individually on the effect of the formation of 3-MCPD and glycidol. The parameters (the change in water content, degree of sprouting, lipid composition, and so on) of those three cultivars of potatoes for 6 months' storage are currently under investigation. Therefore, the current data was not sufficient to analysis and support the effect of storage time between fresh and stored potatoes regarding the formation of 3-MCPD and glycidol. That being said, the results of strips from stored potato have provided more experimental evidence to demonstrate the speculation of the effect of frying time and the migration of 3-MCPD and glycidol equivalents that previously discussed.

2.4.3. The Change of 3-MCPD and Glycidol Using Absorbents

Magnesol (magnesium silicate) and Celite (diatomaceous earth) were applied as absorbents to mitigate the migration of 3-MCPD and glycidol in fried oil. Figure 22 & 23 illustrate the change of 3-MCPD and glycidol equivalent in stored potato strips after using Magnesol. Figure 24 & 25 illustrate the change of 3-MCPD and glycidol equivalent in stored potato strips after using Celite. As can be seen, both absorbents showed a marked reduction in 3-MCPD and glycidol equivalent. In result, Magnesol and Celite were successfully applied as

absorbents and achieved the mitigation of 3-MCPD and glycidol in frying oil. Furthermore,

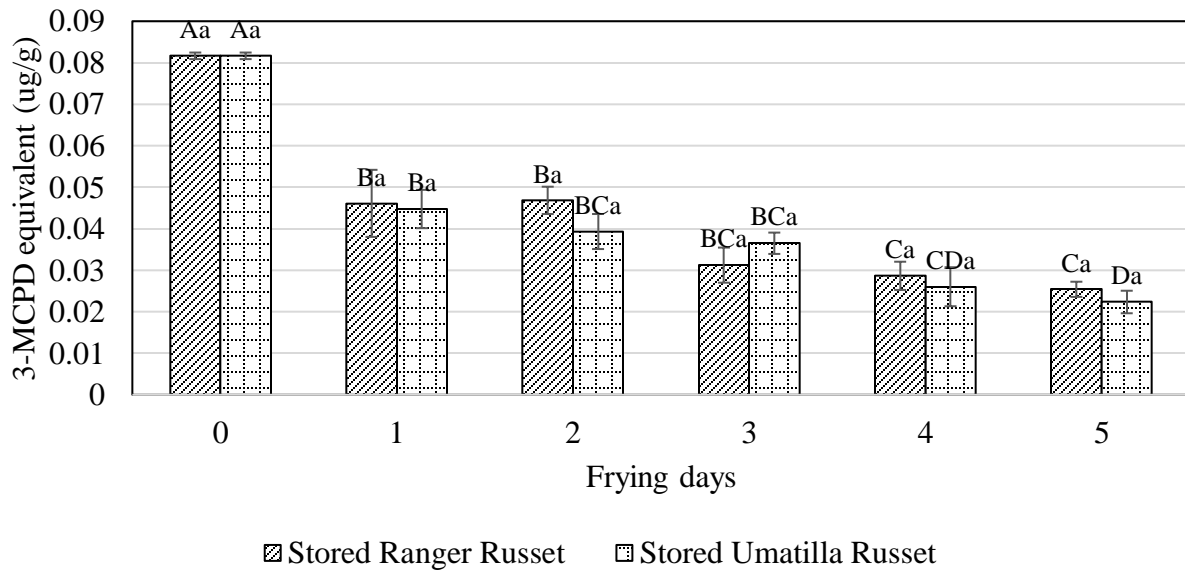Celite showed greater absorption than Magnesol.

Figure 22. The change of 3-MCPD equivalent in oil fried with stored potato strips after using Magnesol
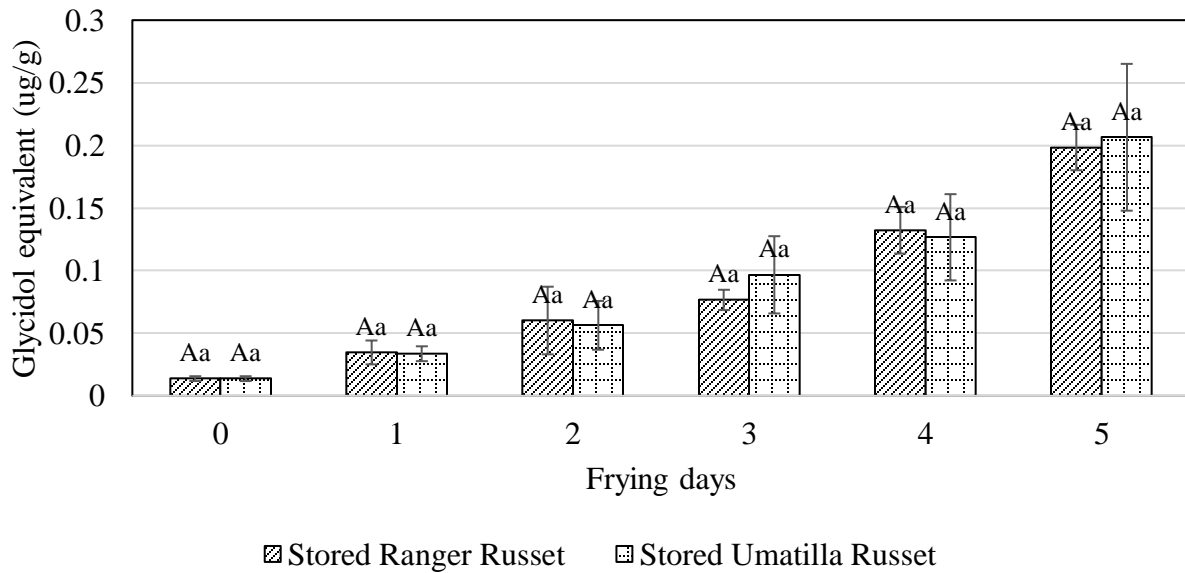
Figure 23. The change of glycidol equivalent in oil fried with stored potato strips after using Magnesol
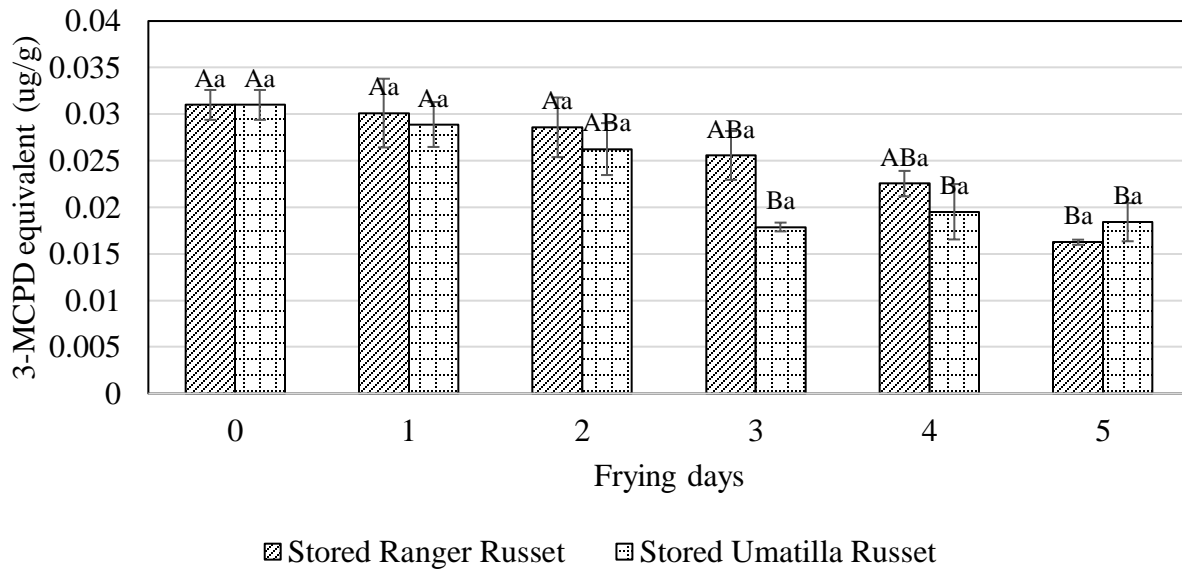
Figure 24. The change of 3-MCPD equivalent in oil fried with stored potato strips after using Celite
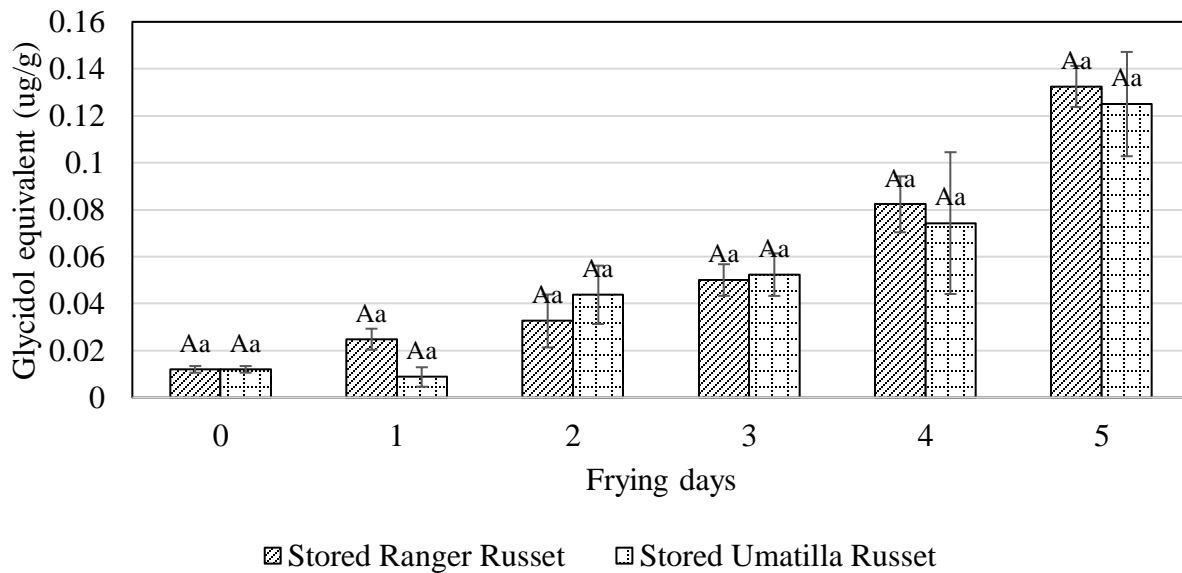
Figure 25. The change of glycidol equivalent in oil fried with stored potato strips after using Celite

Absorbents are believed to absorb polar components including MAG, DAG, FFA, free glycerol and, other impurities in the oil (Banga & Varshney, 2010; Faccini et al., 2011; Lin, Akoh, & Estes Reynolds, 1998; Suseno, Tajul, Nadiah, & Noor, 2012). For example, Faccini et al. (2011) evaluated the efficiency of Magnesol as an absorbent and found that 1% of Magnesol

exhibited remarkable reduction in total glycerol (0.71% to 0.28%), free glycerol (0.26% to 0.02%), MAGs (0.14% to 0.09%), DAGs (0.14% to 0.06%), and TAGs (0.19% to 0.11%) in the unpurified oil sample. The morphology of Magnesol was analyzed by scanning electron microscopy (SEM) which presented spherical particles with diameters ranging from 10 to 50 μm.

Lin et al. (1998) evaluated several commonly used absorbents, including Magnesol and Celite. It can be noted that Celite showed a greater ability to absorb FFA (59.1%), whereas Magnesol showed a greater effect on the oil color, which lightened the oil color by 46.3%. Zhu et al. (1994) summarized that oil absorption of absorbents were affected by pH, polarity, particle size, surface-active sites, moisture, surface area, and porosity. In addition to that, they reported Magnesol has a surface area of 619 m2/g and a pH of 8.5; Celite has a surface area of less than 10 m2/g and a pH of 9.6; Magnesol has 10.8% moisture content, and Celite has 0.1%. Magnesol has the highest total acidic sites of 1.8 mM/g, whereas Celite has less than 0.1 mM/g; therefore, strong surface-active sites were assumed to be mainly responsible for lightening the color. Regarding the effect on the absorption of 3-MCPD and glycidol equivalent, it is speculated that Celite exhibited more effective absorption because of its lower surface area, lower moisture content, and higher pH value.

2.5. Conclusion

Generally, during five frying days, 3-MCPD equivalent in oils decreased with the increased frying time in three different cultivars potatoes. Meanwhile, glycidol equivalent in oils increased with the increased frying days. The results indicated 3-MCPD was degraded, and glycidol was generated during deep-fat frying. Different cultivars did not have significant effects on the change of 3-MCPD and glycidol equivalent overall experiment. Tocopherols and TPM correlated significantly with 3-MCPD and glycidol equivalents during deep-fat frying, and they

44

can be applied as indicators for oil degradation and the levels of 3-MCPD and glycidol equivalents in frying oil. Higher levels of 3-MCPD was found in frying oils and strips prepared from potato after long-term storage (6 months) without any treatment. Both absorbents (Magnesol and Celite) showed a significant reduction of 3-MCPD and glycidol equivalents in frying oils. Celite exhibited greater effect on the mitigation of 3-MCPD and glycidol equivalents than Magnesol.

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