Studies on the Frying Stability and Quick Quality Evaluation Method of Local Rapeseed Kizakinonatane Oil

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ABSTRACT

Kizakinonatane, which was developed by the Tohoku National Agricultural Experimental Station, is one of the major domestic rapeseed cultivars in the Tohoku region. It was released as the first zero-erucic Japanese domestic rapeseed variety in 1989. Recently, the expeller-pressed Kizakinonatane oil (EPKO) has been receiving favorable consumer reviews because it is produced under low heat and without additional chemicals. EPKO has a characteristic taste, specific aroma, and enchanting color. Additionally, EPKO was selected as it is locally available and preferred in the Tohoku region. However, there is minimal literature on the constituents of the EPKO, and its frying stability has yet to be reported. This has raised concern among consumers and begets questions about how efficient and rapid quality control of this oil is currently possible.

The research described in this thesis sought to: a) analyze the characteristics and composition of the fresh EPKO, b) determine its frying stability of EPKO and, c) develop a practical technique for quick quality evaluation of frying oils using near-infrared (NIR) spectroscopy.

In Chapter 1, the research background and a general literature review that is related to the current study are presented.

In Chapter 2, the characteristics and composition of fresh EPKO were analyzed for fatty acid composition, minor components, and physicochemical properties. Commercial refined, bleached, and deodorized canola oil (CO) was used for comparison. Results showed that EPKO contains unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA) such as linoleic acid and linolenic acid, which are different from that of the CO. The EPKO had higher levels of minor compounds such as tocopherol, total phenolic, β-carotene, and chlorophylls as compared to that of the CO. As a result of the large amount of β-carotene in the EPKO, the EPKO was deeper yellow than the CO. Furthermore, the EPKO had a good oxidative stability index (OSI), viscosity, low acid value and peroxide value that are within the limits allowed by the regulations.

In Chapter 3, a comprehensive study was conducted to compare the frying stability of EPKO and CO during the intermittent frying of frozen French fries at 180, 200, and 220°C for 7 h daily over 4 consecutive days, with or without fresh oil replenishment. Results obtained from both frying experiments clearly showed significantly slower rates of total polar compounds (TPC) and carbonyl value (CV) formation in the EPKO samples as compared to that of the CO samples during frying sessions. In other words, the EPKO exhibited a much better oxidative stability than that of the CO. The

EPKO also displayed lower levels of viscosity and comparable color (optical density) values than that of the CO during the frying process. The EPKO showed a significantly higher acid value (AV) level than that of the CO in both frying experiments. The high AV level of EPKO could be attributed to the fact that components which impair the quality of the oil, such as free fatty acids and chlorophyll, were not removed as they did not undergo the refining process. As more free fatty acids were released in the oil, it became more susceptible to thermal oxidation under elevated frying temperatures. The changes in the peroxide value (PV) were irregular during frying in both oil, which could be attributed to the fact that peroxides are unstable compounds and apt to break down to a variety of nonvolatile and volatile products under high temperatures. Replenishment with fresh oil had significant effects on all chemical and physical parameters, except the PV of the frying oils.

Results showed an obvious difference in fatty acid changes between both oils during deep frying. There was a significant decrease in linoleic acid contributions in the EPKO. In the CO, however, the linoleic acid decreased much more significantly than that of the EPKO in the frying experiments with or without replenishment. A deterioration of the linolenic acid was observed in both EPKO and CO, and the CO samples also showed a greater change in the deterioration of the linolenic acid during the frying sessions. The PUFA/SFA

(saturated fatty acid) ratios in the EPKO significantly decreased at the end of the frying sessions. In the case of the CO, the PUFA/SFA ratios were much more pronounced than that of the EPKO after 28 h of frying in both frying experiments. The EPKO showed a significantly slower rate of antioxidants decomposition (tocopherols) than that of the CO during frying at 180 and 200°C. A rapid decrease in total polyphenols content was observed before 14 h of frying in both frying experiments. After 21 h of frying, the total polyphenols could only be quantified in the oil that was fried at 180°C, and had disappeared in all the oil samples after 28 h of frying without replenishment. A similar fast decrease in the polyphenols was also observed in the frying experiment with replenishment. Results show a rapid decrease in chlorophylls content in all EPKO samples when they were subjected to heating at different temperatures in both frying experiments, indicating that the chlorophyll pigments were sensitive to high temperature and that replenishment had little effects in preventing its loss under high temperatures .

Based on the results obtained from this study, the following conclusions were made: 1) EPKO appeared to be more stable during prolonged heating treatments in terms of CV, TPC, fatty acids, and minor components; 2) variations in antioxidants levels (mainly caused by different processing procedure) and degradation rates in oils appeared to explain, in part, some of

the differences in frying stability. 3) fatty acid composition also has an effect on frying stability; 4) oil deterioration increases with increase in frying temperature and time; 5) frequent replenishment with fresh oil significantly improves the frying stability and prolongs the useful life of oils.

In Chapter 4, an investigation into the potential of the NIR spectroscopy as a simple technique for the rapid determination of the degradation of frying oils was conducted using partial least-squares (PLS) regression. There was a strong correlation between the NIR predicted data and reference data in AV results, with coefficient of determination (R^2) values ranging from 0.96 to 0.99 and standard error of prediction (SEP) values from 0.17 to 0.48 mg/g. The most accurate model was the model that involves the first-derivative spectra in the wavelength range of 1800-2200 nm. It used five partial least-squares factors and produced a high coefficient of determination and low values of standard error of calibration (SEC), standard errors of cross-validation (SECV), and SEP. The high RPD (ratio of performance to deviation) value of 12.8 indicates that the model used in this study was useful.

Similar to the AV results, there were strong correlations between the NIR predicted data and the reference data in TPC results with R^2 values of 0.98 and SEP values from 1.04 to 1.4%. The most accurate model was the model that involves the first-derivative spectra in the wavelength range of

1100-1800 nm. It used six PLS factors and generated a high R^2 value and low values for SEC, SECV, and SEP. The RPD value was found to be 7.8. The current results demonstrate that frying oils can be successfully monitored to a very high accuracy using NIR spectroscopy. Furthermore, NIR spectroscopy has significant advantages over other measurement techniques; it is a fast and simple method that requires no sample preparation so it is a very practical method for measuring chemical changes such as AV and TPC values in edible oils during the frying process.

In Chapter 5, a general conclusion, limitations, and a suggestion for future research to this study are included. On the whole, the results presented in chapters 2, 3, and 4 comprising this work can serve as a guide to current literature for those who wish to use domestic rapeseed oil safely, and also indicate that NIR spectroscopy can be used as a rapid and accurate quality control tool for frying oils.

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LIST OF SYMBOLS AND ABBREVIATIONS

>	Greater than
<	Less than
=	Equals
≥	Greater than or equal to
±	Plus or minus
+	Addition
/	Per
–	Minus
×	Times
&	and
°C	Degree Centigrade
µL	Microliter
um	Micrometer
µmol	Micromole
ANN	Artificial neural networks
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
ASTM	American Society for Testing Materials
AV	Acid value
C	Carbon
CA	Cluster analysis
CH	Carbon -Hydrogen bond
cm	Centimeters
CO	Canola oil
CV	Carbonyl value
DNA	Deoxyribonucleic acid
DPLS	Discriminant partial least squares
EPKO	Expeller-pressed Kizakinonatane oil
et al.	Et allii (and others)
etc	Et Cetera
FAME(s)	Fatty acid methyl ester(s)
FFA(s)	Free fatty acid(s)
g	Gram
GC	Gas chromatography
h	Hour

H	Hydrogen
H_2O	Water
HPLC	High performance liquid chromatography
i.d.	Internal diameter
i.e	id est
JOCS	Japan Oil Chemists' Society
kg	Kilogram
KOH	Potassium hydroxide
KNN	K-nearest neighbors
L	Liter or lightness
LDA	Linear discriminant analysis
Ltd	Limited
LWR	Locally weighted regression
m	Meters
meq	Milliequivalent
mg	Milligram
min	Minutes
mL	Milliliter
mm	Millimeters
MRL	Multiple linear regression
MUFA(s)	Monounsaturated fatty acid(s)
N	Normality
nd	Not detected
NIR	Near infrared spectroscopy
No.	Number
NPLS	Non-partial least squares
nm	Nano meter
O	Oxygen
OSC	Orthogonal signal correction
OSI	Oxidative stability index
P	Probability
PCA	principle component analysis
PLS	Partial least squares
PUFA(s)	Polyunsaturated fatty acid(s)
PV	Peroxide value
PRC	Principle component regression
QDA	Quadratic discriminant analysis
r	Correlation coefficient of prediction
R	Replenishment
R^2	Coefficient of Determination

RMSEC	Root mean square error of calibration
RMSECV	Root mean standard error of cross validation
RMSEP	Root mean standard error of prediction
RPD	Ratio of performance deviation
SAE	Sinapic acid equivalents
SD	Standard deviation
SEC	Standard error of the calibration
SECV	Standard errors of cross-validation
SEP	Standard error of the prediction
SFA	Saturated fatty acid
SIMCA	Soft independent modeling of class analogies
SNV	Standard normal variate
SPSS	Statistical Package for the Social Sciences
SVM	Support vector machine
SW-NIR	Short-wave near infrared spectral region
TPC	Total polar compounds
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
v/v	Volume to volume

CHAPTER 1 LITERATURE REVIEW

1.1 Rapeseed/Canola Oil

The global production of Brassica oilseeds has become an important source of vegetable oils over the past two decades, second only to soybeans. Over 66 million metric tons of rapeseed oilseeds have been produced worldwide in 2013 (Table 1-1). The nutritional quality of oil essentially depends on its fatty acid composition. The rapeseed oil contains nutritionally desirable oleic acid that gives stability to the oil, linoleic and linolenic acids, which are essential fatty acids, as well as lower amounts of saturated fatty acids as compared to other vegetable oils. However, early rapeseed cultivars contained high amounts of erucic acid, which has been blamed for producing fatty deposits in the heart, skeletal muscles, and adrenals of rodents as well as impairing growth. It also had high levels of sulphur-containing compounds (glucosinolates) in the meal, which, along with its degradation products, have adverse effects on animals' productivity and is thus considered detrimental for animal feed purposes. In consideration of the adverse effects of diets that are rich in erucic acid and glucosinolates, breeding efforts were initiated at the global level towards improving the nutritional quality of rapeseed oil and

meal. The world's first low erucic acid and low glucosinolates cultivar of Brassica napus was released in 1967 (McLeod, 1974). After significant improvements and modifications of the original high-erucic acid rapeseed cultivars, canola oil (a low erucic acid and glucosinolates rapeseed oil) is now considered one of the most nutritious edible oils. "Canola", which is commonly used in the American continent and Australia (also known as "double-zero"), is a trade name for rapeseed oil containing less than 2% erucic acid and less than 30 μmol/g glucosinolates in meal. The name "rapeseed" is used mainly in Europe and other countries (Przybylski et al., 2005).

Rapeseed is one of the major oilseeds available in Japan. In the late 1930s, the Japanese government established a national program for rapeseed research to strengthen and increase its capacity for rapeseed production. By the early 1950s, Japan significantly increased its rapeseed production, and even exported some of the surplus oil from 1962 to 1972 (Tanaka *et al.*, 1999). However, the volume of domestically produced rapeseed oil quickly waned as a result of a combination of urban migration and cheap imports. Total rapeseed production in 2012 was 1,890 metric tons, which met only 0.08% of Japan's annual consumption demand, according to the reports by United States Department of Agriculture (USDA, 2013). Despite a dwindling agricultural population, the Ministry of Agriculture, Forestry and Fisheries of

Japan recently announced plans to increase domestic rapeseed production to a target volume of 10,000 metric tons, ten times the current level by 2020. In 2013, 2.45 thousand metric tons of rapeseed was consumed in Japan, up by 3% from 2012. Table 1-2 shows a sustained stable demand for rapeseed oil consumption in the world.

Table 1-1 Major oilseeds: world supply and distribution.

(Commodity view) (Million metric tons)

Production / Oilseed	2009/10	2010/11	2011/12	2012/13	Jul 2013/14	Aug 2013/14
Copra	5.88	6.02	5.66	5.96	5.98	5.98
Cottonseed	38.91	43.55	46.41	45.33	44.60	44.01
Palm Kernel	12.44	12.88	13.66	14.70	15.36	15.38
Peanut	35.92	39.52	37.87	39.93	36.43	39.22
Rapeseed	61.06	60.60	61.67	62.28	64.84	66.44
Soybean	260.40	263.92	239.15	267.58	285.89	281.72
Sunflower seed	32.14	33.63	40.64	36.31	39.81	40.34

Source: “Oilseeds: World Markets and Trade” (USDA, August 2013).

Table 1-2 Rapeseed and products: world supply and distribution.

(Thousand metric tons)

Production	Marketing year	Meal, Rapeseed			Oil, Rapeseed			Oilseed, Rapeseed		
		2011/12	2012/13	2013/14	2011/12	2012/13	2013/14	2011/12	2012/13	2013/14
China	Oct-Sep	10,122	10,564	10.187	5,725	5,974	5,761	13,426	14,000	14,200
India	Oct-Sep	3,645	3,645	3,670	2,310	2,310	2,330	6,200	6,800	7,000
Canada	Aug-Jul	3,870	3,720	3,880	3,127	2,930	3,068	14,608	13,310	15,300
Japan	Oct-Sep	1,296	1,325	1,313	1,000	1,015	1,012	1	1	1
European Union	Jul-Jun	12,441	13,057	13,051	8,980	9,424	9,421	19,226	19,161	20,500
Other		4,507	4,341	4,737	3,137	3,002	3,278	7,711	9,003	9,438
Imports	Marketing year	2011/12	2012/13	2013/14	2011/12	2012/13	2013/14	2011/12	2012/13	2013/14
China	Oct-Sep	666	80	100	1,036	1,400	1,100	2,622	2,900	2,500
India	Oct-Sep	0	0	0	98	45	50	0	0	0
Canada	Aug-Jul	8	5	5	92	125	75	97	150	150
Japan	Oct-Sep	14	75	100	29	20	10	2,350	2,380	2,450
European Union	Jul-Jun	231	380	250	605	250	300	3,762	3,400	3,300
Other		4,152	4,518	4,479	2,139	1,842	1,979	4,361	3,231	3,832

Source: “Oilseeds: World Markets and Trade” (USDA, August 2013).

Rapeseed cultivars that have been genetically modified have low levels of erucic acid and glucosinolates, making the oils healthier for human consumption (Yang *et al.*, 2013). Different rapeseed varieties have different fatty acid compositions, and these vary according to the genetic characteristics and environmental conditions. Oils extracted from rapeseed contain significant amounts of monounsaturated fatty acids (44–75%), high amounts of polyunsaturated fatty acids (PUFAs, 22–35%), and small amounts of saturated fatty acids (SFAs, 5–10%) (Szydłowska-Czerniak *et al.*, 2010). In addition to an optimally balanced fatty acid composition, considerable

amounts of bioactive compounds, including tocopherols, polyphenols, sterols, and carotenoids, are present in rapeseed oils (Ratnayake and Daun, 2004, Yang *et al.*, 2011). Because of their potent antioxidative capabilities, these natural antioxidants are believed to provide protection from cardiovascular diseases, cancers, and neurodegenerative diseases (Erkkilä *et al.*, 2008, Yoshie-Stark *et al.*, 2006).

Despite the widespread distribution of various types of oils throughout the world and the fact that Japan basically relies on imports for its oil intake, domestic rapeseed oils are important for Japanese cooking because a specific oil is often selected for use based on local availability and regional preferences.

Kizakinonatane (Brassica napus) (registration No. Natane Norin 47), one of the major domestic rapeseed cultivars in the Tohoku region of Japan developed by the Tohoku National Agricultural Experimental Station, was released as the first zero-erucic Japanese domestic rapeseed variety in 1989 (Okuyama *et al.*, 1994). Kizakinonatane was selected from a cross between “Tohoku-72” and “Rapora” with the purpose of combining the productivity of “Tohoku-72” with the zero erucic acid of “Rapora”. Kizakinonatane is now commonly cultivated throughout the northern part of the Tohoku region in Japan because of its greater intrinsic cold tolerance and higher oil-yielding

ability as compared with other domestic varieties. Expeller-pressed oils have been receiving much attention because expeller-pressed oils usually retain more of the chemical constituents of their natural sources, including the fatty acid contents and minor components, than regular commercial oils (solvent-extracted). Consumers tend to believe that expeller-pressed oils have higher nutritional values than conventionally produced oils because of the lack of chemicals involved in the production process.

1.2 Deep-Fat Frying

Deep-fat frying is a popular and important procedure for both industrial and domestic preparation and manufacture of foods. The flavors, textures, and appearance of food can be enhanced simply by immersing them in hot oil for the specified times required by various food types (Choe and Min, 2007, Warner, 2004). In addition, when compared with other common culinary procedures, frying causes smaller nutrient losses and even enhances the nutritive value of food in a shorter cooking time (Fillion and Henry, 1998, Casal *et al.*, 2010).

During deep-fat frying, the oil is continuously used as a frying medium at elevated temperatures in the presence of air (oxygen), a metal container, and water (moisture). Under these conditions, a variety of deteriorative chemical

reactions take place, including: hydrolysis, oxidation, polymerization, isomerization, and cyclization (Figure 1-1) (Yamsaengsung and Moreira, 2002, Moreira *et al.*, 1999, Gertz, 2000). All of these chemical reactions lead to the decomposition of frying oil and the formation of numerous volatile and nonvolatile products, which may have deleterious effects on human health (Tyagi and Vasishtha, 1996, Dobarganes and Márquez-Ruiz, 2003).

Chemical reactions are initiated by water, steam, and oxygen as food is being fried in oil. The ester linkages of triacylglycerols are hydrolyzed by water and steam, resulting in diacylglycerols and monoacylglycerols, and eventually free fatty acids and glycerol. Part of the glycerol evaporates at temperatures that are higher than 150°C and the remaining glycerol prompts further production of free fatty acids by hydrolysis. The reaction scheme is shown below:

Triacylglycerol + H_2O = Diacylglycerol + Free fatty acid (FFA) (1)

Diacylglycerol + H_2O = Monoacylglycerol + Free fatty acid (FFA) (2)

Monoacylglycerol + H_2O = Glycerol + Free fatty acid (FFA) (3)

Hydrolysis of oil commonly takes place during frying. However, hydrolysis cannot occur unless oil and water form a solution. The presence of a surfactant is also required (Gupta, 2005). Surfactants such as oil

decomposition products and the food itself can prompt the formation of an oil/water solution during frying, which is primarily responsible for the production of free fatty acids in frying oils.

Under high frying temperatures, oxygen, along with the addition of food, reacts with the oil and initiates a variety of oxidative alterations. Hydroperoxides, one of the primary oxidation products, are unstable and apt to further undergo three major types of oxidative degradation (Figure 1-1). The first degradation is the “formation of free radicals”, which produces most of the decomposition products such as oxidized dimers, trimers, epoxides, alcohols, hydrocarbons and non-polar dimers and polymers. The second degradation is the “fission” process, which generates alcohols, aldehydes, acids, and hydrocarbons. The third degradation is the “dehydration” process, which creates ketons (Paul *et al.*, 1997, Zahabi, 2009).

Heating the frying oil causes another thermal oxidation reaction - polymerization, with or without the presence of oxygen (Figure 1-1). The fatty acids found in oils are cleaved by heat, and these cleaved compounds then react with each other, leading to the formation of compounds with high molecular weight and polarity, such as cyclic monomers, dimers, and polymers. As polymerized products increase in the frying oil, the viscosity of

the oil increases, heat transfer reduces, foam generates, and the fried foods turn an undesirable color (Choe and Min, 2007).

Many frying operations involving foods like potato chips, vegetables, and chicken are conducted at temperatures of 160-185°C while extruded products and pellets are typically fried at 195-215°C (Gupta, 2004). During the deep-fat frying process, oils are not only employed as a heat-exchange medium, but also contribute to the quality of fried products as the frying oil is absorbed by the food (Shahidi and Wanasundara, 2002). A wide variety of factors can affect the level of oil deterioration during frying, including type of food, type of oil, type of fryer, unsaturation/saturation of fatty acids, initial oil quality, metals in food or oil, oil temperature, antioxidants, frying time and rate, frying protocols (continuous or intermittent), surface-to-volume ratio of oil, oil replenishment, and oil filtration (Gerde et al., 2007a, Choe and Min, 2007, Gupta et al., 2004, Smith et al., 2007, Artz et al., 2005, Farhoosh and Moosavi, 2008, Mariod et al., 2006, Matthäus, 2006, Paul et al., 1997, Clark and Serbia, 1991, Normand et al., 2006).

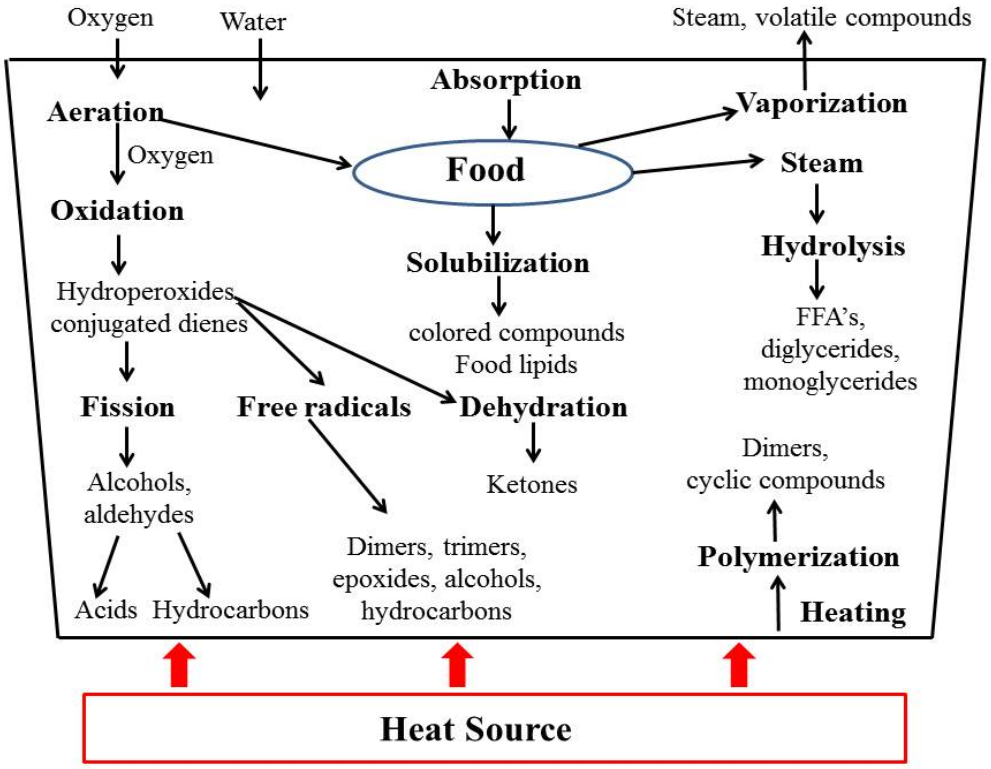

Figure 1-1 Physical and chemical reactions that occur during frying.

Source: (Gupta et al., 2004)

1.3 Near-Infrared (NIR) Spectroscopy and Applications

Near-infrared (NIR) spectroscopy has recently become increasingly important and popular as a rapid and nondestructive analytical technique for industrial quality control and process monitoring. NIR spectroscopy is now widely used in numerous fields, including: chemical, petrochemicals, polymers, textiles, pharmaceuticals, cosmetics, agriculture, food, and medical. (Siessler, 2007, Siesler *et al.*, 2008, Huang *et al.*, 2008, Wang and Paliwal, 2007, Reich, 2005, Luypaert *et al.*, 2007, Macho and Larrechi, 2002). The usefulness and utilization of this technique is primarily attributed to the

following advantages: a) speed of analysis (often takes less than one second to get results), b) minimal or no sample preparation required, c) nondestructive analysis (analyzed samples can be returned to the original lot), d) accuracy and reproducibility (equal to reference testing), e) efficiency and flexibility (multiple constituent per measurement up to 32), f) environmental friendliness (no additional chemicals used), and g) easy and safe operation (no special training is required and measurements can be done in harsh or hostile environments using fiber optic probes) (McClure and Tsuchikawa, 2007).

1.3.1 Basic Principles of Near-Infrared (NIR) Spectroscopy

Infrared radiation is electromagnetic energy (molecular vibration) with wavelengths that are longer than visible light but shorter than microwaves. The region of the electromagnetic radiation is conveniently defined as, both instrumentally and functionally, the near-infrared, covering 780 to 2500 nm (12,821–4000 cm^{-1}); the mid-infrared (or infrared), covering 2500 to 25,000 nm (4000–400 cm^{-1}); and the far-infrared (or terahertz), covering 25,000 to 1,000,000 nm (400–10 cm^{-1}) (Figure 1-2) (Workman and Weyer, 2007).

According to the American Society for Testing Materials (ASTM), the NIR region of the electromagnetic spectrum is defined as the wavelength range of 780-2565 nm corresponding to the wave number range of 12,820-

3959 cm^{-1} (Figure 1-2). It covers the wavelength range adjacent to mid-infrared and extends up to the visible region. The NIR region of the electromagnetic spectrum is usually divided into the short-wave near infrared spectral region (SW-NIR) of 780–1,100 nm (12,820–9091 cm^{-1}) and the long wave near infrared spectral region of 1,100–2,526 nm (9091–3959 cm^{-1}). In the NIR spectral region, most absorption bands are overtones and/or combination bands of the fundamental vibration of chemical bonds (such as -CH, -NH, -OH, -SH, and -CO.) of organic molecules occurring in the mid-infrared region (Reich, 2005, Tan *et al.*, 2012).

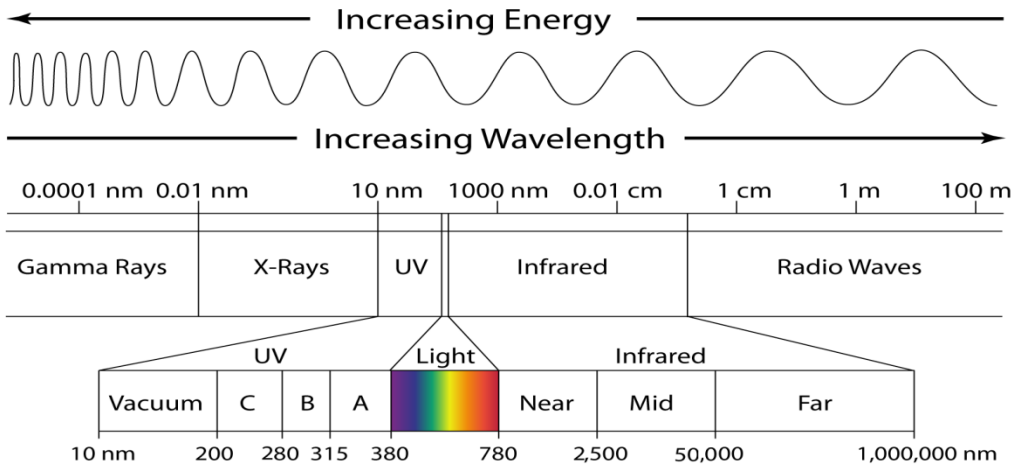

Figure 1-2 Schematic representation of the electromagnetic spectrum.

Source: (Pool, 2013)

When radiation interacts with a sample, the incident radiation may be reflected, absorbed, transmitted, or scattered. Therefore, there are different NIR spectroscopy measurement mode settings for different applications. In

practice, transmittance, transflectance, reflectance, and reactance are the four most commonly used NIR measurement modes (Armenta *et al.*, 2010, Huang *et al.*, 2008). Figure 1-3 depicts the most common measurement modes employed by NIR spectroscopy. Transmittance (Figure 1-3a) mode involves the measurement of radiation that passes through a sample following the Beer-Lambert's law, while reflectance (Figure 1-3c) mode deals with the measurement of radiation reflected from a sample surface. The transflectance mode (Figure 1-3b) is the combination of transmittance with reflectance. The radiation is transmitted through the sample and then scattered back from a diffusely reflecting plate (usually made of ceramic or aluminum). In the case of the interactance mode (Figure 1-3d), a higher probability is given to the incident beam to interact with the sample and the emerging light is detected from a point that is different from the point of incidence on the sample's surface (Pasquini, 2003, Pojić and Mastilović, 2013, Osborne, 2000).

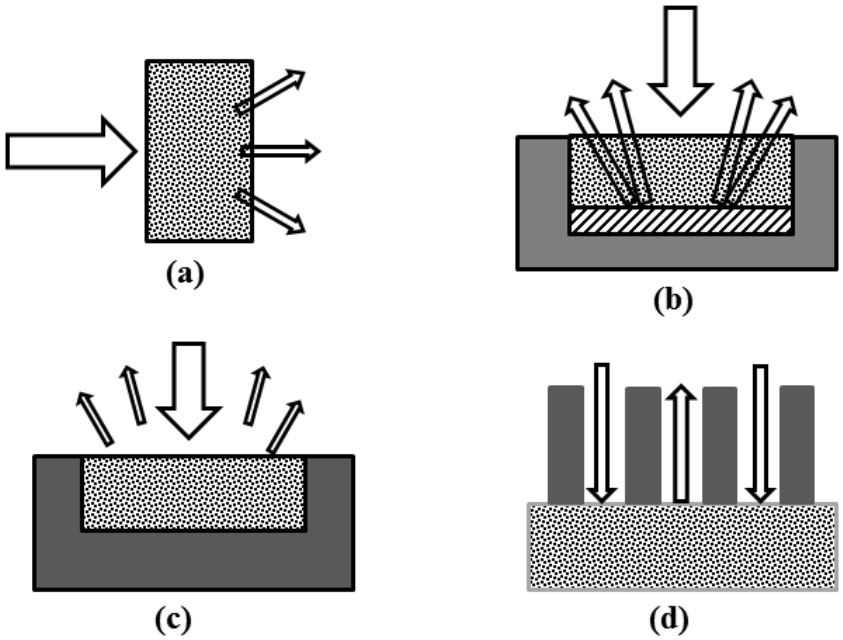

Figure 1-3 Modes of measurements employed in NIR spectroscopy.
(a) transmittance; (b) transflectance; (c) reflectance; (d) interactance
Source: (Tsuchikawa, 2007)

1.3.2 Chemometric Methods in Near-Infrared (NIR) Spectroscopy

As mentioned previously, NIR spectra are weak, broad, heavily overlapping, and it is more difficult to assign the bands in this spectrum as compared to the corresponding mid-infrared spectra. These characteristics severely restrict its sensitivity in the classical spectroscopic sense, and multivariate mathematical and statistical methods, known as chemometrics, are required to relate spectral information to sample properties. However, NIR spectral are still rich in information and its low absorption coefficient, which allows for high penetration depth, can be favorably used to perform direct

chemical and physical analysis of strongly absorbing and even highly scattering samples of interest from one single measurement, with little or no pretreatments (Reich, 2005).

Being an indirect method, NIR technology relies heavily on the quality of the reference method that is used for the basis in the calibration development process. In general, the application of the NIR method for qualitative or quantitative analysis comprises two parts: calibration mode development and prediction (Figure 1-4). A good calibration model has to be built before the NIR can be used to predict future unknowns from their NIR spectra. The calibration process consists of four basic steps: a) selection of calibration sample set; b) collection of spectra and reference data; c) multivariate modeling to relate the spectra to the reference data, and d) validation of the model (Reich, 2005). In other words, this process involves regression techniques and spectra preprocessing.

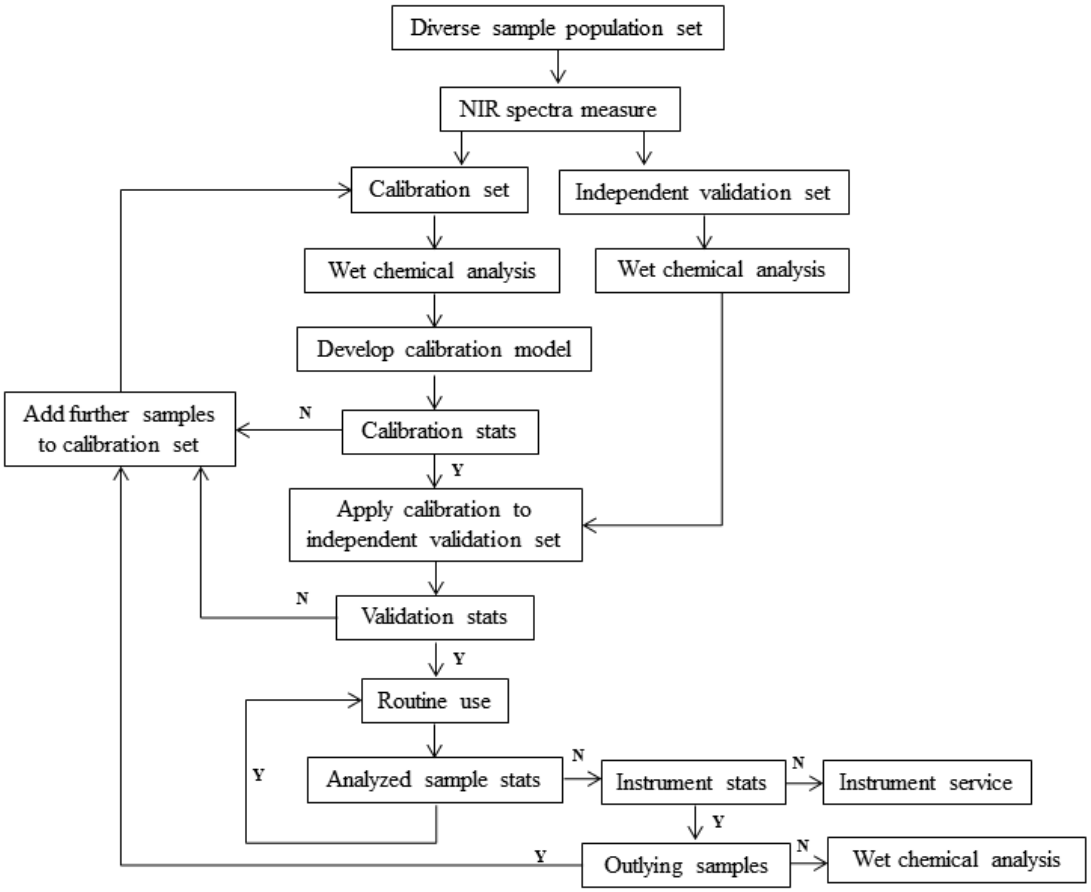

Figure 1-4 NIR spectroscopy calibration validation workflow.

1.3.2.1 Spectral Preprocessing Techniques

Depending on the purpose of analysis, there are three spectra analysis methods that can be used to analyze the NIR spectra: conventional spectral analysis methods, chemometrics, and two-dimensional (2D) correlation spectroscopy. Regardless, it is essential to pretreat or transfer spectral data before modeling because the data acquired contains a lot of background information, noise, and baseline fluctuations. The pretreatment methods can be divided into four general categories as follows: (1) noise reduction

methods, (2) baseline correction methods, (3) resolution enhancement methods, and (4) normalization methods. Smoothing, a noise reduction method that includes the moving-average and Savitzky-Golay methods, is one of the most commonly used methods to eliminate noise (Cen and He, 2007). There are also other methods for noise reduction such as the eigenvector reconstruction, artificial neural networks (ANN), and wavelets, which give a cleaner and more reliable spectrum by only modeling data above a selected threshold (Ozaki *et al.*, 2007, Barclay *et al.*, 1997). The second derivative and multiplicative scatter correction (MSC) are generally the most used methods for baseline correction (Naes *et al.*, 2002). The orthogonal signal correction (OSC), introduced by Wold (Wold *et al.*, 1998), has proved to be a better pretreatment method when used for correcting instrumental drift, bias, and scatter in NIR spectra as compared to other methods (Roggo *et al.*, 2003, Pizarro *et al.*, 2004, Cen and He, 2007). Derivative methods, difference spectra, mean centering, and Fourier self-deconvolution have been commonly used as resolution enhancement methods, which are key in resolving overlapping bands and in elucidating the distinct bands (Cho and Kang, 2011, Ozaki *et al.*, 2007). The standard normal variate (SNV) transformation, robust normal variate (RNV) transformation, de-trend method, and MSC are most frequently employed for spectral normalization (Pojić and Mastilović, 2013).

In summary, the application of pretreatment methods improves the predictive ability of a future calibration model and its selection often involves a trial-and-error approach.

1.3.2.2 Calibration for Quantitative Analysis

The widely accepted and preferred multivariate regression methods multiple linear regression (MRL), principle component regression (PRC), and partial least squares (PLS) have proven their potential in NIR quantitative analysis (Tito *et al.*, 2012, Balabin and Safieva, 2011, Armenta *et al.*, 2007). The application of the above methods is grounded on the assumption that the spectral data and the property of interest are linearly related. However, in some cases, the physic-chemical properties of the sample and/or instrumental effects could result in non-linearity between the spectral data and the target property. Some regression techniques, such as artificial neural networks (ANN), locally weighted regression (LWR), non-partial least squares (NPLS), and support vector machine (SVM), are frequently employed to ameliorate the nonlinear problem (Agelet and Hurburgh Jr, 2010, Wang and Paliwal, 2007).

1.3.2.3 Calibration for qualitative analysis

From a practical point of view, there is often a need for product identification, product composition, and product uniformity. The product

identity or quality, i.e, “good” or “bad”, is presented in the discrete values of the spectral variations of the interest. Multivariate classification methods, also known as pattern recognition methods, are developed for classifying samples with similar characteristics based on a training set of samples with known categories (Luypaert et al., 2007, Cen and He, 2007). The performance of this technique is gauged by the use of a validation that is set by unknown samples. There are a number of multivariate classification methods in NIR qualitative analysis that can be roughly subdivided into two groups: “non-supervised” and “supervised” methods. Cluster analysis (CA), most commonly used as a “non-supervised” method, is capable of producing the grouping or clustering on its own without any a priori information about the group properties (Lee et al., 2005). “Supervised” methods, also known as discriminant analysis, are applied to construct classification rules for a variety of pre-selected subcategories. Figure 1-5 shows the most used quantitative and qualitative multivariate analysis methods in NIR spectroscopy. Methods such as linear discriminant analysis (LDA) (Xanthopoulos et al., 2013), quadratic discriminant analysis (QDA) (Kuhn and Johnson, 2013), soft independent modeling of class analogies (SIMCA) (Xu et al., 2013a), K-nearest neighbors (KNN) (Kramer, 2013), discriminant partial least squares (DPLS) (Jacobsen et al., 1999), principle component analysis (PCA) (Jolliffe, 2005), ANN (Aldrich

and Auret, 2013), and SVM, are frequently used in NIR discriminant analysis (Dai et al., 2014).

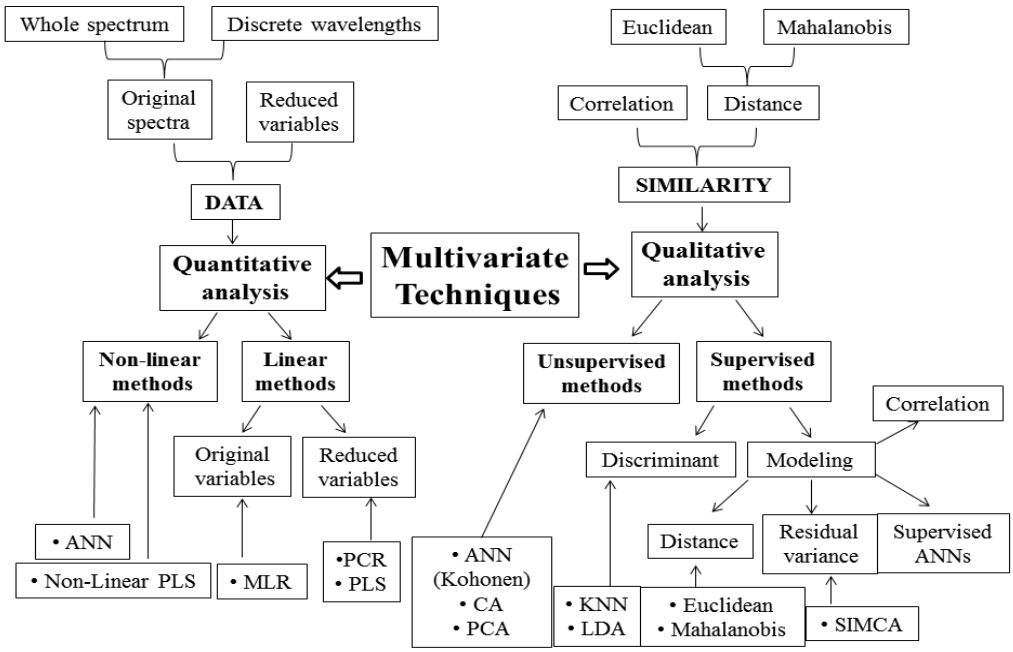

Figure 1-5 Classification of the major quantitative and qualitative multivariate analysis methods used in NIR spectroscopy.
Source: (Barrera-Arellano et al., 2002)

The accuracy of the NIR spectroscopy models for quantity or quality prediction is usually described by coefficient of determination (R^2), root mean square error of calibration (RMSEC), root mean standard error of cross validation (RMSECV), and root mean standard error of prediction (RMSEP). In general, a successful model is typically one with high correlation coefficient (R) or R^2 and low standard error of the calibration (SEC), RMSEC, RMSECV or RMSEP values. Moreover, a good model should have low bias

values (average difference between predicted and measured value), a small difference between the RMSEP and RMSEC, as well as the smallest number of principal components as possible (Magwaza *et al.*, 2012).

1.3.3 Application of Near-Infrared (NIR) Spectroscopy to Food Quality and Quantity Evaluation

Although the discovery of the NIR spectrum was reported by William Herschel as early as 1800, it did not arouse much interest in spectroscopists as an analytical tool. However, the NIR technique has been widely used for the rapid analysis of a variety of agricultural and food products since Karl Norris demonstrated that the NIR spectrometry could be calibrated with multiple linear regression when analyzing agricultural food samples using the NIR technique in the 1960s (McClure and Tsuchikawa, 2007). Since then, NIR spectroscopy has been combined with efficient chemometric data processing techniques, and these techniques have opened up many interesting perspectives for food qualitative and quantitative analysis.

Recently, NIR spectroscopy, with developments in chemometrics and computer programming, has gained wide attention and acceptance from food researchers for raw material testing, product quality control, and process monitoring. In the beverage industry, NIR techniques have been used to

determine the components in nonalcoholic beverages. Chen and his colleagues applied the NIR spectroscopy to determine the sugar composition in raw Ume juice and the major organic acid compositions in raw Japanese apricot fruit juices (Chen *et al.*, 2007, Chen *et al.*, 2006). Several researches were conducted on the determination and discrimination of teas (Chen *et al.*, 2013, Li *et al.*, 2013, Luo *et al.*, 2013, Huo *et al.*, 2013). NIR techniques were also used in alcoholic beverages like wine (Cozzolino *et al.*, 2008, Martelo‐Vidal *et al.*, 2013), beer (Lachenmeier, 2007, Ciaccheri *et al.*, 2014), and distilled spirits (Pontes *et al.*, 2006, Yucesoy and Ozen, 2013). The application of NIR techniques for the quality evaluation of dairy products has become increasingly important and popular. Cevoli *et al* (Cevoli *et al.*, 2013) used NIR spectroscopy to evaluate the authenticity of Parmigiano Reggiano cheese, Kraggerud used NIR to predict the sensory quality of cheese (Kraggerud *et al.*, 2013), and Madalozzo measured fat, protein, and moisture in ricotta cheese using NIR spectroscopy. In addition, NIR analysis has also been used to evaluate the quality of milk, yogurt, and milk powder (Holroyd, 2013, Zhang *et al.*, 2013, Xu *et al.*, 2013b, Botros *et al.*, 2013). A number of previous studies were conducted to detect and qualify the oxidation and adulteration of oil (Gonzaga and Pasquini, 2006, Büning-Pfaue and Kehraus, 2001, Gerde *et al.*, 2007b, Cayuela Sánchez *et al.*, 2013, Mossoba *et al.*, 2013, Nunes, 2013).

Much attention was also given to the evaluation of aquatic and stock products using NIR techniques (Alamprese *et al.*, 2013, Atanassova *et al.*, 2013, Damez and Clerjon, 2013, Liu *et al.*, 2013). In addition, NIR techniques offer potent qualitative and quantitative benefits for other food studies, such as vegetables (Goyal, 2013), fruits (de Oliveira *et al.*, 2014, Blanke, 2013), grain and seeds (Cozzolino *et al.*, 2013, Long *et al.*, 2013), and processed products (Chen *et al.*, 2008, Cozzolino, 2013).

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CHAPTER 2 FATTY ACID COMPOSITION, MINOR COMPONENTS AND PHYSICOCHEMICAL PROPERTIES OF KIZAKINONATANE OIL

2.1 Abstract

Crude expeller-pressed Kizakinonatane oil (EPKO) was analyzed for its fatty acid composition, tocopherols, carotenoids, chlorophyll, total phenolic content, color, oxidative stability, acid value (AV), and peroxide value (PV). Commercial refined, bleached, and deodorized canola oil (CO) was used for comparison. The main fatty acids of the EPKO were oleic acid (C18:1, 63.67%), linoleic acid (C18:2, 19.02%), linolenic acid (C18:3, 7.31%), and palmitic acid (C16:0, 4.06%), with a small amount of erucic acid (C22:1, 1.52%). The major tocopherols of the EPKO were γ-tocopherol (39.75 mg/100 g) and α-tocopherol (20.30 mg/100 g), followed by δ- and β-tocopherols (0.79 and 0.10 mg/100 g, respectively). The carotenoid content of the EPKO (expressed as β-carotene) was found to be 7.15 mg/kg oil, and this is responsible for its deeper yellow color compared with that of the CO (0.13 mg/kg oil). The chlorophyll content and the total phenolic content of the EPKO were 5.60 mg/kg oil and 1245.2 mg sinapic acid equivalents/100 g,

respectively. The viscosity and oxidative stability index (OSI) of the EPKO were comparable to those of the CO. The AV and PV were higher than that of the CO, but still below the limits allowed by general regulations. The results from this study suggest that EPKO could be used to supplement or even replace conventional CO, with benefits to human health.

2.2 Introduction

The global production of *Brassica* oilseed oils is the second largest for vegetable oils, after soybean oil. The annual worldwide production of rapeseed oil is about 23 million tons, and the largest consumer market is China, followed by India and Japan. Before 1940, over 97% of the rapeseed oil consumed in Japan was produced domestically. However, the volume of rapeseed oil produced domestically quickly waned as a result of the import of cheap canola oil, undesirable quality (high levels of erucic acid and glucosinolates were found in the oil and meals), a dwindling agricultural population, and Westernization of the Japanese diet. Over the past two decades, much work has been done in an effort to improve the quality of rapeseed oils produced in Japan. As the nutritional value of rapeseed oil has improved over time, its popularity in Japan has also increased.

Rapeseed cultivars that have been genetically modified have low levels of erucic acid and glucosinolates, making the oils healthier for human consumption (Yang *et al.*, 2013). The quality of domestic rapeseed varieties has been improving along with their nutritional value. Different rapeseed varieties have different fatty acid compositions, and these vary according to the genetic characteristics and environmental conditions. Oils extracted from rapeseed contain significant amounts of monounsaturated fatty acids (44–75%), high amounts of polyunsaturated fatty acids (PUFAs, 22–35%), and small amounts of saturated fatty acids (SAFAs, 5–10%) (Szydłowska-Czerniak *et al.*, 2010). In addition to an optimal balanced fatty acid composition, considerable amounts of bioactive compounds, including tocopherols, polyphenols, sterols, and carotenoids, are present in rapeseed oils (Ratnayake and Daun, 2004, Yang *et al.*, 2011). Because of their potent antioxidative capabilities, these natural antioxidants are believed to provide protection from cardiovascular diseases, cancers, and neurodegenerative diseases (Erkkilä *et al.*, 2008, Yoshie-Stark *et al.*, 2006).

Mechanical pressing is a process in which seed oil is extracted by pressing and grinding the seeds using an extruder or an expeller, without the addition of chemicals (Warner and Dunlap, 2006). Since no additional chemical solvents are used in the production process, expeller-processed oils

retain more of the chemical constituents of their natural sources, including the fatty acid contents and minor components, than regular commercial oils (solvent-extracted). Commercially refined oils are generally used in homes, restaurants, and industrial operations. However, they have bland, neutral flavors, and consumers tend to believe that expeller-pressed oils have higher nutritional values than conventionally produced oils because of the lack of chemicals involved in the production process. They also prefer the characteristic taste, specific aroma, and intense color of expeller-pressed oil. Additionally, despite the widespread distribution of many types of oil, and the fact that canola and soy oils made up 59% of the total Japanese oil market in 2011, a particular type of oil is often selected for culinary use based on local availability and regional preferences. Kizakinonatane (*Brassica napus*) (registration No. Natane Norin 47) was first developed by the Tohoku National Agricultural Experimental Station, and is claimed to be a zero-erucic Japanese domestic rapeseed variety (Okuyama *et al.*, 1994). It is commonly cultivated throughout the northern part of the Tohoku region in Japan because of its greater intrinsic cold tolerance and higher oil-yielding ability compared with other domestic varieties.

Although the quality of an edible oil is crucial to human health and culinary applications, to the best of our knowledge, the physicochemical

properties, fatty acid composition, and bioactive compounds of the expeller-pressed Kizakinonatane oil (EPKO) have yet to be reported. There is minimal data in literature on the constituents of Kizakinonatane seed or oil and this appears to be limited to the work of Embaby et al (Embaby *et al.*, 2010), who determined the minor anti-nutritive components, such as glucosinolates, phytic acid, sinapine, and total phenols in Kizakinonatane meal. The present study was therefore conducted in order to evaluate crude EPKO and determine its fatty acid profile, tocopherol composition, total phenolic content, oxidative stability index (OSI), chlorophyll and carotenoid contents, acid value (AV), peroxide value (PV), and physical properties, such as viscosity and color. The knowledge gained from this study is expected to provide information on the healthy use of EPKO and enable it to be incorporated into food formulations to improve consumer health.

2.3 Materials and Methods

2.3.1 Materials and Reagents

Crude expeller-pressed EPKO and regular commercial CO were both obtained from a local supermarket. Fatty acid methyl standards, Folin-Ciocalteu's phenol reagent, gallic acid monohydrate (purity ≥ 98%), β-carotene, and tocopherol standards (α-, β-, γ-, and δ-T) were purchased from

Wako Pure Chemical Industries Ltd. (Osaka, Japan). Other chemicals and reagents used in the study were analytical grade, unless otherwise stated.

2.3.2 Analytical Methods

2.3.2.1 Determination of Fatty Acids

Fatty acid methyl esters (FAMEs) were prepared using the American Oil Chemists' Society (AOCS) Official Method Ce 2-66 (AOCS, 1997). Oil (0.2 g) was reacted with 2 mL of *n*-hexane, followed by 0.2 mL of methanolic KOH (2 N). The mixture was well shaken for 60 s and centrifuged for 5 min at 1500 rpm. The top layer (1 µL) was injected into a gas chromatograph (Shimadzu GC-2010) equipped with a flame ionization detector and a Shimadzu AOC 20Si autosampler. An Agilent high-polarity HP-88 column (100 m × 0.25 mm i.d., with a 0.25 µm film thickness) was used with helium as the carrier gas, at a flow rate of 2.0 mL/min. The detector temperature was 280°C; the column temperature was held at 50°C for 1 min, increased at a rate of 10°C/min to 170°C and held for 10 min, and then increased at a rate of 5°C/min to 210°C and held for 8 min. The total run time was 38 min. Individual FAME peaks were identified by comparing their retention times against FAME standards. The relative percentage of each fatty acid was

determined by dividing the area under the individual peak by the total peak area of all fatty acids in the oil sample.

2.3.2.2 Determination of Tocopherols

Prior to high-performance liquid chromatography (HPLC) analysis, 0.5 g of oil sample was saponified and extracted using a modified version of Lee's method (Lee *et al.*, 2000). The treated sample (50 μL) was analyzed using a Shimadzu LC-20AT HPLC system consisting of a guard-column (Mightysil Si 60, 5 μm, 5 mm × 4.6 mm, Kanto Chemical Co., Inc., Tokyo, Japan), an analytical column (YMC-Pack SIL-06, 5 μm, 250 mm × 4.6 mm, YMC Co., Kyoto, Japan), an LC-20AT pump, an autosampler (SIL-20A, Shimadzu Co., Japan), and a Shimadzu RF-10AXL fluorescence detector set at an excitation wavelength of 298 nm and an emission wavelength of 325 nm. The flow rate of the mobile phase (hexane–propanol–acetic acid, 1000:6:5, v/v/v, with 5 μg/mL butylated hydroxytoluene) was maintained at 1.5 mL/min. The concentration of each tocopherol was calculated from the calibration curves prepared for α-, β-, γ-, and δ-tocopherol isomers.

2.3.2.3 Determination of Carotenoid Content

The Association of Official Analytical Chemists (AOAC) Official Method 958.05 was used to determine the carotenoids in the oils (AOAC,

1990). The carotenoid content was measured using a calibration curve obtained by preparing β-carotene solutions in hexane in the concentration range of 0.1–3.0 µg/mL. The absorbance at 440 nm was read against a hexane blank on a Benchmark Plus microplate reader (Bio-Rad, Hercules, USA). The amount of total carotenoids in the oil samples was quantified and expressed in terms of β-carotene (mg/kg oil).

2.3.2.4 Determination of Chlorophyll Content

The chlorophyll pigments in the EPKO were determined using the American Oil Chemists' Society (AOCS) Official Method Cc 13i-96 (AOCS, 1998, Revised 2011). The absorbances of the samples were determined at 630, 670, and 710 nm using a UV-3100 spectrophotometer (Shimadzu Co., Kyoto, Japan). The chlorophyll content was calculated using the absorptivity of pheophytin a, which is the main chlorophyll pigment in crude vegetable oils. The chlorophyll contents of the commercial CO were not measured using this method since maximum absorption does not occur at 670 nm in processed oils.

2.3.2.5 Determination of Total Phenolic Content

The total phenolic contents of the oil samples were determined spectrophotometrically using the Folin–Ciocalteu reagent, according to a

previous method (Koski *et al.*, 2002, Gutfinger, 1981), with some modifications. An oil sample (2.5 g) was dissolved in 2.5 mL of hexane, extracted by centrifugation at 5000 rpm for 5 min (Himac CR21F, Hitachi Co., Ltd, Japan) with 2.5 mL of aqueous methanol (80:20 v/v), and the supernatant was collected. This procedure was repeated twice. All three extractions were combined in a 50 mL volumetric flask with 2.5 mL of Folin–Ciocalteu reagent and 5 mL of 7.5% sodium carbonate. Distilled water was added to the reaction mixture so that the total volume was 50 mL. After 2 h, the mixture was measured spectrophotometrically at 765 nm against a reagent blank. Sinapic acid in assay solutions in the concentration range of 0–400 µg/mL was used to prepare the standard curve for the total phenolic content. The results were expressed as mg sinapic acid equivalents (SAE)/100 g of oil.

2.3.2.6 Determination of Acid Value

The acid value of the oil sample was established using an automatic potentiometric titrator AT-500N (Kyoto Electronics Manufacturing, Kyoto, Japan), according to the AOCS Official Method Cd 3d-63 (AOCS, 1997, revised 2003). All acid value analysis results were expressed in mg KOH/g oil.

2.3.2.7 Determination of Peroxide Value

The peroxide value was measured with an AT-500N, using the Japan Oil Chemists' Society (JOCS) Official Method 2.5.2.1 (JOCS, 2003). An oil sample (5 g oil) was dissolved in a mixture of acetic acid and isooctane (3:2 v/v ratio) and then left to react with a saturated potassium iodide solution. The released iodine was then titrated with 0.01 mol/L sodium thiosulfate solution. All peroxide value analysis results were expressed in meq O_2/kg oil.

2.3.2.8 Determination of Viscosity

The flow characteristics of the oil samples were measured using a sine-wave vibro-viscometer (SV-10; A&D Company, Ltd., Tokyo, Japan). Measurements were made at 25°C. The viscosities were expressed in mPa s.

2.3.2.9 Determination of Color

The colors of the oil samples were determined using a portable spectrophotometer (CM-700d, Konica Minolta Sensing Inc., Osaka, Japan). An oil sample (30 mL) was transferred to a 50 mL glass container and the lens was placed over the container to exclude external light. The colors were expressed as L^* [$L^* = 0$ (blank), $L^* = 100$ (white)], a^* ($+a^*$ = red, $-a^*$ = green), and b^* ($+b^*$ = yellow, $-b^*$ = blue).

2.3.2.10 Determination of Oxidative Stability

The oxidative stability of the EPKO was determined with a Rancimat apparatus (743, Metrohm KEBO Lab AB, Herisau, Switzerland), which was used to measure the induction period of a 3 g sample of oil heated to 110°C with an air flow of 10 L/h. During the oxidation process, volatile products were formed in the distilled water and were measured conductometrically.

2.3.3 Statistical Analysis

Analyses were carried out in triplicate. The values of different parameters were expressed as the mean ± standard deviation ($\bar{\chi}$ ± SD).

2.4 Results and Discussion

2.4.1 Fatty Acid Composition

The fatty acid profiles of the EPKO and commercial CO are presented in Table 2-1. The results show that unsaturated fatty acids were the predominant fatty acids, and accounted for about 93.15% and 93.29% of the total fatty acids in the EPKO and CO, respectively. The main unsaturated fatty acid was oleic acid, and its content was found to be 63.67% in the EPKO and 63.79% in the commercial CO. The oleic acid contents observed in this study were within the previously reported range of contents (50–85%) for rapeseed

oils (Przybylski, 2011, Abidi *et al.*, 1999). It is known that monounsaturated fatty acids are effective in lowering total and low-density lipoprotein cholesterol of plasma (Rivellese *et al.*, 2003). Linoleic acid was found to be the major PUFA, followed by linolenic acid, at levels of 19.02% and 7.31%, respectively, in the EPKO, and 19.28% and 9.09%, respectively, in the commercial CO. PUFAs are well known for their role as essential fatty acids as well as their role in reducing plasma cholesterol levels and helping to regulate prostaglandin production (Tapiero *et al.*, 2002). The major SFA in both the EPKO and CO was palmitic acid (C16:0) at levels of 4.06% and 3.47% of total fatty acids, respectively. The total SFA content of the EPKO (6.21%) was a little higher than that of the commercial CO (6.02%). With respect to the flavor and health aspects of the oil, a fried product of optimal quality can be produced, without the presence of *trans* isomers, by using oils with high oleic acid and low linolenic acid contents (Ackman, 1983). The ratio of n-6 to n-3 of the EPKO (2.60) was a little higher than that of the commercial CO (2.12). Previous studies have shown that the optimal ratio of n-6 to n-3 (2:1) is beneficial in preventing cardiovascular diseases, cancer, inflammatory diseases and autoimmune diseases (Hu, 2001, Simopoulos, 2002). It is worth mentioning that the amount of erucic acid in EPKO was 1.52%, rather than zero as previously claimed; however, its content was less

than 2%, which is the maximum level set for erucic acid content in edible oils by the Codex Alimentarius Commission (Codex.Alimentarius.Commission, 2005).

Table 2-1 Fatty acid compositions (%) of EPKO and commercial CO.

Fatty acid	EPKO	CO
Palmitic acid (C16:0)	4.06 ± 0.02	3.47 ± 0.03
Palmitoleic acid (C16:1)	0.14 ± 0.00	0.13 ± 0.00
Stearic acid (C18:0)	1.45 ± 0.01	1.31 ± 0.01
Oleic acid (C18:1)	63.67 ± 0.02	63.79 ± 0.08
Linoleic acid (C18:2 n-6)	19.02 ± 0.03	19.28 ± 0.03
Linolenic acid (C18:3 n-3)	7.31 ± 0.01	9.09 ± 0.06
Arachidic acid (C20:0)	0.48 ± 0.01	0.98 ± 0.01
Eicosenoic acid (C20:1)	1.49 ± 0.03	0.98 ± 0.03
Behenic acid (C22:0)	0.22 ± 0.01	0.26 ± 0.01
Erucic acid (C22:1)	1.52 ± 0.05	0.02 ± 0.00
SFA	6.21 ± 0.00	6.02 ± 0.04
MUFA	66.81 ± 0.06	64.92 ± 0.10
PUFA	26.34 ± 0.04	28.37 ± 0.08
n-6/n-3	2.6 ± 0.00	2.12 ± 0.01

Data expressed as means ± standard deviations (n = 3). EPKO: Expeller-pressed Kizakinonatane oil; CO: Canola oil; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

2.4.2 Minor Components

Tocopherols are a group of chemical compounds, many of which have vitamin E activity, which are recognized as very efficient natural antioxidants and free-radical quenchers. Tocopherols have various physiological effects, most of which are particularly favorable to human health (Lee et al., 2005, Packer and Landvik, 2006). The types and amounts of tocopherols in the EPKO and CO are presented in Table 2-2. The major types of tocopherols in the EPKO were γ-tocopherol (47.03 mg/100 g) and α-tocopherol (17.40 mg/100 g), followed by β-tocopherol (3.61 mg/100 g) and δ-tocopherol (0.93 mg/100 g). The tocopherol profile of the CO was similar to that of the EPKO. The γ- and α-tocopherol contents found in the CO were 44.38 and 13.21 mg/100 g, respectively. Both the β- and δ-tocopherol contents (6.97 and 0.96 mg/100 g, respectively) were a little higher than that in the EPKO. The types and amounts of tocopherols vary with the type of oil, and it has been reported in previous studies that rapeseed oil has high amounts of α- and γ-tocopherols (usually in a 1:2 ratio), with trace amounts of β- and δ-tocopherols (Schwartz *et al.*, 2008, Yang *et al.*, 2013, Sodeif *et al.*, 2010). α-Tocopherol and γ-tocopherol have similar singlet-oxygen-quenching abilities and have beneficial effects on the prevention of prostate and colon cancers (Gysin *et al.*, 2002).

Table 2-2 Minor components of oil samples.

Parameter	EPKO	CO
α-tocopherol (mg/100 g)	17.40 ± 0.27	13.21 ± 0.15
β-tocopherol (mg/100 g)	3.61 ± 0.06	6.97 ± 0.28
γ-tocopherol (mg/100 g)	47.03 ± 0.14	44.38 ± 0.83
δ-tocopherol (mg/100 g)	0.93 ± 0.02	0.96 ± 0.02
β-carotene (mg/kg)	7.15 ± 0.04	0.13 ± 0.02
Chlorophyll (mg/kg)	5.60 ± 0.25	nd
Total phenolic content (mg SAE/100 g)	1245.25 ± 18.53	766.86 ± 19.56

Data expressed as means ± standard deviations (n = 3). EPKO: Expeller-pressed Kizakinonatane oil; CO: Canola oil; nd: not detected.

The β-carotene contents of both oils are shown in Table 2-2. The EPKO possessed a higher level of β-carotene (7.15 mg/kg), which is responsible for its darker yellow color compared with that of the CO (0.13 mg/kg). The β-carotene content in the EPKO was within the range reported in previous studies for canola/rapeseed oils, i.e., 1.4–25.5 mg/kg (Koski *et al.*, 2002, Goulson and Warthesen, 1999). The low β-carotene content of the CO is mainly attributed to the refining process, during which the amount of carotenoids is greatly reduced (Čmolík *et al.*, 2000). In addition to carotenoids, chlorophyll pigments were also found in rapeseed oils. The amount of chlorophyll in the EPKO was 5.60 mg/kg, which was within the range of chlorophyll contents previously reported for canola/rapeseed oils (5-

55 mg/kg) (Przybylski, 2011). β-Carotene is an important terpenoid compound that is, known for its powerful antioxidant functions; it scavenges free-radicals in the body and prevents DNA damage and subsequent mutations (Liebler and McClure, 1996, Krinsky, 2006). β-Carotene has also been reported to have a positive association with decreased risk of cardiovascular diseases and cancers (Lee *et al.*, 1999). Chlorophyll is an important factor in oil stability because of its potent antioxidative effect in the dark (Endo *et al.*, 1985). However, chlorophyll in rapeseeds imparts an undesirable color to the oil and is a strong pro-oxidant, acting as a sensitizer for singlet-oxygen production under light (Rukmini and Raharjo, 2010).

Phenolic compounds, a large group of phytochemicals in plants, are now receiving considerable attention because of their potent effects in the prevention of both heart disease and cancer (Cartea *et al.*, 2010). The results presented in Table 2-2 show that the EPKO had a level of total phenolic content (1245.2 mg SAE/100 g) that was 1.6 times higher than that of the commercial CO (766.8 mg SAE/100 g). Yang et al. reported total phenolic contents ranging from 9 to 100 mg of tannic acid/100 g in 203 cold-pressed oils of Chinese cultivars (Yang *et al.*, 2013), and Gruzdienė et al. detected total phenolic contents ranging from 74 to 843 mg gallic acid equivalents/100 g in 15 oil samples from Lithuanian rapeseed cultivars (Gruzdienė and

Anelauskaitė). The differences between our total phenolic content results and previously published results might have been caused by factors such as the cultivars, degree of maturity, sample preparation, and the analytical method used.

2.4.3 Physicochemical Characteristics

The physicochemical characteristics of the oils are shown in Table 2-3. The acid value (AV) is a measure of the free fatty acids (FFAs) present in the oil. An increase in the amount of FFAs in an oil sample indicates the hydrolysis of triglycerides. The results show that the acid values for the EPKO and CO were 1.66 and 0.10 mg KOH/g, respectively. The high acid value of EPKO could be attributed to the fact that FFAs and moisture in the EPKO were not removed as the oil did not undergo a refining process. As FFAs are very susceptible to lipid oxidation, this could explain the high peroxide value (PV) (8.69 meq O_2/kg) of the EPKO compared with that of the CO (1.30 meq O_2/kg). This shows that the EPKO maintained its good quality since its AV was lower than the maximum AV that is permitted in mechanical-pressed oils by the regulations, i.e., 4.0 mg KOH/g, and its PV was lower than the maximum PV that is permitted in mechanical-pressed oils by the regulations, i.e., 15.0 meq O_2/kg (Codex.Alimentarius.Commission, 2005). The viscosity

of the EPKO was higher than that of the CO. Kim et al. (Kim *et al.*, 2010) observed that a decrease in the oil viscosity was associated with an increase in the amount of 18:2 fatty acids and a decrease in the amount of 18:1 fatty acids. The fatty acid profiles obtained in this study were in agreement with their observations. The flow behavior of an oil is not only affected by the main components (C18:1 and C18:2 fatty acids), but also by impurities. The oil color results show that the EPKO had a lower L^* value (19.19) and higher a^* (9.08) and b^* (32.88) values than that of the CO, indicating that the EPKO was reddish in color and darker than the CO (Table 2-3). The intense yellowish color of the EPKO was probably a result of the β-carotene content (Table 2-2).

The oxidative stability index (OSI) is a measure of the resistance of lipids to oxidation under accelerated heating and aeration conditions. Oils with higher OSI values are usually regarded as having better oxidative stability or a longer shelf-life. The OSI value of the EPKO was determined and compared with that of the commercial CO (Table 2-3). The OSI value of the EPKO was 6.47 h, which is shorter than the 8.57 h that was observed in the commercial CO. It is not surprising that commercial CO has a higher OSI value since constituents such as FFAs, copper, iron, and chlorophyll and its decomposition products, which act as catalysts in oxidation processes, were

largely removed during the refining, bleaching, and deodorizing processes (Przybylski et al., 2005). The chlorophyll content may play an important role in the oxidative stability of the EPKO, especially if the oil is exposed to light. The oxidative stability of a vegetable oil is dependent on many factors, including the presence of light, oxygen partial pressure, the degree of unsaturation of the fatty acids, the presence of antioxidants and pro-oxidants, and the processing and storage conditions (Garcia-Mesa *et al.*, 1993).

Table 2-3 Comparison of physicochemical properties of EPKO and CO.

Parameter	EPKO	CO
Acid value (mg KOH/g)	1.66 ± 0.04	0.10 ± 0.01
Peroxide value (meq O_2/kg)	8.69 ± 0.29	1.30 ± 0.02
Viscosity (mPa·s)	67.73 ± 0.21	56.70 ± 0.17
OSI (h)	6.47 ± 0.06	8.57 ± 0.06
Color		
*L**	19.19 ± 0.01	30.80 ± 0.04
*a**	9.08 ± 0.01	-1.94 ± 0.01
*b**	32.88 ± 0.02	6.26 ± 0.02

Data expressed as means ± standard deviations (n = 3). EPKO: Expeller-pressed Kizakinonatane oil; CO: Canola oil; OSI: Oxidative stability index.

2.5 Conclusions

The fatty acid profile of the EPKO, which is similar to that of the CO, presented in this study indicated that EPKO is a healthy oil as it is rich in

unsaturated fatty acids, namely oleic acid (C18:1), linoleic acid (C18:2; n-6), and linolenic acid (C18:3; n-3). The EPKO had higher levels of minor compounds such as tocopherol, total phenolics, β-carotene, and chlorophyll compared with the commercial CO. As a result of the large amount of β-carotene in the EPKO, the EPKO is a deeper yellow and darker than the CO. Furthermore, the EPKO has a good OSI and viscosity, and low AV and PV that are within the limits allowed by regulations. Despite the fact that mechanical-pressing gives a relatively low recovery of oil from oilseeds, which leads to a higher price, more and more consumers are shifting their focus in oil consumption from quantity to quality. Based on the data obtained in this study, we concluded that domestic EPKO could be used to supplement or even replace conventional CO, and meet part of the nutritional requirements in human diets as well as improve human health.

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CHAPTER 3 FRYING STABILITY OF KIZAKINONATANE OIL IN COMPARISON WITH CANOLA OIL

3.1 Abstract

This study was carried out to investigate the frying performance of expeller-pressed Kizakinonatane (Brassica napus) oil (EPKO) during deep-fat frying of frozen French fries with/without replenishment. Commercial regular canola oil (CO) was used for comparison. The frying oils were used during intermittent frying of frozen French fries at 180, 200, and 220°C for 7 h daily over 4 consecutive days. The EPKO exhibited lower levels of total polar compounds (TPC), carbonyl value (CV), and viscosity as well as comparable color (optical density) values to that of the CO. The changes in monounsaturated fatty acid/polyunsaturated fatty acid (MUFA/PUFA) ratios in the EPKO were lower than that of the CO, while the PUFA/SFA (saturated fatty acid) ratios were higher than that of the CO by the end of the frying session. The EPKO showed a significantly slower rate of antioxidants decomposition (tocopherols) than the CO during frying at 180 and 200°C. A trend of initial decrease followed by a gradual increase in carotenoids content

was observed in both the EPKO and CO during the frying process. The chlorophyll was sensitive to high temperature and was significantly destroyed in the EPKO when subjected to frying. Results show that fresh EPKO contains higher levels of acid value (AV), peroxide value (PV), viscosity, and optical density values than that of CO. Replenishment with fresh oil had significant effects on all chemical and physical parameters, except the PV of the CO and EPKO and the AV of the EPKO during the frying processes. Based on the results, the EPKO is inherently suited for preparing deep-fried foods at high temperatures.

3.2 Introduction

Deep-fat frying is a popular and important procedure for both industrial and domestic preparation and manufacture of foods. The flavors, textures, and appearance of food can be enhanced simply by immersing them in hot oil for the specified times required by various food types (Choe and Min, 2007). In addition, when compared with other common culinary procedures, frying causes smaller nutrient losses and even enhances the food nutritive value in a shorter cooking time (Casal *et al.*, 2010).

During deep-fat frying, the oil is continuously used as a frying medium at elevated temperatures in the presence of air (oxygen), a metal container, and

water (moisture). Under these conditions, a variety of chemical reactions take place, including oxidation, hydrolysis, oxidative polymerization, isomerization, and cyclization (Gertz, 2000). All of these chemical reactions lead to the decomposition of the frying oil and the formation of numerous volatile and nonvolatile products, which may have deleterious effects on human health (Dobarganes and Márquez-Ruiz, 2003). During the deep-fat frying process, oils are not only employed as a heat-exchange medium, but also contribute to the quality of fried products as the frying oil is absorbed by the food (Shahidi and Wanasundara, 2002). Therefore, oil frying stability is of great importance.

Deep-fried foods, such as French fries and tempura, are very popular in industries, restaurants, and homes in Japan. Although canola oil remains popular with Japanese consumers (40% of total Japan retail sales in 2011), oil products from other seeds are gaining popularity (Euromonitor.International, 2012). Kizakinonatane *(Brassica napus*) (registration No. Natane Norin 47) was developed by the Tohoku National Agricultural Experiment Station and claimed as a zero erucic Japanese rape variety. It is commonly cultivated throughout the northern part of the Tohoku region because of its greater intrinsic cold tolerance and higher oil yielding ability compared with other domestic varieties (Okuyama *et al.*, 1994). EPKO obtained by expeller

pressing has received favorable consumer reviews because of its characteristic taste, superior fragrance, and light yellow color. Consumers also tend to believe that expeller oil has a higher nutritive value than conventionally produced oils because of the lack of heat and chemicals involved in the production process (Koski *et al.*, 2002). Additionally, despite the widespread distribution of many types of oil, a particular oil is often selected for frying based on local availability and regional preferences.

Different oils have been reported to possess different thermal stability. Many frying operations involving foods like potato chips, vegetables, and chicken are conducted at temperatures of 160-185°C while extruded products and pellets are typically fried at 195-215°C (Gupta, 2004). To the best of our knowledge, there have been no reports concerning the frying stability of the EPKO during a deep-fat frying process. The current work is focused on investigating the frying stability of EPKO during the deep-fat frying of French fries by measuring the AV, PV, CV, TPC, color, viscosity, fatty acid composition, tocopherols, carotenoids, chlorophylls, and polyphenols before and after frying. Frying cycles were also carried out with commercial CO for comparison. In this study, the higher temperatures of 200 and 220°C have also been applied in order to investigate the maximum frying performance of EPKO.

3.3 Materials and Methods

3.3.1 Materials

EPKO was purchased from a local oil supplier and commercial regular CO was obtained from a local supermarket. Frozen par-fried French fries in an institutional pack were purchased from a local supermarket and stored at -18°C until use. All chemicals and solvents used in the study were of analytical reagent grade unless otherwise stated and purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan).

3.3.2 Frying Procedure and Oil Sampling

Two frying experiments were carried out to fry the frozen French fries: without oil replenishment (Frying Experiment I) and with frequent oil replenishment (Frying Experiment II). The frying was conducted in a restaurant style stainless steel electric fryer TF-40A (Taiji & Co., Ltd., Kanagawa, Japan). In these experiments, 4 kg of fresh oil (CO or EPKO) was heated for 7 h daily for 4 days at 180, 200, and 220°C, respectively. When the temperature of the oil reached the appointed temperature, a batch of 100 g of frozen French fries was fried for 3 min at 22 min intervals for a period of 7 h per day over 4 consecutive days. This was equivalent to frying 17 batches per day and a total of 68 batches for the whole frying cycle. During the process,

200 mL of heated oil was drawn after every 3.5 h of frying and kept frozen at -18°C until analysis. At the end of each frying day, the fryer was switched off, covered with the fryer lid and left to cool overnight. In frying experiment I, no fresh oil was added to the fryer throughout the entire frying process. In frying experiment II, experiment I was replicated, except that 400 mL of fresh oil was replenished on every second day of frying. In industrial frying (i.e., high frying load), the general recommendation for food to oil ratio is 1:6. Nevertheless, since the main purpose of this study is to compare the frying stability of EPKO to that of CO, the food to oil ratio was set at 1:40, which is common to catering/restaurant applications (i.e., low frying load) (Kalogianni *et al.*, 2009).

3.3.3 Analytical Methods

3.3.3.1 Determination of Acid Value

The AV of the oil samples were determined using an automatic potentiometric titrator AT-500N (Kyoto Electronics Manufacturing, Kyoto, Japan) according to the AOCS Official Method Cd 3d-63 (AOCS, 1997, revised 2003). All AV analysis results were expressed in mg KOH g/oil.

3.3.3.2 Determination of Peroxide Value

PV was measured with an AT-500N following the Japan Oil Chemist's Society (JOCS) Official Method 2.5.2.1 (JOCS, 2003a). All PV analysis results were expressed in meq O_2/kg oil.

3.3.3.3 Determination of Carbonyl Value

The CV of the oil samples was determined according to the JOCS Official Method Tentative 13-2003 Carbonyl Value (Butanol Method) (JOCS, 2003b). All CV analysis results were expressed in μmol/g.

3.3.3.4 Determination of Total Polar Compounds

The TPC estimation of oil samples were based on the dielectric constant change, measured directly in the hot oil, with a food oil monitor FOM 310 (Ebro Electronics, Ingolstadt, Germany), according to the manufacturer's instructions.

3.3.3.5 Determination of Color (absorbance)

The color index of oil samples were determined according to the AOCS official method Cc 13c-50 (AOCS, Reapproved 2009) using a UV-3100PC spectrophotometer (Shimadzu Co., Kyoto, Japan). Oil samples were placed in a standard disposable cuvette (1 cm optical path) and warmed to 25°C in a

water bath for 30 min prior to measuring the spectrophotometric absorbance. The spectrophotometer absorbance was zeroed against air.

3.3.3.6 Determination of Viscosity

The flow characteristics of the oil samples were measured using a sine-wave vibro viscometer (SV-10; A&D Company, Ltd., Tokyo, Japan). Measurements were taken at 25°C. Viscosity analysis results were expressed in mPa·s.

3.3.3.7 Determination of Fatty Acids

Fatty acid methyl esters (FAMEs) were prepared using the American Oil Chemists' Society (AOCS) Official Method Ce 2-66 (AOCS, 1997). Oil (0.2 g) was reacted with 2 mL of *n*-hexane, followed by 0.2 mL of methanolic KOH (2 N). The mixture was well shaken for 60 s and centrifuged for 5 min at 1500 rpm. The top layer (1 µL) was injected into a gas chromatograph (Shimadzu GC-2010) equipped with a flame ionization detector and a Shimadzu AOC 20Si autosampler. An Agilent high-polarity HP-88 column (100 m × 0.25 mm i.d., with a 0.25 µm film thickness) was used with helium as the carrier gas, at a flow rate of 2.0 mL/min. The detector temperature was 280°C; the column temperature was held at 50°C for 1 min, increased at a rate of 10°C/min to 170°C and held for 10 min, and then increased at a rate of

5°C/min to 210°C and held for 8 min. The total run time was 38 min. Individual FAME peaks were identified by comparing their retention times against FAME standards. The relative percentage of each fatty acid was determined by dividing the area under the individual peak by the total peak area of all fatty acids in the oil sample.

3.3.3.8 Determination of Tocopherols

Prior to high-performance liquid chromatography (HPLC) analysis, 0.5 g of oil was saponified and extracted using a modified version of Lee's method (Lee *et al.*, 2000). The treated sample (50 μL) was analyzed using a Shimadzu LC-20AT HPLC system consisting of a guard-column (Mightysil Si 60, 5 μm, 5 mm × 4.6 mm, Kanto Chemical Co., Inc., Tokyo, Japan), an analytical column (YMC-Pack SIL-06, 5 μm, 250 mm × 4.6 mm, YMC Co., Kyoto, Japan), an LC-20AT pump, an autosampler (SIL-20A, Shimadzu Co., Japan), and a Shimadzu RF-10AXL fluorescence detector set at an excitation wavelength of 298 nm and an emission wavelength of 325 nm. The flow rate of the mobile phase (hexane–propanol–acetic acid, 1000:6:5, v/v/v, with 5 μg/mL butylated hydroxytoluene) was maintained at 1.5 mL/min. The concentration of each tocopherol was calculated from the calibration curves prepared for α-, β-, γ-, and δ-tocopherol isomers.

3.3.3.9 Determination of Carotenoid Content

The AOAC Official Method 958.05 was used to determine the carotenoids in the oils (AOAC, 1990). The carotenoid content was measured using a calibration curve obtained by preparing β-carotene solutions in hexane, in the concentration range of 0.1–3.0 µg/mL. The absorbance at 440 nm was read against a hexane blank on a Benchmark Plus microplate reader (Bio-Rad, Hercules, USA). The amounts of total carotenoids in the oil samples were quantified and expressed in terms of β-carotene (mg/kg oil).

3.3.3.10 Determination of Chlorophyll Content

The chlorophyll pigments in the EPKO were determined using the AOCS Official Method Cc 13i-96 (AOCS, 1998, Revised 2011). The absorbances of the samples were determined at 630, 670, and 710 nm using a UV-3100 spectrophotometer (Shimadzu Co., Kyoto, Japan). The chlorophyll content was calculated using the absorptivity of pheophytin a, which is the main chlorophyll pigment in crude vegetable oils. The chlorophyll contents of the commercial CO were not measured using this method since maximum absorption does not occur at 670 nm in processed oils.

3.3.3.11 Determination of Total Phenolic Content

The total phenolic contents of the oil samples were determined spectrophotometrically using the Folin–Ciocalteu reagent, according to a modified version of a previous method (Koski *et al.*, 2002, Gutfinger, 1981). An oil sample (2.5 g) was dissolved in 2.5 mL of hexane, extracted by centrifugation at 5000 rpm for 5 min (Himac CR21F, Hitachi Co., Ltd, Japan) with 2.5 mL of aqueous methanol (80:20 v/v), and the supernatant was collected. This procedure was repeated twice. All three extractions were combined in a 50 mL volumetric flask with 2.5 mL of Folin–Ciocalteu reagent and 5 mL of 7.5% sodium carbonate. The volume of the reaction mixture was made up to 50 mL with distilled water. After 2 h, the mixture was measured spectrophotometrically at 765 nm against a reagent blank. Sinapic acid in assay solutions in the concentration range of 0–400 µg/mL was used to prepare the standard curve for the total phenolic content. The results were expressed as mg sinapic acid equivalents (SAE)/100 g of oil.

3.3.4 Statistical Analysis

All measurements were carried out at least in triplicate. Data were analyzed by one-way analysis of variance (ANOVA) and regression analyses using SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL, USA).

Statistically significant differences between means were determined by Duncan's multiple range tests. Statistically significant differences were determined at the $P < 0.05$ level.

3.4 Results and Discussion

3.4.1 Changes in Acid Value

The free fatty acid in oils is formed from hydrolysis, oxidation due to free radical formation, and cleavage of double bonds during frying. Changes in the AV of the oil samples in experiment I are given in Figure 3-1. The AV significantly increased ($P < 0.05$) with increasing frying temperature and time in all treatments. The AV of the EPKO sharply increased from an initial value of 1.69 mg KOH g/oil to 5.50, 6.97, and 6.57 mg KOH g/oil after 28 h of frying at 180, 200, and 220°C, respectively. In comparison, the AV of the CO samples gradually increased from an initial value of 0.09 mg KOH/g oil to 1.02, 1.32, and 2.05 mg KOH g/oil after 28 h of frying at 180, 200, and 220°C, respectively.

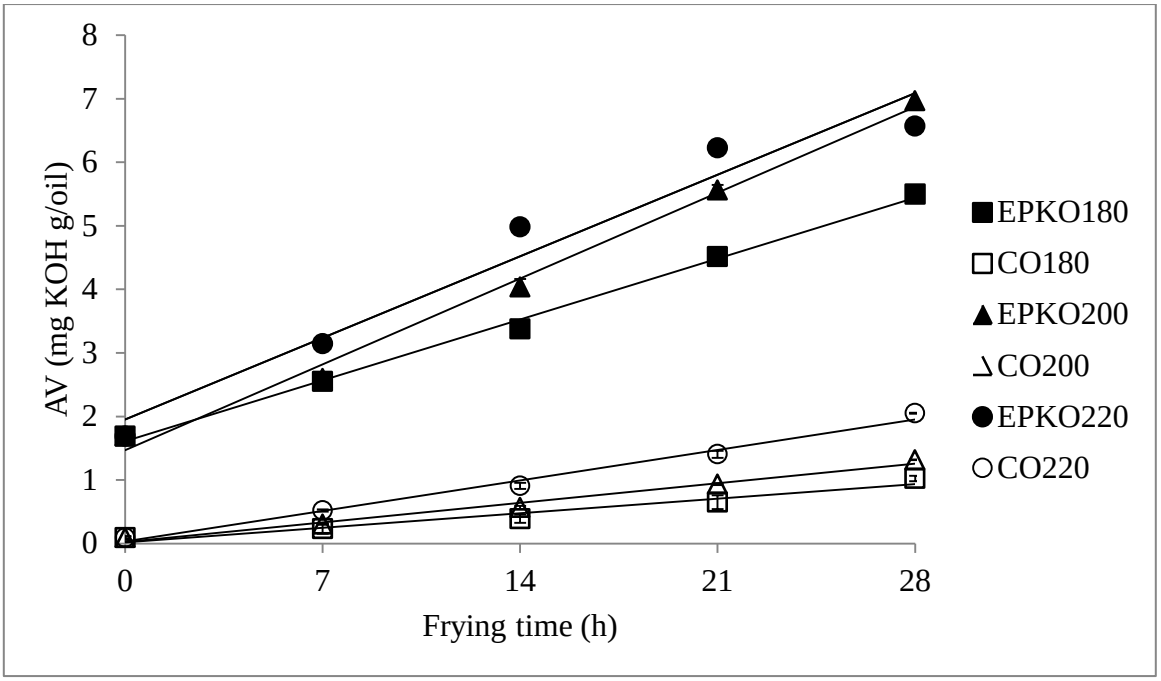

Figure 3-1 Changes in the AV during frying at different temperatures without oil replenishment.

Changes in the AV of all the treatments in frying experiment II are shown in Figure 3-2. The results indicate that the AV of all oil samples significantly increased ($P < 0.05$) with elevated temperatures and prolonged frying time. At the end of 28 h of frying, the respective AV at 180, 200, and 220°C were found to be 5.97, 7.28, and 8.73 mg KOH g/oil for the EPKO samples and 0.85, 1.07, and 1.28 mg KOH g/oil for the CO samples.

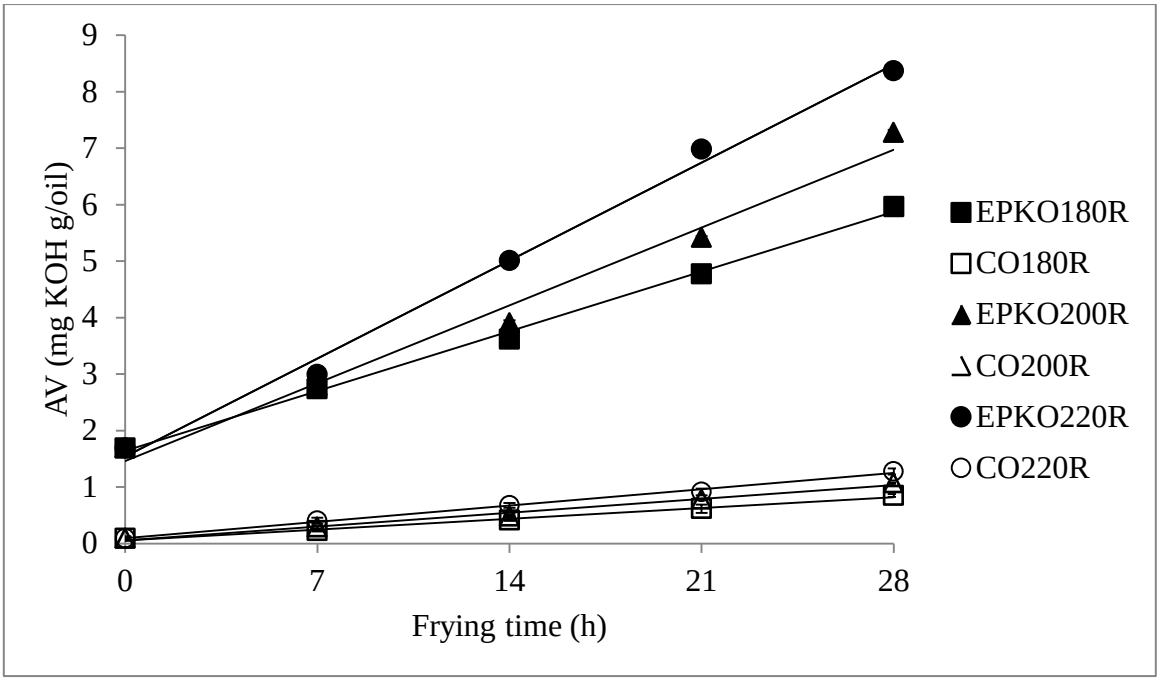

Figure 3-2 Changes in the AV during frying at different temperatures with oil replenishment.

The expeller- or extruded-pressed oils are usually produced by pressing rapeseed using a screw press and then purified by sedimentation or filtration which only removes turbid matters. The high initial AV level of the EPKO could be attributed to the fact that components that impair the quality of the oil, such as free fatty acids, chlorophyll or products of oxidative degradation, were not removed as the oil had not been subjected to refining processes (Matthäus and Brühl, 2008). As more free fatty acids are released in oil, it becomes more susceptible to thermal oxidation under elevated frying temperatures. Therefore, the EPKO with a higher level of AV showed a higher susceptibility to the hydrolysis of fatty acids than the CO during the frying

processes. This observation is in agreement with that reported in previous similar studies (Petukhov *et al.*, 1999, Tekin *et al.*, 2009).

The slope of regression also show that the extent of hydrolytic deterioration of the CO in experiment I, as measured by the AV level, were 1.2, 1.3, and 1.7 times faster during frying at 180, 200, and 220°C than those in experiment II, respectively. Clearly, replenishment played an important role in AV changes. In other words, frequent replenishment of frying oil with fresh oil seems to slow down the hydrolysis of CO (Romero *et al.*, 1998). The increment rate of AV in EPKO samples in experiment II was expected to decrease due to the effect of replenishment with fresh oil; however, none of the oils had reductions in AV level. The unexpected increase in AV levels might be attributed to higher amounts of short and unsaturated fatty acids present in the EPKO as well as the presence of moisture (from frozen fries and oil). In most deep-fat frying operations, the amount of free fatty acid produced by hydrolysis is too small to affect the quality of the food; adverse effects are typically due to oxidation of unsaturated fatty acids (Ogunsina *et al.*, 2011).

3.4.2 Changes in Peroxide Value

The PV, which represents the total hydroperoxide content, is considered to be one of the most common quality parameters of oils during production,

storage, and marketing (Antolovich *et al.*, 2001). The PV of the frying medium EPKO dramatically decreased ($P < 0.05$) in both experiments I (from 10.21 O_2/kg oil to 4.85, 4.12, and 4.96 O_2/kg oil at 180, 200, and 220°C, respectively) (Figure 3-3), and II (from 10.21 O_2/kg oil to 5.55, 4.39, and 4.77 O_2/kg oil at 180, 200, and 220°C, respectively) during the preliminary stages of frying (Figure 3-4), followed by a slight increase and then a decrease as frying continued. This observation might be explained by the fact that the high initial level of hydroperoxides, which resulted from the accumulation of poor quality seeds in the fresh EPKO because of a lack of refining processes (Daun and Burch, 1984, Paul *et al.*, 1997, Codex.Alimentarius.Commission, 2008), were rapidly destroyed by high temperature during frying. The decrease in PV during the initial stages of frying indicates that the decomposition rate of hydroperoxides surpassed their rate of formation, and that the prolonged frying time could have played a role in decomposing the hydroperoxides as well.

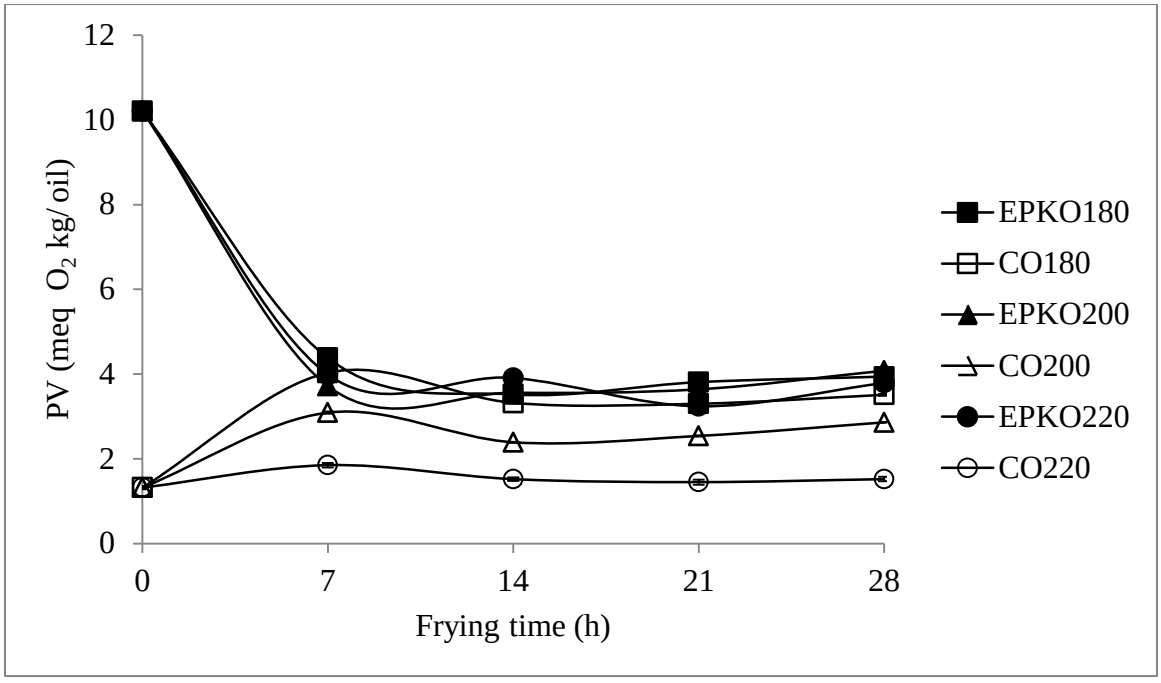

Figure 3-3 Changes in the PV during frying at different temperatures without oil replenishment.

Although the same temperatures were applied to both the EPKO and CO samples, the results obtained from the CO samples showed a sharp increase in PV ($P < 0.05$) during the initial stages of frying in experiment I (from 1.32 O_2/kg oil to 4.69, 4.78, and 3.42 O_2/kg oil at 180, 200, and 220°C, respectively), followed by a decrease (Figure 3-3). Further frying led to a new slight increase in PV. Similar patterns of change were also observed in experiment II. An increase during the early stages of frying was observed (from 1.32 O_2/kg oil to 4.72, 5.05, and 4.10 O_2/kg oil at 180, 200, and 220°C, respectively), followed by a decrease after further frying (Figure 3-4). The results in experiment II also indicate that frequent replenishment with fresh oil

has little impact on the PV during deep-fat frying, which is similar to the case for experiment I.

An increase in the PV during the frying process implies the formation of peroxides through oxidation. However, peroxides are unstable compounds and will break down to a variety of nonvolatile and volatile products under high temperatures (Dobarganes and Velasco, 2002), thus leading to the formation of fewer hydroperoxides as frying continued. The late increase in the PV could be attributed to new hydroperoxides that were formed once more during the cooling period. The same patterns of change were reported in many previous deep-fat frying studies (Abdulkarim *et al.*, 2007, Man *et al.*, 1999).

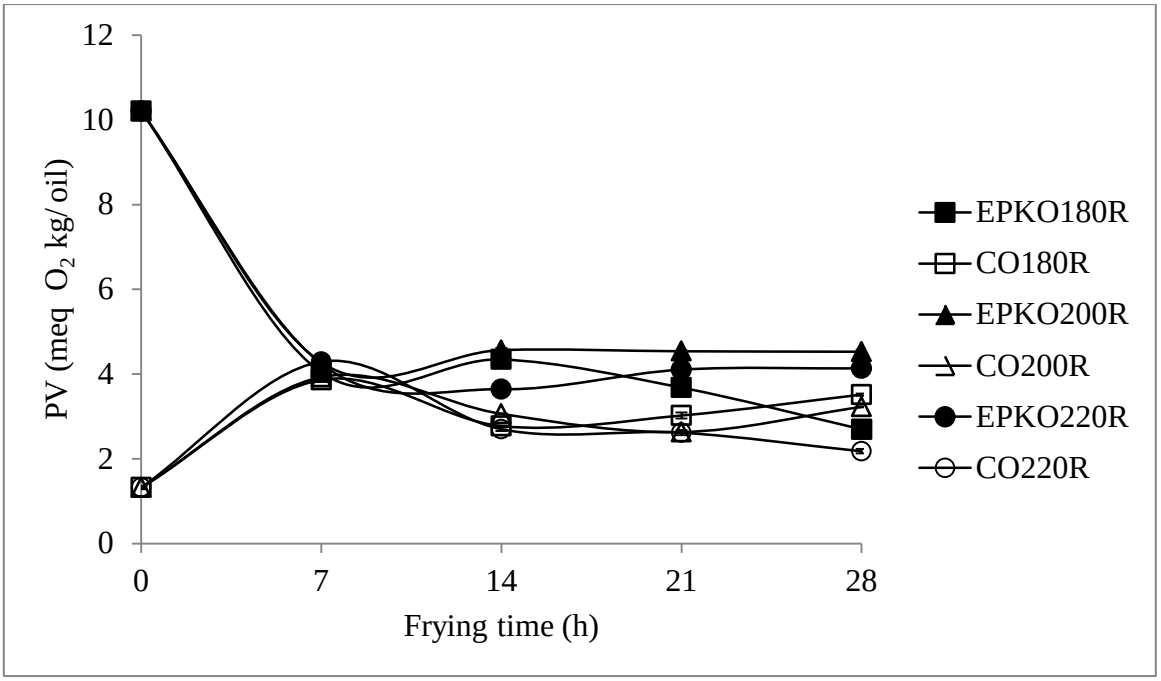

Figure 3-4 Changes in the PV during frying at different temperatures with oil replenishment.

3.4.3 Changes in Carbonyl Value

The CV measures the secondary oxidation products generated from the degradation of hydroperoxides, such as aldehydes and ketones, which are more stable than peroxides. Because these secondary products are considered to be the major contributors to rancidity and unpleasant flavors and decrease the nutritional value of fried foods (Antolovich *et al.*, 2001), the determination of the CV is very important for evaluating the quality of frying oils.

The changes in the CV during frying experiment I over 4 consecutive days of frying at 180, 200, and 220°C are shown in Figure 3-5. The results

show that the CV of the EPKO samples significantly increased ($P < 0.05$) from 2.63 to 15.99 at 180°C, 19.36 at 200°C, and 47.21 μmol/g at 220°C at the end of the frying process, which indicate that even after 28 h of frying at 220°C, none of the CV of the EPKO samples exceeded the discard level of 50 μmol/g set in Japan (Hara *et al.*, 2006). A rapid increase ($P < 0.05$) in the CV of all the CO samples was observed with the increase in frying time. The CV of the CO samples increased from 1.99 to 57.43 at 180°C, 61.89 at 200°, and 65.12 μmol/g at 220°C after 28 h of frying at different temperatures. The slopes of regression show that changes in the CV of the CO samples were 4.6, 3.7, and 1.4 times faster during frying at 180, 200, and 220°C, respectively, than the EPKO samples.

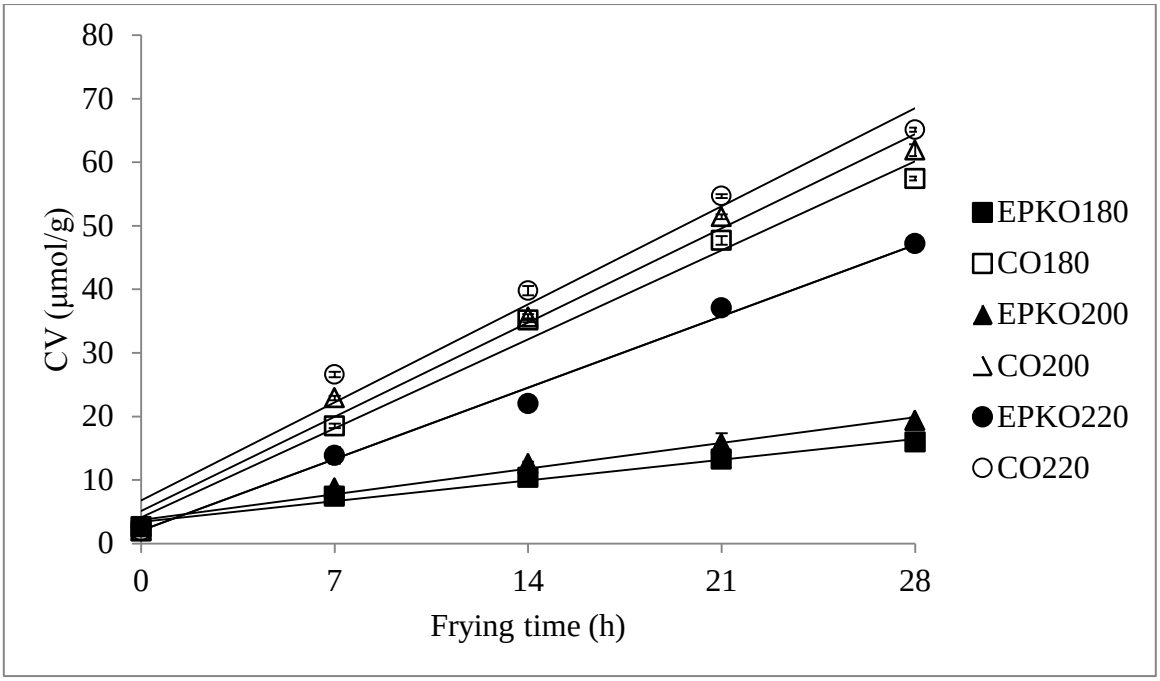

Figure 3-5 Changes in the CV during frying at different temperatures without oil replenishment.

As expected, similar patterns of change in the CV were observed in experiment II. The CV of all the CO samples markedly increased ($P < 0.05$) from 1.99 to 43.60 at 180°C, 52.92 at 200°C, and 61.72 μmol/g at 220°C while the CV of all the EPKO samples showed moderate increases from 2.62 to 12.27 at 180°C, 14.45 at 200°C, and 38.11 μmol/g at 220°C during frying (Figure 3-6). The lower increment of the CV shown in experiment II compared with experiment I was mainly a result of the frequent replenishment with fresh oil. Results obtained from experiment I and II show significantly low levels of CV ($P < 0.01$) in the EPKO samples after 28 h of frying at 180, 200, and 220°C as compared to that of the CO samples. In other words, the

EPKO exhibited much better oxidative stability than CO when frying at 180°C, as well as at higher temperatures of 200 and 220°C.

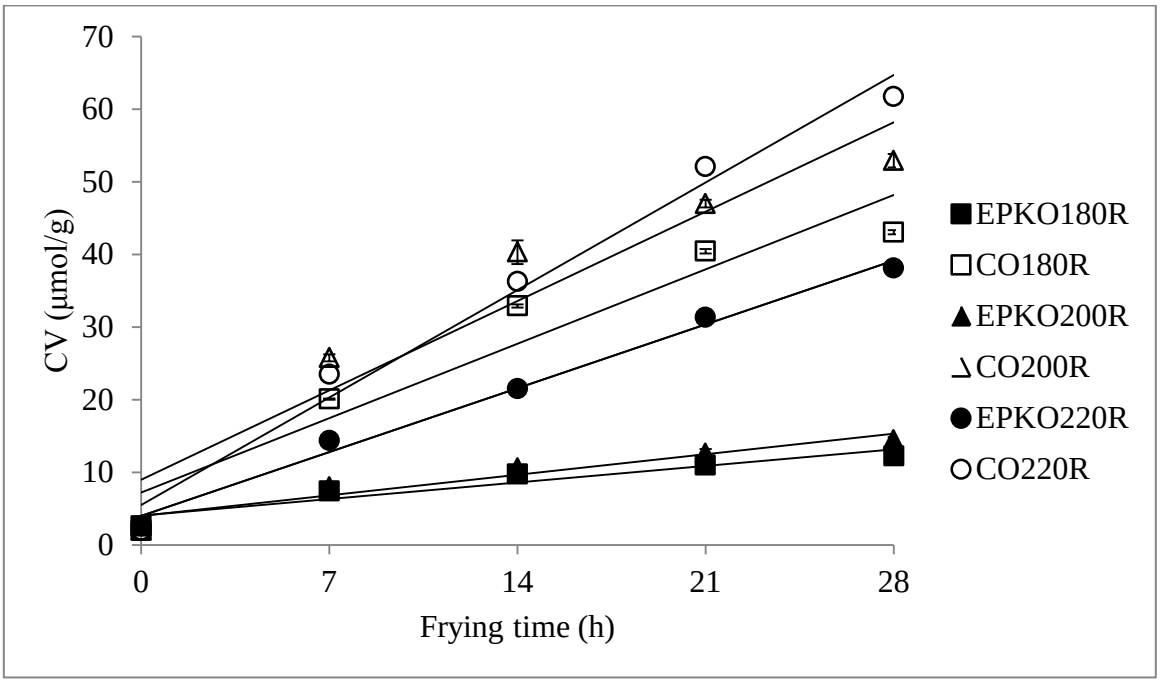

Figure 3-6 Changes in the CV during frying at different temperatures with oil replenishment.

Many factors affect the formation and accumulation of carbonyl compounds during frying, including the frying temperature and time, type of frying foodstuffs and frying oil, antioxidants in fresh oil, and refining process (Farhoosh *et al.*, 2009a). Tonani *et al* (Totani *et al.*, 2007) reported that low levels of CV in recovered oil were mainly due to the vaporization of carbonyl compounds with steam generated from the water content of frying foodstuffs. Moreover, the low levels of CV might also partly be due to the presence of more natural minor compounds that could be acting as antioxidants in pressed

oils than the corresponding refined, bleached, and deodorized oils (Warner and Knowlton, 1997).

3.4.4 Changes in Total Polar Compounds

TPC consist of degraded products and nonvolatile oxidized derivatives, polymeric, and cyclic substances formed during frying as well as those from food material contaminants present in the frying oil. The determination of TPC is one of the most reliable methods for assessing the extent of deterioration in frying oils (Fritsch, 1981). The TPC content indicates the total amount of new compounds formed during frying that have higher polarity than triacylglycerols, thus providing a good indicator of the quality of used frying oils. The maximal values for TPC in many European countries have been set to be between 24 and 27% (Mariod *et al.*, 2006), while Japan has not established a specific level for polar compounds to date.

The initial levels of TPC were found to be 1.0 and 3.0% for the CO and EPKO samples, respectively (Figure 3-7). In this study, the TPC contents increased almost linearly with frying time for all the oils, with high correlation coefficients ($R^2 > 0.94$). Assuming the maximum permissible amount of TPC is 24%, the time required to reach this amount was used as a measure of the frying stability (t_{24}) of the oils. As shown in Figure 3-7, the highest t_{24} value

observed in the EPKO in experiment I was at 180°C (82.7 h), followed by temperatures of 200°C (58.2 h) and 220°C (26.5 h). The highest t_{24} value found in CO was at 180°C (24.8 h), followed by temperatures of 200°C (22.4 h) and 220°C (18.6 h). A similar pattern of change in the TPC was also observed in frying experiment II. The EPKO fried at 180°C showed the highest t_{24} value (89.3 h) while the highest t_{24} value found for the CO was fried at 180°C (33.1 h). The results show that the EPKO exhibited a frying stability that was significantly higher than that of the CO and that frequent replenishment of fresh oil decreases the formation of polar compounds.

These results of a low TPC percentage indicate a high oxidative stability of the EPKO with regards to changes in triacylglycerols that occurred during the frying processes. The lower TPC formation in the EPKO samples was likely due to the fatty acid composition and the ratio of oleic to linolenic acid (Abdulkarim and Ghazali, 2012). In a related study, Matthäus B (Matthäus, 2006) determined the frying stability of high-oleic rapeseed oils and found that high-oleic rapeseed oils had lower TPC levels after 72 h of frying when compared to other commonly used edible oils. Warner *et al* (Warner and Knowlton, 1997) reported that genetically modified corn oils, which contain 65% oleic acid, had remarkably lower TPC levels after 20 h of frying at 190°C as compared to normal corn oils. Similar findings were also

reported in Abdulkarim's work. The high-oleic Moringa oleifera seed oils showed a lower TPC level after 30 h of frying at 185°C as compared to other vegetable oils (Abdulkarim *et al.*, 2007).

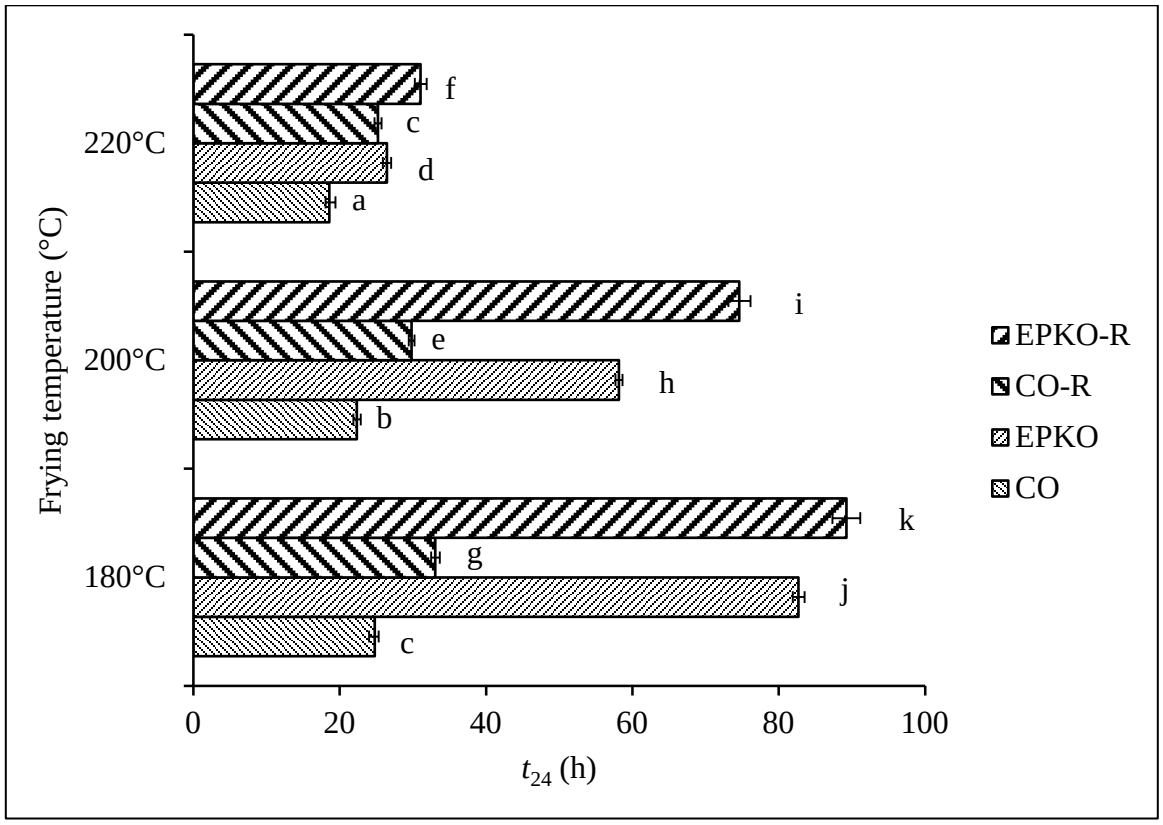

Figure 3-7 The time required to reach a TPC content of 24% (t_{24}) for oil samples that were fried at different temperatures.

R represents frying oil that was replenished with fresh oil on every second day of frying. Mean ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

3.4.5 Changes in Color

The color of frying oil is one of the major parameters of acceptance to be evaluated, because color development is an indication of oxidation, polymerization, formation of carbonyl compounds, and other chemical changes (Paul *et al.*, 1997).

The changes in color for all treatments in experiment I during 4 consecutive days of frying are given in Figure 3-8. The results show that the optical density of the CO samples significantly increased ($P < 0.05$) with an increase in frying temperature and time. The optical density of the CO samples increased from an initial value of 0.06 to 0.78, 0.95, and 1.67 after 28 h of frying at 180, 200, and 220°C, respectively. The EPKO samples show a high initial optical density value of 1.19 and the lowest optical density of EPKO samples were found to be 0.41 after 10.5 h of frying at 180°C, 0.25 after 7 h of frying at 200°C, and 0.25 after 3.5 h of frying at 220°C. The optical density values of the EPKO samples were found to be 1.02, 1.15, and 1.36 at the end of frying at 180, 200, and 220°C, respectively.

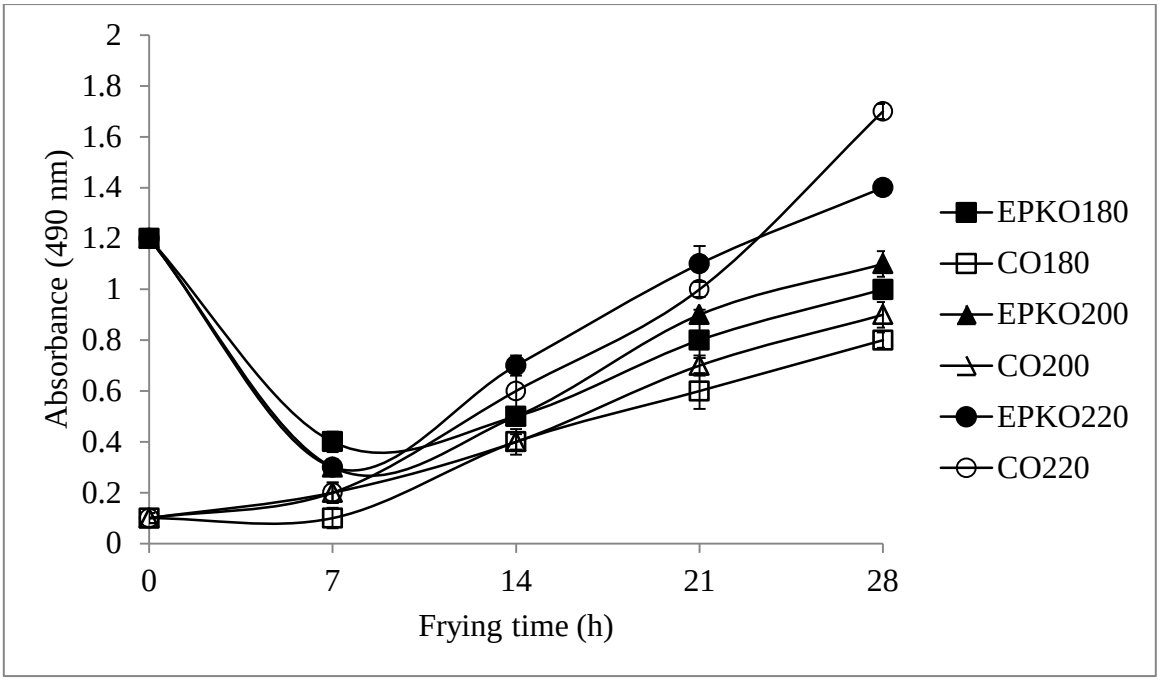

Figure 3-8 Changes in the color during frying at different temperatures without oil replenishment.

A similar pattern of change in the optical density of all oil samples fried in experiment II was observed (Figure 3-9). After 28 h of frying, the optical density values of the CO were found to be 0.67, 0.92, and 0.96 at 180, 200, and 220°C, respectively, while the values for the EPKO were 0.81, 0.80, and 1.15 at 180, 200, and 220°C, respectively.

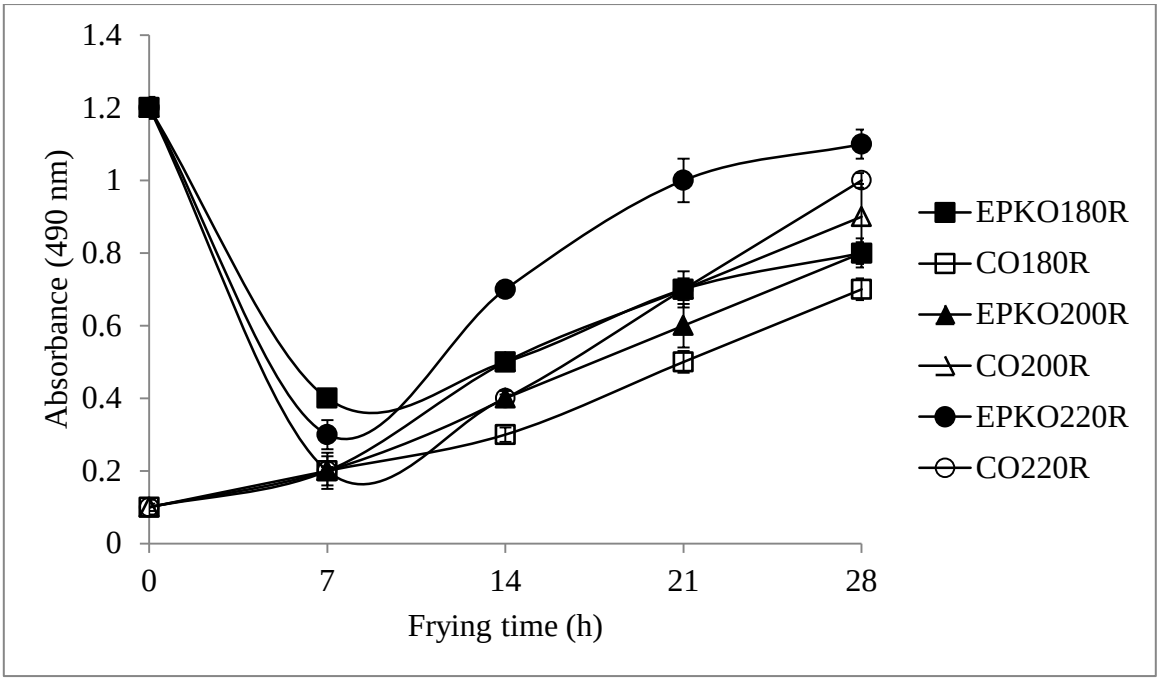

Figure 3-9 Changes in the color during frying at different temperatures with oil replenishment.

The increased optical density indicates that the oil samples became darker with an increase in frying time and temperature. This could be due to the oxidative reactions, the formation of polymers, and browning pigments from food (Mazza and Qi, 1992, Goburdhun *et al.*, 2000). As described by Blumenthal in his "frying oil quality curve", frying oil usually goes through five phases during the degradation process (Blumenthal, 1991). Based on the phases described in the frying oil quality curve, an early decrease in the optical density in the EPKO samples could be due to the fact that the frying oil underwent a breaking-in period (mainly due to a rapid breakdown of carotenoids and chlorophylls), during which the frying oil became quite clear.

The high optical density value in the fresh EPKO samples could be attributed to the presence of turbid matter and plant pigments (carotenoids and chlorophylls) as the oil did not undergo any refining process (Przybylski *et al.*, 2005a).

3.4.6 Changes in Viscosity

The changes in viscosity in experiment I are shown in Figure 3-10. The results show that viscosity significantly increased ($P < 0.05$) with frying time for the samples at all measurement temperatures. Although the EPKO samples had a higher initial viscosity, they had a lower increase in viscosity (from 67.6 mPa·s to 127.7, 126.6, and 191.9 mPa·s frying at 180, 200, and 220°C, respectively) than the CO samples after 28 h of frying (from 56.6 mPa·s to 177.0, 187.7, and 235.0 mPa·s frying at 180, 200, and 220°C, respectively).

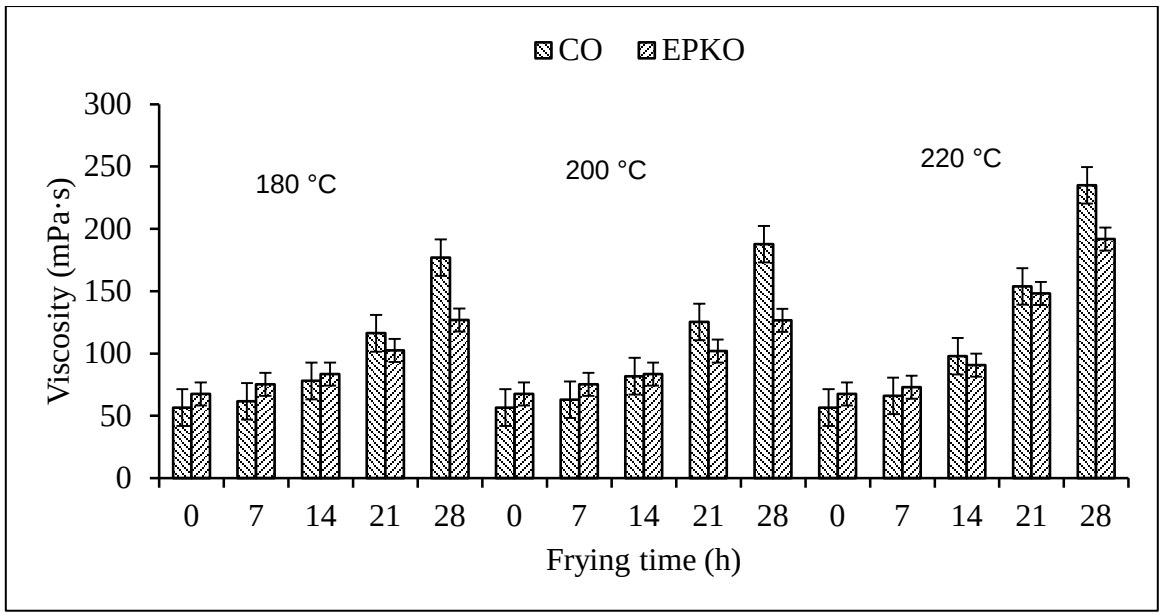

Figure 3-10 Changes in the viscosity during frying at different temperatures without oil replenishment.

All oil samples fried in experiment II (Figure 3-11) seemed to follow a similar pattern of change in viscosity, and the CO samples were found to have a higher increase in viscosity (from 56.6 mPa·s to 124.6 and 150.5 mPa·s at 200 and 220°C, respectively) after 28 h of frying than that of the EPKO samples (from 67.6 mPa·s to 116.7 and 112.7 mPa·s at 200 and 220°C, respectively). The higher initial viscosity of the EPKO samples than that of the CO samples was probably due to the impurities that remained in the oil after the pressing process.

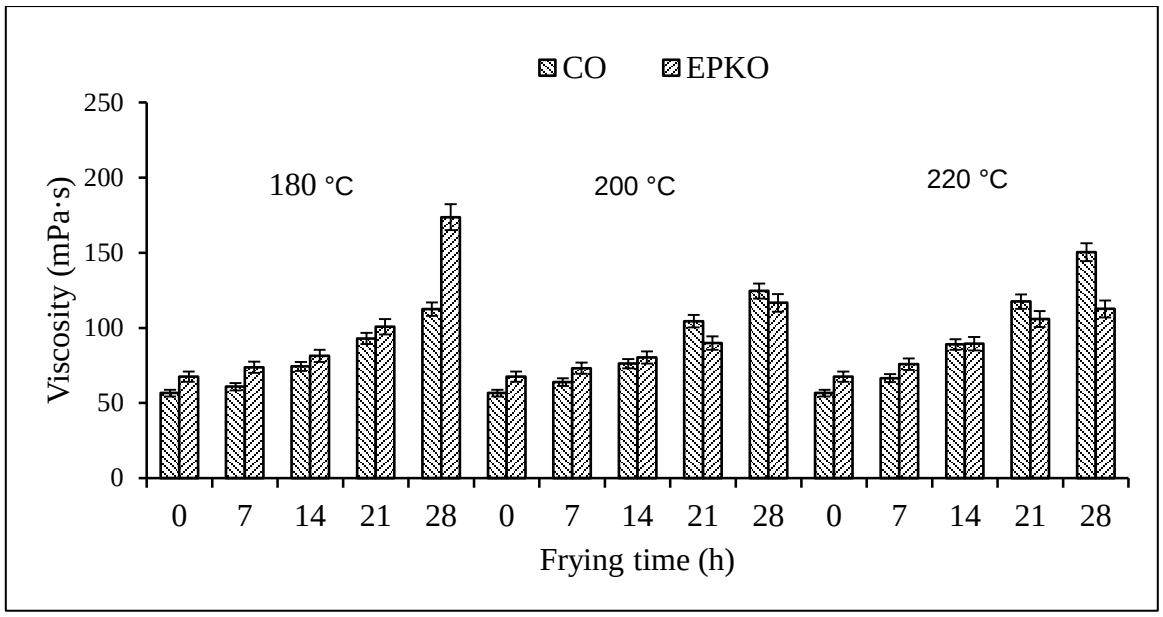

Figure 3-11 Changes in the viscosity during frying at different temperatures with oil replenishment.

The increase in viscosity of frying oils has been related to thermal oxidation and polymerization reactions. These reactions result in the formation of high molecular weight polymer compounds, leading to an increase in viscosity (Al-Harbi and Al-Kahtani, 1993). It has also been reported that oil viscosity may be related to differences in saturated fatty acids, with consequently higher melting points (Fasina *et al.*, 2006). Since the viscosity is the result of thermal polymerization, the excessive increases in viscosity observed in the EPKO (173.7 mPa·s) at the end of the frying process in experiment II at 180°C were unexpected, as oils were fried at a lower temperature and should have been less susceptible to thermal polymerization, as compared to that of oil fried at higher temperatures. Further investigation is

being conducted in our laboratory in order to better understand the factors that contribute to the viscosity of EPKO.

3.4.7 Changes in the Main Fatty Acids Composition

The changes in the main fatty acids composition during frying experiment I over 4 consecutive days of frying at 180, 200, and 220°C are shown in Table 3-1 and Table 3-2. When the relative percentages of fatty acids for both oils during the frying period were compared, a progressive decrease in both linoleic and linolenic acids contributions was observed. The linoleic acid of the CO decreased by 23.9, 27.2, and 29.0% after 28 h of frying at 180, 200, and 220°C, respectively. In comparison, the linoleic acid of the EPKO decreased by 16.5, 17.7, and 24.2% after frying at 180, 200, and 220°C, respectively. The deterioration of the linolenic acid of the oil samples was more pronounced during the frying sessions. The linolenic acid of the CO was reduced by 53, 64, and 70.2%, while the linolenic acid of the EPKO was reduced by 29.6, 38, and 60.1% after 28 h of frying at 180, 200, and 220°C, respectively. Losses of these two fatty acids was expected since they are present in plentiful amounts in vegetable oils and are more susceptible to oxidative changes than any of the other major fatty acids. The percentage contribution of the oleic acid to total fatty acid composition increased for most

oil samples at the end of 28 h of frying, with the increase ranging from 0.9% for the EPKO to 6.5% for the CO. There was a statistically significant increase in saturated fatty acid in both the EPKO and the CO. At the end of the frying cycles, the increment in the saturated fatty acid content in the EPKO were found to be 90.7 and 110.9% when heated at 200 and 220°C, respectively, which are lower than that of the corresponding CO samples (116.7 and 123.2%), despite the initial higher amounts of saturated fatty acids. The excessive increase in saturated fatty acids (126.2%) observed in the EPKO at the end of the frying experiment fried at 180°C as oils were fried at a lower temperature and should have been much less susceptible to oxidative changes as compared to that of oil fried at higher temperatures. The PUFA/SFA ratio, known as the polyene index, is often regarded as a measure of the extent of the polyunsaturation of an oil and of its tendency to undergo autoxidation (Farhoosh et al., 2009b). The PUFA/SFA ratios in both the EPKO and the CO significantly decreased ($P < 0.05$) after 28 h of frying at 180, 200 and 220°C. Results show that the PUFA/SFA ratios of the CO decreased more rapidly than that of the EPKO, indicating that the CO was more susceptible to oxidation than EPKO under heating.

Table 3-1 Changes in contribution of CO fatty acids at different frying temperatures without replenishment.

Frying time (h)	Contribution (relative percentage)						
	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	PUFA/SFA
180℃							
7	4.31 ± 0.10	1.38 ± 0.01	65.00 ± 0.07	18.33 ± 0.32	1.42 ± 0.52	7.46 ± 0.20	3.46
14	5.44 ± 0.14	1.44 ± 0.02	66.20 ± 0.80	17.09 ± 0.10	1.05 ± 0.03	6.14 ± 0.04	2.80
21	6.56 ± 0.22	1.56 ± 0.02	67.48 ± 0.18	15.69 ± 0.48	1.11 ± 0.11	5.16 ± 0.07	2.11
28	7.81 ± 0.42	1.71 ± 0.02	67.94 ± 0.76	14.66 ± 0.19	1.06 ± 0.05	4.27 ± 0.02	1.69
200℃							
7	4.90 ± 0.26	1.31 ± 0.02	66.60 ± 0.64	17.58 ± 0.44	1.11 ± 0.04	6.52 ± 0.34	3.13
14	5.80 ± 0.12	1.47 ± 0.04	66.06 ± 0.42	17.14 ± 0.17	1.19 ± 0.04	5.91 ± 0.20	2.59
21	7.06 ± 0.16	1.56 ± 0.02	68.03 ± 0.02	15.28 ± 0.08	1.26 ± 0.01	4.35 ± 0.06	1.86
28	9.16 ± 0.35	1.75 ± 0.10	67.29 ± 0.59	14.03 ± 0.08	1.39 ± 0.21	3.28 ± 0.43	1.33
220℃							
7	4.67 ± 0.04	1.41 ± 0.02	65.38 ± 0.07	18.03 ± 0.03	1.40 ± 0.02	6.63 ± 0.02	3.14
14	5.88 ± 0.15	1.49 ± 0.04	66.40 ± 0.33	16.72 ± 0.07	1.58 ± 0.05	5.07 ± 0.09	2.32
21	7.71 ± 0.64	1.61 ± 0.03	67.07 ± 0.72	15.15 ± 0.33	1.62 ± 0.14	3.69 ± 0.27	1.63
28	9.34 ± 0.38	1.79 ± 0.09	67.43 ± 0.49	13.69 ± 0.42	1.60 ± 0.08	2.71 ± 0.12	1.22

Table 3-2 Changes in contribution of EPKO fatty acids at different frying temperatures without replenishment.

Frying time (h)	Contribution (relative percentage)						
	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	PUFA/SFA
180℃							
7	6.28 ± 0.85	1.40 ± 0.06	64.19 ± 0.60	17.32 ± 0.21	0.48 ± 0.10	6.26 ± 0.16	2.80
14	5.89 ± 0.00	1.57 ± 0.01	63.17 ± 0.01	18.30 ± 0.02	0.58 ± 0.01	6.61 ± 0.02	2.99
21	9.67 ± 0.40	1.45 ± 0.14	65.34 ± 2.78	17.12 ± 0.21	0.41 ± 0.08	5.34 ± 0.00	1.85
28	11.39 ± 0.78	1.40 ± 0.06	61.54 ± 0.42	15.83 ± 0.10	0.40 ± 0.06	5.15 ± 0.10	1.50
200℃							
7	6.82 ± 0.54	1.27 ± 0.08	64.63 ± 0.34	17.38 ± 0.10	0.41 ± 0.10	5.89 ± 0.12	2.64
14	6.37 ± 0.82	1.51 ± 0.10	64.91 ± 0.32	16.68 ± 0.15	0.84 ± 0.11	5.55 ± 0.08	2.39
21	7.45 ± 0.38	1.56 ± 0.06	64.78 ± 0.24	16.14 ± 0.14	0.90 ± 0.10	4.99 ± 0.03	2.02
28	8.60 ± 0.79	1.63 ± 0.03	64.07 ± 1.02	15.59 ± 0.51	0.94 ± 0.02	4.53 ± 0.07	1.71
220℃							
7	4.72 ± 0.15	1.48 ± 0.01	65.06 ± 0.69	18.15 ± 0.33	0.85 ± 0.03	5.87 ± 0.26	3.26
14	5.57 ± 0.03	1.55 ± 0.01	65.76 ± 0.16	17.27 ± 0.12	1.13 ± 0.01	4.76 ± 0.05	2.57
21	6.85 ± 0.33	1.69 ± 0.02	65.96 ± 1.02	16.03 ± 0.36	1.37 ± 0.01	3.73 ± 0.19	1.91
28	8.96 ± 0.61	1.83 ± 0.05	65.97 ± 0.21	14.37 ± 0.59	1.43 ± 0.03	2.92 ± 0.04	1.33

Changes in the fatty acids of all the treatments in frying experiment II are depicted in Table 3-3 and Table 3-4. Similar trends of change in fatty acids were observed. There was a marked decrease of both the linoleic and the linolenic acids in the CO. The linoleic acid had an initial value of 19.3%, which decreased to 16.5, 16.1, and 15.2% after 28 h of frying at 180, 200, and 220°C, respectively, while the linolenic acid had an initial value of 9.1%, which decreased to 5.6, 4.8, and 3.6 % after frying at the same temperatures. The percentage contributions of the linoleic and linolenic acids in the EPKO were found to be 17.2, 16.9, and 15.3% after 4 days of frying at 180, 200, and 220°C, respectively, while the percentage contributions of the linolenic acid were 5.8, 5.3, and 3.6% after frying at the same temperatures. The saturated fatty acids significantly increased in both the CO and the EPKO ($P < 0.05$), while the PUFA/SFA ratios greatly decreased in all cases ($P < 0.05$). The decrement was smaller than that of the PUFA/SFA ratio in experiment I. These results indicate that frequent replenishment with fresh oils has an obvious effect on the changes of fatty acids during heating. In all cases, the trend of change in the fatty acids composition was that more saturated fatty acids was generated and less unsaturated fatty acids remained due to the effects of oxidation and/or hydrolysis.

Table 3-3 Changes in contribution of CO fatty acids at different frying temperatures with replenishment.

Frying time (h)	Contribution (relative percentage)						
	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	PUFA/SFA
180℃							
7	4.30 ± 0.04	1.34 ± 0.01	65.27 ± 0.11	18.54 ± 0.03	0.99 ± 0.01	7.55 ± 0.07	3.77
14	5.19 ± 0.01	1.46 ± 0.06	65.74 ± 0.15	17.78 ± 0.01	1.01 ± 0.01	6.76 ± 0.04	3.07
21	6.08 ± 0.08	1.50 ± 0.01	66.03 ± 0.26	17.14 ± 0.06	0.99 ± 0.01	6.12 ± 0.10	2.60
28	6.83 ± 0.06	1.59 ± 0.02	66.16 ± 0.11	16.52 ± 0.04	1.01 ± 0.02	5.58 ± 0.06	2.25
200℃							
7	4.25 ± 0.07	1.32 ± 0.01	65.76 ± 0.33	18.32 ± 0.13	1.11 ± 0.01	7.10 ± 0.16	3.63
14	5.25 ± 0.02	1.45 ± 0.00	66.16 ± 0.07	17.55 ± 0.03	1.15 ± 0.01	6.21 ± 0.03	2.90
21	6.20 ± 0.09	1.54 ± 0.01	66.58 ± 0.31	16.69 ± 0.12	1.20 ± 0.01	5.40 ± 0.13	2.36
28	7.07 ± 0.09	1.62 ± 0.02	66.79 ± 0.35	16.07 ± 0.12	1.24 ± 0.03	4.82 ± 0.13	2.02
220℃							
7	3.83 ± 0.09	1.52 ± 0.23	67.95 ± 0.75	16.69 ± 0.52	1.51 ± 0.18	5.68 ± 0.23	3.09
14	4.63 ± 0.19	1.44 ± 0.01	68.58 ± 0.53	16.46 ± 0.23	1.50 ± 0.04	4.92 ± 0.09	2.68
21	5.88 ± 0.42	1.54 ± 0.04	68.35 ± 0.85	15.80 ± 0.37	1.61 ± 0.05	4.18 ± 0.13	2.11
28	7.11 ± 0.17	1.67 ± 0.02	67.65 ± 0.29	15.17 ± 0.10	1.66 ± 0.02	3.60 ± 0.06	1.72

Table 3-4 Changes in contribution of EPKO fatty acids at different frying temperatures with replenishment.

Frying time (h)	Contribution (relative percentage)						
	C16:0	C18:0	C18:1	C18:2	C20:0	C18:3	PUFA/SFA
180℃							
7	4.61 ± 0.11	1.51 ± 0.10	63.96 ± 1.13	17.70 ± 0.35	0.58 ± 0.04	6.31 ± 0.11	3.42
14	4.89 ± 0.44	1.47 ± 0.06	63.99 ± 1.41	18.21 ± 0.49	0.49 ± 0.01	6.14 ± 0.10	3.35
21	5.94 ± 0.32	0.19 ± 0.09	65.92 ± 0.42	16.73 ± 0.11	0.36 ± 0.17	5.97 ± 0.03	3.37
28	6.39 ± 0.37	1.34 ± 0.05	67.09 ± 0.31	17.24 ± 0.15	0.24 ± 0.07	5.83 ± 0.13	2.86
200℃							
7	4.26 ± 0.31	1.47 ± 0.08	66.34 ± 1.07	18.22 ± 0.77	0.58 ± 0.04	6.30 ± 0.28	3.76
14	5.11 ± 0.15	1.35 ± 0.02	66.50 ± 0.42	17.62 ± 0.21	0.52 ± 0.05	5.82 ± 0.05	3.26
21	4.98 ± 0.87	1.51 ± 0.09	66.31 ± 0.34	16.54 ± 0.73	0.75 ± 0.08	5.46 ± 0.14	2.89
28	5.82 ± 0.06	1.42 ± 0.04	66.77 ± 0.17	16.91 ± 0.15	0.69 ± 0.08	5.25 ± 0.15	2.75
220℃							
7	3.96 ± 0.07	1.42 ± 0.01	68.08 ± 0.43	17.40 ± 0.11	0.80 ± 0.03	5.32 ± 0.07	3.59
14	4.76 ± 0.26	1.40 ± 0.02	68.02 ± 0.32	17.10 ± 0.12	0.91 ± 0.05	4.96 ± 0.10	3.00
21	5.93 ± 1.14	1.62 ± 0.07	67.94 ± 1.68	15.70 ± 0.35	1.37 ± 0.09	3.74 ± 0.21	2.09
28	6.30 ± 0.55	1.65 ± 0.08	66.02 ± 2.06	15.27 ± 0.76	1.33 ± 0.11	3.63 ± 0.28	1.93

The tendency observed in this study is in accordance with many previous reports. In a recent similar study, Aladedunye and Przybylski (Aladedunye and Przybylski, 2009) monitored the changes in the fatty acids composition of regular canola oil after 49 h of frying at 185 and 215°C. There were an 8.5 and 13.3% loss of linoleic acid in the oil samples that were fried at 185 and 215°C, respectively. The linolenic acid content significantly decreased in both oil samples, ranging from 24.0% to 47.1%. They noticed an increase in both saturated fatty acids and oleic acid during the frying sessions in their study. In another similar research, White (White, 1991) found 7-11.5% losses of linoleic acid and 27-46% losses of linolenic acid in soybean oils after 40 h of heating at 180°C. Petersen et al. (Petersen *et al.*, 2013) examined changes in the fatty acids composition of rapeseed oil, high-oleic rapeseed oil, sunflower oil, high-oleic sunflower oil, and palm olein after 36 h of frying at 170°C. They reported a significant decrease in linolenic acid in the rapeseed oil varieties during the deep-frying trial (from 7.6% to 5.9% in rapeseed oil and from 3.3% to 2.4% in high-oleic rapeseed oil). Wang et al. (Wang *et al.*, 2013) reported significant increases in saturated fatty acids and oleic acid while the linoleic and linolenic acids were markedly reduced in three types of soybean oils after 32 h of frying French fries at 180-185°C. Juárez et al. (Juárez *et al.*, 2011) conducted a discontinuous deep frying experiment on

potatoes in soybean and sunflower oil at 180°C with fresh oil replenishment, and found a significant decrease in polyunsaturated fatty acids and increase in saturated fatty acids in all oil samples.

3.4.8 Changes in Tocopherols

The fatty acid composition of oils plays an important role in determining their oxidative stability during storage or frying processes. However, other factors, such as amounts and types of chemical and natural minor components, have significant effects on the stability and quality of frying oil and fried food as well (Warner *et al.*, 2004). Tocopherols are considered one of the most important minor constituents in oils and most widely used antioxidants. The rate of loss of tocopherols during repeated or continuous frying has been used for assessing the progressive degradation of rapeseed oil (Gordon and Kourimska, 1995), modified canola oils (Normand *et al.*, 2001), sunflower oil (Andrikopoulos *et al.*, 2002), olive oil (Casal *et al.*, 2010), palm olein (Bansal *et al.*, 2010), and a frying oil blend of canola and palm olein (Al-Khusaibi *et al.*, 2012).

The deterioration effects of the three frying temperatures on the tocopherols (α-, β-, γ-, and δ-Tocopherol) in the two examined oils in experiment I, as measured using HPLC analysis, are shown in Figure 3-12 (α-

tocopherol), Figure 3-13 (β-tocopherol), Figure 3-14 (γ-tocopherol), and Figure 3-15 (δ-tocopherol).

As shown in Figure 3-12, α-tocopherol in the CO samples that were fried at 180 and 200°C degraded at significantly faster rates than that of the α-tocopherol in the EPKO samples. Within 7-14 h, the initial concentration of α-tocopherol was reduced by over 50% for the CO samples fried at 180 and 200°C, while the level of α-tocopherol degradation in the EPKO had not reached that amount even by the end of the 28-h frying period. The results clearly show that the α-tocopherol in the CO degraded at a faster rate than that of the EPKO at 180 and 200°C. However, the order was reversed during frying at 220°C. The amount of α-tocopherol was found to be 18.67 and 6.44 mg/kg for the CO and EPKO after 28 h of frying, respectively.

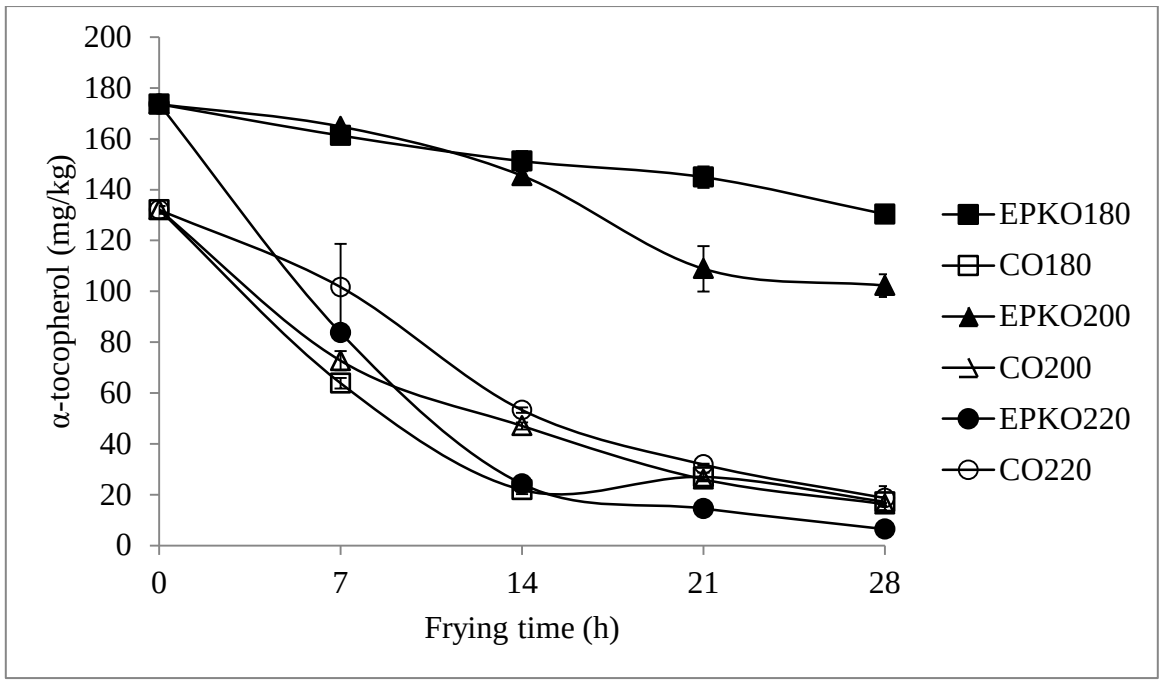

Figure 3-12 Changes in α-tocopherol content during frying at different temperatures without oil replenishment.

Although the initial β-tocopherol content in the CO was higher than that of the EPKO, the β-tocopherol level of the CO decreased at a faster rate than that of the EPKO when oil samples were subjected to frying at 180 and 200°C. By the end of 4 d of frying, the concentration of β-tocopherol was found to be 11.81 and 9.46 mg/kg for the EPKO after frying at 180 and 200°C, respectively, and 4.82 and 0.69 mg/kg for the CO, after frying at 180 and 200°C, respectively. The β-tocopherol content in the EPKO that was fried at 220°C was undetectable and trace amounts remained in the CO at the end of the frying session (Figure 3-13).

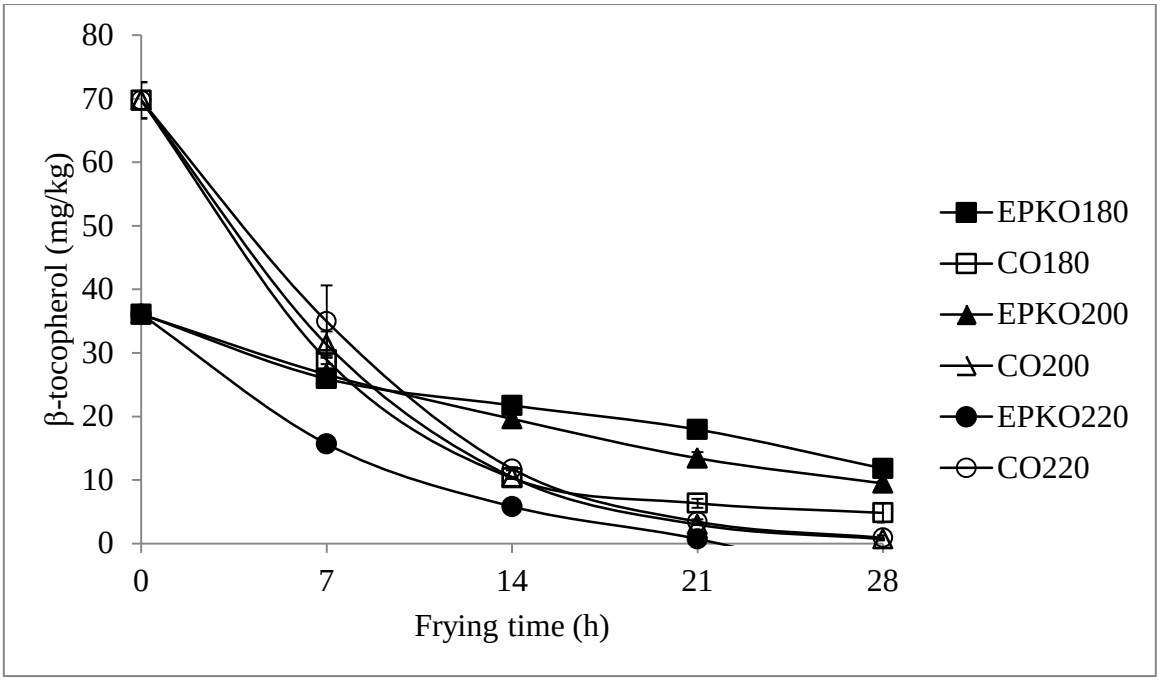

Figure 3-13 Changes in β-tocopherol content during frying at different temperatures without oil replenishment.

Within 7 h, the initial concentrations of γ-tocopherol were reduced by over half in the CO samples that were fried at 180 and 200°C. The same level of γ-tocopherol degradation was seen between 14 and 21 h for the EPKO. In the case of oil samples that were fried at 220°C, a more rapid loss of γ-tocopherol was observed. The amount of γ-tocopherol was found to be 150.07, 60.77, and 10.88 mg/kg for the EPKO at the end of frying at 180, 200, and 220°C, respectively, and 24.51, 38.57, and 12.74 mg/kg for the CO at the end of frying at 180, 200, and 220°C, respectively (Figure 3-14).

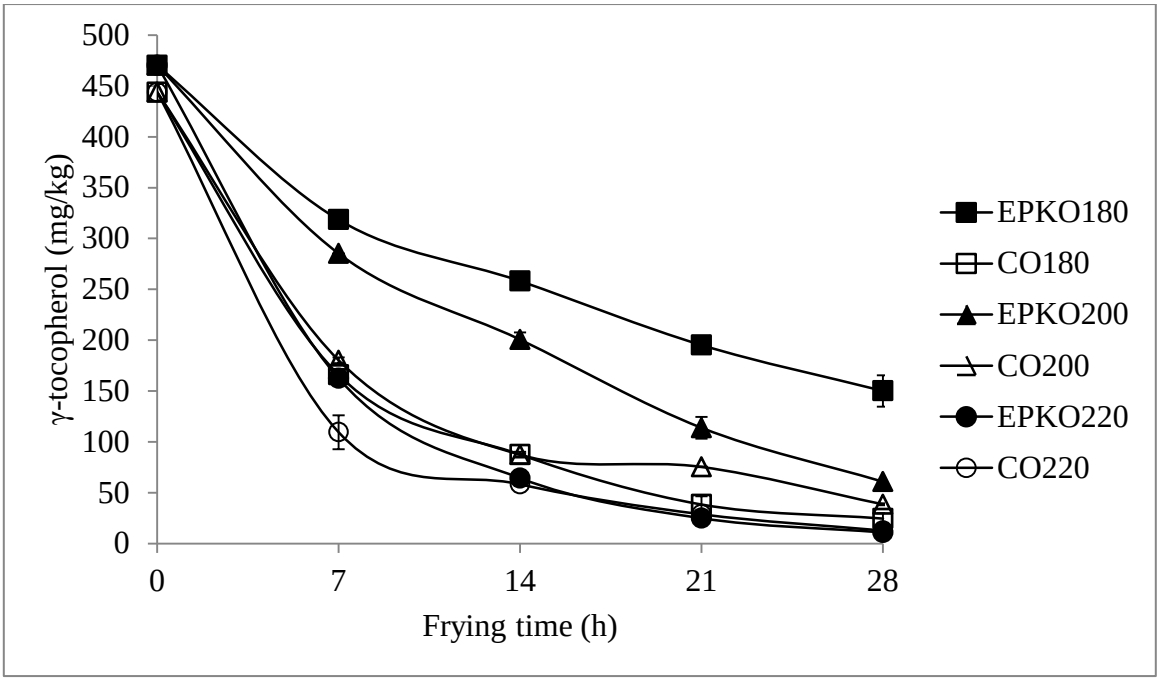

Figure 3-14 Changes in γ-tocopherol content during frying at different temperatures without oil replenishment.

Prior to frying, both the EPKO and the CO presented with similar amounts of δ-tocopherol, 9.27 and 9.65 mg/kg, respectively. The EPKO displayed a slower rate of δ-tocopherol degradation as compared to the CO during the frying session at 180 and 200°C. However, the order of the rate of degradation was reversed when the oil samples were subjected to frying at 220°C. At the end of 28 h of frying at 180, 200, and 220°C, the amount of δ-tocopherol that was retained in the EPKO was found to be 4.97, 3.86, and 0.81 mg/kg, respectively, while the amount of δ-tocopherol that was retained in the CO after frying at 180, 200, and 220°C for 28 h was 2.56, 1.19, and 2.20 mg/kg, respectively (Figure 3-15).

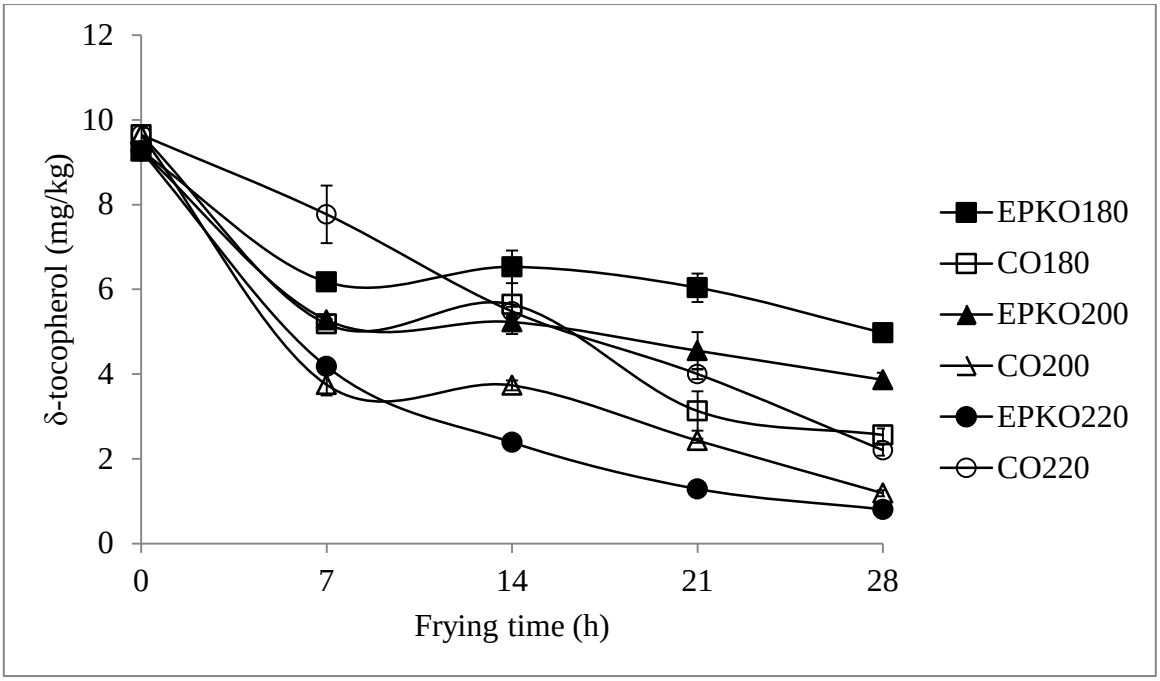

Figure 3-15 Changes in δ-tocopherol content during frying at different temperatures without oil replenishment.

As expected, similar trends of change in the tocopherols were observed in frying experiment II. The level of α-tocopherol in the CO samples markedly decreased from 132.07 mg/kg to 19.05, 30.85, and 33.08 mg/kg after frying at 180, 200, and 220°C, respectively. On the other hand, the level of α-tocopherol in the EPKO samples showed slight decreases from 173.62 mg/kg to 160.54 and 111.85 mg/kg at the end of the frying session at 180 and 200°C, respectively. The lowest α-tocopherol content in the EPKO was found to be 9.33 mg/kg, at the end of the 220°C frying session (Figure 3-16). There was a significant degradation of β-tocopherol in the CO samples. By the end of the frying session at 180, 200, and 220°C, the amount of β-tocopherol that was

retained was 3.85, 4.79, and 4.76 mg/kg, respectively. However, the EPKO displayed a much slower rate of β-tocopherol degradation than that of the CO when oils were subjected to frying at 180 and 200°C. Similar to that of frying experiment I, the lowest β-tocopherol retention level was observed in the EPKO samples at the end of the frying period at 220°C (Figure 3-17). Figure 3-18 illustrates the changes in the γ-tocopherol during experiment II. Somewhere between 14 and 21 h, the initial concentrations of γ-tocopherol were reduced by over half in the EPKO samples that were frying at 180 and 200°C, while the same level of γ-tocopherol degradation was seen within 7 h for the CO. In the case of oil samples that were fried at 220°C, a more rapid loss of γ-tocopherol was observed in the EPKO than in the CO. A moderate degradation of δ-tocopherol was observed in both the EPKO and the CO during the frying process. The EPKO displayed slower rates of δ-tocopherol degradation during the deep frying of French fries at 180 and 200°C as compared to that of the CO (Figure 3-19).

Oils in which tocopherols degrade slowly are expected to show higher stability as tocopherols can act as antioxidants. The lower rate of degradation of the tocopherols shown in experiment II as compared to experiment I was mainly a result of the frequent replenishment with fresh oil. Results obtained from experiment I and II show significantly high levels of tocopherol retention

($P < 0.05$) in the EPKO samples after 28 h of frying at 180 and 200°C as compared to that of the CO samples. In other words, the EPKO exhibited a much better oxidative stability than the CO when fried at 180°C and 200°C.

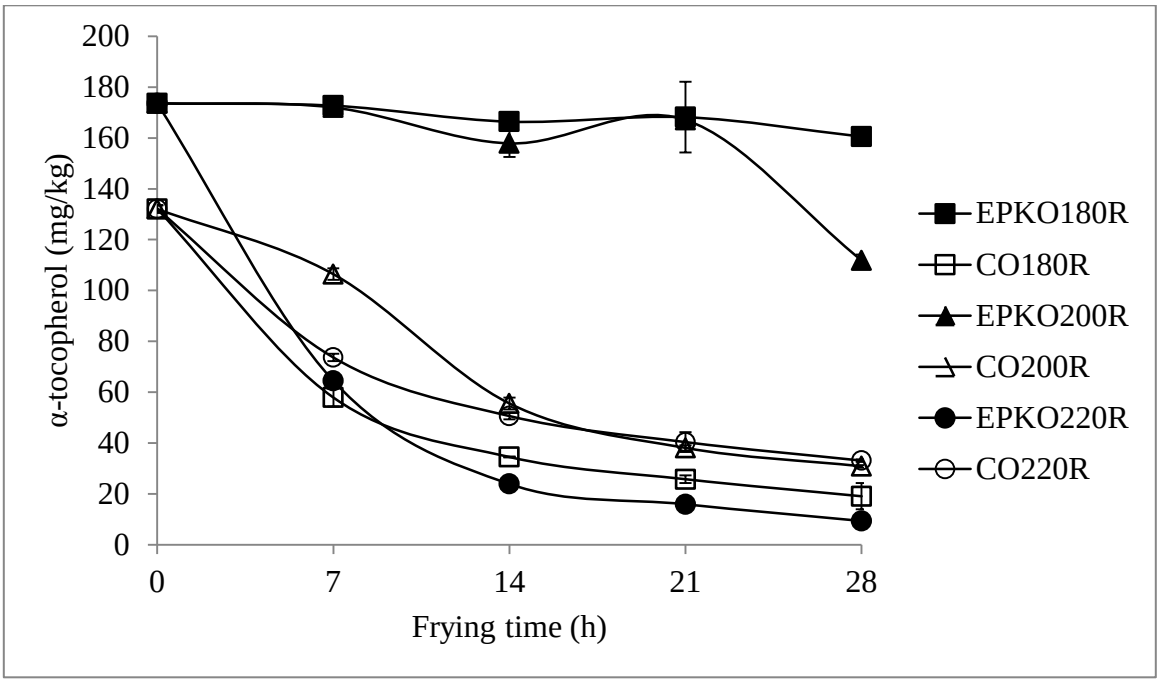

Figure 3-16 Changes in α-tocopherol content during frying at different temperatures with oil replenishment.

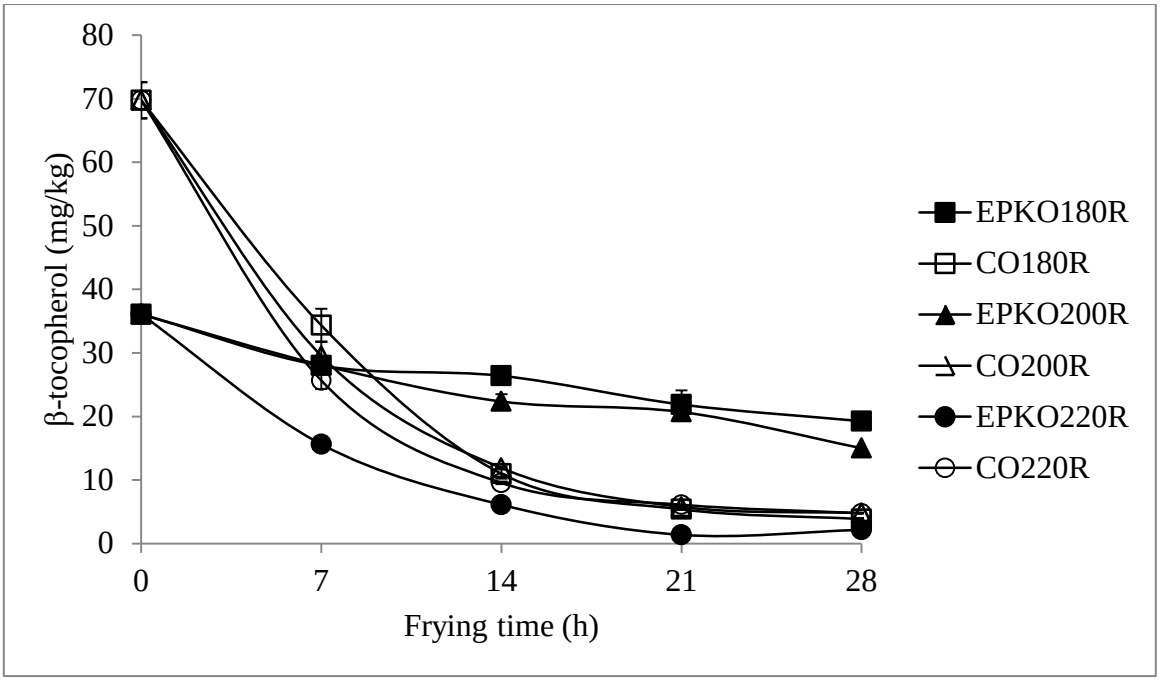

Figure 3-17 Changes in β-tocopherol content during frying at different temperatures with oil replenishment.

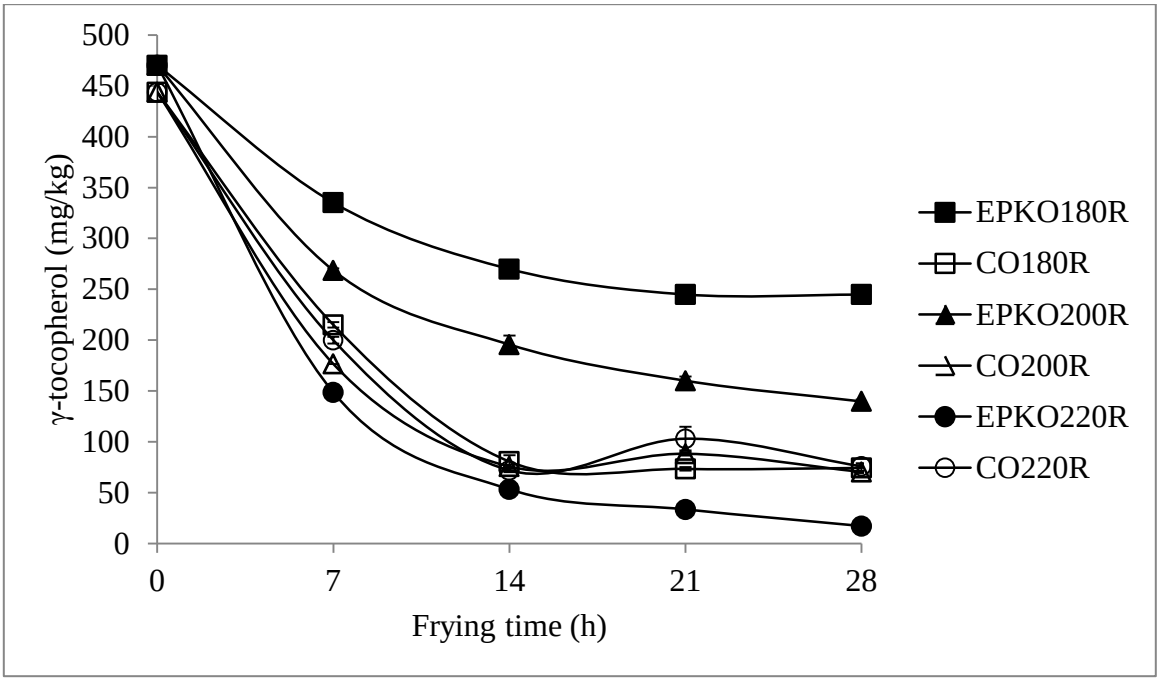

Figure 3-18 Changes in γ-tocopherol content during frying at different temperatures with oil replenishment.

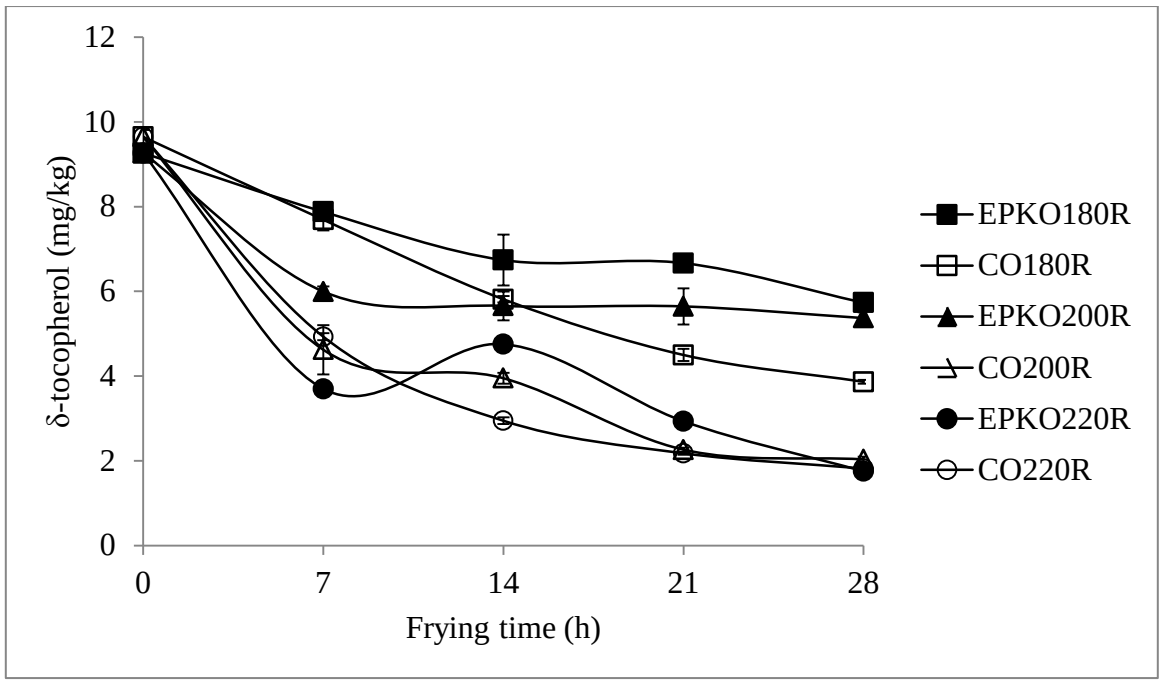

Figure 3-19 Changes in δ-tocopherol content during frying at different temperatures with oil replenishment.

Tocopherols are well-known for its efficient antioxidative ability to protect oils from oxidation during processing, storage, and later in the fryer, which largely depends on the temperature, presence of oxygen, light, metals, and synergists (Lalas, 2010). It has been proven that vegetable oils that contain higher levels of tocopherols than animal fats have shown greater stability under the same oxidative conditions (Carpenter, 1979).

Results obtained in this study show that the order of the tocopherols decomposition of the EPKO and the CO was, in most cases, $\gamma > \beta > \delta > \alpha$ after 4 d of frying under different temperatures. These observation are in agreement with the findings by Miyagawa and colleagues, who reported that the order of

degradation of tocopherols was γ > δ >α, after frying 32 batches of a mixture of soybean and rapeseed oils (Miyagawa *et al.*, 1991). The same decomposition order was also observed by Carlson and Tabacchi in a frying study that they conducted where they used soybean oil for the deep frying of French fries (Carlson and Tabacchi, 1986). Tsaknis and Lalas found that there were rapid γ-tocopherol losses when they used Moringa oleifera seed oil to fry potatoes and cod (Tsaknis and Lalas, 2002). In a frying study conducted by Aladedunye and Przybylski using refined regular canola oil, they reported that γ-tocopherol degraded at a faster rate than α-tocopherol at a lower frying temperature of 185°C, although the order was reversed when the oils were subjected to frying at 215°C (Aladedunye and Przybylski, 2009). In another frying study involving four types of frying oils, high-oleic rapeseed oil, palm olein, high-oleic sunflower oil, and partially hydrogenated rapeseed oil, Matthäus (Matthäus, 2006) also observed that γ-tocopherol degraded at a faster rate than α-tocopherol during the 72 h frying period.

On the other hand, Gordon and Kourimska (Gordon and Kourimska, 1995) reported that α-tocopherol had a higher rate of loss than β-, γ- and δ-tocopherol in rapeseed oil during the deep frying of potato chips at 162°C. Barrera-Arellano et al., who came to the same conclusion, reported that the

decomposition rate of α-tocopherol was faster than that of other tocopherols (Barrera-Arellano *et al.*, 1999, Barrera-Arellano *et al.*, 2002).

Much of the researches indicate that the loss of tocopherols can be inhibited by combining other synergists/additives with tocopherols in the frying oils. For instance, in a study by Gordon and Kourimska (Gordon and Kourimska, 1995), it took 7 and 7-8 fryings for α-tocopherol to reach its 50% depletion when γ- and δ-tocopherols were added, while the oil that had no antioxidants added had the same level of α-tocopherol degradation after the oil was fried 4-5 times. As demonstrated by Gupta (Gupta, 2005), the oxidative stability of a frying oil can be significantly improved by supplementing the oil with γ- and/or δ-tocopherols.

3.4.9 Changes in Polyphenols

Phenolic compounds are reported to have a large number of beneficial physiological properties, including antiallergic, anti-inflammatory, antimicrobial, and cardioprotective effects (SzydłOwska-Czerniak, 2013). These bioactive compounds, which act as antioxidants, play a vital role in human health by scavenging free radicals, quenching reactive oxygen species, and inhibiting oxidative enzymes (Antolovich *et al.*, 2002). The changes in total polyphenols content of the EPKO during the frying cycles are presented

in Figure 3-20 and Figure 3-21. A rapid decrease in total polyphenols content was observed before the 14th of frying in both frying experiments was reached when oils were subjected to heating at 180, 200, and 220°C. The total rate of loss of polyphenols increased with an increase in frying time and temperature. After 21 h of frying, the polyphenols could only be quantified in the oil that was fried at 180°C, and had disappeared in all the oil samples by the 28th h of frying experiment I. A similar initial fast decrease in the amount of total polyphenols was also observed in frying experiment II. A small quantity of total polyphenols remained after 28 h of frying at 180°C while the polyphenols were absent after 21 and 14 h of frying at 200 and 220°C, respectively.

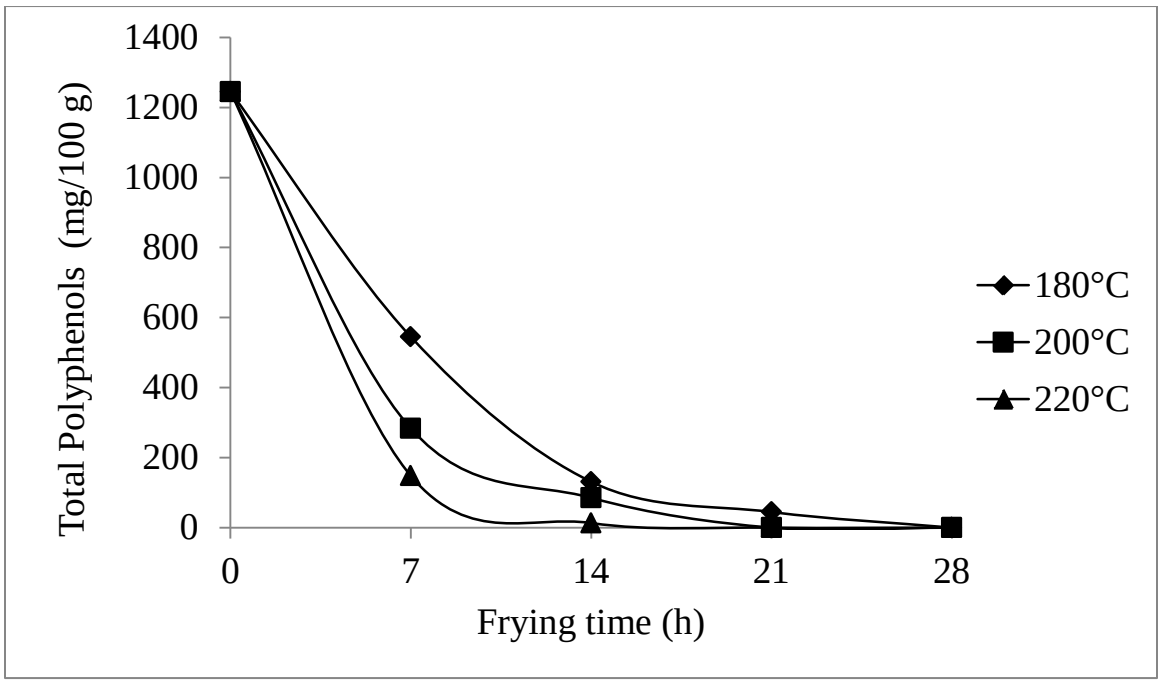

Figure 3-20 Changes in total polyphenols content of the EPKO during frying at different temperatures without oil replenishment.

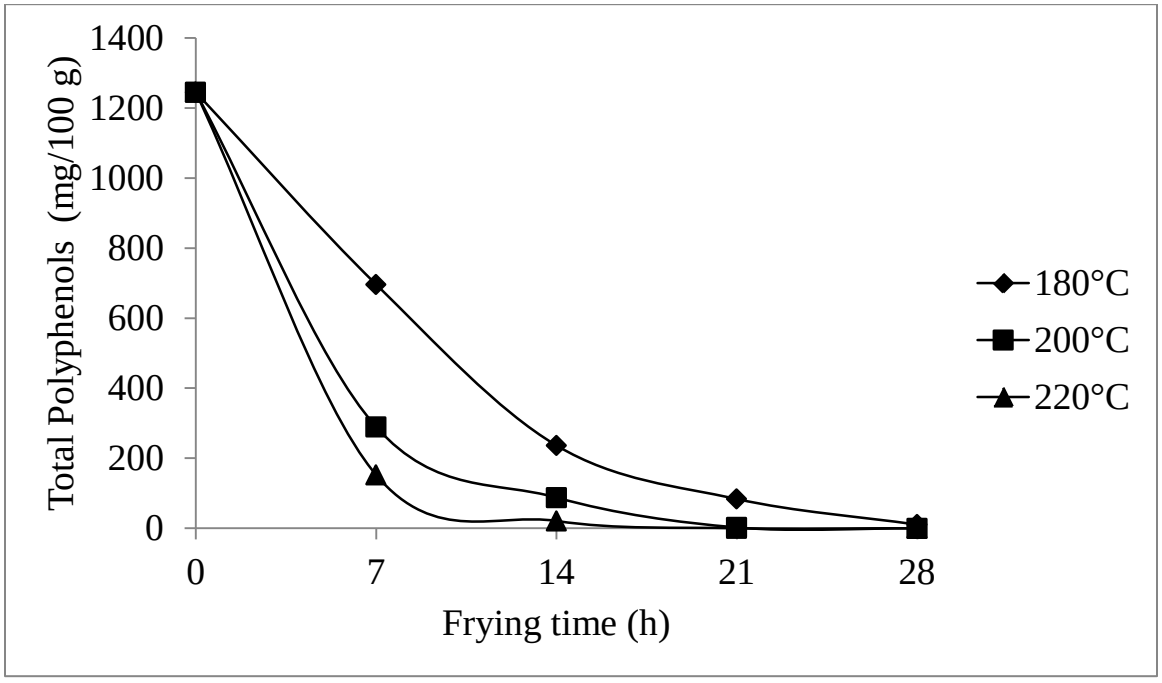

Figure 3-21 Changes in total polyphenols content of the EPKO during frying at different temperatures with oil replenishment.

The changes in the content of polyphenols in the CO during 4 d of frying are shown in Figure 3-22 and Figure 3-23. From Figure 3-22, it can be seen that total polyphenols were only present in a small amount (5.3 mg/100 g oil) after 21 h of frying at 180°C and was absent in all oil samples at the 28th h. With respect to frying experiment II, a negligible amount of total polyphenols (0.3 mg/100 g oil) remained at the end of the frying session at 180°C, while the total polyphenols were undetectable by the current method employed after 14 h of frying at 200 and 220°C (Figure 3-23).

As results obtained from both frying experiment I and II have shown, more than 50% of the polyphenols decomposed rapidly within 7 h of frying, suggesting that the frying resistance of the polyphenols towards thermal oxidation is mainly restricted to the first few hours of frying. This observation is consistent with previously published results. Casal et al (Casal *et al.*, 2010) investigated the stability of commercial olive oils that were used to fry potato chips at 170°C for 27 h and found that the polyphenols had completely decomposed after 9 h of frying. Similar results were also reported by Gómez-Alonso et al. (Gómez-Alonso *et al.*, 2003). The concentration of the phenolic compounds was reduced to 50-60% of the initial value by the end of the first frying process. In a study involving the comparison of the effects of pan-frying and deep-frying on the frying stability of different oils, Andrikopoulos

et al. (Andrikopoulos *et al.*, 2002) found that the retention of total phenolics in oils ranged from 70-80% (first frying) to 20-30% (eighth frying). However, a previous study conducted by Karakaya et al. (Karakaya and Şimşek, 2011) reported that there was no significant reduction in the total polyphenols of oils after 125 min of frying at 190°C.

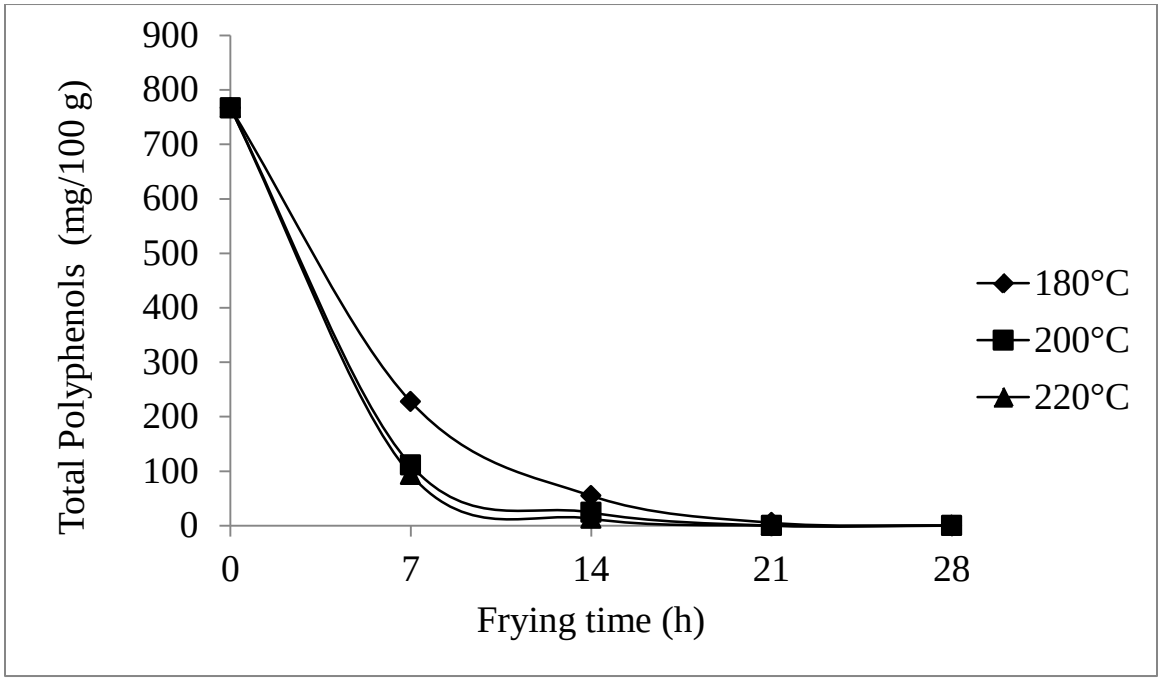

Figure 3-22 Changes in total polyphenol content of the CO during frying at different temperatures without oil replenishment.

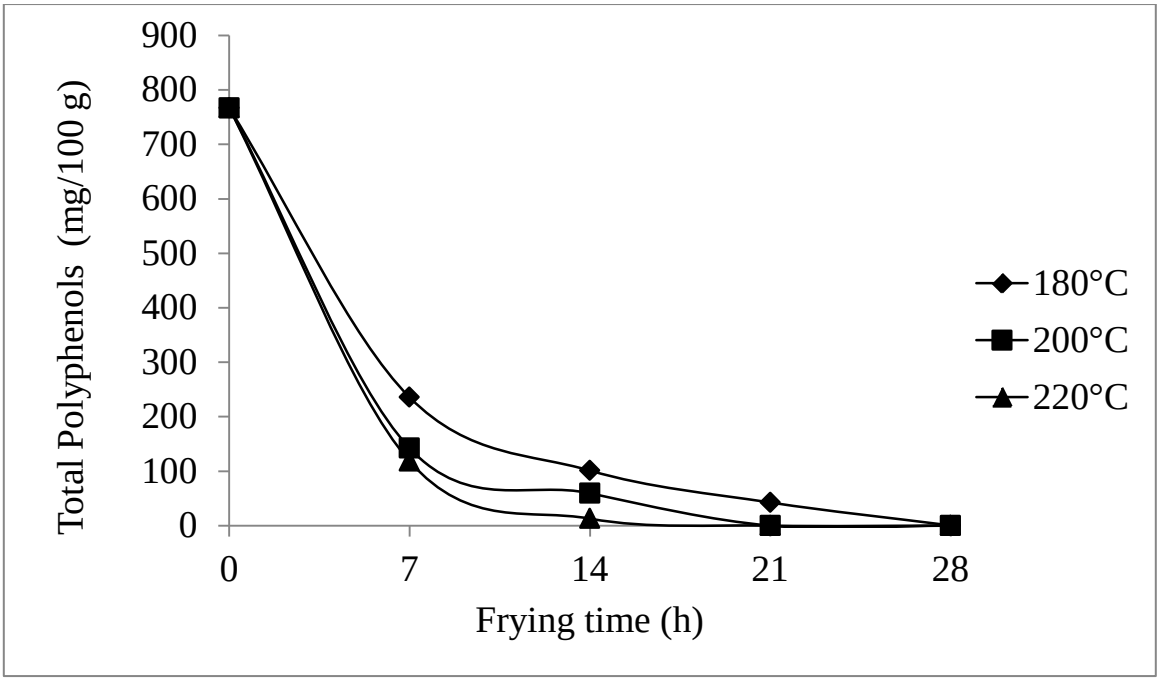

Figure 3-23 Changes in total polyphenols content of the CO during frying at different temperatures with oil replenishment.

3.4.10 Changes in Carotenoids

Carotenoids are natural pigments which are produced by a large variety of plants and are known to have important physiological properties like its free radical scavenging and singlet oxygen quenching abilities (Lee *et al.*, 2003). Table 3-5 reports the changes that occurred in the β-carotene content of the oils that were subjected to the frying treatment in frying experiment I. Carotenoids are naturally present in substantial amounts in crude rapeseed oils but is negligible in refined, bleached and deodorized oils. During frying, there was an initial sharp decrease of the β-carotene content, with a continuous decrease between the 14-21st h, followed by a slight increase in all EPKO

samples that were fried at different temperatures. However, the β-carotene content in the CO steadily increased as the frying time and temperatures increased (Table 3-5). The increase in the β-carotene content in the frying oil samples is probably due to its dissolution from potatoes containing carotenoids and the smaller food to oil ratio as the frying was repeated (Casal *et al.*, 2010). Loss of carotenoids in foods is mainly dependent on heating time and temperature, its concentration, and the presence of oxidized lipids (Lee *et al.*, 2003, Onyewu *et al.*, 1986). Generally, carotenes are believed to protect the oil from thermal degradation. However, their effects in preventing thermal oxidation would be minimal or non-existent in the absence of other antioxidants (Choe and Min, 2007). It was reported that the oxidative stability of frying oil synergistically increased when carotenes and tocotrienols combined while potato chips are being fried (Schroeder *et al.*, 2006).

Table 3-5 Changes in the β-carotene during frying at different temperatures without replenishment.

Frying time (h)	EPKO	CO
180℃		
0	7.31 ± 0.26	0.13 ± 0.02
7	1.34 ± 0.18	0.40 ± 0.04
14	1.10 ± 0.08	0.82 ± 0.11
21	1.39 ± 0.11	1.28 ± 0.18
28	1.64 ± 0.08	2.10 ± 0.09
200℃		
7	1.02 ± 0.02	0.56 ± 0.02
14	1.32 ± 0.03	1.17 ± 0.10
21	1.75 ± 0.04	2.04 ± 0.03
28	2.40 ± 0.08	2.76 ± 0.29
220℃		
7	1.12 ± 0.09	0.63 ± 0.03
14	1.89 ± 0.01	1.70 ± 0.11
21	2.90 ± 0.07	3.93 ± 0.19
28	3.76 ± 0.24	6.87 ± 0.41

3.4.11 Changes in Chlorophylls

Chlorophylls are present in rapeseed/canola crude oils in varied amounts (5-55 mg/kg) and the composition and content of chlorophyll pigments are mainly related to the maturity of the oil seed (Przybylski, 2011). The chlorophyll content is greatly reduced during refining and bleaching processes (Gunstone, 2004). Figure 3-24 shows the changes in the chlorophyll content in the EPKO that had no fresh oil replenishment under different heating temperatures. The initial chlorophyll content of the EPKO was 5.6 mg/kg, which is close to the lowest level of chlorophylls as stated in previous

reports (Przybylski *et al.*, 2005b). Bearing in mind that chlorophylls contained in oil can easily break down into derivatives such as pheophytin (Niewiadomski *et al.*, 1965), the results obtained in this study were within expectation since the oils were investigated a few months after their extraction. Pigments present in rapeseed/canola usually impart undesirable color to the oil, thus they are often removed from the oil. During oil processing, the amount of chlorophyll is usually reduced to less than 0.025 mg/kg in commercial refined, bleached and deodorized oils (Przybylski *et al.*, 2005b). Since maximum absorption does not occur at 670 nm in processed oils, the chlorophyll content of the commercial CO used in this study were not measured.

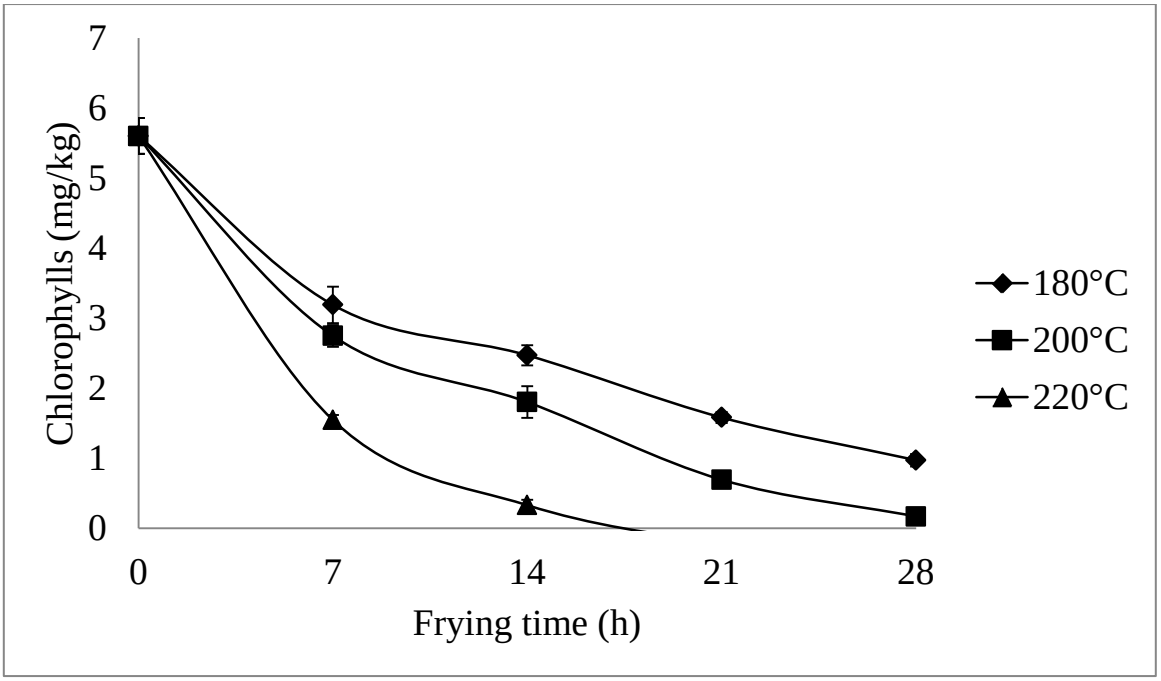

Figure 3-24 Changes in chlorophyll content of EPKO during frying at different temperatures without oil replenishment.

A rapid decrease in chlorophyll content was observed in all the samples when they were subjected to heating at 180, 200, and 220°C. The rate of loss of the chlorophylls increased as the frying time and temperature increased. At the end of the frying session, the chlorophyll content was found to be 0.9 and 0.1 mg/kg in the oil samples that were heated at 180 and 200°C, respectively. The chlorophylls disappeared after 21 h of frying at 220°C. Figure 3-25 depicts the changes in the chlorophyll content of the EPKO that had oil replenishment under different heating temperatures. A similar pattern of change was also observed in experiment II. The amount of chlorophyll was reduced to 1.1 and 0.1 mg/kg in the oil samples after 28 h of frying at 180 and

200°C, respectively. The chlorophylls vanished after 21 h of frying at 220°C. The changes in the chlorophyll content observed in both frying experiment I and II indicate that the chlorophyll pigments are sensitive to high temperatures and that replenishment did little to prevent its loss under high temperatures. These findings are in agreement with previous studies. Karoui et al. (Karoui *et al.*, 2011) reported that chlorophyll contents decreased in corn oil when oils were heated from 25 to 200°C for 30 min. They also found that the chlorophyll losses were greater in deep-fried oils than in heated oils. Taha et al. (Taha *et al.*, 1988) reported that chlorophylls were greatly destroyed at 180°C for 60 min in cottonseed oil. In short, based on previous studies and the current results, the rate of degradation of chlorophylls depends on the temperature and length of heating/frying treatment.

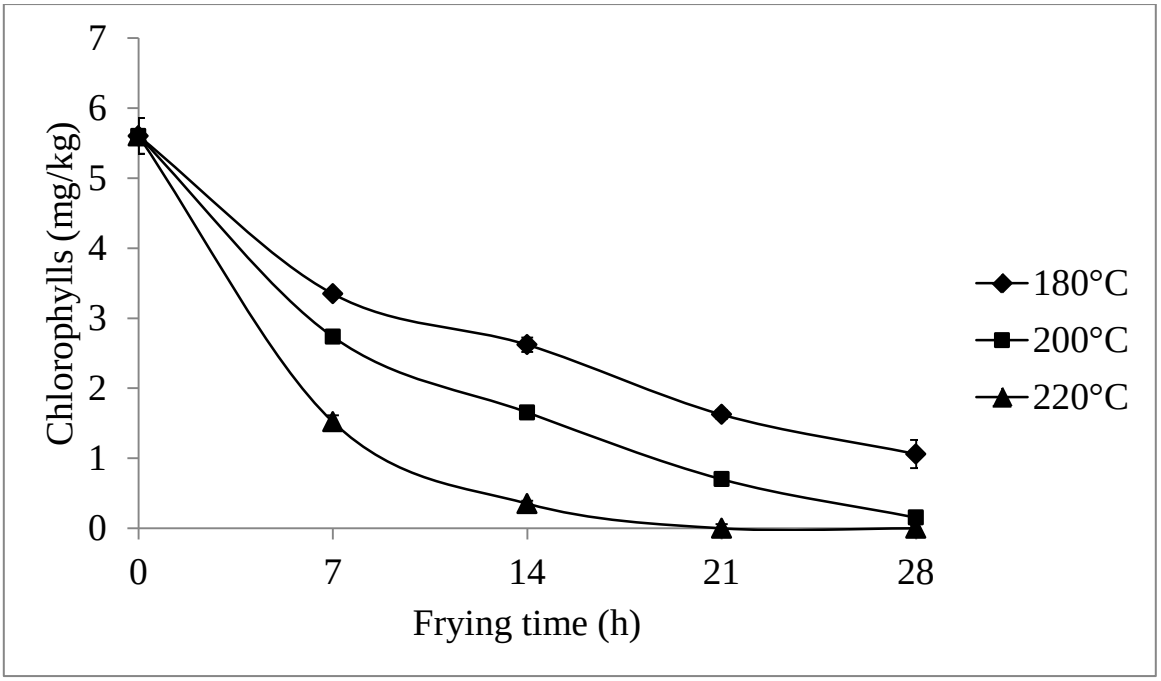

Figure 3-25 Changes in chlorophyll content of EPKO during frying at different temperatures with oil replenishment.

3.5 Conclusions

Based on the results for CV, TPC, color, viscosity, and minor components in this study, there was an appreciable distinction between the frying stability of the EPKO and the commercial regular CO. The EPKO appeared to be more stable during prolonged heating treatments of 28 h at 180, 200, and 220°C because of lower levels of TPC, CV, and viscosity as well as comparable optical density values to that of the CO. The changes in the monounsaturated fatty acid/polyunsaturated fatty acid (MUFA/PUFA) ratios in the EPKO were lower than that of the CO, while the PUFA/SFA (saturated fatty acid) ratios were higher than that of the CO by the end of the frying

session. Oils with relatively slow rates of tocopherol decomposition had a tendency to display greater oxidative stability, indicating that tocopherol may play an important role in determining the frying stability of vegetable oils during deep frying. The carotenoid content was affected by both the frying oils and the fried material during the frying process. The chlorophylls and polyphenols were sensitive to high temperature and were significantly destroyed when oils were subjected to frying. Fresh EPKO, however, contains higher levels of AV, PV, viscosity, and optical density values than that of CO. The results also show that frequent replenishment with fresh oil and lower frying temperatures retarded the deterioration of the oils during frying and prolonged the useful life of frying oils.

In summary, it can be concluded that EPKO is suitable for preparing deep-fried foods at high temperatures, with some nutritional advantages, such as high amounts of unsaturated fatty acids and low amounts of saturated fatty acids. Nevertheless, further investigations are needed for a better understanding of the constituents of the EPKO and the possible mechanisms involved in its oxidative stability during frying.

3.6 References

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CHAPTER 4 DETERMINE THE DEGRADATION OF FRYING OIL BY NEAR-INFRARED SPECTROSCOPY

4.1 Abstract

The degradation of frying oil was determined using near-infrared (NIR) spectroscopy and partial least-squares (PLS) regression. A total of 156 (104 in a calibration set and 52 in a validation set) frying oil samples were obtained from an actual potato frying process. The NIR transmission spectra of the samples were acquired directly from glass test tubes (13 mm dia.) using a NIR spectrometer. Calibration models with very high accuracy were developed for predicting acid values (AV) and total polar compounds values (TPC) using PLS regression with full cross-validation. The coefficients of determination for calibration (R^2) and standard errors of cross-validation (SECV) were 0.99 (SECV: 0.17 mg KOH/g) and 0.98 (SECV: 1.25%) for AV and TPC, respectively. The accuracy of the NIR calibration models was tested using the validation set, yielding values for the root-mean square of the prediction (SEP) of

0.17 mg KOH/g and 1.04% for AV and TPC values, respectively. The research results demonstrate that frying oils can be successfully monitored to a very high accuracy using NIR spectroscopy combined with glass tubes of 13 mm diameter as cells.

4.2 Introduction

Deep-fat fried foods are popular because of their unique flavors and textural characteristics qualities that are closely connected to the quality of the frying oil (Blumenthal, 1991). During frying, oil is subjected to prolonged periods of heating at high temperatures in the presence of air and water. This leads to a wide range of complex chemical reactions, such as thermal oxidation, hydrolysis and polymerization (White, 1991, Clark and Serbia, 1991). The compounds generated from these chemical reactions not only have negative effects on the flavor but also add undesirable constituents to fried foods (Tyagi and Vasishtha, 1996). Therefore, quality control for frying oil and a rapid technique for its analysis are both very important.

Various quality attributes are used to evaluate the quality of frying oil: acid value (AV) or free fatty acid (FFA), total polar compounds (TPC) or total polar materials (TPM) using traditional chemical methods. Most standard analytical methods used for oil analysis are expensive, need lengthy sample

preparation times, and often depend on advanced instrumentation (Fritsch, 1981, Orthoefer, 1988, Osawa *et al.*, 2007). Thus, physical methods based on colorimetric reactions, refractive index, density, and viscosity have also been proposed (Fritsch, 1981, Gracian Tous, 1968, Bansal *et al.*, 2010). These methods are relatively easy to use but are not suitable for monitoring the chemical changes in frying oils during deep-fat frying. Furthermore, techniques such as high performance liquid chromatography (HPLC) and gas chromatography (GC) have also been used for identifying adulterants but are expensive and time-consuming (Andrikopoulos *et al.*, 2001).

NIR spectroscopy is a rapid, reliable, and non-destructive technique that is widely used for quality and process control for the quantitative characterization of various products without using reagents or solvents (Chen *et al.*, 2007). Previous studies have reported the determination of FFA and TPM in heated soy-based oils with water added using NIR spectroscopy with a quartz cuvette of 2 mm path length combined with data analysis using partial least square (PLS) and forward stepwise multiple linear regressions (Ng *et al.*, 2007) Kazemi et al. (Kazemi *et al.*, 2005) also used visible/near-infrared reflectance spectroscopy combined with PLS techniques to predict viscosity, AV, and TPC values in frying oils. These studies have demonstrated the ability of NIR spectroscopy to successfully monitor the thermal degradation of

edible oils, but were limited either to oils heated in the presence of pure water or blends of hydrogenated and non-hydrogenated oils. Thus these studies did not realistically evaluate the oils used to fry foods.

Ng et al. (Ng *et al.*, 2011) used NIR spectroscopy with a quartz cuvette of 2 mm path length to predict the TPM and FFA content of soy-based frying oils actually used to fry foods. Ogutcu et al. (Ogutcu *et al.*, 2012) also used NIR spectroscopy with PLS techniques to predict the viscosity, FFA, and TPM values in frying oil samples generated during the dough frying process. Relatively high correlations between wet chemical analysis and NIR spectroscopy data were reported. However, these studies were limited to samples from commercially refined oils and used only one constant temperature in the frying process. This would, in itself, generate a strong correlation between NIR predictions and analytically derived values for AV and TPC from the frying oil samples, and would thus not assist in making a practical NIR model for predicting AV or TPC values.

The objectives of the present study were to test the use of NIR spectroscopy to determine the AV and TPC values of frying oils generated from refined and unrefined oils at different frying temperatures and to investigate the applicability of the performance of degradation estimation by

NIR spectroscopy using separate disposable test tube cells (5 ml, 13 mm diameter).

4.3 Materials and Methods

4.3.1 Samples Preparation

Two kinds of rapeseed oil (commercially refined canola oil from the Nisshin Oillio Group Ltd, Tokyo, Japan, and unrefined rapeseed Kizakinonatane oil from the Akita New Bio Farm Co., Ltd, Akita, Japan) were used as frying oil samples. Frozen par-fried French fries in an institutional pack were purchased from a local supermarket and used for deep-frying. The frying was conducted in a restaurant-style stainless steel electric fryer TF-40A (Taiji & Co., Ltd., Kanagawa, Japan). Using frying temperatures of 180, 200 or 220°C, batches of 100 g of frozen French fries were fried for 3 min at 22 min intervals, when the temperature of the oil reached the desired temperature, for a period of 7 h each day for 4 consecutive days. This is equivalent to frying 17 batches per day and a total of 68 batches for the whole experiment. During the frying processes, 200 ml of heated oil was drawn off every 3.5 h and stored at -18°C until analysis for AV and TPC values and acquisition of NIR spectral data. The frying experiments were carried out once using canola oil and twice using rapeseed Kizakinonatane oil. A total of 156 frying oil

samples, degraded to different degrees, were obtained from the food frying processes.

4.3.2 Reference Analysis

The acid value (AV) of the frying oil samples was determined in triplicate using an automatic potentiometric titrator (AT-500N, Kyoto Electronics Manufacturing, Kyoto, Japan) according to AOCS Official Method Cd 3d-63 (AOCS, 1997, revised 2003). All results from the AV analysis were expressed as mg KOH/g oil. The total polar compounds (TPC) values from the frying oil samples were determined directly with a food oil monitor (FOM 310, Ebro Electronics, Ingolstadt, Germany) by measuring the change in dielectric constant.

4.3.3 Spectral Acquisition

Spectroscopic data from the frying oil samples were collected with a Foss NIRSystems model 6500 scanning spectrometer (NIRSystems Division of Foss Electric, Silver Spring, MD, USA). A spectral analysis software (NSAS, version 3.53; Foss NIRSystems, Inc.) was used in this study to collect near-infrared spectroscopic data. Disposable glass test tubes (5 ml, 13 mm dia.) containing the frying oil samples were used as sample measurement cells. Transmission spectra over the 700–2500 nm range at a resolution of 2 nm

were collected from each frying oil sample at room temperature (25°C). All samples were heated to 25°C in a water bath prior to collection of spectra, then placed in the circular hollow of an aluminum block maintained at 25°C with an electric thermostat. An empty glass tube was used as a reference before obtaining the spectral measurements. Oil samples were scanned 32 times in the sample compartment mode for each spectrum.

4.3.4 Calibration Development and Validation

The 156 oil samples generated from the experiment were divided into calibration and validation sets: 104 samples for the calibration set and 52 samples for the validation set. Statistics for the AV and TPC values from the frying oil samples selected for the calibration and validation sets are shown in Table 4-1.

Table 4-1 Statistics of the reference data for calibration and validation sets.

	Calibration set (n=104)			Validation set (n=52)		
	AVG	Range	SD	AVG	Range	SD
AV (mg KOH/g)	2.82	0.09-8.37	2.06	2.80	0.09-7.47	2.02
TPC (%)	11.00	0.50-40.00	8.21	10.92	0.50-35.00	7.97

The calibration models were created by PLS regression from the log (1/T) spectra and its first and second derivatives, where T is the transmittance of a sample at a specific wavelength. The coefficient of determination (R^2),

standard error of calibration (SEC), and mean square error of cross-validation (SECV) were used to determine the terms included in the calibration model. The models generated were validated using the validation sample set. The correlation coefficient of prediction (r) and standard error of the prediction (SEP) were used to choose the best model; SEP measures how well the model predicts sample values in the validation set.

4.4 Results and Discussion

4.4.1 NIR Spectra of Frying Oils

Figure 4-1 shows the raw NIR spectrum (700–2500 nm) of a frying oil sample. Intense absorbance peaks from the frying oils can be observed in the NIR region depicted. The peaks evolving around 926 nm are caused by the C-H stretching 3rd overtone of CH_2 groups. The peaks evolving around 1210 nm are caused by the C-H stretching 2nd overtone of CH_3 and CH_2 groups, and those around 1390 and 1414 nm are caused by the 2C-H stretching and C-H deformation vibration of CH_3 and CH_2 groups, respectively. Peaks at 1728 and 1760 nm are observed because of the C-H 1st overtone of CH_2 groups. A shoulder around 2144 nm can be attributed to the C-H and C=C stretching vibrations of CH=CH groups. Furthermore, the peak observed at 2178 nm is

due to the asymmetric C-H stretching and the C=C stretching vibrations from CH=CH groups (Osborne *et al.*, 1993, Christy *et al.*, 2004).

To evaluate spectral changes observed during the frying process and to estimate useful spectral regions in the NIR range for determining AV and TPC values, the variance spectrum was obtained from the spectra of all frying oil samples (Figure 4-1). In this plot, peaks at around 1212, 1428, 1728, 1760, 2040, 2078, 2144, and 2178 nm were also observed corresponding with those shown for the raw NIR spectrum in Figure 4-1. In particular, peaks and troughs were observed in the range from 2000–2200 nm. Osborne et al. (Osborne *et al.*, 1993) have reported that the absorption patterns obtained between 2000 and 2200 nm were related to the degree of oxidation in oils. Therefore it can be seen that there is much information about the degradation of frying oils that can be obtained from the NIR spectra.

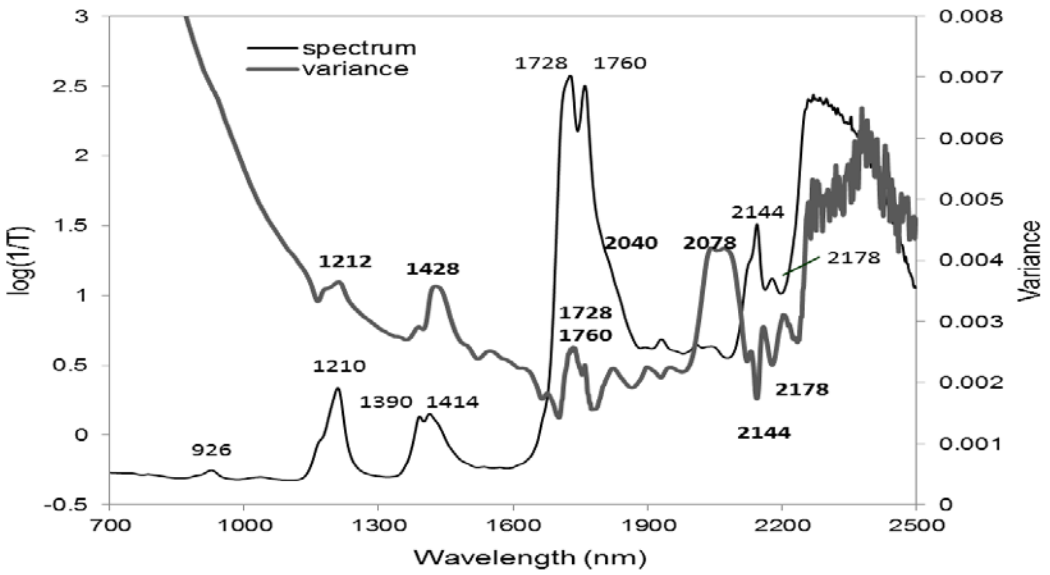

Figure 4-1 NIR spectrum of a frying oil sample and variance based on the spectra of all frying oil samples.

4.4.2 NIR Models for Acid Values

PLS regression results for predicting AV values in frying oils using NIR spectra are shown in Table 4-2. A total of 15 PLS calibration models were developed for analyzing frying oils using the calibration and validation sample sets. There was a strong correlation between the NIR predicted data and validation data, with R^2 values ranging from 0.95 to 0.99 and SEP values from 0.17 to 0.48 mg KOH/g. Relatively good results were found when the results from the short wavelength (700–1100 nm), middle wavelength (1100–1800 nm) and long wavelength regions (1800–2200 nm) were compared. The models based on the longer wavelength region had a relatively high

correlation between the predicted values and the actual AV values for frying oils.

Table 4-2 Partial least squares analysis results for acid values.

		F	R^2	SEC	SECV	SEP	Bias	RPD
Raw spectra	700-1100 nm	10	0.96	0.42	0.53	0.47	0.09	4.3
	1100-1800 nm	6	0.97	0.37	0.43	0.39	0.05	5.3
	1800-2200 nm	6	0.99	0.17	0.21	0.17	0.05	12.0
	1100-2200 nm	6	0.99	0.19	0.23	0.19	0.05	10.7
	700-2200 nm	7	0.99	0.22	0.28	0.22	0.05	9.4
First derivative spectra (segment 20nm, gap 0nm)	700-1100 nm	9	0.96	0.42	0.54	0.48	0.12	4.3
	1100-1800 nm	6	0.96	0.39	0.44	0.38	0.05	5.4
	1800-2200 nm	5	0.99	0.15	0.17	**0.17**	0.04	12.8
	1100-2200 nm	5	0.99	0.21	0.23	0.19	0.04	10.9
	700-2200 nm	5	0.99	0.21	0.23	0.20	0.06	10.1
Second derivative spectra (segment 20nm, gap 0nm)	700-1100 nm	10	0.98	0.27	0.34	0.46	0.08	4.6
	1100-1800 nm	7	0.98	0.32	0.40	0.35	0.03	5.9
	1800-2200 nm	5	0.99	0.17	0.20	0.19	0.02	11.0
	1100-2200 nm	5	0.99	0.20	0.23	0.22	0.02	9.3
	700-2200 nm	5	0.99	0.23	0.26	0.21	0.03	9.9

F: number of factors; R: coefficient of determination; SEC, standard error of calibration; SECV: standard of cross validation; SEP: standard error of prediction; Bias: average of difference between reference value and NIR value; RPD: ratio of performance to deviation

The most accurate model involved the first-derivative spectra in the wavelength range of 1800–2200 nm. It used five PLS factors and produced a high coefficient of determination (R^2), low values of standard error of calibration (SEC), standard error of cross-validation (SECV), and standard error of prediction (SEP) of 0.99, 0.15 mg KOH/g, 0.17 mg KOH/g, and 0.17 mg KOH/g, respectively. The cross validation and prediction (validation) results, represented graphically by plotting the reference analysis AV values

against the predicted values, showed a strong linearity as shown in Figure 4-2. Furthermore, all the prediction models had ratio performance deviation (RPD) values above 4.3, with the model involving first-derivative spectra in the wavelength range of 1800–2200 nm exhibiting an RPD value of 12.8. Generally, an RPD value above 3 indicates a useful model where good quantitative predictions are possible (Williams, 2001, Chen *et al.*, 2004, Chen *et al.*, 2005, Chen, 2007). It can be concluded that the NIR spectra provided a good estimation of the AV value in frying oils, particularly for spectra that showed a low SEP value and a high RPD value.

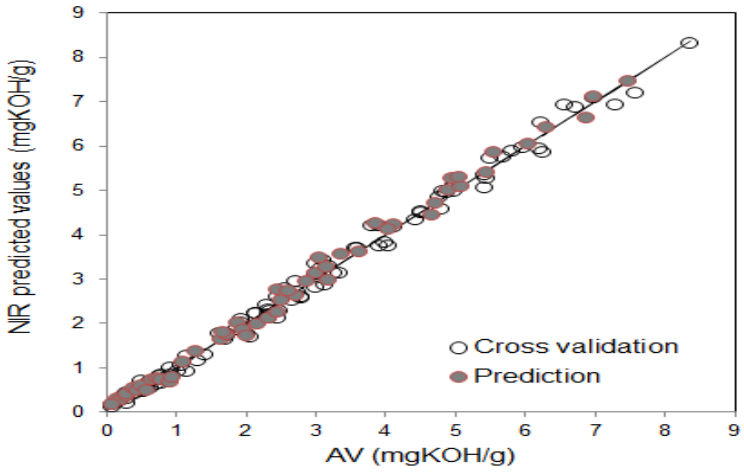

Figure 4-2 Scatter plot diagram comparing actual AV values and NIR predicted values.

4.4.3 NIR Models for Total Polar Compounds Values

PLS regression results for predicting TPC values in frying oils using NIR spectra are shown in Table 4-3. Similar to the AV results, there were strong

correlations between the NIR predicted data and the validation data with R^2 values ranging from 0.96 to 0.98, and SEP values from 1.04 to 1.67%. By observing and comparing the NIR results from short wavelength (700–1100 nm), middle wavelength (1100–1800 nm) and long wavelength regions (1800-2200 nm), it was found that relatively good results were provided by the middle wavelength region, which showed a relatively high association between the predicted values and the actual TPC values of the frying oils.

Table 4-3 Partial least squares analysis results for TPC values.

		F	R^2	SEC	SECV	SEP	Bias	RPD
Raw spectra	700-1100 nm	9	0.98	1.16	1.82	1.35	-0.04	6.0
	1100-1800 nm	5	0.98	1.02	1.25	1.21	0.19	6.7
	1800-2200 nm	5	0.98	1.08	1.43	1.23	0.21	6.6
	1100-2200 nm	5	0.99	1.01	1.21	1.21	0.18	6.7
	700-2200 nm	5	0.98	1.16	1.34	1.33	0.20	6.1
First derivative spectra (segment 20nm, gap 0nm)	700-1100 nm	9	0.98	1.14	1.51	1.40	-0.01	5.8
	1100-1800 nm	5	0.98	1.03	1.25	**1.04**	0.05	7.8
	1800-2200 nm	5	0.98	1.07	1.24	1.19	0.15	6.8
	1100-2200 nm	3	0.98	1.05	1.23	1.04	0.10	7.7
	700-2200 nm	3	0.98	1.12	1.28	1.28	0.11	6.3
Second derivative spectra (segment 20nm, gap 0nm)	700-1100 nm	7	0.98	1.29	1.58	1.67	0.14	4.9
	1100-1800 nm	6	0.99	0.93	1.23	1.11	0.12	7.3
	1800-2200 nm	6	0.99	0.94	1.28	1.19	0.03	6.8
	1100-2200 nm	5	0.98	1.12	1.31	1.09	0.13	7.5
	700-2200 nm	5	0.98	1.17	1.40	1.21	0.06	6.6

F: number of factors; R: coefficient of determination; SEC, standard error of calibration; SECV: standard of cross validation; SEP: standard error of prediction; Bias: average of difference between reference value and NIR value; RPD: ratio of performance to deviation

The most accurate model involved first-derivative spectra in the wavelength range from 1100-1800 nm. It used five PLS factors and produced

a high coefficient of determination (R^2), low values for standard error of calibration (SEC), standard error of cross-validation (SECV), and standard error of prediction (SEP) of 0.98, 1.03, 1.25, and 1.04%, respectively. The cross validation and prediction results, represented graphically by plotting the reference TPC values against the predicted values, showed a strong linearity as shown in Figure 4-3. Furthermore, all prediction models had ratio performance deviation (RPD) values above 4.9, with the model involving first-derivative spectra in the wavelength range of 1100–1800 nm exhibiting an RPD value of 7.8. It can also be concluded that the NIR spectra provided a good estimation of TPC values in the frying oils.

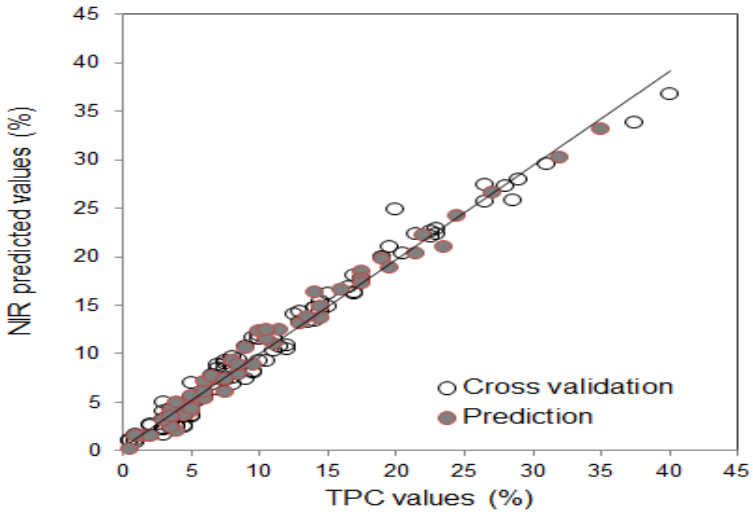

Figure 4-3 Scatter plot diagram comparing actual TPC values and NIR predicted values.

Generally, the AV value of oil increases not only with increasing frying time and temperature, but also with the presence of moisture, oxygen, and

other contaminants transferred from the immersed food to the oil. The amount of secondary oxidation products such as aldehydes or ketones also increase as the frying oil degrades. The measured TPC value based on the secondary oxidation products increases with frying time. There may be a high correlation between the AV and TPC values in the frying oil samples, which might have helped to make the NIR models for AV or TPC. However, in the present study, we used refined and unrefined original oil samples combined with a range of frying oil temperatures. This could explain the low correlation value of 0.05 between the AV and TPC values of the frying oil samples, compared with a value of 0.84 in the research by Ogutcu et al. (Ogutcu et al., 2012). Therefore, from the present research, it may be said that the NIR models derived for predicting AV and TPC values are practical, but were not related to each other.

4.5 Conclusions

NIR spectroscopy can be successfully applied to the measurement of AV and TPC values in frying oils. PLS regression analysis for spectra recorded at 1800-2200 nm gave a low SEP value of 0.17 mg KOH/g and a very high RPD value of 12.8 for predicting AV values. PLS regression analysis for spectra recorded at 1100-1800 nm gave a low SEP value of 1.04% and a relatively

high RPD value of 7.8 for predicting TPC values. These prediction results showed that NIR spectroscopy using separate disposable 5 mL test tube cells is a very useful method for measuring the degradation of frying oils. Furthermore, NIR spectroscopy has significant advantages over other measurement techniques in that it is a fast and simple method that requires no sample preparation, so it is a very practical method for measuring AV and TPC values in edible oils during the frying process.

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CHAPTER 5 OVERALL CONCLUSIONS, LIMITATIONS AND IMPLICATIONS FOR FUTURE WORK

5.1 Conclusions

Based on the results from the studies presented in this dissertation, the following conclusions were made:

1. EPKO, as one of the domestic rapeseed cultivars, is a good source of healthy unsaturated fatty acids and important minor compounds. This suggests that EPKO could be used to supplement or even replace conventional CO and satisfy part of the nutritional requirements in human diets (Chapter 2).
2. The results of the present study indicate that tocopherol is the major determinant of frying stability of vegetable oils. Oils with relatively slow rates of tocopherol decomposition have a tendency to display greater oxidative stability during deep frying (Chapter 3).
3. Fatty acid composition has an effect on frying stability. Oils with a lower content of polyunsaturated fatty acids showed notable improvements in frying stability (Chapter 3).

4. Oil deterioration increases with an increase in frying temperature and prolonged frying time. Frequent replenishment with fresh oil significantly improves the frying stability and prolongs the useful life of oils (Chapter 3).
5. Other minor components such as polyphenols, carotenoids, and chlorophylls appeare to have a negligible effect on the frying stability of oils during deep frying (Chapter 3).
6. Frying oils should be evaluated by combining the results of multiple measurements, and not evaluated on a sole index in order to confirm abuse (Chapter 3).
7. NIR spectroscopy is an effective, simple, rapid and practical method for determining oxidation level in both crude and commercial rapeseed oils during deep frying (Chapter 4).
8. A universal calibration for both AV and TPC of the crude or refined, bleached, and deodorized rapeseed oils during deep frying can be applied successfully (Chapter 4).

5.2 Limitations and Implications for Future Work

The results of the current research indicated that oil deterioration is dependent on many factors, such as fatty acid composition of an oil, type and

content of minor components, frying temperature and time, etc. The presence of polyunsaturated fatty acids and the decomposition rate of tocopherol could explain some of the significant differences between the EPKO and CO during frying (especially when fried at 180 and 200°C). Future work is needed in order to investigate other possible factors and how their effects are exerted to affect the frying stability.

In addition to fatty acid and tocopherols, other minor oil components such as sterols, FFAs, and mono- and di-glycerides, as well as their degradation or oxidation products, may play an important role in the oxidation stability of frying oils. The interactive effects of different minor components in different types, contents, and combinations of oils may also play a role in frying stability and are worth further investigation. For example, carotenes, in the presence of other antioxidants (tocotrienols), are able to perform its role in protecting oil from thermal oxidation during frying of potato chips. Therefore, studies should be carried out to determine whether a single minor component or in combination with other minor components results in less oil deterioration during frying.

In the current study, comparisons of the frying stability between crude rapeseed oil and refined, bleached, and deodorized canola oil were made. However, many factors such as seed cultivar, planting location, degree of seed

maturity, and oil processing methods, which may have affected their oxidation stability, were not matched between the two frying oils. In general, an ideal frying study would conduct studies of oils that have different characteristics but undergo the same production methods. Additionally, the same type of oil samples should be prepared from different suppliers.

Unlike frying methods that are frequently employed in homes, restaurants, or large industrial operations, the results reported in the present study were obtained under laboratory conditions with higher frying temperature, longer frying time, and lower frying load. Therefore, a future frying study should be set up to assess the frying stability of oils under more realistic conditions.

Finally, NIR spectroscopy is a simple and reproducible method for measuring oxidation frying oils. Further investigation should be conducted to evaluate the applicability of the NIR on a variety of other oil samples and to establish a more universal NIR model for oils from different sources. In addition, further work is also needed to investigate the potential of NIR spectroscopy for predicting other degradation products during frying in a more complex food system.