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EFFECT OF MINIMAL REFINING ON THE MINOR CONSTITUENTS AND OXIDATION STABILITY OF SUNFLOWER SEED OIL

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in

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Supervisor

Prof. Dr. Medeni MASKAN

by

Pınar GÜMÜŞ

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Pınar GÜMÜŞ

ABSTRACT

EFFECT OF MINIMAL REFINING ON THE MINOR CONSTITUENTS AND OXIDATION STABILITY OF SUNFLOWER SEED OIL

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Question of thesis was "if minimal refining process should be accomplished with the minimum losses of desirable substances for production of sunflower seed oil rich with micronutrient". The overall objectives of the study were to produce oxidatively stable minimal neutralized sunflower seed oil using three chemicals ($Ca(OH)_2$, MgO and Na_2SiO_3) as alternatives to strong alkali (NaOH), which is used in conventional neutralization method; to optimize minimal neutralization operations by the response surface methodology; to determine optimal process conditions for sunflower seed oil. First part of this study suggested that the minimal operation is possible to obtain sunflower seed oil having high nutrition value by altering the neutralization process. Second part indicated that sunflower seed oils obtained by minimal neutralization having lower free fatty acids (FFAs) content and better oxidative stability than oil neutralized by traditional method. Third part focused on which weak alkaline has higher oxidation stability, minimum FFA content and maximum α-tocopherol content. The estimated optimal values for minimal neutralization of sunflower seed oil were an amount of $Ca(OH)_2$ of 0.30 %, 53.0 ºC and 19.7 minutes; an amount of MgO of 0.38 %, 57.6 ºC and 17.8 minutes; an amount of Na_2SiO_3 of 0.81 %, 55.7 ºC and 19.1 minutes. It was concluded that the oil neutralized by NaOH had the shortest hydroperoxide and hexanal lag phases, thus, were the least stable oil and oils neutralized by $Ca(OH)_2$, MgO and Na_2SiO_3 had lower FFA and higher oxidative stability than oil neutralized by NaOH. The oil neutralized by $Ca(OH)_2$ had the lowest FFA, anisidine value and highest total phenolics and α-tocopherol contents and had better oxidative stability than oil neutralized by NaOH. It suggests that $Ca(OH)_2$ could be more effective in producing a high quality oil.

Key Words: Sunflower Seed Oil, Minimal Refining, Antioxidant Activity, Lipid Oxidation, Tocopherol

ÖZET

MİNİMAL RAFİNASYONUN AYÇİÇEĞİ ÇEKİRDEĞİ YAĞININ MİNÖR BİLEŞENLERİ VE OKSİDASYON DAYANIKLILIĞI ÜZERİNE ETKİSİ

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Tezin temel sorusu, "mikro besin ögeleri açısından zengin ayçiçek yağı üretimi için minimal rafinasyon işleminin minimum düzeyde yararlı minör bileşen kayıplarıyla gerçekleştirilip gerçekleştirilmeyeceğidir". Çalışmanın genel amaçları; geleneksel nötralizasyon yönteminde kullanılan kuvvetli alkali NaOH'a alternatif olarak üç kimyasal $Ca(OH)_2$, MgO ve Na_2SiO_3 kullanarak oksidatif dayanıklı minimal nötralize ayçiçek yağı üretmek ve minimal nötralizasyon işlemlerini yanıt yüzey yöntemi ile optimize ederek ayçiçek yağı için optimum proses koşullarını belirlemekti. Çalışmanın ilk bölümü, nötralizasyon işlemi değiştirilerek elde edilen minimal işlem ile yüksek besin değerine sahip ayçiçek yağı elde etmenin mümkün olduğunu ileri sürmüştür. İkinci bölüm minimal nötralizasyon ile elde edilen ayçiçek yağların, geleneksel yöntem ile nötralize edilen yağdan daha düşük serbest yağ asitleri (SYA) içerdiği ve daha iyi oksidatif dayanıklılığa sahip olduğunu göstermiştir. Üçüncü bölümde hangi zayıf alkalinin daha yüksek oksidasyon stabilitesine, en düşük SYA değerine ve maksimum α-tokoferol miktarına sahip olduğuna odaklanılmıştır. Ayçiçek yağının minimal nötralizasyonu için elde edilen optimal değerler $Ca(OH)_2$ için 0.30 %, 53.0ºC ve 19.7 dk; MgO için 0.38 %, 57.6 ºC ve 17.8 dk; Na_2SiO_3 için 0.81 %, 55.7 ºC ve 19.1 dakikadır. NaOH ile nötralize edilen yağın en kısa hidroperoksit ve heksanal lag fazlarına sahip olmasından dolayı daha az kararlı olduğu ve $Ca(OH)_2$, MgO ve Na_2SiO_3 ile nötralize edilen yağların sodyum hidroksit ile nötralize edilen yağa göre daha düşük SYA değerine ve daha yüksek oksidatif stabiliteye sahip olduğu sonucuna varılmıştır. $Ca(OH)_2$ ile nötralize edilen yağın en düşük SYA, anisidin değeri ve en yüksek toplam fenolik ve α-tokoferol içeriğine sahip olmasi ve NaOH ile nötralize edilen yağa göre daha iyi oksidatif dayanıklılığa sahip olduğundan dolayı yüksek kaliteli bir yağ üretiminde $Ca(OH)_2$'in daha etkili olabileceği düşünülmektedir.

Anahtar Kelimeler: Ayçiçek Yağı, Minimal Rafinasyon, Antioksidan Aktivite, Lipit Oksidasyonu, Tokoferol

Dedicated to my beloved parents

Fadime & Halil

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LIST OF SYMBOLS

α	Alfa
β	Beta
γ	Gama
δ	Sigma

LIST OF ABBREVIATIONS

ABTS	2,2′-azino-bis (3-ethylbenzothiazoline- 6-sulfonate)
ANOVA	Analysis of variance
AV	Anisidine value
CAE	Caffeic acid equivalent
CD	Conjugen Diene
CT	Conjugen Triene
DPPH	2,2-diphenyl-1- picrylhydrazyl
DVB	Divinylbenzene
E-E	Extrusion-Expelling
FA	Fatty Acid
FFA	Free Fatty Acid
GC	Gas Chromatography
HPLC	High Pressure Liquid Chromatography
PDMS	Polydimethylsiloxane
PPM	Parts per million
PV	Peroxide value
PTFE	Polytetrafluoroethylene
RSM	Response Surface Methodology
SPME	Solid phase microextraction
SPSS	Statistical package for the social sciences
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant activity
TBARS	Thiobarbituric acid reactive substance
TPC	Total phenolics content
UV-VIS	Ultraviolet visible

CHAPTER I

INTRODUCTION

Sunflower oil is widely used in cooking, salad and frying, margarine and nonfood that has high nutritional quality and diversity of fatty acids profiles. It contains both desirable minor compounds such as tocopherols, carotenoids, phytosterols and undesirable micronutrients (e.g. FFAs, coloring pigments, pesticide residues, gums, oxidation yields). It is reported that sunflower seed includes various minor components having health and nutritional benefits and giving to specific taste and color. Refining conditions and type of oil influenced minor compounds found in oil. Undesirable components that lead to darkening, foaming, smoking, improvement of off-flavors may decrease the quality of oils and oxidative stability. Therefore, these components must be removed by refining. During traditional oil manufacturing process, many minor components are lost [1-7].

Crude oil has undesirable impurities (free fatty acids, phospholipids, pigments) impacting negatively appearance, odor and flavor of refined oil and consumer health. Oil refining is essential to eliminate these impurities; keep desirable portion of oil (e.g., tocopherols and sterols); develop the oil quality, increase the shelf life of oil and obtain an oxidatively stable refined oil with light color, desirable odor and taste for consumer acceptance [8-12]. Refining process of edible oils contain several steps. Degumming is used for removal of phospholipids and mucilaginous substances, and trace metals. Neutralization is generally carried out by chemical way or by physical way. Some alkalis (NaOH, KOH, $NaHCO_3$, Na_2CO_3) can be used to neutralize oil for removal of the free fatty acids in this second stage of refining. The purpose of bleaching is to remove colorings, oxidation products by using bleaching clays. Deodorization step is performed at high temperature and low pressures for the removal of odor creating components [9-11].

There are several innovative approaches in edible oil literature such as minimal refining, green vegetable oil manufacturing and soft oil processing. Minimal refining process for edible oils aims to give less damage to chemical form of triglycerides and retain desirable health promoting micronutrients, such as phytosterols, tocopherols and phenolic components, while decreasing the level of undesirable compounds (FFAs, oxidative products, pigments) to acceptable levels [5, 13, 14].

Oxidative stability is the period of time essential to reach the important point of oxidation, throughout processing and storage and it is needed to indicate oil quality and shelf life. It leads to the development of rancid odors and unpleasant flavors also alters texture and color and thus limits the shelf life and cause nutritional loss. It can be monitored by delaying the generation of lipid hydroperoxides and free radicals or by scavenging the free radicals created in food systems. Strategies to retard lipid oxidation are necessary to develop food security by lowering spoilage, reducing the amount of oxygen within foods and to yield healthier foods [15-20].

There are several indicators for the evaluation of lipid oxidation of edible oils; peroxide value, conjuge dien and trien value as markers of primary oxidation reactions and AV and TBARS as indicators of secondary oxidation reactions. Many gas chromatographic methods were improved in order to measure oxidation stability such as headspace solid phase micro extraction [19, 21, 22]. Some factors affecting lipid oxidation are transition metal, oil composition, water activity, prooxidants, antioxidants and environmental factors such as light, oxygen and temperature. Prooxidant accelerate lipid oxidation while antioxidants inhibit lipid oxidation. Antioxidants are divided into two groups; primary and secondary antioxidants. Several antioxidant applications provide for stabilization of foods apt to oxidative deterioration. These applications consist of control of prooxidants and decreasing the damaging impacts of free radicals [16, 23].

1.1 Aims and Significance of The Current Study

There were three main parts in this PhD thesis. In the first part, it was aimed to produce minimal neutralized sunflower oil as an alternative to conventional neutralization by using three weak neutralizing agents. In the second part, the effect of traditional neutralized oil produced by NaOH and minimal neutralized oils by $Ca(OH)_2$, MgO, and Na_2SiO_3 on FFA and total phenolic content, free radical scavenging activity, lipid hydroperoxide and hexanal was compared.

The third part consist of three main purposes; (i) to produce minimally neutralized sunflower oil using weak alkalis as an alternative to sodium hydroxide used in conventional neutralization, (ii) to optimize minimal neutralization operation by the Response Surface Methodology, (iii) to investigate storage stability of sunflower seed oils at 55°C for 28 days. The results of the present study are useful to produce sunflower oil containing more bioactive components which are important for health. This PhD thesis supply important knowledge to science to develop minimal refining, an innovative refinement approach in which industrial costs are reduced and sustainability will become possible.

CHAPTER II

LITERATURE REVIEW

2.1 Sunflower Seed

Sunflower seed (*Helianthus annuus L.*) that belongs to the Compositae (Asteraceae) family is one of the most significant growing crops around the world. It was traditionally cultivated in North America. Sunflower is divided into two groups; the oilseed and non-oilseed. The chemical content of the sunflower oil depends on the genetic, environmental situations and the maturity of the seed [24, 25]. The quality and composition of sunflower seed that adapted to high temperature and limited water surrounding, is impacted by maturity of the seed, growing conditions and storage parameters, [6, 24, 26]. Sunflower seeds are abundant in minor components such as tocopherols and phytosterols having different health advantages [2].

Abitogun et al. [25] studied the sunflower seeds were prepared. The oil was extracted by solvent extraction. The oils were examined for their physico-chemical parameters. The free fatty acid level (1.40%) and the percentage yield was 41.3% makes it an excellent source of oil.

Zlatanov et al. [27] investigated the alterations in the composition of total fatty acids, phospholipids and sterol esters, and their fatty acids, and of free sterols and tocopherols in developing seeds of a selection of high oleic acid sunflower varieties grown in Bulgaria were examined over a period of 15th to 90th day after flowering by means of various chromatographic methods. The yield is a good quality oil with a useful FA content and has good for a salad and cooking.

Yıldırım et al. [28] studied the impact of refining process on fatty acid, sterol and volatile composition of sunflower oil. Crude sunflower oil was refined through neutralization, dewaxing, bleaching, winterization and deodorization steps. Results demonstrated that total polyunsaturated fatty acids decreased together with linoleic acid, the refining and a similar reduction was recorded for β- sitosterol, campesterol and stigmasterol. Total amount of volatiles decreased by 14.51% during refining and the highest content of total volatile compounds were defined in the bleaching.

2.2 Sunflower Seed Oil

Sunflower is cultivated for edible oil production. Ukraine, Russia, European Union and Argentina are leading countries for production. Ukraine and Russia yield almost half of the world sunflower seeds [29]. Sunflower oil is known as a rich source of natural micronutrients having light color, mild flavor and low level of saturated fat [3, 30]. Crude edible oil is a primary nutrient containing undesirable substances such as free fatty acids, gummy and coloring substances. To enhance the nutritional quality of edible oils is needed to increase the oxidation stability and decrease the amount of undesirable fatty acids of the oil [6, 31, 32]. Crude sunflower oil needs to refine and eliminate undesirable minor components before consumption [11, 33, 34]. Major components constitute 95-99 % of the oil, while minor components constitute 1-5 %of the oil. Major components in vegetable oils are shown in Figure 2.1[35].

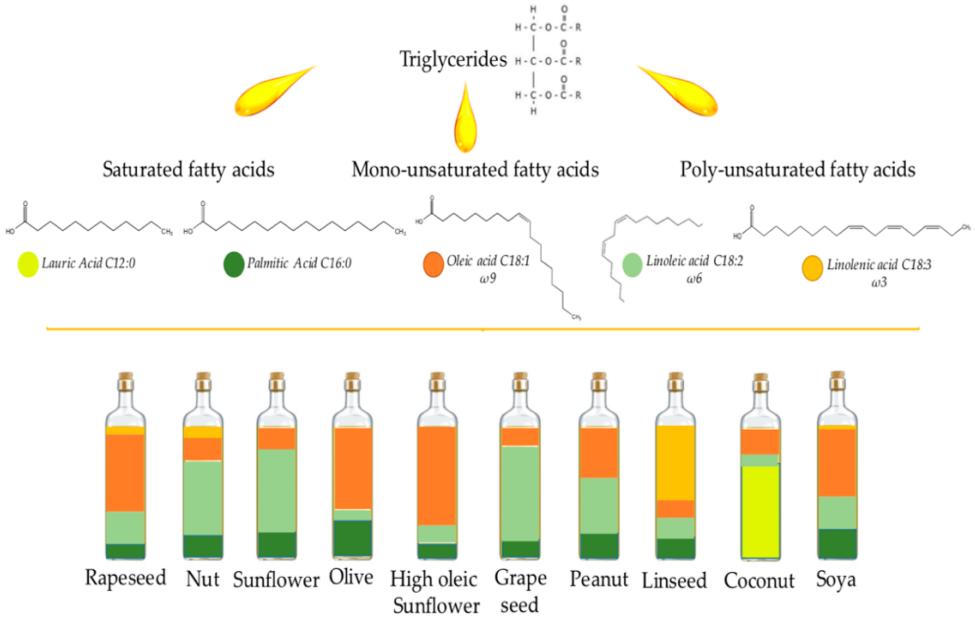

Figure 2.1 Major components in vegetable oils

Sunflower has been cultivated on about 675.983 ha with production of around 1.9 million tons annually. The average yield of the sunflower is 289 kg/da in Turkey (Table 2.1). Sunflower is produced primarily as an oil crop in Turkey and in the USA. The production and consumption of sunflower oil in Turkey and in the USA has shown in Table 2.2 and 2.3. Oil production in Turkey and in the USA is 1.007.000 and 201.000 ton, respectively in 2018. Oil consumption in Turkey and in the USA is 1.117.000 and 218.000 ton, respectively in 2018. The nutrient and fatty acid composition (per 100 g) of sunflower oil has shown in Table 2.4 and 2.5 [36].

Table 2.1 Turkey's Sunflower Seed Area-Production and Yield in 2014-2019

Years	Area (ha)	Production (ton)	Yield (kg/da)
2014	554.265	1.480.000	269
2015	568.995	1.500.000	264
2016	616.780	1.500.000	244
2017	681.397	1.800.000	264
2018	648.934	1.800.000	277
2019	675.983	1.950.000	289

[37]

Table 2.2 Turkey's Sunflower Oil Production and Consumption in 2014-2019

Years	Production (ton)	Consumption (ton)
2014/15	650.000	827.000
2015/16	690.000	876.000
2016/17	869.000	1.042.000
2017/18	1.004.000	1.107.000
2018/19	1.007.000	1.117.000
2019/20*	1.104.000	1.170.000

*Forecast [37]

Table 2.3 USA's Sunflower Oil Production and Consumption in 2014-2019

Years	Production (ton)	Consumption (ton)
2014/15	146.000	180.000
2015/16	204.000	206.000
2016/17	218.000	232.000
2017/18	197.000	237.000
2018/19	201.000	218.000
2019/20*	185.000	213.000

*Forecast [37]

Table 2.4 The nutrient composition (per 100 g) of sunflower oil

Component	Unit	Average
Energy	kcal	900
Energy	kJ	3766
Water	g	0.00
Ash	g	0.00
Protein	g	0.00
Nitrogen	g	0.00
Fat, total	g	100.00
Carbohydrate	g	0.00
Fiber, total dietary	g	0.00
Retinol (preformed vitamin A)	µg	0.00
Vitamin D, IU	IU	0.00
Vitamin D-3 (cholecalciferol)	µg	0.00
Vitamin E	α-TE	47.83
Vitamin E, IU	IU	71.27
Alpha-tocopherol	mg	47.83

Table 2.5 Fatty acid composition (per 100 g) of sunflower oil

Component	Unit	Average
Fatty acids, total saturated	g	10.382
Fatty acids, total monounsaturated	g	30.917
Fatty acids, total polyunsaturated	g	54.071
Fatty acid 14:0 (myristic acid)	g	0.067
Fatty acid 16:0 (palmitic acid)	g	5.774
Fatty acid 18:0 (stearic acid)	g	3.308
Fatty acid 20:0 (arachidic acid)	g	0.249
Fatty acid 22:0 (behenic acid)	g	0.717
Fatty acid 24:0 (lignoceric acid)	g	0.268
Fatty acid 16:1 n-7 cis (palmitoleic acid)	g	0.105
Fatty acid 18:1 n-9 cis (oleic acid)	g	30.649
Fatty acid 20:1 n-9 cis	g	0.163
Fatty acid 18:2 n-6 cis, cis	g	53.985
Fatty acid 18:3 n-3 all-cis	g	0.086

Rade et al. [1] studied non-refined sunflower oils taken from industry and laboratory by pressing and hexane extraction and sunflower oils from refining process. Free fatty acid content, carotenoids, peroxide value, sterols, chlorophylls and oxidative stability were carried out. According to the results among all sunflower oils, the laboratory extracted oil showed the best oxidative stability while the fully refined one were more stable than cold and hot-pressed oils.

Poiana et al. [3] observed the impact of storage conditions on the oxidative stability of sunflower and pumpkin unrefined oil. The physicochemical properties of these oils were estimated. The pumpkin oil indicated the highest oxidative stability due to availability of antioxidants in unrefined pumpkin oil. The susceptibility to oxidation influenced by the storage conditions. The susceptibility of pumpkin oil to oxidation indicated that it was more stable than sunflower oil during these storage conditions.

Taşan et al. [38] reported that the impacts of oilseed extraction ways on the characteristics and stability of unrefined sunflower oil by measuring free fatty acid content, peroxide value, total and individual tocopherol contents and their oxidation stability. The unrefined oils were stored for four months at 40±2°C for monthly analyses. This study also recommended that it is essential for the oil industry to revalorize the full pressing method besides the solvent extraction terms utilized for sunflower seeds to obtain nutrition quality and oxidative stability.

Naz et al. [39] stated that the alterations of total and individual tocopherols during different sunflower oil production steps by reverse-phase HPLC. The results showed that tocopherol content was decreased after the refining processes. In order to decrease the loss of tocopherols for longer shelf life of edible oils, the processing technology should be developed.

On the other hand, Suliman et al. [40] studied the impact of chemical refining on oil stability index of sunflower oil, FFA, tocopherol content and color. The results showed that all these quality parameters are reduced during the chemical refining process, except oil stability. Deodorization step influenced all of these quality attributes. Most tocopherols were lost during deodorization step. Processors want essential developments in process technology to protect tocopherol content and to enhance nutrition quality of processed oil.

Ergonul and Koseoglu [41] investigated impact of chemical and physical refining on tocopherol contents of sunflower oil. They concluded that significant amount of tocopherol was eliminated during the refining processes for all oil types. It is important to reduce the loss of tocopherols for nutrition aspects.

2.2 Refining of Vegetable Oils

2.2.1 Traditional Refining

Extraction of oils from seeds can be conducted by two main steps; pressing or solvent extraction. There are two kinds of oil pressing: cold press and hot press. Pretreatment of seeds by heat in hot press is needed. On the contrary, in cold press thermal treatment is not used. In extraction of oils, firstly extraction by pressure with an expeller is applied, then the resulting solid matter is subjected to a solvent extraction and finally pressed and solvent extracted oils are mixed before refining. Oil extraction by solvent method is used for low content of oil seeds in contrast to oil extraction by pressing method is applied for high content of oil seeds. Oil extraction by pressing compared with solvent extraction has lower cost, safer and simpler. Solvent extraction leads to several problems such as emitting of volatile organic compounds into atmosphere and high process cost [42, 43].

Crude oil is obtained by mechanical extraction or solvent extraction methods before four main stages of refining. Degumming is the initial step of refining process which removes phospholipids, and trace metals. Free fatty acids in edible oil are one of the important parameters to determine the acceptability and quality of oils. It is desirable to decrease FFA as much as possible as they negatively impact smoke point, cause foaming and accelerate lipid oxidation. Neutralization step removes free fatty acids by the addition of NaOH to produce FFA soaps that can be removed with water. Alternately, FFA can be removed by elevated temperature under vacuum as is done in physical refining. Bleaching step of refining process eliminates pigments and oxidation products. Deodorization is the final step in the refining is used for removal of volatile odiferous components to produce bland and odorless oil [7, 44, 45].

Physical refining that composed of degumming, bleaching, winterization and distillation. It is more economical and has positive effect on oil stability than chemical refining. Distillation is known as removal of free fatty acids from oils without using alkali. Chemical refining comprises of degumming, neutralizing, bleaching, winterizing and deodorizing. Physical refining is an environment-friendly operation. Extraction of crude sunflower oil is insufficient for obtaining edible oils since it includes a lot of undesirable materials such as gums, odoriferous and color materials, free fatty acids etc. Therefore, most consumers interest in minimally processed oils has recently encouraged the manufacturing of cold pressed oils [9, 40, 43, 46].

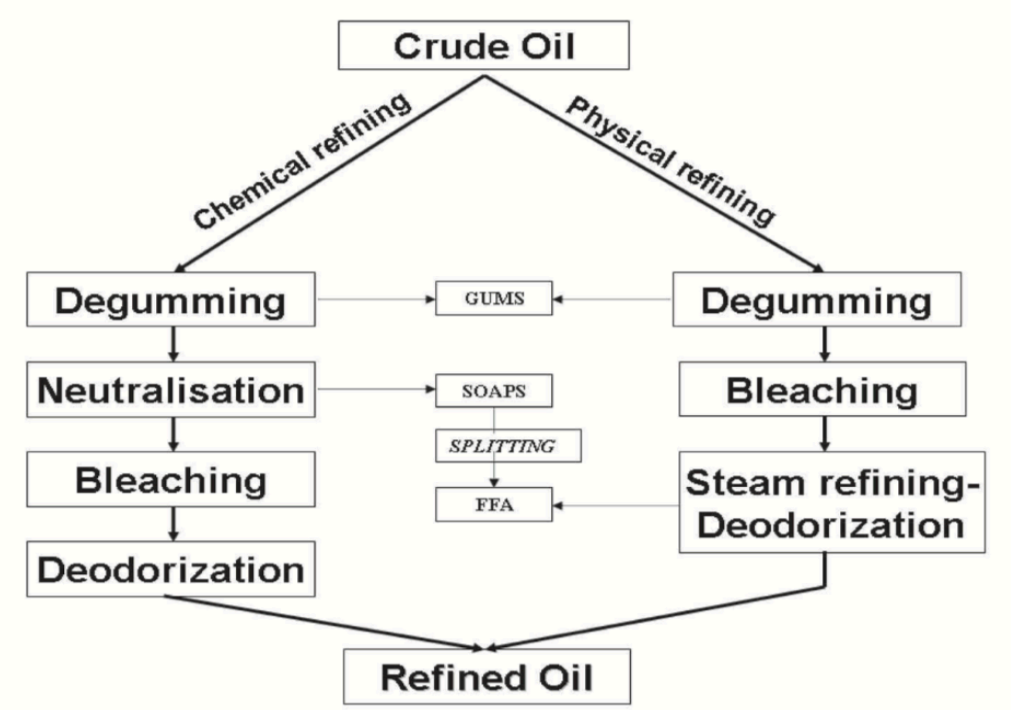

Figure 2.2 General overview of chemical and physical refining lines [47]

Refining Stage:	Compounds removed or reduced
Degumming:	Phospholipids, trace metals, pigments, carbohydrates, and proteins
↓	
Neutralization:	Free fatty acids, phospholipids, pigments, trace metals, sulfur, and insoluble matter
↓	
Washing :	Soap (form by free fatty acids or glycerols with sodium hydroxide)
↓	
Drying:	Water
↓	
Bleaching:	Pigments, oxidation products, trace metals, and traces of soap
↓	
Filtration:	Spent bleaching earth
↓	
Deodorization:	Free fatty acids, mono- and diacylglycerols, oxidation products, pigments, decomposition products, pesticides, sterols, sterol ester, tocopherols, and other antioxidants
↓	
Physical refining:	Free fatty acids, mono- and diacylglycerols, oxidation products, pigments, decomposition products, and pesticides
↓	
Polishing:	Any residual traces of oil insoluble

Figure 2.3 Traditional refining stages of edible oils [16]

Pal et al. [44] studied an experimental oil refining unit to compare between crude and refined oil in terms of quality and oil composition. The results showed that reduction in phosphorous content from 6.15 ppm to 0, FFA content from 1.1 to 0.24 % (oleic acid), peroxide value from 22.5 to 7.9 meq/kg were obtained from crude to refined oil.

Zacchi et al. [48] studied the effect of pretreatment, pressing and solvent extraction processes on the properties of rapeseed oil. The oils were refined by chemical and physical refining procedures, and the effect of refining stage on the composition of minor components was estimated. They concluded that contents of polyphenols and phospholipids in the oil obtaining from pretreated seeds enhanced. The content of polyphenols first decreased during degumming and complete removal was observed during neutralization. The polyphenols were affected by the type and amount of bleaching clay in physical refining. After deodorization, there were a significant amount of polyphenols retained in these oils. These samples had more oxidative stability than chemically refined oils.

Later, Farhoosh et al. [49] studied the influence of refining stages on the PV, acid value, carbonyl value, total polar compounds content, polar compounds distribution and oxidative stability index of soybean and canola oils in order to figure out influence of the refining stages. The acid value, oxidative stability index and total polar compounds contents decreased at the neutralization step and peroxide value changed, carbonyl value increased during the refining steps. Results indicated more importantly alterations in diglycerides and triglyceride dimers contents than in the FFA during the deodorization step.

Vaisali et al. [10] reported that crude oil obtained from vegetable and fish sources includes mono-, di-, triacylglycerols along with contaminants. They aimed to eliminate the impurities negatively affecting the oil quality and decreasing the shelf life of oil. Refining technology was developed by physical and chemical refining or untraditional processes containing biological and membrane techniques. In their review study it was aimed to show the technological deficiencies in the current methods of research to overcome the mentioned lacks.

On the other hand, some researchers reported that the effect on refining steps of sunflower oil such as neutralization, bleaching and deodorization on physicochemical features was controlled. Commercial refining had large effect on quality features of sunflower oil. Among four different refining steps, the most alteration was observed in some parameters such as color, soap content, smoke point, peroxide value in the neutralization, bleaching and deodorization. This study showed that among all refining steps deodorization had greater effect on physicochemical properties on the stability and nutrition quality of sunflower oil [27].

Liu et al. [50] claimed the impact of refining treatment on physicochemical parameters, chemical compositions and antioxidant capacities of rice bran oil. They showed that acid and peroxide values decreased after the refining. Antioxidant activities of these oils decreased during the refining. They also concluded that neutralization step leads to the greatest losses of phytochemicals and antioxidant activities.

Wu et al. [51] investigated the physicochemical attributes, micronutrients and oxidative stability of the oil obtained from the five types of rapeseeds through refining process. According to results, an increase in the acid value, PV and AV were observed while concentration of tocopherols, ß-sterols, carotene and phenols decreased during refining.

In summary, Pan et al. [52] examined the impact of chemical refining treatment on the physicochemical features and lipid oxidation of perilla seed oil. 5 samples corresponding to degumming, neutralization, bleaching, deodorization, and refined oil were investigated. They concluded that the acid and PV, tocopherol content and total phenolic content decreased, while the saponification and p-anisidine value increased considerably.

2.2.2 A New Approach: Minimal Refining

In recent years, consumer interest in healthy oils has been increasing. While undesirable minor components such as free fatty acids, pigments and gums and phospholipids are removed or decreased to acceptable levels by refining, some healthy desirable minor components such as phytosterols, tocopherols, carotenoids and polyphenolic components are also removed. Less refining can retain the healthy components but can also result in increased susceptibility of lipid oxidation by

components such as free fatty acids. Minimally refined oils became most popular day by day. Less-processed oils are healthier having higher nutritional value than refined oils. In order to obtain the minimum oil loss and most stable refined oil retaining more desirable nutrient minimal refining has been improved as alternative to conventional refining [5,13,14].

Refining is an essential treatment to eliminate unwanted minor constituents from unrefined oils. However, traditional treatment utilizes strong alkali throughout neutralization and use of activated clays in the bleaching step and also high temperature and low pressure in the deodorization step may result in the degradation of desirable components such as tocopherols. In minimal refining, magnesol is used instead of the hot-water washing step in traditional refining since it absorbs the soap. Another reason is that traditional hot water washing step leads to production of waste water. Consequently, no energy is needed to heat water and no waste water is generated during minimal refining. Similarly, this minimal refining provides to protect desirable minor components by omitting deodorization step and using wet bleaching instead of conventional dry bleaching [53]

Essid et al. [54] recommended that the neutralization is performed in a solid-liquid biphasic medium by replacing $Ca(OH)_2$ for NaOH as the neutralizing agent. Lime that is used as a neutralizing agent makes it potential to protect nearly 80% of the α-tocopherol. The results also showed that oils neutralized with lime retaining natural antioxidants had greater oxidative stability.

Kuleasan and Tekin [55] reported that the impact of sodium hydroxide in neutralization of soybean oil was enhanced by using different adsorbents. NaOH in different concentrations was attached to the parts of kieselguhr, celite and bentonite. The neutralization was carried out at ambient temperature and different reaction times. The residual soap was eliminated by centrifugation and washing and drying was omitted. It was concluded that free fatty acid neutralization in crude oil was performed by kieselguhr application. Adsorbents used enhanced the efficacy of NaOH and removal of soap from the neutralized oil through neutralization. These applications were energy saving and more practical.

De and Patel [56] investigated the feasibility of calcium hydroxide in the neutralization of rice bran oil. Crude oil samples of three different free fatty acids content (3.5–8.4 wt %) were refined by neutralizing with lime. Caustic soda neutralization (at 80–90°C) of FFA was replaced by a high temperature (150–210 °C) low pressure (2–4 mm Hg) reaction with lime. Neutralization of oil was also accomplished by using $NaHCO_3$ and Na_2CO_3, nontraditional alkalis and these alkalis were compared with NaOH and $Ca(OH)_2$. Total recovery of oil in lime refining was observed to be more than other competitive processes worked.

In a review by Yemiscioglu et al. [14], it has been reported that vegetable oils containing mainly triacylglycerols, important amount of desirable and undesirable minor components were exposed to chemical or physical refining to eliminate undesirable compounds (gums, free fatty acids, oxidative products, and pigments). Because desirable health promoting bioactive compounds also lost. This review aimed to figure out chemical characteristic of minor components and alterations in bioactive components during refining and describe a minimal refining in oil refining giving less hazard to structure of oil and bioactive compounds.

In another study, Wang et al. [57] studied commodity (normal) and high-oleic soybean oils extracted with extrusion-expelling (E-E). The extracted oils were minimally processed by water degumming and adsorptive deacidification to obtain edible oil. Deodorization was performed at 150°C for 1 h by purging with nitrogen (N_2), carbon dioxide (CO_2), or steam. The lipid oxidation results of the gas-purged normal oils were better than that of the traditionally deodorized oils. This study also indicated that tocopherol contents, FFA and colors of the deodorized oils were not importantly different among the treatments.

2.3 Minor Components

Some nutritional minor bioactive components of unsaponifiable fraction in crude vegetable oils are tocopherols, sterols, polyphenols etc. [4, 58]. Tocopherols that present in varying amounts in vegetable oils, retards lipid oxidation in food systems. These natural phenolic antioxidants which improve food quality decrease during refining process [41, 59].

These constituents exhibiting antioxidant activity in sunflower seeds play important role as a protection against many diseases and contribution to necessary activities against oxidative stress. Tocopherols known as vitamin E especially α-tocopherol having antioxidant activity decreases the risk of cardiovascular disease and shows anti-carcinogenic activities. Tocopherols occur as four forms named α-, β-, γ-, and δ-tocopherol [6, 7, 30, 60]. Sterols are another minor constituent of fat and oils improving oil oxidative stability at high temperature, exhibiting anti-inflammatory and antitumor activity and leading to a reduction of cholesterol levels in human blood. Most of the sterols (free sterols and sterol esters) which contain most of the unsaponifiable substance are removed during neutralization and deodorization stage [2, 5, 8]. One of the most important minor components is polyphenols having antioxidative and anti-microbial characteristics. They are also removed during neutralization step in refining process [5].

Alpaslan et al. [46] investigated tocopherol contents of sunflower oil, produced by chemical and physical refining or soft column deodorization. They concluded that total tocopherol content reduced after the deodorization stage. Both refining processes led to greater losses in the tocopherol content when compared with the soft column deodorization method. The concentration of α-, β, γ- and δ-tocopherols also decreased after the refining process.

In another study, tocopherols of sunflower oils at all steps of chemical and physical refining processes were evaluated by HPLC. The study indicated that total and individual tocopherol contents gradually reduced after the refining treatments [61].

Chen et al. [62] reported that triacylglycerols and minor constituents in edible oils mainly affect their physical and chemical properties. This review focused on the effect of these minor components on lipid oxidation in bulk oils and oil-in-water emulsions and also contributed to design food product with oxidative stability.

Fine et al. [6] reported that oilseeds such as sunflower, rape, and soybean containing tocopherols, phytosterols, polyphenols contributed to aroma and color, protect against health disorders. These micronutrients showed antioxidant activity limiting the lipid oxidation. This review also described the impact of refining processes on the minor component of different vegetable oils.

Xie et al. [63] aimed to estimate alterations of some minor compounds (tocopherols and phytosterols) in soybean oil and to decrease dangerous compounds such as trans FAs when retain the minor micronutrients throughout refining. It can be concluded that neutralization was an important stage to decrease phytosterols and polycyclic aromatic hydrocarbons.

2.4 Oxidation Stability

Lipid oxidation that is a complex chain of chemical interactions between unsaturated fatty acyl groups in lipids with active oxygen species consists of three steps [15, 16, 64]:

1. Initiation- the formation of free radicals;

   RH➔R• + H•

2. Propagation- the free-radical chain reactions;

   R• + O_2 ➔ ROO•

   ROO• + RH ➔ ROOH + R•

3. Termination- the formation of non-radical products:

   ROO• + ROO• ➔ ROOR + O_2

   ROO• + R• ➔ ROOR

   R• + R• ➔RR

Lipid oxidation decreases shelf life and quality of food and yields toxic products. These products can cause cancer and some inflammatory diseases [64, 65]. Important indicators for determining lipid oxidation are peroxide value, thiobarbituric acid value, acid value and anisidine value. These parameters are not sufficient when used alone for the assessment of lipid oxidation. Unsaturated lipids are oxidized to yield alkyl radicals these alkyl radicals react with oxygen then yield peroxyl radicals. The peroxyl radicals can react with more lipids to form the primary oxidation products of unsaturated fatty acids (known as hydroperoxides). The lipid hydroperoxide degrades to yields volatile compounds referred to as secondary oxidation products such as hexanal and propanal, impacting the sensory quality of foods. Hexanal that is a one of the important oxidation products, is known as an alternative indicator for lipid oxidation [21, 52, 54].

Lipid hydroperoxides are known as a primary oxidation product which have no flavor and odor however break down quickly to form secondary products, many of which have a strong flavor and odor. Rancidity occurs while unsaturated fatty acids break down into volatile components. These compounds are obtained from the decomposition of fatty acid hydroperoxides. Aldehydes, hydrocarbons, ketones, and epoxides are secondary oxidation product that negatively affect aroma are produced from the decomposition of lipid hydroperoxides. Higher oleic concentrations are required for advancing production of hydroperoxide at first step and then decomposition of hydroperoxide immediately after hydroperoxides arrived the highest level [16, 62, 67, 68]. Acid value indicates deterioration of oils; primary oxidative reactions are expressed by peroxide value, CD and CT. Secondary oxidative reactions are expressed by TBARS and anisidine value using Spectrophotometric methods with UV-visible [22, 69].

Lipid researches focused on how to prevent lipid oxidation in foods. Most strategies to enhance oxidative stability in bulk oils are needed both to improve food security and to obtain healthier foods. The addition of antioxidants into foods is the most popular way to inhibit lipid oxidation. Oxidative degradation of the oil can be controlled by avoiding the generation of lipid hydroperoxides and by scavenging the free radicals in foods. Retarding lipid oxidation both extends shelf life of oil-containing products and increases nutritional value [16, 18, 20, 62].

Antioxidants which are compounds inhibit lipid oxidation in foods, are divided into two groups according to their chemical mechanisms. Primary antioxidants postpone lipid oxidation by breaking chain mechanism and by inactivating the free radicals that promote oxidation. Secondary antioxidants reduce the rate of oxidation by decreasing prooxidative factors during chain mechanisms. Use of natural and synthetic antioxidants is effective to enhance oxidative stability and quality of food products. Ethylene diamine tetra acetic acid, butylated hydroxy anisole and butylated hydroxy toluene are common examples of synthetic antioxidants [17, 18, 23].

Many factors affecting oxidative reactions in bulk oils are environmental factors such as temperature, oxygen availability, light, antioxidants and prooxidants, water activity negatively impacts oil refining and transition metals that cause decreasing nutritional value and formation off-flavors [23, 70]. Oxygen that presents in the

atmosphere is a diatomic gaseous molecule. Two forms of oxygen are responsible for oxidative reactions that cause rancidity in lipid-containing food products. Oxygen either present in the headspace or dissolved in the bulk oils. Reducing the amount of oxygen within foods has been known as effective way to enhance oxidative stability. Various methods are improved to remove oxygen within foods, e.g. modified atmosphere, active and vacuum packaging and flushing by nitrogen [71, 72].

Tuberoso et al. [73] analyzed fatty acids, tocopherols, β-carotene, and phenolic compounds of edible oilseeds. It was concluded that autoxidation of oils through processing and storage is the important responsible for food deterioration, sensory and nutrition quality. Development of the nutritive value of oilseeds should be supported.

Azarbad et al. [19] investigated that lipid oxidation is a harmful process during food, storage, Hexanal determination is important way to evaluate lipid oxidation. The improved method exhibited an easy way to detect hexanal as an indicator for lipid oxidation in oils.

Saeed and Naz [74] investigated the impacts of traditional and microwave heating on the oxidation features of corn and soy bean oils. They observed that that acid value, PV, oxidative indices, total oxidation value and AV altered importantly. Rising in temperature and microwave heating had a greater influence on the chemical degradation of the fatty acids of the oil. Soybean oil indicated less oxidative alterations compared to corn oil.

Sadeghi et al. [75] estimated the oxidative stability of olive, sesame, and sunflower oils at various temperatures (4, 25, and 37 °C) and in subject to light for three months. Acidity, peroxide, anisidine, conjugated dienes, and conjugated trienes and oil stability index were determined to examine the stability of the oils. Results indicated that cold-pressed oils had a high allowance against the creation of the oxidation secondary products.

Recently, Cui et al. [76] investigated the oxidative characteristics, fatty acids and primary minor components in hazelnut oil through oxidation for 40 days. Tocopherols reduced until they could not be determined. This work also provided a

reference for the selection of natural antioxidants to enhance the shelf by finding a better understanding of the relationships between bioactive compounds and their oxidation properties.

2.5 Response Surface Methodology (RSM)

Response surface methodology (RSM) has become widespread optimization method in last years. Optimization is an essential term used in many works such as chemical and biochemical process for the proficiency of the processing and high suitability of the processes yield and obtaining the best possible response from process. The parameters impacting the process are named as independent variables while the responses are arranged as dependent variables. RSM is preferred to define the relationships between dependent (output) variables and the independent (input) variables affecting the process by using a limited number of experiments [12, 77, 78]. The aim of RSM is to optimize a response and design of experiments, select the points that the response should be estimated. Several researches have been carried out on the optimization processes of refining of edible oils.

Martincic et al. [79] focused on optimization of industrial-scale deodorization of high-oleic sunflower oil by RSM. The results of an experimental program carried out. The deodorization manner of some minor components was measured on a pilot-scale deodorizer. The results of this study exhibited that the response surface methodology supplied the tools needed to define the optimal deodorization values.

Ondrejovic et al. [80] investigated suitable conditions for the flaxseed oil bleaching process using the central composite design response surface methodology to examine the content of three independent variables. The results revealed that optimum parameters for the bleaching: 50°C, time 77 min and solid-liquid ratio 56 g of bleaching agent to 1 liter of oil.

Wei et al. [81] examined the relationship between temperature of refining and the loss of fatty acids and bioactive components in tea seed oil. The optimal values were 35 °C in the degumming stage, 45 °C in the neutralization stage, 85 °C in the bleaching stage, 150 °C at a pressure of 0.3 MPa in the deodorization and 7 °C in the dewaxing step. It was concluded that the characteristic of the refined oil using the optimized temperatures was similar to that of conventional refined oil and optimal refined oil more retained bioactive compounds than that of conventional method.

Some researchers stated that caustic neutralization obtains large amounts of soap-stock that is a byproduct and quite costly to dispose. Use of water in caustic neutralization is also high. Therefore, they aimed to use the high voltage electric field method for neutralization of sunflower oil under laboratory condition omitting drawbacks of caustic neutralization and to determine the optimum conditions by response surface methodology. This study also contributed to the improvement of green refining of oil for the food industries [82].

Finally, Nekouei and Rezaei [83] reported that acid degumming was examined by RSM using four degumming parameters. They concluded that peroxide and anisidine value of the almond oil reduced, tocopherols and the oxidative stability of the oil increased during the degumming process.

CHAPTER III

MINIMAL NEUTRALIZATION FOR SUNFLOWER SEED OIL

3.1 Introduction

Most people currently prefer to consume healthier, oxidatively and physically stable oils. Refining process is applied to develop quality parameters of oil and obtain more oxidatively stable oil. Most researchers studied how minor components in bulk oils affect lipid oxidation have concentrated on their physical and chemical attributes [16, 62, 84]. Lipids can be divided into fats and oils that are a significant role in the food quality and human growth. Lipids that are one of the major components in foods, are one of the primary targets of oxidation reactions [18,23,67,85]. Lipid oxidation that is one of the major reasons of quality deterioration in food products. It not only decreases nutrition value but also leads to loss of product shelf life because it yields rancid odors and flavors, alters color and textures in oil-containing foods [18, 71, 86].

Refining processing steps are used to eliminate undesirable compounds (free fatty acids, oxidative products and other contaminants pesticides, heavy metals). Nevertheless, substantial amounts of valuable minor components having nutritional benefits and health related features are destroyed such as antioxidants, vitamins, provitamins, tocopherols, phenolic components and plant sterols are also being removed [1, 7, 14].

Non-refined or minimally processed oils became important in the whole world. Most people think that less-processed foods are healthier and virgin oils have extra nutritional and health benefits compared to refined oils. Consequently, in order to reach the maximum oil quality that is acceptable for human consumption. Refining should be accomplished with the minimum losses of desirable substances. Minimal refining that is a new approach for health was developed [5, 13, 38, 87].

The aims of this part of study are to produce minimal neutralized sunflower oil by using $Ca(OH)_2$, Na_2SiO_3 and MgO and to indicate minimal neutralization process accomplished for sunflower oil with more oxidative stability and lower free fatty acid content than crude oil.

3.2 Material and Method

3.2.1 Material

Unrefined Sunflower Oil 100% Pure was used for minimal refining process. This oil was obtained from Mikho papa, Georgia, USA. Weak alkalis such as calcium hydroxide powder (Acros Organics., New Jersey, USA), magnesium oxide powder (Acros Organics., New Jersey, USA) and 41° Baume sodium silicate solution (J.T. Baker, Avantor., PA, USA) were used**.** Magnesol R60 was purchased from Dallas Group of America, Jefferson-ville, IN, USA. Methanol, 1-butanol, hydrochloric acid was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Ethanol, phenolphthalein, DPPH (2,2-diphenyl-1- picrylhydrazyl), ethyl acetate, trolox, citric acid, sodium hydroxide, ammonium thiocyanate, iron (II) sulfate heptahydrate, cumene hydroperoxide, barium chloride dihydrate, hexanal, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Double distilled water was utilized in all experiments. Glassware was soaked in 2 M hydrochloric acid (HCl) overnight to remove metals, followed by rinsing with double-distilled water before use.

3.2.2 Methods

3.2.2.1 Degumming

Degumming and neutralization were conducted according to described previous studies with some modifications [13, 55, 88, 89].

Water degumming with acid pretreatment was carried on the crude sunflower oil. Temperature of crude oil was set up to 70 °C while stirring at 250 rpm on a magnetic stirrer (Isotemp, Fisher Scientific). A 64 % citric acid solution was mixed with the oil at 0.7 % level for 30 minutes at 250 rpm on a magnetic stirrer. Secondly distilled water was mixed with the oil (2 % of total mass) at 250 rpm for 30 minutes. The gums and phospholipids were eliminated by centrifugation (Sorvall Lynx 4000, Thermo Scientific) at 3,750 g for 10 minutes by separating the aqueous and oils phases by decantation.

3.2.2.2 Minimal Neutralization

Degummed sunflower oil was neutralized with some other alkali metal compounds according to an earlier described procedure with some alterations by Ghazani et al. [13]. Three different weak alkalis as neutralizing agents: calcium hydroxide, magnesium oxide and a 41° Baume (38%) sodium silicate solution were used to neutralize degummed sunflower oil. Temperature of degummed oil was first set up to 55 °C and 2.5 % (w/w) of distilled water agitated at 600 rpm for 3 minutes then blended by a Bio homogenizer (Biospec products Inc, Bartlesville, OK) at 20,000 rpm for 30 s and separated into 3 parts. A 0.15 % calcium hydroxide, 0.5 % magnesium oxide and 0.5 % 41° Baume sodium silicate solution were mixed with oil separately. These concentrations were recommended by Ghazani et al. [13]. The oil samples were blended at 20,000 rpm for 1 minute and agitated strongly at 600 rpm for 20 minutes. Then, temperature of the mixtures was changed to 60 °C for centrifugation at 5,200 g for 10 minutes and filtered (Whatman-42). Magnesol R60 was used instead of the hot-water washing step. Temperature of clear oil was adjusted to 80 °C and stirred at 600 rpm for 20 minutes with 1 % Magnesol R60. It was centrifuged at 5,200 g for 10 minutes and then filtered (Whatman-42). Oil samples (0.5 mL) were placed into 10 mL glass vials and capped with aluminum caps with polytetrafluoroethylene (PTFE)/silicone septa and were withdrawn periodically from the incubator at 55°C for 28 days (two oxidation tests: lipid hydroperoxides and hexanal). According to type of analysis, oil samples were stored at 4°C, -18°C or -80 °C until analyzed.

3.2.2.3 Determination of Free Fatty Acids

Oil samples (28.2 g) were weighed in an erlenmeyer, followed by addition of 50 ml ethanol, a few drops phenolphthalein indicator and titrated with 0.1 N NaOH [90]. Free fatty acid content was calculated by formula below (Equation 3.1).

$$\%FFA=\frac{ml\times N\times F\times 100}{M\times 1000} \qquad (3.1)$$

Where, V = ml of NaOH titrated, N = normality of NaOH solution, M = weight of sample (g), F = equivalent weight of FFA (as oleic acid = 282).

3.2.2.4 Measurement of Lipid Hydroperoxides

Lipid hydroperoxides were detected using a procedure adapted from Shantha and Decker [91]. Sunflower oil (0.2 g) was weighed and dissolved in 2.8 mL of a methanol-butanol (2:1, v/v) mixture.

Next 15 μ L of 3.94 M ammonium thiocyanate and 15 μ L of 0.072 M Fe^{2+} (ferrous sulfate) was added as an indicator. The ferrous solution was made by mixing 0.132 M $BaCl_2$ in 0.4 M HCl and 0.144 M $FeSO_4$. After a waiting period of 20 minutes, the absorbance was read at 510 nm using a UV-Vis spectrophotometer (Genesys 20, Thermo Spectronic, Waltham, MA, USA). The calculations were made from a standard curve of cumene hydroperoxide ($y = 2.6595x + 0.0067$; $R^2 = 0.9994$).

3.2.2.5 Measurement of Headspace Hexanal

Head- space hexanal was detected according to the method described by Panya et al. [92] with some alteration for bulk oils. Hexanal was determined with a gas chromatograph (GC-2014 Shimadzu, Marlborough, MA) equipped with a solid phase microextraction auto injector (AOC-5000, Shimadzu, Tokyo, Japan). The oil in the 10 mL glass vials capped with aluminum caps with polytetrafluoroethylene /silicone septa was heated at 55°C for 10 minutes in the auto sampler heating sampler heating block before measurement.

A 50/30 μm divinylbenzene (DVB) / carboxen / polydimethylsiloxane (PDMS) stable flex) fiber (Supelco Co., Bellefonte, PA) was then inserted into the vial headspace to absorb volatiles for 2 minutes. The fiber was transferred to the GC injector port (250°C) for 3 minutes. The injection port was operated in split mode, and the split ratio was set at 1:7. Volatiles were separated on a fused-silica capillary Equity-1 Supelco column (30 $\times$ 0.32 mm inner diameter $\times$ μm) coated with 100% PDMS. The temperatures of the injector, oven and flame ionization detector were 250, 65, and 250°C, respectively. Peak integration was calculated using Shimadzu EZstart (version 7.4). Sample run time was 10 minutes. Hexanal concentrations were detected from peak areas using a standard curve ($y = 7146.5x$; $R^2 = 0.9865$) from hexanal in oil [71].

3.2.2.6 Free Radical Scavenging Capacity

The total free radical scavenging capacity of the oil was measured 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [93]. DPPH was dissolved in a small amount of ethyl acetate and diluted with ethyl acetate to an absorbance of 0.700 ± 0.020 at 520 nm. A 20 mg oil was weighed in a test tube, and 80 μ L ethyl acetate as well as 2.9 ml DPPH solution was added. Then, the sample was mixed with a vortex (Genie 2, Fisher Scientific, MA, USA) for 20 s. The sample was incubated for 30 minutes in the dark and absorbance was read by UV-Vis spectrophotometer (Genesys 20, Thermo Spectronic, and Waltham, MA, USA) at 520 nm against ethyl acetate. The results were calculated from a standard curve of Trolox in ethyl acetate ($y=-0.0298x + 0.669$; $R^2= 0.9997$ and then stated as μ g trolox equivalent (TE)/g oil.

3.3 Results and Discussion

3.3.1 Free Fatty Acid Content

The Free fatty acids levels of sunflower oil during refining processes with different alkali materials are shown in Figure 3.1. Initial FFA contents for crude sunflower oil (CB) was 1.01%. It can be seen that the FFA of crude sunflower oil decreased after neutralization. The different amounts of calcium hydroxide (CC), magnesium oxide (CM) and sodium silicate (CN) were effective for lowering the FFA content. The FFA content (0.15 % $Ca(OH)_2$, 0.5 % MgO and 0.5 % Na_2SiO_3) were 0.57, 0.47, and 0.74 %, respectively. The concentration of each neutralizing agent was recommended by Ghazani et al. [13]. There were no significant differences between MgO and $Ca(OH)_2$ ($P>0.05$). Some previous studies showed that FFA content decreased after neutralization step. Pal et al. [44] reported that FFA content of crude sunflower oil was found to be reduced from 1.1 to 0.24 after neutralization. Suliman et al. [40] observed that reduction in FFA content from 0.5 to 0.1 % during neutralization of crude sunflower oil.

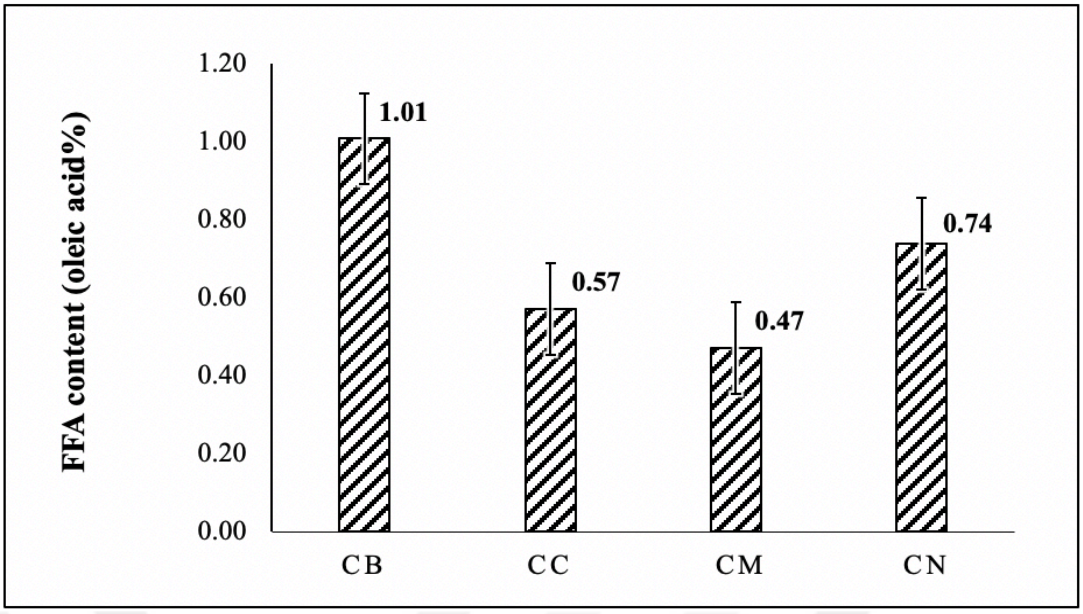

Figure 3.1 Free fatty acid values of crude and minimal neutralized sunflower oils

3.3.2 Lipid Oxidation

The effect of refining by different alkalis on oxidative stability of sunflower oil was investigated. In order to figure out the impact of type of alkali on lipid oxidation in bulk oils, 3 weak alkali types were used in bulk oils (0.15 % $Ca(OH)_2$, 0.5 % MgO and 0.5 % Na_2SiO_3).These concentrations were recommended by Ghazani et al. [13]. The oil samples were incubated at 55°C and extent of lipid hydroperoxide and hexanal concentration were measured. These variations of lipid hydroperoxides and hexanal of the crude and refined sunflower oils by different alkali materials during 28 days are shown in Figure 3.2. Minimal neutralized sunflower oils had same hydroperoxide lag phases meaning they showed similar oxidative stability.

Shah et al. [34] claimed that there was no any major influence observed on PV in the neutralization stage for sunflower oil. Since PV of crude sunflower oil and neutralized sunflower oil 3.2 and 2.9 meq O_2/kg of oil. Another study compared between conventional and minimal refined canola oils. They also found that conventionally refined canola oil oxidized very fast than minimal refined canola oils [13].

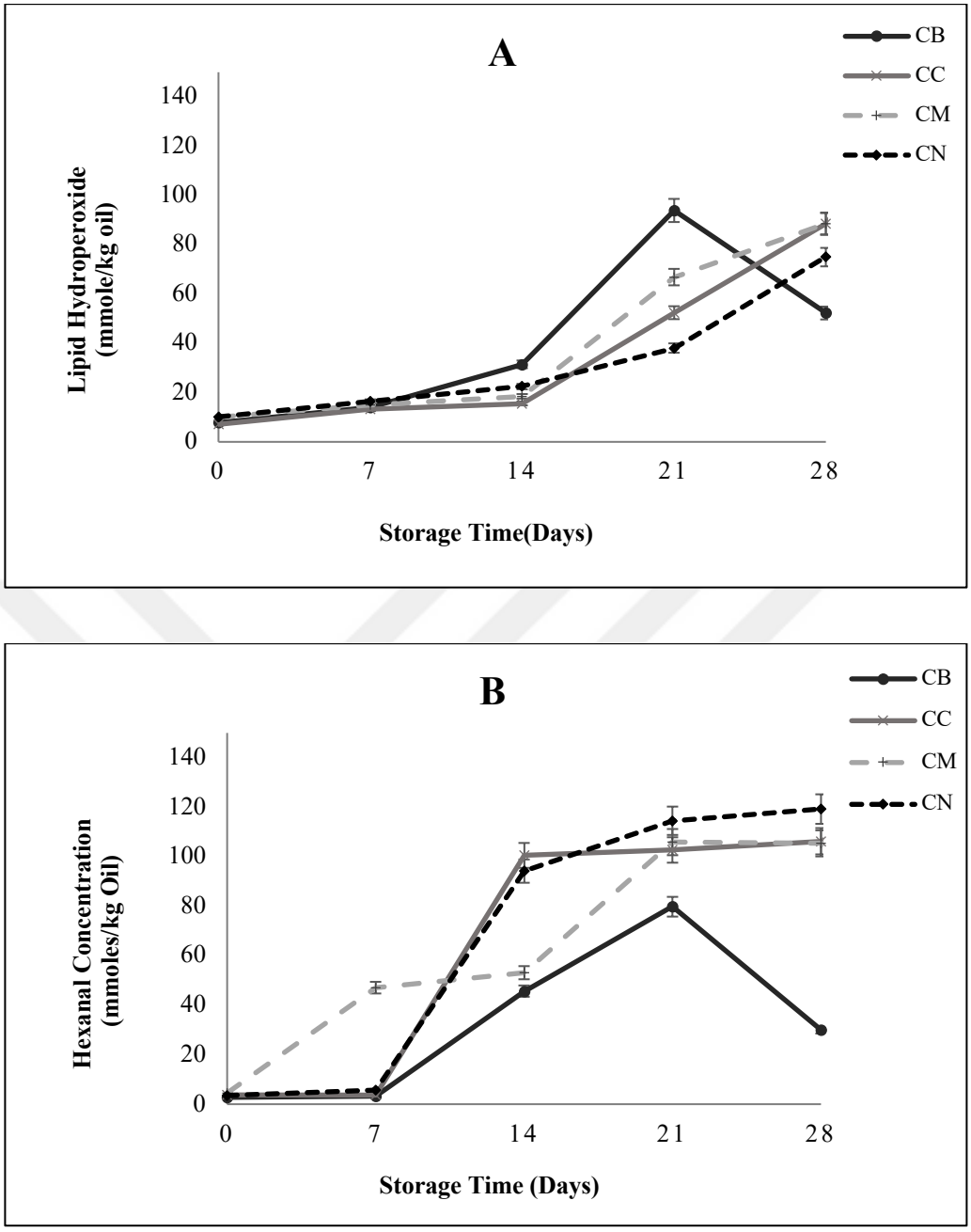

Figure 3.2 Formation of lipid hydroperoxides (**A**) and hexanal (**B**) in sunflower oil neutralizing different alkali materials (CB: Crude oil, CC: 0.15 % $Ca(OH)_2$, CM:0.5 % MgO and CN: 0.5 % Na_2SiO_3 during storage at 55 °C

3.3.3 Free Radical Scavenging Capacity

The free radical scavenging capacity of the sunflower oils was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method assay that is a spectrophotometric method to evaluate the free radical scavenging potential of a sample. The free radical scavenging capacities of crude, degummed and refined sunflower oils after neutralization are shown in Figure 3.3. The free radical scavenging capacities of crude, degummed and sunflower oils neutralized with 0.15 % $Ca(OH)_2$, 0.5 % MgO, 0.5 % Na_2SiO_3 ranged from 13.67 to 13.69 mmole TE/kg oil. Free radical scavenging activity of crude oil was significantly decreased ($P<0.05$) compared to the others. There were no statistically differences ($P>0.05$) between MgO and $Ca(OH)_2$ treatments. There were also no significant differences between Na_2SiO_3 and $Ca(OH)_2$ ($P>0.05$) treatments with respect to radical scavenging activity (Figure 3.3).

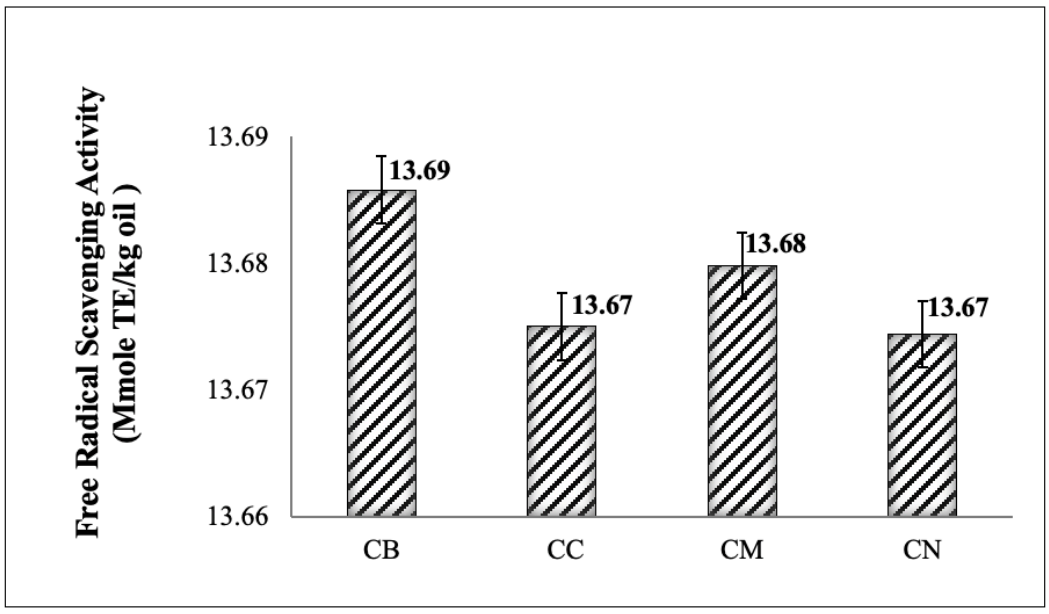

Figure 3.3 Free radical scavenging capacities of crude and minimal neutralized sunflower oils by $Ca(OH)_2$, MgO and Na_2SiO_3 respectively

The both have similar effect on free radical scavenging activity and significantly decreased. Castelo-branco et al. [94] reported that total antioxidant capacity of refined sunflower oil is 2.44 mmole Trolox eq /kg. Karamac et al. [95] showed that ABTS cation radical-reducing ability of sunflower seed fractions was expressed as TEAC values The TEAC values varied from 0.10 mmol Trolox eq/g to 2.21 mmol Trolox eq/g.

3.4 Conclusion

New minimal neutralization approach was improved as alternatives to conventional neutralization. This method was aimed to neutralize crude sunflower seed oil with $Ca(OH)_2$, MgO, and Na_2SiO_3 instead of strong alkali NaOH. The FFA content after neutralizing with 0.15 % $Ca(OH)_2$, 0.5 % MgO and 0.5 % Na_2SiO_3 were 0.57, 0.47, and 0.74 %, respectively. It was revealed that use of weak alkali in the minimal neutralization has lowering effect on FFA content of sunflower seed oil compared to crude sunflower seed oil. These results suggested that the minimal operation possible to obtain edible sunflower oil having high nutrition value by altering the neutralization process.

CHAPTER IV

COMPARISON OF TRADITIONAL AND MINIMAL NEUTRALIZATION METHODS FOR SUNFLOWER SEED OIL

4.1 Introduction

One of the most significant oil seeds around the world is sunflower (*Helianthus annuus* L.). During the 19th and 20th centuries sunflower was one of the largest vegetable oil products cultivated in the world. Argentina, USA and Ukraine are the leading centers in the production of sunflower. This crop can be adapted to be grown successfully with limited water availability and under high environmental temperatures. Sunflower oil contains high amount of linoleic acid which has useful effects on heart health and bioactive components such as tocopherols, sterols, phenolics which have also been postulated to improve health [2, 3, 24, 26, 96].

Refining of crude oils is an essential method to produce high quality edible oil that has positive sensory attributes and a long shelf-life. Refining is used to reduce undesirable compounds such as gums, free fatty acids, oxidative products, pigments and contaminants (pesticides, heavy metals) to ensure the oil is safe for consumption and can be used in multiple food processing application (e.g. refrigerated emulsions and frying oils). However, considerable amounts of minor components with nutritional benefits are also removed such as antioxidants, vitamins, provitamins, tocopherols, phenolic components and plant sterols [1, 4, 7, 14]. Refining process is composed of degumming, neutralization, bleaching and deodorization [87, 97, 98].

Degumming removes phospholipid and gums. Neutralization is known as deacidification. In this step primarily free fatty acids remove. During neutralization the fatty acids are converted into soaps with the addition a caustic agent and are then removed by decantation or centrifugation [7, 87]. Bleaching uses absorbents to remove off-colors and deodorization removes off-flavors under reduced pressures. Physical refining also uses the degumming and bleaching but uses high temperature

distillation to remove both free fatty acids and off-flavors at the same time. Sodium hydroxide used in neutralization and activated clays in bleaching or physical conditions in deodorization such as high temperature and low pressure leads to neutral oil loss during traditional refining as well as the healthy minor components [40, 46, 53]. A previous research suggests the minimal operations possible to obtain edible oil having high nutrition value by altering the oil refining processes. For example, the high pH of refining has been shown to degrade oil bioactive compounds so switching to a weaker alkali might produce healthier oil [13]. Several weaker alkalis such as sodium carbonate, ammonia hydroxide, calcium hydroxide and magnesium oxide have been used as an alternative to sodium hydroxide to reduce neutralization loss in the past studies. These studies claimed that calcium hydroxide is cheaper and weaker than sodium hydroxide. So that, it is more economical to use and the refining loss is lower [53, 99].

Another study showed the effects of magnesium silicate, aluminum hydroxide, calcium hydroxide, magnesium oxide, magnesium hydroxide and calcium silicate gel solutions on free fatty acid removal from frying oils. They found that magnesium oxide was effective in removing FFA because of its high solubility in oil [100]. Hernandez et al. [101] applied 40% (w/w) sodium silicate instead of sodium hydroxide to neutralize crude oils. They stated that after the reaction of sodium silicate with free fatty acids, soaps can be removed by filtration.

Lipid oxidation is characterized as a process of complex chemical reactions between active oxygen species with unsaturated fatty acids. Sunflower oil comprises high amounts of unsaturated fatty acids making it sensitive to oxidation which can cause consumer rejection of the product. Free fatty acids are prooxidants so they need to be removed to obtain the desired shelf-life of the oil. This suggests that minimally processed oils might still require a neutralization step to increase shelf-life [16, 23, 57, 102].

In this part of the study, the effect of neutralizing a degummed oil by $Ca(OH)_2$, MgO and Na_2SiO_3 as an alternative to NaOH on bioactive compounds and lipid oxidation was compared with the idea that refining with only 2 of the typical 4 steps could produce healthier oil with similar oxidative stability.

4.2 Materials and Methods

4.2.1 Materials

Crude sunflower oil was used in all experiments (Dr Adorable Inc., Chicago, IL, USA). Methanol was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Folin-ciocalteau reagent, sodium carbonate, caffeic acid, citric acid, sodium hydroxide, were supplied from Sigma-Aldrich (St. Louis, MO, USA). Double distilled water was utilized in all experiments.

4.2.2 Methods

Degumming and neutralization were conducted according to previously described studies with some modifications [13, 55, 88, 89] as shown in Figure 4.1. For traditional neutralization, temperature of degummed sunflower oil was set up to 40°C. 16° Baume (11.60 % w/v) NaOH solution at 0.20 % level was added to the oil and agitated at 250 rpm for 15 minutes. After temperature of mixture reached 70°C, it was centrifuged at 5,200 g for 10 minutes and then filtered (Whatman-42). The neutralized oil was heated up to 85°C. The residual soap was removed by centrifugation at 5,200 g for 10 minutes and finally filtered through Whatman-42 filter paper [13, 55]. Degummed sunflower oil was neutralized with other alkali metal compounds according to an earlier described procedure with some alterations by Ghazani et al. [13]. Minimal Neutralization was made according to the method described in Chapter 3. (Figure 4.1) [53]. Storage conditions were applied according to the method described in Chapter 3.

4.2.2.1 Determination of Free Fatty Acids

Measurement of FFAs was done according to the method described in Chapter 3.

4.2.2.2 Free Radical Scavenging Capacity

Free radical scavenging capacity was detected according to the method described in Chapter 3.

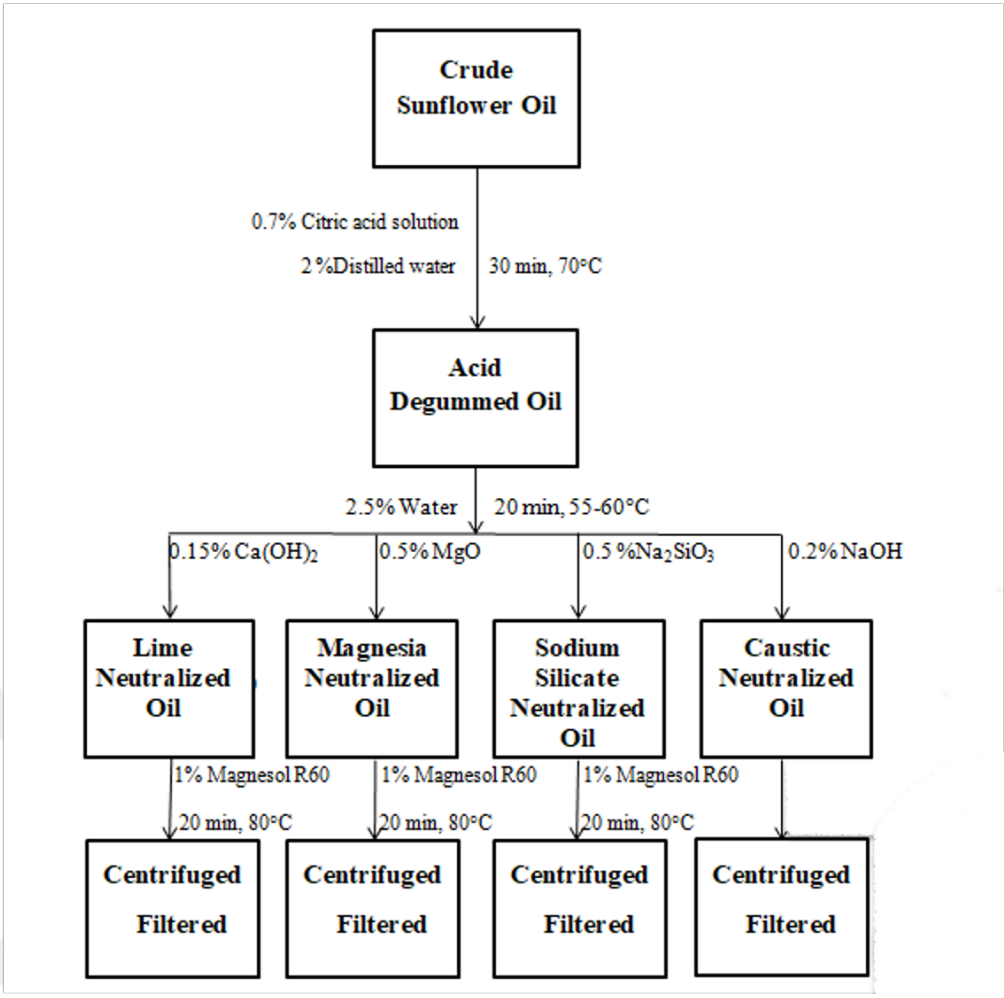

Figure 4.1 Flow chart of conventional and minimal neutralization for crude sunflower oil

4.2.2.3 Determination of Total Phenolics Content.

A 0.5 gram sample of sunflower oil was dissolved in 0.5 mL of methanol in 2 ml test tubes. After mixing vigorously, the vials were centrifuged at 5,000 rpm for 2 minutes [103]. The upper methanolic phase was collected and the total phenolics content was measured with Folin-Ciocalteau reagent. The procedure comprised of dilution of 0.1 mL or a suitable aliquot of the methanol extract with water to 2 mL and addition of 0.5 ml Folin-Ciocalteau reagent. After 3 minutes, 1 mL of Na_2CO_3 solution was added. The content was shaken and brought up to 10 mL with water in a volumetric flask. The absorbance was read after 1 hour at 725 nm against a reagent blank. Total phenolics content was calculated from standard curves of caffeic acid ($y = 0.0126x - 0.0279$; $R^2 = 0.9951$) and stated as mg caffeic acid equivalent (CAE)/100 g oil [104].

4.2.2.4 Lipid Oxidation Studies

Measurement of lipid hydroperoxides and headspace hexanal concentration was made was made according to the method described in Chapter 3.

4.2.2.5 Statistical Analysis

One way ANOVA and Duncan's test were performed for FFA and TPC content to determine the significant differences amongst the means of the three replications ($P<0.05$) using the SPSS version 23.0. (IBM Corp. 2015, IBM SPSS, Armonk, NY) [105].

4.3 Results and Discussion

4.3.1 Free fatty acids content

A variety of NaOH alternatives were tested for their ability to remove FFA in degummed sunflower oils. The Free fatty acid levels of crude oil, degummed oil and oils refined by different alkalis are shown in Table 4.1. Initial FFA contents for crude and degummed oils were 0.30 and 0.18 %, respectively. The Free fatty acid content of the degummed oil reduced from 0.18 to 0.12 % after neutralizing with 0.2 % NaOH. Shah et al. [34] reported that FFA concentration in crude sunflower oil reduced from 0.56 to 0.14% after neutralizing with NaOH. Pal et al. [44] stated that FFA content of crude sunflower oil decreased from 1.1 to 0.24 after NaOH neutralization. Suliman et al. [40] also observed a reduction in FFA content from 0.50 to 0.10 % during NaOH neutralization of crude sunflower oil.

This study determined the effectiveness of removing FFA from degummed sunflower oil with calcium hydroxide, magnesium oxide and sodium silicate. These neutralizing agents were chosen because they provide to decrease refining loss; magnesium oxide is effective in eliminating FFA because of its high solubility in oil and calcium hydroxide more economical than sodium hydroxide [53]. The concentration of each neutralizing agent was recommended by Ghazani et al. [13]. The FFA content (0.15 % $Ca(OH)_2$, 0.5 % MgO and 0.5 % Na_2SiO_3) were 0.05, 0.07 and 0.11 % respectively. There were no significantly different between calcium hydroxide and magnesium oxide ($P>0.05$). Calcium hydroxide and magnesium oxide produced the lowest FFA concentrations ($P>0.05$). Our results consistent with Ghazani et al. [13]. They stated that calcium hydroxide is the most effective base in

minimal neutralization. It is understood that a less amount of calcium hydroxide contributes adequate neutralization of the degummed oil and also found that after neutralizing with 0.15 % Ca(OH)2, 0.5 % MgO and 0.5 % Na_2SiO_3, the FFA content of degummed oil reduced from 0.55 to 0.03, 0.08, and 0.07 %, respectively.

Table 4.1 FFA and TPC values of crude, degummed and minimal-neutralized sunflower oils

Variable	Crude Oil	Degummed Oil	$Ca(OH)_2$	MgO	Na_2SiO_3	NaOH
FFA	0.30 ± 0.01^{D}	0.18 ± 0.03^{C}	0.05 ± 0.01^{A}	0.07 ± 0.01^{A}	0.11 ± 0.01^{B}	0.12 ± 0.02^{B}
TPC	1.40 ± 0.01^{D}	1.28 ± 0.09^{CD}	0.89 ± 0.02^{A}	1.14 ± 0.01^{B}	1.23 ± 0.02^{BC}	1.38 ± 0.04^{D}

A, B, C, D: Means in the same row followed by different uppercase letters represent significant differences at a level α =0.05 level.

4.3.2 Free Radical Scavenging Capacity and Total Phenolics Content

Endogenous phenolics are typically found to provide protection against lipid oxidation in oils. However, non-phenolic compounds can also inhibit lipid oxidation in oils (e.g. carotenoids). Therefore, measuring both total phenolics and free radical scavenging capacity can provide some insights to how refining steps can impact antioxidants in the sunflower oil. DPPH radical scavenging capacity is often used to determine antioxidant capacity as it is a lipid soluble free radical that changes color upon interaction with an antioxidant [106-109]. The DPPH radical scavenging capacities of crude, degummed and refined sunflower oils after neutralization are shown in Figure 4.2. DPPH-scavenging capacities of crude (AB), degummed (AD) and neutralized sunflower oils ranged from 13.67 to 13.69 mmole TE/kg oil. Free radical scavenging activity of degummed oil was significantly decreased ($P<0.05$) compared to the others.

There were no statistically differences ($P>0.05$) between MgO (AM) and $Ca(OH)_2$ (AC) treatments. There were also no significant differences between Na_2SiO_3 (AN) and NaOH (AT) ($P>0.05$) treatments with respect to radical scavenging activity. The both have similar effect on free radical scavenging activity and have not changed significantly ($P>0.05$). Unlike the others, MgO and $Ca(OH)_2$ have the same effect in free radical scavenging activity and significantly increased ($P<0.05$).

Castelo-Branco et al. [94] showed that total antioxidant capacity of refined sunflower oil is 2.44 mmole TE/kg. Pellegrini et al. [110] observed the total antioxidant capacity of refined sunflower oil (1.17 mmol TE/kg) using TEAC (trolox equivalent antioxidant capacity) assay. The amount of phenolic compounds is a significant quality factor in oils because of their role in oxidation stability. Total phenolics content of crude, degummed and degummed-neutralized sunflower oils during refining processes with different alkali materials treatments ranged from 0.89 to 1.40 mg CAE/100 g oil (Table 4.1). There is an important linear relationship between phenolic contents and antioxidant activity [108]. Like DPPH results, Total phenolics content of degummed oils significantly decreased compared to crude oil. There was also no statistically difference ($P>0.05$) between crude oil and refined oil while treated by NaOH. Neutralizing with sodium hydroxide and crude oil had the highest total phenolic contents and $Ca(OH)_2$ had the lowest total phenolics content. Similar TPC results have been observed elsewhere. The total phenolic content of refined sunflower oil using the Folin-Ciocalteu reagent method was found 1.55 mg of gallic acid equivalent/100 g of oil [94]. Siger et al. [111] reported that the total phenolics content of cold-pressed sunflower oil to be 1.20 mg CAE/100 g.

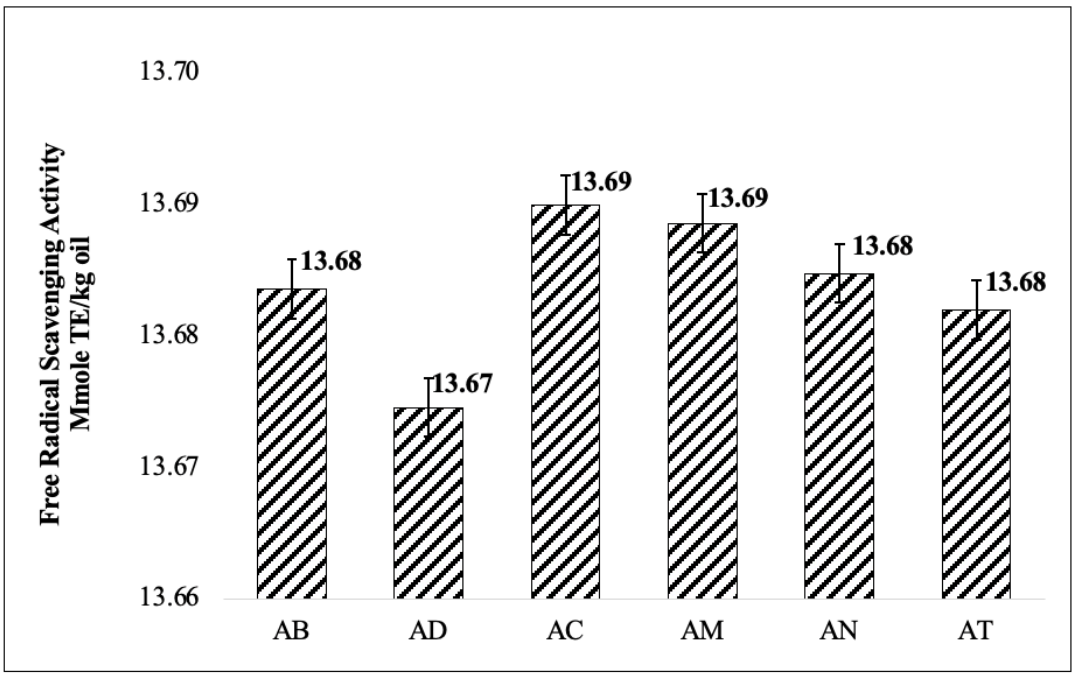

Figure 4.2 Free Radical Scavenging Capacities of crude, degummed and minimal-neutralized sunflower oils

4.3.3 Lipid Oxidation

The effect of neutralization by different alkali materials on lipid oxidation of sunflower oil was investigated. (Figure 4.3). The degummed and NaOH neutralized oils had the shortest hydroperoxide and hexanal lag phases meaning they were the least stable oils. FFA and total phenolics would be two important factors in the oxidative stability of the oil with FFA accelerating oxidation and phenolics inhibiting oxidation. The degummed oil and NaOH neutralized oil had the highest FFA and phenolic concentrations yet were least oxidatively stable oils. This suggests that the prooxidant activity of the FFA could have overcome the protection of the higher levels of phenolics. $Ca(OH)_2$ and MgO treated oils exhibited low FFA content and high antioxidant activity. Therefore, both alkalis were oxidatively stable than NaOH. According to Figures 4.2 and 4.3, it was observed that increase in peroxide value in degummed oil may be due to their low free radical scavenging activity and high FFA content. Unlike sodium hydroxide, all weak alkalis had similar effect on the peroxide value. There were not big differences among lipid oxidation of refined oils by $Ca(OH)_2$, MgO and Na_2SiO_3.

Ghazani et al. [13] stated that according to PV results of refined oils, traditionally refined oil oxidized very fast among all refined oils. The least lag phase observed in oils refined by sodium hydroxide. As it can be seen from Table 4.1, traditionally refined oil exhibited higher FFA content than the other refined oils. It showed a greater increase in PV which comes from the higher amounts of free fatty acids in this oil. These observations are in accord with Yi et al. [67]. They studied the effect of FFA on oxidation stability in the water in stripped walnut oil system. They found that enhancing levels of oleic acid accelerated lipid oxidation.

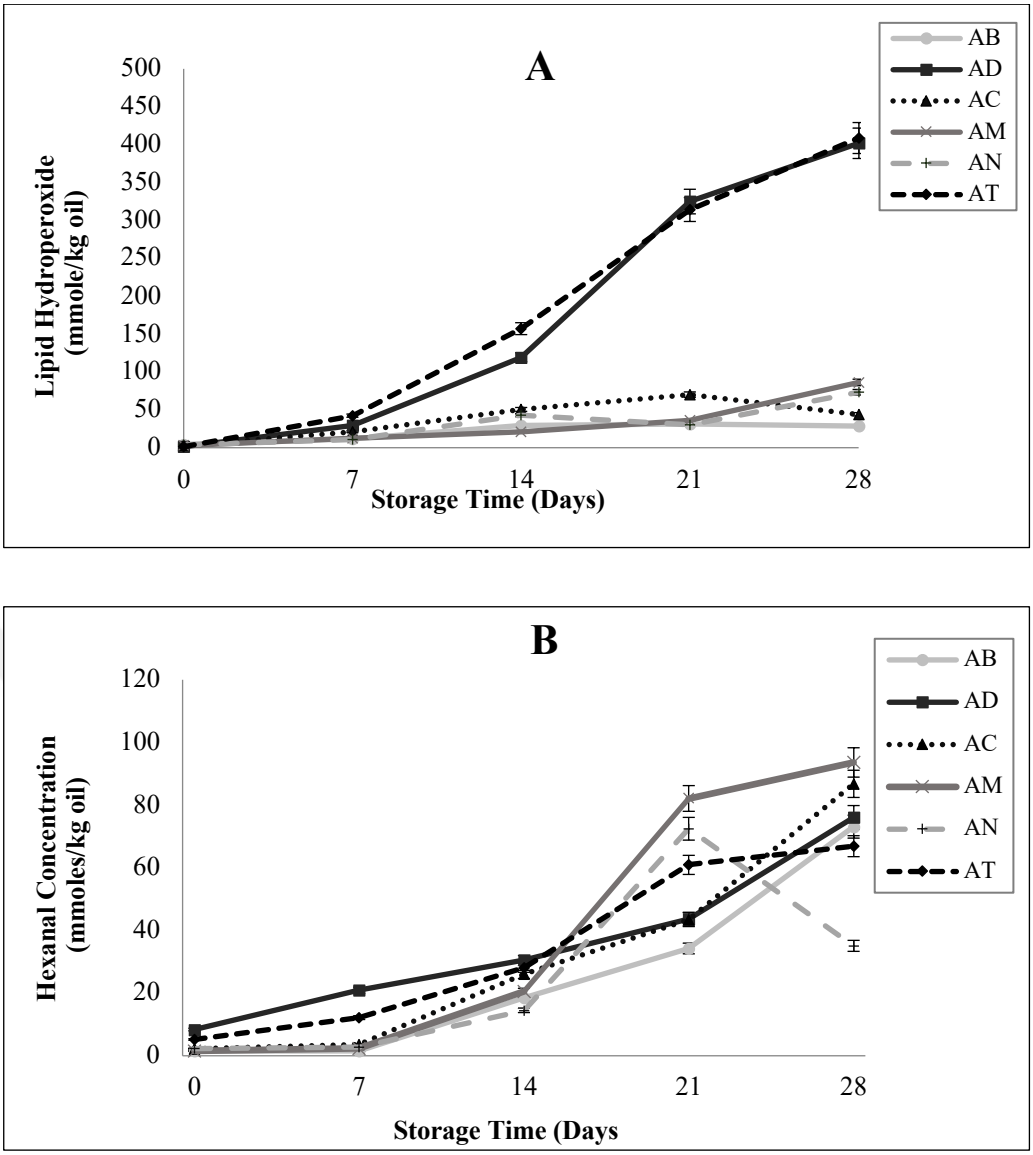

Figure 4.3 Formation of lipid hydroperoxides (**A**) and hexanal concentration (**B**) in sunflower oil neutralizing different alkali materials (AB: Crude oil, AD: Degummed oil, AC: 0.15 % $Ca(OH)_2$, AM: 0.5 % MgO, AN: 0.5 % Na_2SiO_3 and AT: 0.2 % NaOH during storage at 55 °C)

4.4 Conclusion

In this study, weak alkalines such as $Ca(OH)_2$, MgO and Na_2SiO_3 were used to neutralize degummed sunflower oil instead of strong alkali (NaOH) which is used in conventional neutralization. The free fatty acid content after neutralizing with 0.15 % $Ca(OH)_2$, 0.5 % MgO, 0.5 % Na_2SiO_3 and 0.2 % NaOH were 0.05, 0.07, 0.11 and 0,12 %, respectively. Oils neutralized by $Ca(OH)_2$, MgO and Na_2SiO_3 had lower FFA content (free fatty acid) than oils neutralized by NaOH and also had better oxidative stability suggesting that they could be more effective in producing a high quality oil. Second part indicated that sunflower oil obtained by minimal neutralization having lower FFA content and more oxidative stable than sunflower oil produced by traditional neutralization.

CHAPTER V

EFFECT OF MINIMAL REFINING PROCESS ON MINOR COMPONENTS AND OXIDATION STABILITY OF SUNFLOWER OIL AND PROCESS OPTIMIZATION

5.1 Introduction

Crude vegetable oils are not only are composed of desirable components such as phospholipids, tocopherols, tocotrienols, flavonoids, carotenoids and sterols but also are composed of undesirable components such as free fatty acids, gums and coloring matters. Preservation of the components that present nutritional properties with a positive effect on human health is important factor in the quality and identification of vegetable oils. Sunflower oil which plays a significant role in the food sectors is used for industrial and nutritional aims in the edible oil production [1, 7, 27, 32, 43, 112, 113].

There is an increasing interest in several minor compounds with health and nutritional benefits present in sunflower oil. These micronutrients can protect against cardiovascular diseases, chronic diseases and aging disorders and are also important for oil stability [2, 6, 41]. Sunflower seed has high amounts of tocopherols which are natural antioxidants. Sunflower seeds primarily comprise α-tocopherol which represents more than 90% of the total tocopherols. β- and γ-tocopherol present in amounts less than 5% of the total tocopherols in sunflower seeds. Edible oils are exposed to air at different rates and consequently oxidation and nutritional deterioration is common. The degree of unsaturation of fatty acids is one of the prominent parameters that affect lipid oxidation. Modification of the fatty acid composition and the composition of minor components, can influence oxidative stability and quality of edible oils [6, 24, 58, 114, 115].

Lipid oxidation is the most important reaction in the deterioration of oils. This reaction is associated with unsaturation of the oils and it causes the formation of hydroperoxides that are the primary oxidation products. Lipid oxidation reduces the nutritional quality of oil by creating rancid odors and unpleasant flavors and also negatively impacts human health. Concentration of minor components with antioxidant or prooxidant properties affect on oxidative stability of oils. For instance, presence of tocopherols which are oil soluble natural antioxidant is important for oxidative stability while free fatty acids are prooxidative. Alteration of free fatty acid composition and addition of natural antioxidants to the oil provide strategies for improvement of the oxidative stability of oils [1, 46, 49, 106, 116, 117].

In order to produce minimally neutralized sunflower oil using weak alkalis as an alternative to sodium hydroxide used in conventional neutralization, it will be necessary to better define processing conditions. This chapter aimed to optimize neutralization operation using RSM to determine optimal processing conditions which would allow obtaining an oxidatively stable sunflower seed oil that is lower in FFA and higher in α-tocopherol.

5.2 Materials and Methods

5.2.1 Materials

Crude sunflower oil was used in all experiments (A&M Gourmet Foods Inc., Toronto, ON, Canada). Methanol, hexane, dioxane was purchased from Fisher Scientific (Fair Lawn, NJ, USA). p-anisidine, acetic acid, and iso-octane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Double distilled water was utilized in all experiments.

5.2.2 Degumming

Degumming was carried out according to the method described in Chapter 3.

5.2.3 Traditional Neutralization

Traditional neutralization was made according to the method described in Chapter 4.

5.2.4 Minimal Neutralization

Minimal neutralization was made according to the method described in Chapter 4.

5.2.5 Experimental Design for Optimization of Minimal Neutralization Process

Response Surface Methodology (RSM) was used to the experimental data using the statistical software (Design-Expert version 12.05.0, Minneapolis, MN, USA). For each dependent variable obtained from a mathematical model was created by using multiple regression analysis method. The independent variables were the process temperature (A) (40–70°C), the amount of alkali (B) (0.10-1.00%) and the reaction time (C) (10-30 minutes) for all three alkaline applications. FFA and tocopherol content were the two dependent variables in this design.

Design-Expert software [118] used the numerical optimization method to search for the optimum point to optimize process variables. The optimization of minimal neutralization process was carried out by RSM was used to determine optimal process conditions for each weak alkali and produce minimal neutralized oil with minimum FFA content and maximum α-tocopherol retention. The determined independent variable values and the neutralization process of the oil were performed with 3 replications. The dependent variables were examined and optimization was verified experimentally. Thus, due to the experimental design, a total of 69 neutralization production experiments were carried out for the production of minimally neutral oil. These are 20 tests per alkali application and 3 tests under optimum production conditions. Response surface plots and regression analysis were generated with the same software to find optimal conditions and figure out how the relations of independent variables impacted the response. RSM including three independent factors and five-level central composite design was preferred to optimize the three independent variables, specifically temperature (°C), amount of alkali (%) and reaction time (minute) (Tables 5.1 and 5.2). The five coded levels (-1.68, -1, 0, 1, 1.68) were applied to each of the three variables in the design (Table 5.1), resulting in 20 experimental runs (Table 5.2).

Table 5.1 Coded and actual levels of independent variables used for central composite design in the minimal neutralization of sunflower seed oil

		Independent variable level codes				
Independent variables	**Code**	-1.68	-1	0	1	1.68
Process Temperature(°C)	A	39.86	46	55	64	70.14
Amount of Alkali (%)	B	0.10	0.28	0.55	0.82	1.00
Reaction Time(min)	C	9.91	14	20	26	30.09

Table 5.2 Experimental design with different combinations of temperature, alkali amount and time in the minimal neutralization of sunflower seed oil

	Coded variables			Actual variable values		
Run no	**A**	**B**	**C**	**Process Temperature (°C)**	**Amount of Alkali (%)**	**Reaction Time (min)**
1	-1	-1	-1	46	0.28	14
2	1	-1	-1	64	0.28	14
3	-1	1	-1	46	0.82	14
4	1	1	-1	64	0.82	14
5	-1	-1	1	46	0.28	26
6	1	-1	1	64	0.28	26
7	-1	1	1	46	0.82	26
8	1	1	1	64	0.82	26
9	-1.68	0	0	39.86	0.55	20
10	1.68	0	0	70.14	0.55	20
11	0	-1.68	0	55	0.10	20
12	0	1.68	0	55	1.00	20
13	0	0	-1.68	55	0.55	9.91
14	0	0	1.68	55	0.55	30.09
15	0	0	0	55	0.55	20
16	0	0	0	55	0.55	20
18	0	0	0	55	0.55	20
18	0	0	0	55	0.55	20
19	0	0	0	55	0.55	20
20	0	0	0	55	0.55	20

5.2.6 Methods of Analysis

5.2.6.1 Determination of Free Fatty Acids

Determination of Free Fatty Acids was made according to the method described in Chapter 3.

5.2.6.2 Determination of α-Tocopherol Content

Fat samples (50-60 mg) were dissolved in 10 ml hexane and filtered with a 0.2 µm syringe filter. Each sample (20µL) was injected into HPLC system (LC-2030 Shimadzu, Marlborough, MA) system equipped with a supelcosil LC- Diol column (250mm x 4.0mm, 5 µm). The mobile phase was a mixture of hexane and dioxane (19:1 v/v). Flow rate was 1mL/min. A Shimadzu RF-20A fluorescence detector was used to determine tocopherol homologs at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Peak integration was conducted using Shimadzu EZstart software (version 7.2). Quantification of alpha-tocopherol in the samples was calculated based on an α-Tocopherol of the standard curve ($y = 19269x – 20495$; $R^2 =0.9402$) [119-121].

5.2.6.3 Free Radical Scavenging Capacity

Free radical scavenging capacity was determined according to the method described in Chapter 3.

5.2.6.4 Determination of Total Phenolics Content

Total phenolics content was determined according to the method described in Chapter 4.

5.2.6.5 Measurement of Lipid Hydroperoxides

Lipid hydroperoxides was detected according to the method described in Chapter 3.

5.2.6.6 Measurement of Headspace Hexanal

Headspace hexanal was measured according to the method described in Chapter 3.

5.2.6.7 Determination of Para-Anisidine Value (p-AV)

Sunflower oils (2 g) were dissolved in 25 ml iso-octane. This mixture was called as fat solution. Absorbance of this solution was determined at 350 nm using UV-visible spectrophotometer (Genesys 150, Thermo Scientific, Waltham, MA, USA). Five millilitres of the above mixture was mixed with 1 ml 0.25% p-anisidine in acetic acid (w/v). After 10 minutes incubation of absorbance was measured at 350 nm using a spectrophotometer. p-AV was calculated according to the following equation 5.2:

$$p - AV = \frac{25 * (1.2 * As - Ab)}{m} \quad (5.1)$$

where, A_s is the absorbance of the fat solution after reaction with the p-anisidine reagent; A_b is the absorbance of the fat solution without anisidine; m is the mass of sunflower oil (g) sample [106].

5.2.6.8 Statistical Analysis

One way ANOVA and Duncan's test were performed for α-tocopherol content, p-Anisidine value, FFA and TPC content to determine the significant differences amongst the means of the three replications ($P<0.05$) using the SPSS version 23.0. (IBM Corp. 2015, IBM SPSS, Armonk, NY). One sample t-test was conducted to compare the mean actual values of the responses with the predicted values [105]. Response surface methodology was used for experimental design. ANOVA was applied to evaluate the significant effects of independent variables on the responses. The significance of coefficients of fitted models was assessed by using p value, lack of fit and coefficient of variation. A mathematical model was created by using multiple regression analysis describing the effect of each variable on the responses. RSM was successfully established with regression analyses to estimate the impacts of different conditions on the minimal refining process [118].

5.3 Results and Discussion

In this chapter sunflower seed oil was neutralized by using NaOH (as alkali used in traditional neutralization process) and $Ca(OH)_2$, MgO, and Na_2SiO_3. Traditionally neutralized oil and those that are neutralized by alternative alkalis were compared to each other with respect to the following variables. Also, the oils neutralized at optimal

processing conditions were stored at 55°C for lipid oxidation stability studies. The effect of neutralization process independent variables (process temperature, amount of alkali used, reaction time) on free fatty acids content, α- tocopherol content, free radical scavenging capacity, total phenolics content, hydroperoxide content, headspace hexanal content and p-anisidine value were investigated. Multiple regression analyses were carried out using response surface analysis to define the relationship between independent variables and responses.

5.3.1 Experimental Design and Fitting the Models

Twenty experiment runs were performed with the central composite design and the responses (FFA and α-tocopherol content) are demonstrated in Table 5.3. The FFA of neutralized sunflower oil by $Ca(OH)_2$ ranged from 0.02 to 0.2 % while α-tocopherol content of neutralized sunflower oil by $Ca(OH)_2$ changed from 87.13 to 175.9 ppm. The FFA of neutralized sunflower oil by MgO changed from 0.10 to 0.30 % while α-tocopherol content of neutralized sunflower oil by MgO varied from 103.43 to 181.22 ppm. The FFA of neutralized sunflower oil by Na_2SiO_3 changed from 0.07 to 0.38 % while α-tocopherol content of neutralized sunflower oil by Na_2SiO_3 ranged from 180.59 to 229.25 ppm.

The FFA and α-tocopherol content of the minimal neutralized sunflower oils were analyzed by multiple regression models. These responses were defined by the regression analysis to estimate the dependent variable as a function of different levels of three independent factors and the impacts of these factors on the minimal neutralization.

The mathematical models between FFA, α-tocopherol concentration and the process parameters suggested the quadratic model. That means the process variables have quadratic effects on the responses significantly ($p<0.05$). Six polynomial quadratic models for FFA and α-tocopherol contents were given in Table 5.4 while treated with $Ca(OH)_2$ (CF), MgO (MF), Na_2SiO_3 (NF) and $Ca(OH)_2$ (CT), MgO (MT) and Na_2SiO_3 (NT), respectively. Regression analysis showed that the fitted models had a coefficient of determination (R^2) that varied from 0.7760 to 0.9836 (Table 5.4).

Coefficients (R^2 and adjusted R^2) were utilized to evaluate the accuracy of the models. Adequate precision is a measure of the signal to noise ratio which must be bigger than 4. The R^2 and the adjusted R^2 were 0.7760 and 0.5744 respectively for FFA in neutralized sunflower oil by $Ca(OH)_2$. The observed adequate precision of 7.562 indicated an adequate signal. The R^2 value was 0.8662 and the adjusted R^2 was 0.7458 for FFA in neutralized sunflower oil by MgO. The observed adequate precision of 10.577 indicated an adequate signal and the R^2 value was 0.9836 and the adjusted R^2 was 0.9688 for FFA in neutralized sunflower oil by Na_2SiO_3. The observed adequate precision of 30.533 indicated an adequate signal.

The R^2 and the adjusted R^2 were 0.8527 and 0.7200 respectively for α-tocopherol content in neutralized sunflower oil by $Ca(OH)_2$. The observed adequate precision of 7.374 indicated an adequate signal. The R^2 and the adjusted R^2 were 0.7780 and 0.5782 respectively for α-tocopherol content in neutralized sunflower oil by MgO. The observed adequate precision of 7.902 indicated an adequate signal. the R^2 was 0.8295 and the adjusted R^2 was 0.6760 for α-tocopherol content in neutralized sunflower oil by Na_2SiO_3. The observed adequate precision of 6.730 indicated an adequate signal.

Table 5.3 Experimental data with different combinations of temperature, amount of alkali and time in the minimal neutralization of sunflower oils

Run no	Actual variable values			Responses					
				FFA content (oleic acid %)			α-Tocopherol content (ppm)		
	Process Temperature (°C)	Amount of Alkali (%)	Reaction Time (min)	$Ca(OH)_2$	MgO	Na_2SiO_3	$Ca(OH)_2$	MgO	Na_2SiO_3
1	46	0.28	14	0.05	0.17	0.37	144.36	138.75	199.17
2	64	0.28	14	0.04	0.2	0.35	87.44	146.1	216.01
3	46	0.82	14	0.02	0.13	0.15	133.54	103.43	210.99
4	64	0.82	14	0.03	0.1	0.16	87.13	140.92	211.86
5	46	0.28	26	0.05	0.16	0.37	100.65	181.22	180.59
6	64	0.28	26	0.05	0.13	0.35	164.04	160.06	211.09
7	46	0.82	26	0.02	0.15	0.13	119.55	175.7	181.14
8	64	0.82	26	0.02	0.1	0.16	175.9	159.26	211.63
9	39.86	0.55	20	0.04	0.1	0.21	131.97	178.39	197.37
10	70.14	0.55	20	0.02	0.11	0.22	145.61	165.6	195.58
11	55	0.10	20	0.2	0.3	0.38	117.69	131.47	229.25
12	55	1.00	20	0.03	0.1	0.07	122.68	114.79	204.16
13	55	0.55	9.91	0.02	0.12	0.28	134.51	111.17	199.41
14	55	0.55	30.09	0.03	0.12	0.25	96.59	110.54	184.24
15	55	0.55	20	0.02	0.12	0.28	160.36	142.42	219.94
16	55	0.55	20	0.03	0.12	0.29	160.97	143.15	218.14
18	55	0.55	20	0.02	0.11	0.28	159.96	143.09	218.88
18	55	0.55	20	0.03	0.11	0.27	160.06	141.82	219.54
19	55	0.55	20	0.03	0.12	0.28	161.64	142.35	217.98
20	55	0.55	20	0.02	0.12	0.27	160.79	142.20	218.50

Table 5.4 The regression models obtained by using independent variables temperature (A), amount alkali (B) and reaction time (C)

Response	Regression Model	R^2
CF	0.0208-0.0025A-0.0283B+0.0012C+0.0025AB -0.0025BC-0.0015A^2+0.0285B^2-0.0033C^2	0.7760
MF	0.1200-0.0046A-0.0378B-0.0044C-0.0100AB -0.0100AC+0.0125BC-0.0054A^2+0.0282B^2-0.0001C^2	0.8662
NF	0.2793+0.0012A-0.0997B-0.0052C+0.0100AB +0.0025AC-0.0025BC-0.0182A^2-0.0146B^2-0.0005C^2	0.9836
CT	160.23+2.88A+2.05B+3.21C+0.44AB +27.88AC+5.24BC-6.81A^2-13.38B^2-15.02C^2	0.8527
MT	141.91-1.04A-5.48B+10.69C+4.36AB -10.31AC+4.27BC+13.79A^2-3.49B^2-7.83C^2	0.7780
NT	219.87+5.54A-2.45B-5.79C-2.00AB +5.41AC-0.82BC-7.82A^2-0.67B^2-9.47C^2	0.8295

5.3.2 Evaluation of the Effects of Process Variables on Responses

Two responses (FFA and α-tocopherol content) were chosen for determining the most useful and acceptable oil product. Sunflower oil is very rich in α-tocopherol content and has high FFA content. Due to this fact, α-tocopherol and FFA content of the oil product were measured. The effects of independent variables (temperature, amount of alkali and time) on responses were estimated to detect optimum conditions. The regression models described the impacts of process variables by quadratic equation. The influences of process variables were indicated by 3D response surface graph.

5.3.2.1 Effect of neutralization parameters on free fatty acid content

FFA value is one of the significant factors to show the acceptability and quality of oils [112]. The refining removes FFA content so FFA content was the one of two responses that measured to evaluate the effects of the process temperature, time and amount of alkali parameters for optimizing reduction of FFA in sunflower oil. ANOVA results suggested that the quadratic models were more suitable for the response of FFA content for all minimally neutralized sunflower seed oil.

Table 5.5 indicates the ANOVA results and estimated coefficients for FFA content of minimal neutralized sunflower oils by $Ca(OH)_2$. According to the Table 5.5, quadratic model was significant ($p<0.05$). The lack of fit of the model was non-significant ($p>0.05$). That is the model chosen "properly" describes the experimental result. Well-fitting model for FFA for minimal sunflower seed oil refined by $Ca(OH)_2$ were successfully generated. According to the ANOVA results, temperature (A) and time (C) is not significant ($p>0.05$). However, amount of alkali (B) affected FFA content of the sunflower seed oil refined by $Ca(OH)_2$ significantly ($p<0.05$). It means that increase in alkali amount of sunflower oil refined by $Ca(OH)_2$ results in increased FFA content. The interactive effect between independent variables was not significant ($p>0.05$).

Table 5.5 Analysis of variance results and estimated coefficients for quadratic model on FFA content of sunflower oil neutralized by $Ca(OH)_2$ (CF) [A: Temperature (°C), B: Amount of Alkali (%), C: Time (min)]

Source	CF					
	Coefficients	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**
Model: Quadratic		0.0236	9	0.0026	3.85	0.0236*
Intercept	+0.0208					
A	-0.0025[b]	0.0001	1	0.0001	0.1216	0.7346
B	-0.0283[a]	0.0109	1	0.0109	16.00	0.0025
C	+0.0012[b]	0.0000	1	0.0000	0.0304	0.8651
AB	+0.0025[b]	0.0000	1	0.0000	0.0734	0.7920
AC	+0.0000[b]	0.0000	1	0.0000	0.0000	1.0000
BC	-0.0025[b]	0.0001	1	0.0001	0.0734	0.7920
A^2	-0.0015[b]	0.0000	1	0.0000	0.0480	0.8311
B^2	+0.0285[a]	0.0117	1	0.0117	17.23	0.0020
C^2	-0.0033[b]	0.0002	1	0.0002	0.2266	0.6443
Residual		0.0068	10	0.0007		
Lack of Fit		0.0068	5	0.0014	2	0.1642**

[a]Significant at p-value<0.05[b]Not significant at p-value>0.05
*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.7760

Response surface plots for the effect of independent parameters on the FFA values of sunflower seed oils refined by $Ca(OH)_2$ are shown in Figures 5.1-5.3. FFA content of sunflower oil refined by $Ca(OH)_2$ remained constant while temperature was increased from 46 to 64°C (Figure 5.1). In a similar way, FFA content did not change while time was increased from 14 to 26 min (Figure 5.2). FFA content decreased when alkali amount was increased from 0.26 to 0.55 % while FFA content remain constant when alkali amount was increased from 0.55 to 0.82 % as indicated in Figure 5.1 and 5.3.

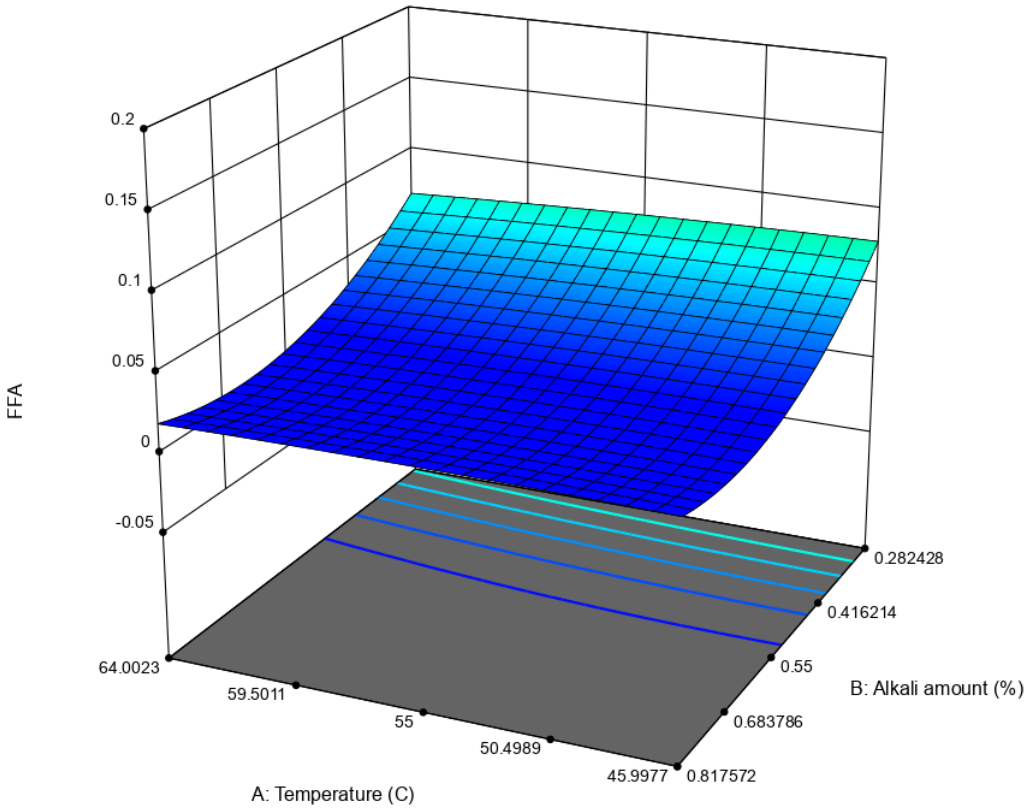

Figure 5.1 Response surface plot for the effect of temperature and alkali amount on the free fatty acid content of sunflower oil refined by Ca(OH)

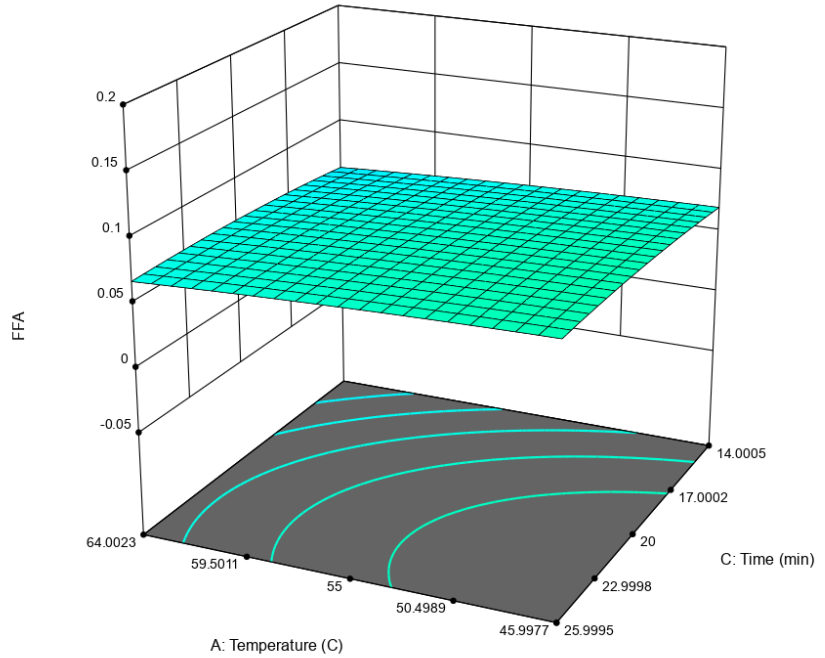

Figure 5.2 Response surface plot for the effect of temperature and time on the free fatty acid content of sunflower oil refined by $Ca(OH)_2$

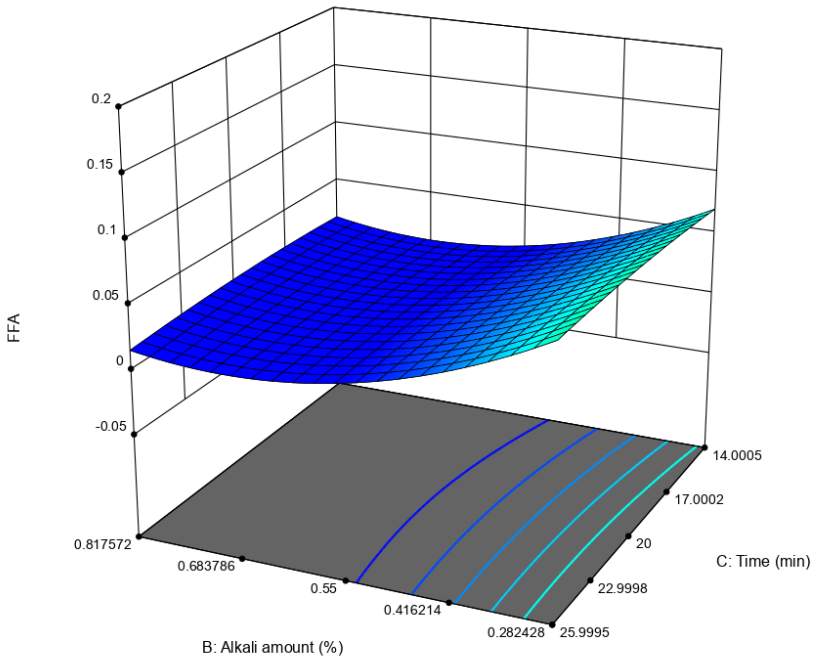

Figure 5.3 Response surface plot for the effect of time and alkali amount on the free fatty acid content of sunflower oil refined by $Ca(OH)_2$

Table 5.6 indicates the ANOVA results and estimated coefficients for FFA content of minimally neutralized sunflower oils by MgO. According to the Table 5.6, quadratic model was significant ($p<0.05$) indicates the excellent fit of the experimental data and the lack of fitness of the model were non-significant ($p>0.05$). Lack of fit test for the response of FFA content were insignificant ($p>0.05$). That is the model sufficiently chosen describes the experimental result. Thus, well-fitting model for FFA for minimal sunflower seed oil refined by MgO was generated successfully. ANOVA results showed that time (C) and temperature (A) had no effect on the FFA content of sunflower oil refined by MgO at the 95% confidence level. However, FFA content of sunflower oil refined by MgO changed quadratically with alkali amount (B). The interactive effect between independent variables was not significant ($p>0.05$).

Table 5.6 Analysis of variance results and estimated coefficients for quadratic model on FFA content of sunflower oil neutralized by MgO (MF) [A: Temperature (°C), B: Amount of Alkali (%), C: Time (min)]

Source	MF					
	Coefficients	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**
Model: Quadratic		0.0354	9	0.0039	7.19	0.0024*
Intercept	+0.1200					
A	-0.0046[b]	0.0003	1	0.0003	0.5342	0.4816
B	-0.0378[a]	0.0195	1	0.0195	35.68	0.0001
C	-0.0044[b]	0.0003	1	0.0003	0.4818	0.5034
AB	-0.0100[b]	0.0008	1	0.0008	1.46	0.2544
AC	-0.0100[b]	0.0008	1	0.0008	1.46	0.2544
BC	+0.0125[b]	0.0013	1	0.0013	2.28	0.1616
A^2	-0.0054[b]	0.0004	1	0.0004	0.7694	0.4010
B^2	+0.0282[a]	0.0114	1	0.0114	20.92	0.0010
C^2	-0.0001[b]	1.485E-07	1	1.485E-07	0.0003	0.9872
Residual		0.0055	10	0.0005		
Lack of Fit		0.0055	5	0.0011	2.2	0.1352**

[a]Significant at p-value<0.05[b]Not significant at p-value>0.05
*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.8662

Response surface plots for the effect of independent parameters on the FFA values of sunflower seed oils refined by MgO are shown in Figures 5.4-5.6. A slight decrease was observed in FFA content of sunflower oil refined by MgO with increasing time while FFA stayed the constant while the temperature was enhanced from 46 to 64°C (Figure 5.5). FFA content decreased with increasing alkali amount as indicated in Figure 5.4 and 5.6.

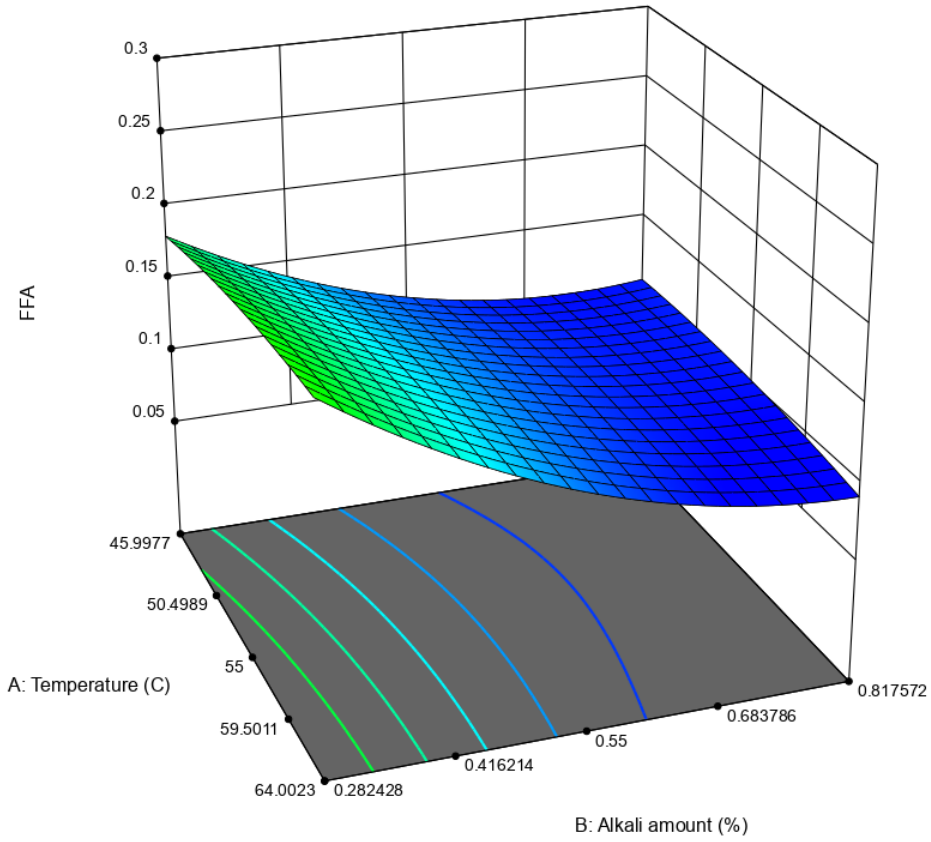

Figure 5.4 Response surface plot for the effect of temperature and alkali amount on the free fatty acid content of sunflower oil refined by MgO

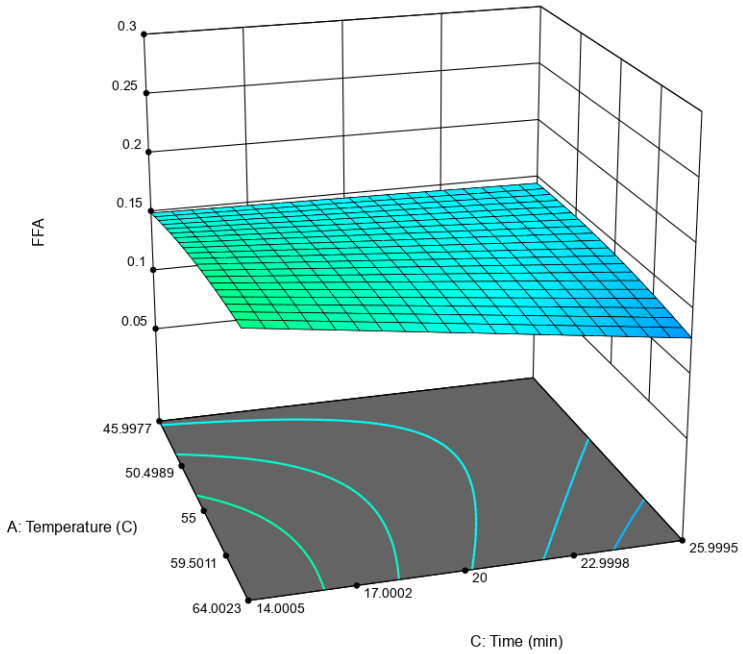

Figure 5.5 Response surface plot for the effect of temperature and time on the free fatty acid content of sunflower oil refined by MgO

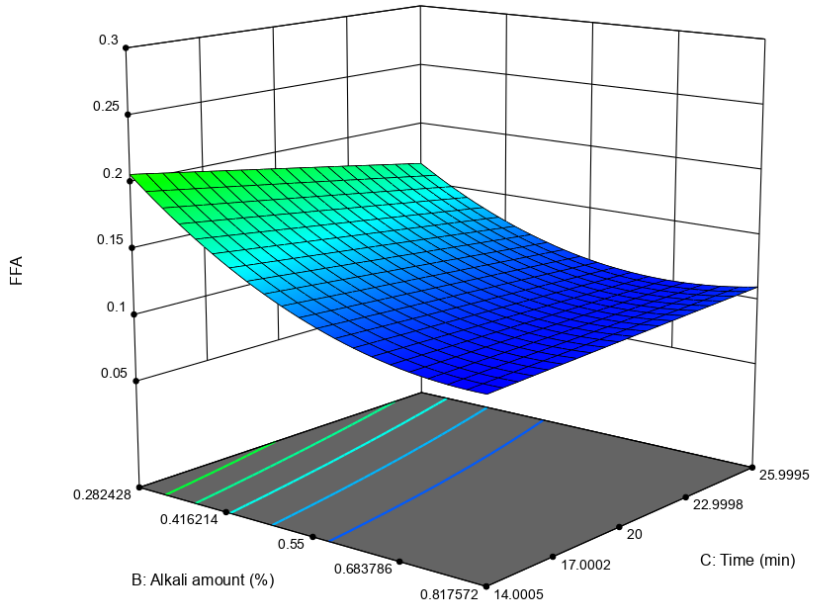

Figure 5.6 Response surface plot for the effect of time and alkali amount on the free fatty acid content of sunflower oil refined by MgO

Table 5.7 indicates the ANOVA results and estimated coefficients for FFA content of minimal neutralized sunflower seed oils by Na_2SiO_3. According to the Table 5.7, quadratic model was significant ($p<0.05$) indicates the excellent fit of the experimental data and the lack of fitness of the model were non-significant ($p>0.05$). Lack of fit test for the response of FFA content were insignificant ($p>0.05$). That is the model properly chosen describes the experimental result. Thus, well-fitting model for FFA for minimal sunflower seed oil refined by Na_2SiO_3 were successfully generated. ANOVA results showed that the linear terms for temperature (A) and time (C), the second-order terms of time (C^2), The interaction terms between temperature and alkali amount (AB), temperature and time (AC), and alkali amount and time (BC) exerted statistically no significant impacts ($p>0.05$) on the FFA content of minimal neutralized sunflower oils while alkali amount (B), the second-order terms of temperature (A^2) and time (C^2) were found to be statistically significant ($p<0.05$) terms affecting FFA content.

Table 5.7 Analysis of variance results and estimated coefficients for quadratic model on FFA content of sunflower oil neutralized by Na_2SiO_3 (NF) [A: Temperature (°C), B: Amount of Alkali (%), C: Time (minute)]

Source	NF					
	Coefficients	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**
Model: Quadratic		0.1442	9	0.0160	66.45	<0.0001*
Intercept	+0.2793					
A	+0.0012[b]	0.0000	1	0.0000	0.0859	0.7755
B	-0.0997[a]	0.1357	1	0.1357	562.65	<0.0001
C	-0.0052[b]	0.0004	1	0.0004	1.51	0.2477
AB	+0.0100[b]	0.0008	1	0.0008	3.32	0.0986
AC	+0.0025[b]	0.0000	1	0.0000	0.2073	0.6586
BC	-0.0025[b]	0.0000	1	0.0000	0.2073	0.6586
A^2	-0.0182[a]	0.0048	1	0.0048	19.76	0.0012
B^2	-0.0146[a]	0.0031	1	0.0031	12.82	0.0050
C^2	-0.0005[b]	3.701E-06	1	3.701E-06	0.0153	0.9039
Residual		0.0024	10	0.0002		
Lack of Fit		0.0024	5	0.0005	2.5	0.1020**

[a]Significant at p-value<0.05[b]Not significant at p-value>0.05
*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.9836

According to overall ANOVA results, it was concluded that the the alkali amount had the strongest effect on the decrease of the FFA content. While temperature and time could have no significant effect on the FFA content for all type refined sunflower oils (Table 5.5-5.7). FFA content of sunflower seed oil refined by Na_2SiO_3 decreased with increasing alkali amount and while FFA stayed at the same level when the temperature was raised from 46 to 64°C (Figure 5.7). Finally, FFA content did not change when time changed as shown in Figure 5.8 and 5.9.

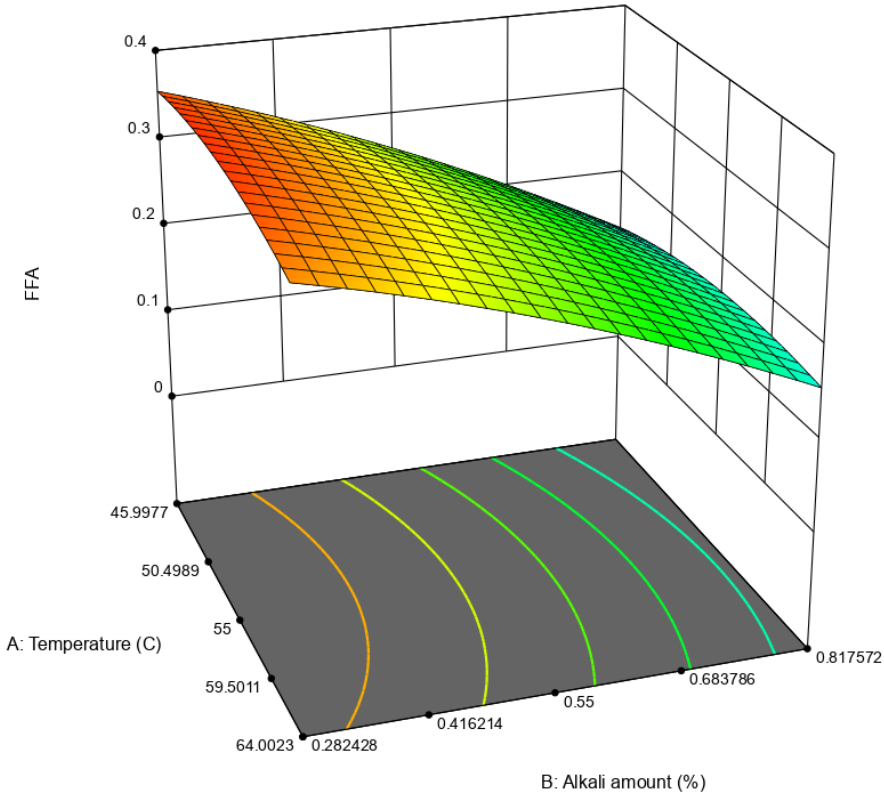

Figure 5.7 Response surface plot for the effect of temperature and alkali amount on the free fatty acid content of sunflower oil refined by Na_2SiO_3

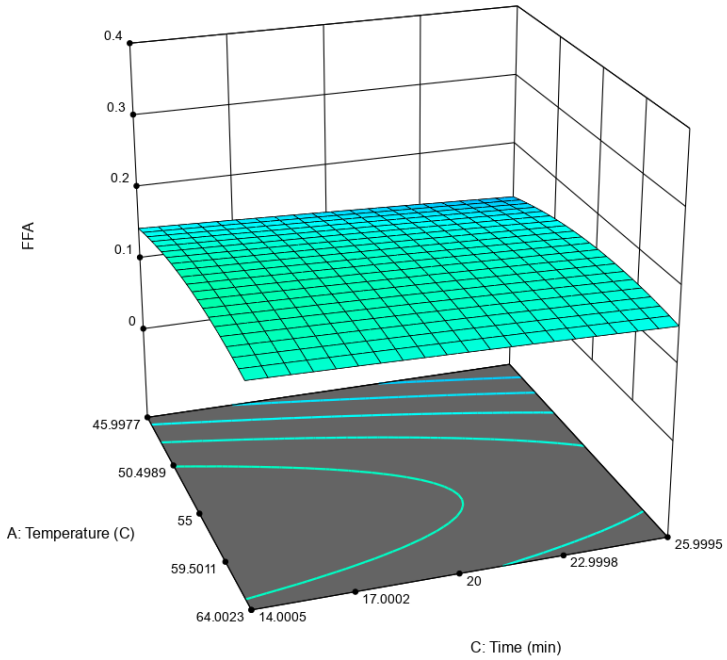

Figure 5.8 Response surface plot for the effect of temperature and time on the free fatty acid content of sunflower oil refined by Na_2SiO_3

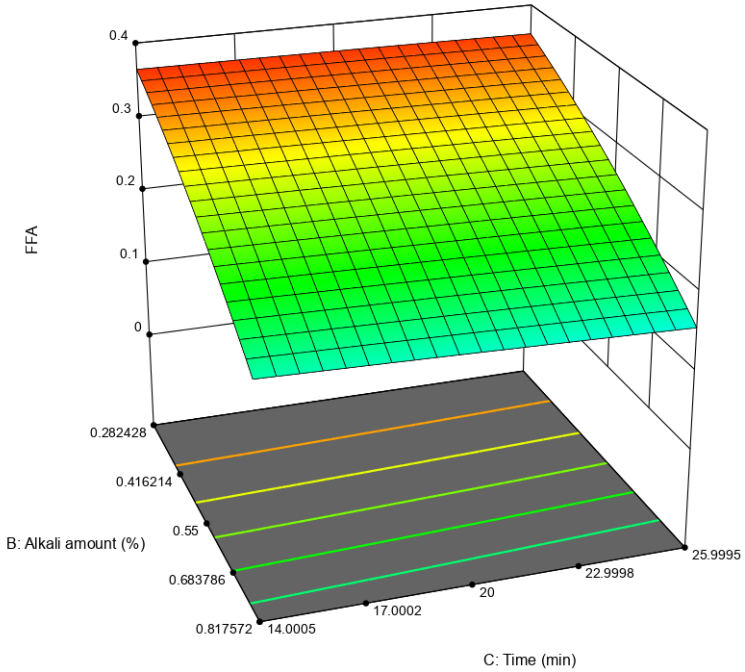

Figure 5.9 Response surface plot for the effect of time and alkali amount on the free fatty acid content of sunflower oil refined by Na_2SiO_3

Results of these response surface plots demonstrated that alkali amount is a powerful variable for changing the FFA content of the sunflower seed oil. As the alkali amount is increased this would decrease FFA content giving rise to obtain a better sunflower seed oil.

5.3.2.2 Effect of neutralization parameters on α-tocopherol content

Tocopherols, also known as Vitamin E, are well known antioxidants molecules naturally found in vegetable oils. There are four forms α-, β-, γ- and δ-tocopherol [7]. Since the most abundant tocopherol in sunflower oil is α- tocopherol, its content was the one of two responses that measured to evaluate the effects of the process temperature, time and amount of alkali parameters and interactions between these parameters for optimizing α-tocopherol retention in sunflower seed oil. ANOVA results offered that the quadratic models were more convenient for the response of α-tocopherol content for all minimally neutralized sunflower seed oil.

Table 5.8 shows the ANOVA results and estimated coefficients for the α-tocopherol content of minimal neutralized sunflower oils by $Ca(OH)_2$. According to the Table 5.8, quadratic model was significant ($p<0.05$). The lack of fitness of the model was insignificant ($p>0.05$). That is the model properly chosen describes the experimental data. Well-fitting model for α-tocopherol for minimal sunflower seed oil refined by $Ca(OH)_2$ were successfully generated. Among the three parameters, the first-order terms for temperature (A) and alkali amount (B) and time (C), the interaction terms between temperature and alkali amount (AB) and the interaction terms alkali amount and time (BC) exerted statistically no significant impacts ($p>0.05$) on the α-tocopherol content of minimal neutralized sunflower oil. However, the quadratic terms for temperature (B^2), temperature and time (AC) statistically ($p<0.05$) affected α-tocopherol concentration in sunflower oil refined by $Ca(OH)_2$.

Table 5.8 Analysis of variance results and estimated coefficients for quadratic model on α-tocopherol content of sunflower oil neutralized by $Ca(OH)_2$ (CT) [A: Temperature (°C), B: Amount of Alkali (%), C: Time (min)]

Source	CT					
	Coefficients	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**
Model: Quadratic		12326.93	9	1369.66	6.43	0.0038*
Intercept	+160.23					
A	+2.88[b]	113.38	1	113.38	0.5322	0.4824
B	+2.05[b]	57.50	1	57.50	0.2699	0.6147
C	+3.21[b]	141.09	1	141.09	0.6623	0.4347
AB	+0.4338[b]	1.51	1	1.51	0.0071	0.9347
AC	+27.88[a]	6220.03	1	6220.03	29.20	0.0003
BC	+5.24[b]	219.35	1	219.35	1.03	0.3342
A^2	-6.81[b]	667.45	1	667.45	3.13	0.1071
B^2	-13.38[a]	2581.25	1	2581.25	12.12	0.0059
C^2	-15.02[b]	3252.07	1	3252.07	15.27	0.0029
Residual		2130.21	10	213.02		
Lack of Fit		2130.21	5	426.04	2	0.1642**

[a]Significant at p-value<0.05[b]Not significant at p-value>0.05
*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.8527

The 3-D response surface plots for the effect of independent parameters on α-tocopherol content of sunflower seed oils refined by $Ca(OH)_2$ are shown in Figures 5.10-5.12. The α-tocopherol content of sunflower oil increased when alkali amount was increased from 0.26 to 0.55 %, while α-tocopherol content decreased when alkali amount was increased from 0.55 to 0.82 % (Figure 5.10).

Similarly, α-tocopherol content of sunflower oil refined by $Ca(OH)_2$ increased when time was raised from 14 to 20 minutes, while α-tocopherol content reduced when time was enhanced from 20 to 26 minutes (Figure 5.12). According to Figure 5.11 α-tocopherol content reduced when temperature was raised from 55 to 64°C.

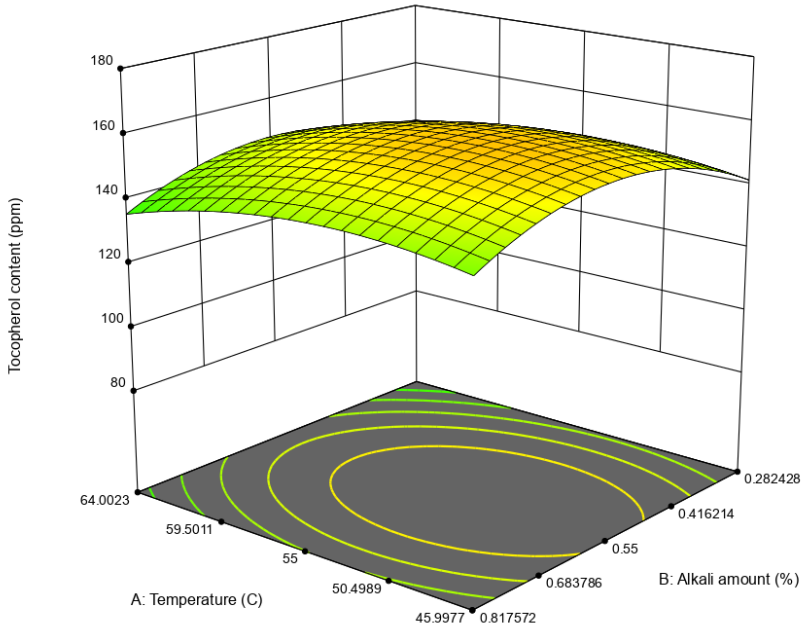

Figure 5.10 Response surface plot for the effect of temperature and alkali amount on the α-tocopherol content of sunflower oil refined by $Ca(OH)_2$

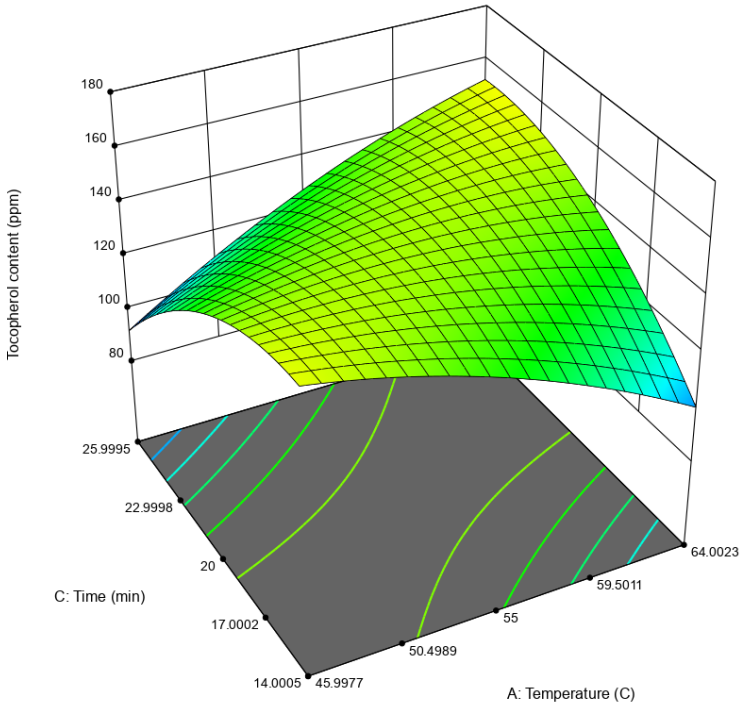

Figure 5.11 Response surface plot for the effect of temperature and time on the α-tocopherol content of sunflower oil refined by $Ca(OH)_2$

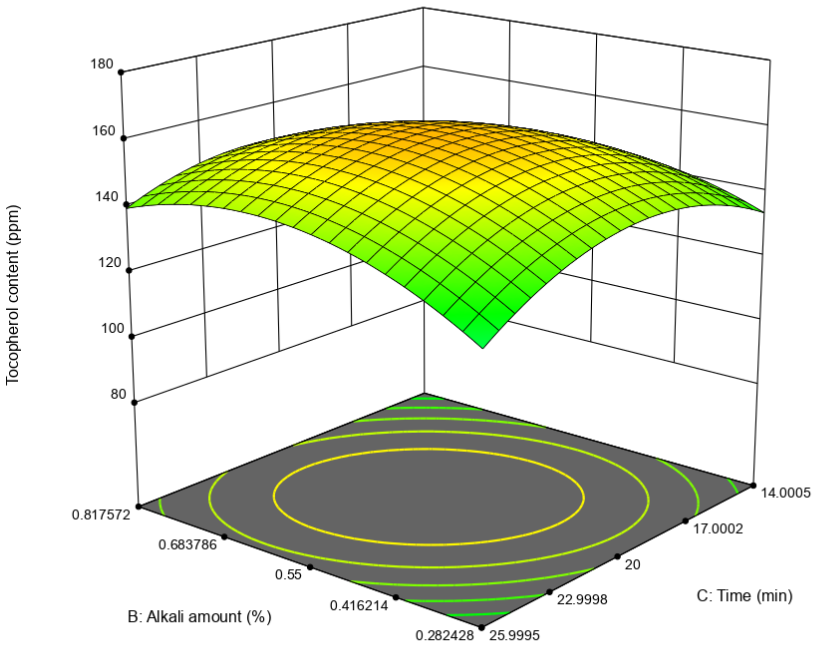

Figure 5.12 Response surface plot for the effect of time and alkali amount on the α-tocopherol content of sunflower oil refined by $Ca(OH)_2$

Table 5.9 indicates the ANOVA results and estimated coefficients for the α-tocopherol content of minimal neutralized sunflower oils by MgO. According to the Table 5.9, quadratic model was significant ($p<0.05$) indicates the excellent fit of the experimental data and the lack of fitness of the model were insignificant ($p>0.05$) for the response of α-tocopherol content. That is the model properly chosen describes the experimental data. Well-fitting model for α-tocopherol for minimal sunflower seed oil refined by MgO were successfully generated. Among the three parameters, the first-order terms for temperature (A) and alkali amount (B), the cross-product terms between temperature and alkali amount (AB) and the interaction terms alkali amount and time (BC) had no significant effects ($p>0.05$) on the α-tocopherol content of minimal neutralized sunflower oil. On the other hand, the linear terms for time (C) highly significantly affected ($p<0.05$) α-tocopherol content of sunflower oil refined by MgO.

Table 5.9 Analysis of variance results and estimated coefficients for quadratic model on α-tocopherol content of sunflower oil neutralized by MgO (MT) [A: Temperature (°C), B: Amount of Alkali (%), C: Time (min)]

Source		MT				
	Coefficients	Sum of Squares	Df	Mean Square	F-value	p-value
Model: Quadratic		7342.08	9	815.79	3.89	0.0227*
Intercept	+141.91					
A	-1.04[b]	14.91	1	14.91	0.0712	0.7950
B	-5.48[b]	410.48	1	410.48	1.96	0.1918
C	+10.69[a]	1560.41	1	1560.41	7.45	0.0212
AB	+4.36[b]	151.90	1	151.90	0.7251	0.4144
AC	-10.31[b]	849.54	1	849.54	4.06	0.0717
BC	+4.27[b]	146.03	1	146.03	0.6971	0.4233
A^2	+13.79[a]	2739.34	1	2739.34	13.08	0.0047
B^2	-3.49[b]	175.46	1	175.46	0.8376	0.3816
C^2	-7.83[b]	883.36	1	883.36	4.22	0.0671
Residual		2094.86	10	209.49		
Lack of Fit		2094.86	5	418.97	1.99	0.1658**

[a]Significant at p-value<0.05[b]Not significant at p-value>0.05
*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.7780

The effect of independent parameters on α-tocopherol content of sunflower seed oils refined by MgO are shown in Figures 5.13-5.15. Figure 5.13 indicated that α-tocopherol content reduced when temperature was raised from 46 to 55°C, however it increased while temperature was enhanced from 55 to 64°C. α-tocopherol content enhanced when time was raised from 14 to 20 minutes, while α-tocopherol content reduced when time was raised from 20 to 26 minutes (Figure 5.14). According to Figure 5.15, α-tocopherol content of sunflower oil refined by MgO slightly decreased with increasing alkali amount.

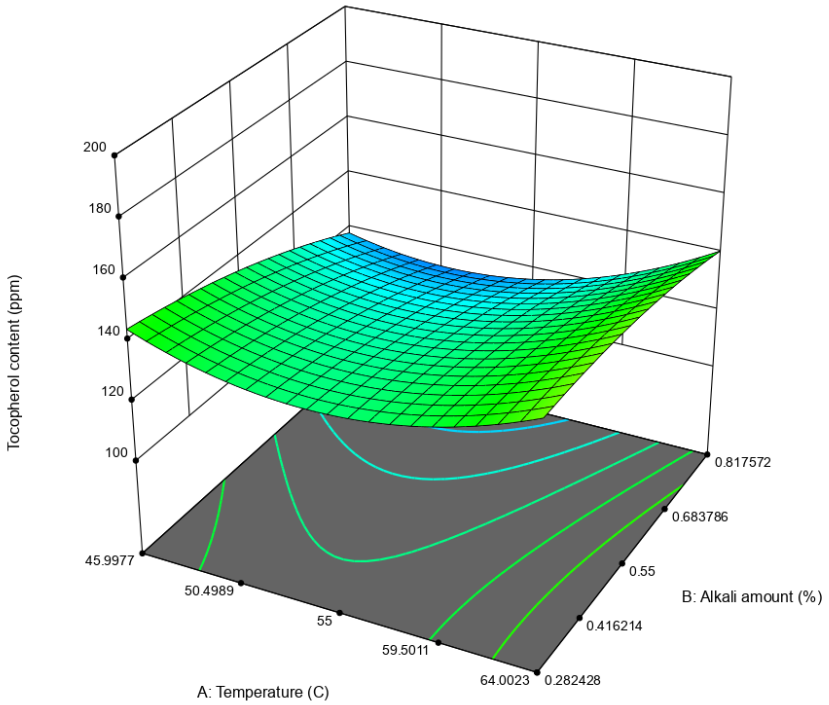

Figure 5.13 Response surface plot for the effect of temperature and alkali amount on the α-tocopherol content of sunflower oil refined by MgO

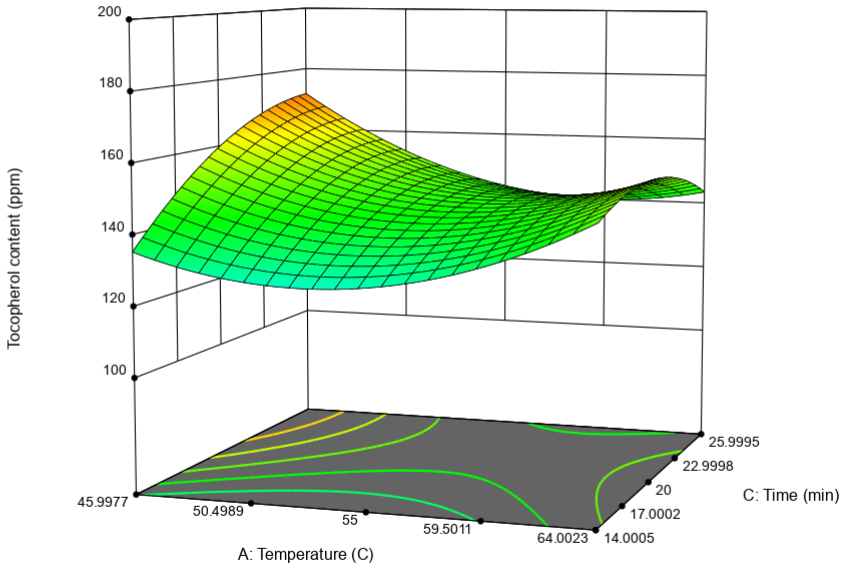

Figure 5.14 Response surface plot for the effect of temperature and time on the α-tocopherol content of sunflower oil refined by MgO

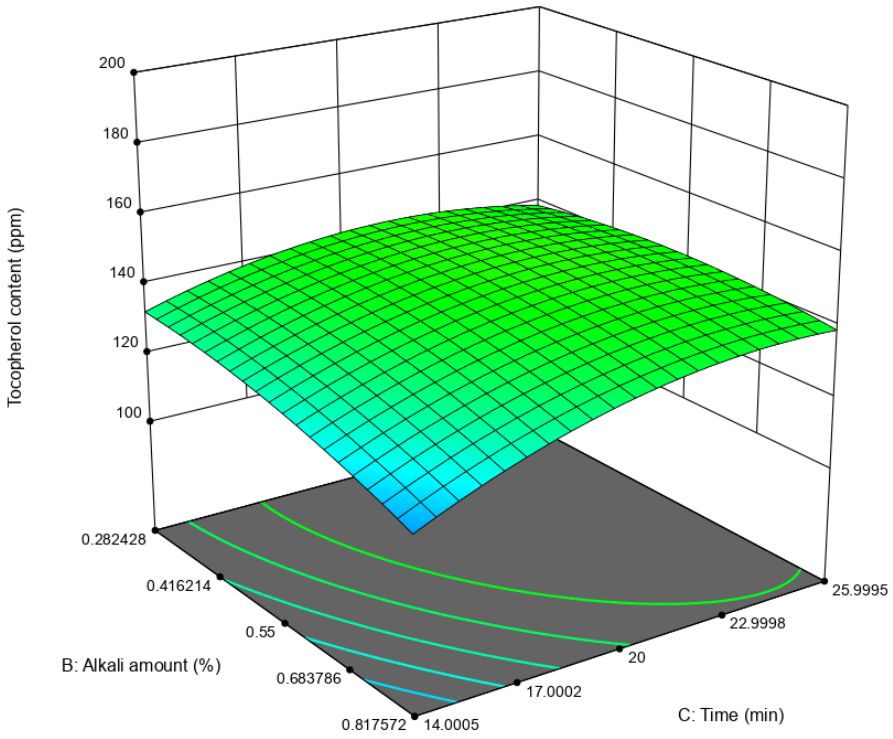

Figure 5.15 Response surface plot for the effect of time and alkali amount on the α-tocopherol content of sunflower oil refined by MgO

Table 5.10 indicates the ANOVA results and estimated coefficients for α-tocopherol content of minimal neutralized sunflower oils by Na_2SiO_3. According to the Table 5.10, quadratic model was significant ($p<0.05$) indicates the excellent fit of the experimental data and the lack of fitness of the model were insignificant. Lack of fit test for the response of α-tocopherol content were insignificant ($p>0.05$). That is the model properly chosen describes the experimental result. Well-fitting model for α-tocopherol for minimal sunflower seed oil refined by Na_2SiO_3 were successfully generated. Among the three parameters, the first-order terms for alkali amount (B), the cross-product terms between temperature and alkali amount (AB), the interaction terms temperature and time (AC) and the interaction terms alkali amount and time (BC) exerted statistically no significant impacts ($p>0.05$) on the α-tocopherol content of minimal neutralized sunflower seed oil. However, the linear terms for temperature (A) and time (C) significantly ($p<0.05$) affected α-tocopherol concentration in sunflower oil refined by Na_2SiO_3.

Table 5.10 Analysis of variance results and estimated coefficients for quadratic model on α-tocopherol content of sunflower oil neutralized by Na_2SiO_3 (NT) [A: Temperature (°C), B: Amount of Alkali (%), C: Time (minute)]

Source	NT					
	Coefficients	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**
Model: Quadratic		3222.96	9	358.11	5.40	0.0072*
Intercept	+219.87					
A	+5.54[a]	419.49	1	419.49	6.33	0.0306
B	-2.45[b]	81.86	1	81.86	1.24	0.2924
C	-5.79[a]	458.06	1	458.06	6.91	0.0252
AB	-2.00[b]	31.92	1	31.92	0.4817	0.5035
AC	+5.41[b]	234.14	1	234.14	3.53	0.0896
BC	-0.8225[b]	5.41	1	5.41	0.0817	0.7809
A^2	-7.82[a]	881.58	1	881.58	13.30	0.0045
B^2	-0.6689[b]	6.45	1	6.45	0.0973	0.7615
C^2	-9.47[a]	1291.15	1	1291.15	19.48	0.0013
Residual		662.67	10	66.27		
Lack of Fit		662.67	5	132.53	1.99	0.1658**

[a]Significant at p-value<0.05[b]Not significant at p-value>0.05
*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.8295

The effect of independent parameters on α-tocopherol content of sunflower seed oils refined by Na_2SiO_3 are shown in Figures 5.16-5.18. It was observed that α-tocopherol content of sunflower oil refined by Na_2SiO_3 stayed fairly constant with alkali amount (Figure 5.16 and 5.18). On the other hand, α-tocopherol content increased as temperature increased from 46 to 64°C, it also tended to decrease with increasing time (Figure 5.17). Results of these response surface plots indicated that time and temperature are more effective variables for altering the α-tocopherol content of the sunflower oil than alkali amount variable.

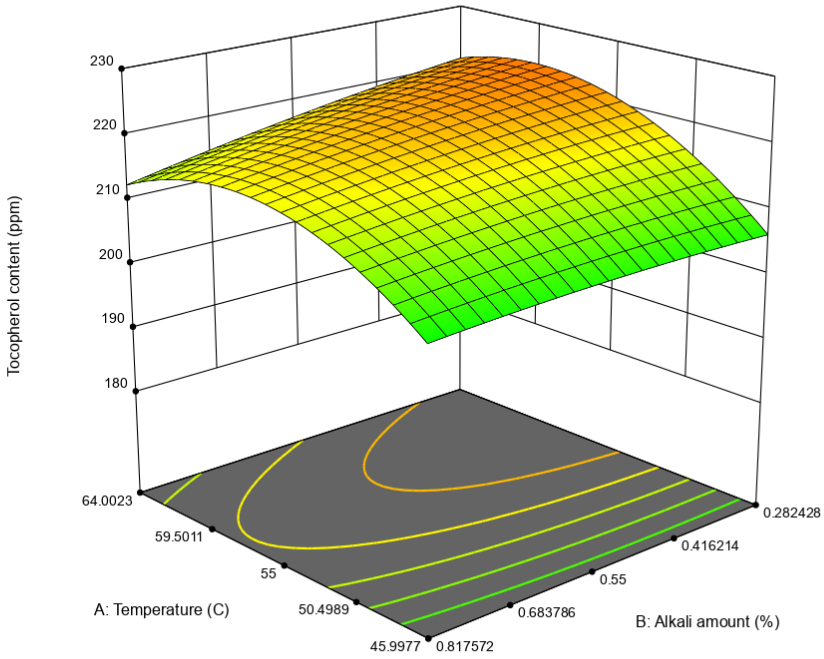

Figure 5.16 Response surface plot for the effect of temperature and alkali amount on the α-tocopherol content of sunflower oil refined by Na_2SiO_3

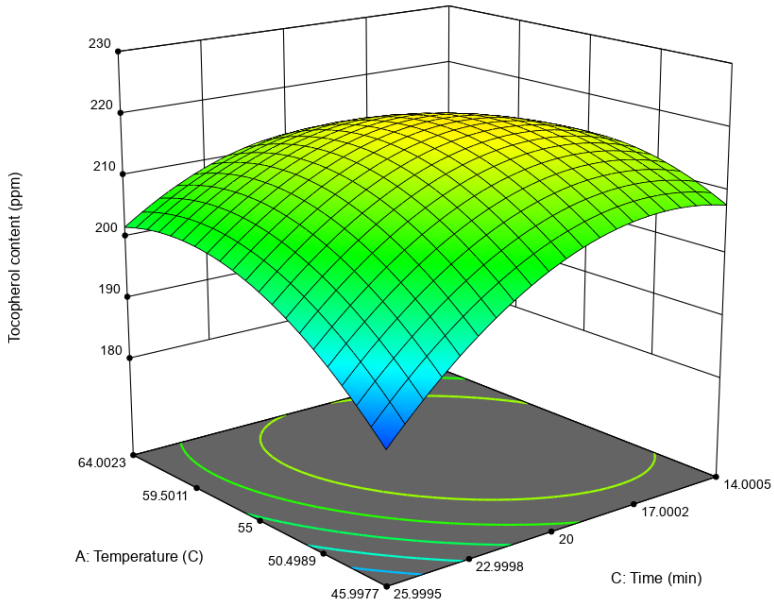

Figure 5.17 Response surface plot for the effect of temperature and time on the α-tocopherol content of sunflower oil refined by Na_2SiO_3

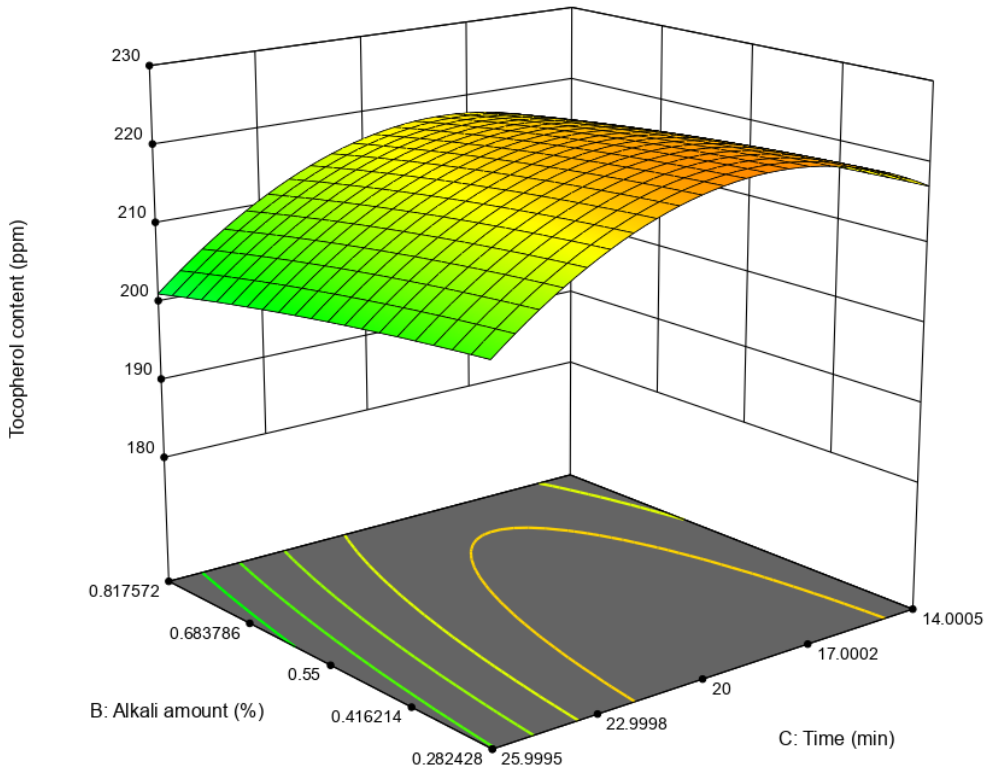

Figure 5.18 Response surface plot for the effect of alkali amount and time on the α-tocopherol content of sunflower oil refined by Na_2SiO_3

According to overall ANOVA results analysis, it was concluded that while temperature had a significant impact on the α-tocopherol content of refined sunflower seed oil by Na_2SiO_3, the effect of temperature was not found to be significant on the α-tocopherol content of refined sunflower seed oil by $Ca(OH)_2$ and MgO at 95% confidence interval. Although alkali amount had no significant effect on the α-tocopherol content for all type refined sunflower seed oils, both time and temperature had an increasing impact on the α-tocopherol content of sunflower oil refined by Na_2SiO_3. Also, it can be found that time had an increasing impact on the α-tocopherol content of sunflower oil refined by MgO. The results also show that time had no significant impact on the α-tocopherol content of refined sunflower oil by $Ca(OH)_2$.

5.3.3 Model Optimization and Verification

Minimal neutralization with the central composite design estimated the effects of the process temperature, time and amount of alkali for optimizing reduction of FFA and α-tocopherol retention in sunflower oil. According to some studies indicated that the temperature of neutralization was generally 65-90°C [8, 49, 122, 123]. Other studies claimed that the neutralization was occurred in 20 to 30 min [40, 123]. In this study, time changed from 10 to 30 minutes for reaction of FFA with weak alkalis and 54-58°C was used for neutralization. In order to obtain minimal neutralized sunflower oil with minimizing FFA content and maximizing α-tocopherol retention, the optimal parameters of the neutralization step were determined by the association of two responses. Optimization was conducted to figure out optimal independent variables with desirable responses.

The optimal processing conditions for neutralization of sunflower oil were $Ca(OH)_2$ at 0.30 %, 53.0 °C and 19.7 minutes, MgO at 0.38 %, 57.6 °C and 17.8 minutes, Na_2SiO_3 at 0.81 %, 55.7 °C and 19.1 minutes. According to response values, response surface methodology offers solutions. These solutions have desirability values. The desirability is the closeness of responses to the ideal value and ranges from 0 to 1. As desirability approaches to 1, response gets close to ideality. Three desirability were 1, 1 and 0.724 as shown in Table 5.11.

The optimal process values obtained for two responses (FFA and α-tocopherol content) by the RSM. Minimal FFA and maximum α-tocopherol content values were predicted at optimal conditions. The predicted and experimental results were presented in Table 5.11. One-sample t-test was made to confirm the sufficiency of the current model. A mean value for FFA and α-tocopherol concentration was acquired from three experiments, which indicated validation of the model.

While using $Ca(OH)_2$ as neutralization agent, a mean value was 0.07 ± 0.01 % for FFA content under optimum parameters. At the same conditions, the predicted value was 0.07 % for FFA content. The test indicated that there were no important differences ($P>0.05$) in these values for FFA content in minimal refined sunflower seed oil by $Ca(OH)_2$.

In the case of MgO treatment, a mean value was 0.15 ± 0.03 % for FFA content at optimal parameters. For the same parameter, the predicted value was 0.16 % for FFA content. The test revealed that there were no important differences (P>0.05) between the values for FFA content in minimal refined sunflower seed oil by MgO.

While using Na_2SiO_3 as neutralization agent, a mean value was 0.13 ± 0.02 % for FFA content under optimum parameters. The predicted value was 0.15 % for FFA content at the same conditions. The test indicated that there were no statistically differences (P>0.05) between the values for FFA content in minimal refined sunflower seed oil by Na_2SiO_3. It was seen that the experimental values were compatible with the predicted values. The good correlation between these results were adequate to estimate the decrease of FFA for sunflower oil after neutralization. These verification results for FFA content showed that the predicted values from models were accurate.

Table 5.11 Comparison of experimental and predicted values of FFA content and α-Tocopherol content at optimal conditions

Alkali Types	Process Variables			Responses				
	Temperature (°C)	Alkali Amount (%)	Time (min)	FFA Content (%)		α-Tocopherol content (ppm)		
	Optimum Values			Predicted	Experimented	Predicted	Experimented	Desirability
$Ca(OH)_2$	53.0	0.30	19.7	0.07	0.07 ± 0.01	146.78	209.1 ± 1.0	1
MgO	57.6	0.38	17.8	0.16	0.15 ± 0.03	141.21	191.2 ± 4.0	1
Na_2SiO_3	55.7	0.81	19.1	0.15	0.13 ± 0.02	214.17	205.6 ± 10.0	0.724

While using $Ca(OH)_2$ as neutralization agent, a mean value of 209.1 ± 1.0 ppm was obtained experimentally for α-tocopherol content and the predicted α-tocopherol content was 146.78 ppm under optimum parameters. One-sample t-test was made to confirm the sufficiency of the current model. The test showed that there were significant differences ($P<0.05$) between the experimental and predicted values for α-tocopherol content in minimal refined sunflower seed oil by $Ca(OH)_2$. While using MgO as neutralization agent, a mean value of 191.2 ± 4.0 ppm was obtained experimentally for α-tocopherol content and the predicted α-tocopherol content was 141.21 ppm under optimum parameters. The test indicated that there were statistical differences ($P<0.05$) between these results for α-tocopherol content in minimal refined sunflower seed oil by MgO.

While Na_2SiO_3 was used as neutralization agent, a mean value was 205.6 ±10.0 ppm was experimentally obtained for α-tocopherol content. The predicted α-tocopherol content was 214.17 ppm under optimum parameters. The test revealed that there were no significant differences ($P>0.05$) between these results for α-tocopherol content in minimal refined sunflower seed oil by Na_2SiO_3. The good correlation for these results were sufficient for estimating more α-tocopherol retention for minimal neutralized sunflower oil with Na_2SiO_3. However, it was seen that difference between the experimental and predicted values for α-tocopherol content in the neutralization of the minimal refining process for sunflower oil by $Ca(OH)_2$ and MgO, respectively. Neutralization conditions were slightly different for predicted and experimental results and these differences can be caused by experimental errors.

Free fatty acid content of neutralized sunflower oil by $Ca(OH)_2$, MgO and Na_2SiO_3 to 0.07%,0.15% and 0.13% respectively and retained the α-tocopherol to 209.1, 191.2 and 205.6 ppm respectively under optimal conditions. The results of this study indicated that it is possible to obtain a minimally neutralized sunflower seed oil with lower FFA content and higher α-tocopherol content. The conditions obtained in this study for maximum α-tocopherol and minimum FFA content are the most adequate for the process of minimal neutralization. This is important to improve the nutritional quality of refined sunflower seed oils. There are not so many studies about optimization of minimal neutralization for sunflower oil or other vegetable oils so far. However, there are several studies on the optimization of traditional refining for some vegetable oils.

Chew et al. [118] suggested that central composite design was used to study the effect of the parameters in the neutralization, sodium hydroxide (0–5%), temperature (25–85°C) and time (10–50 minutes). The optimum parameters were recommended at 3.75% (NaOH), 40°C (temperature) and 20 min (time) to provide the neutralized kenaf seed oil with decreasing free fatty acids. Under these parameters, free fatty acids of 0.12% was obtained

Another study [119] found that degumming for kenaf seed oil were optimized using RSM with central composite design. Temperature (25–85°C), phosphoric acid dosage (0–0.2%) and water dosage (0–40%), on the phosphorus content in degummed oil were investigated. The optimal parameters were detected optimal conditions (temperature of 40°C, a phosphoric acid dosage of 0.09% w/w and a water dosage of 22.4% w/w). Phosphorus content of degummed kenaf seed oil was 6.70 mg/kg.

Boroujeni et al. [12] revealed that the optimization of bleaching conditions can be increased sunflower oil quality. The model optimization using the RSM method determined that the optimal values were (37.31 min, 92.7 °C, and clay concentration 1.18%.

Ortega-Garcia et al. [120] studied the impact of temperature (76.4–143.6°C), time (6.4–73.6 minute) and clay amount (0.16–1.84% w/w) on tocopherol concentration and quality of soybean oils were examined. The optimal bleaching parameters were found as 96°C; 23 min; 1.4%. with 0.1meq/kg of peroxide value, 91.74% of tocopherol retention, and color 1.53 red value units was detected for bleached soybean oil at these optimal conditions.

Another study revealed that optimization of the bleaching parameters in the chemical refining process for kenaf seed oil, concentration of bleaching earth (0.5-2.5% w/w), temperature (30-110 °C) and time (5-65 min). Total oxidation value and color reduction used as responses. The optimal parameters were: 1.5%, 70 °C, and 40 min for bleaching. Total oxidation value of 8.09 and color reduction of 32.95% was found for bleached kenaf seed oil at these optimal conditions [121].

5.3.4 Effect of Minimal Neutralization at Optimal Conditions on Minor Components and Oxidation Stability of Sunflower Oil

5.3.4.1 Free Fatty Acids Content

Free fatty acid content is a good indicator for estimation the proficiency of the neutralization step and the oil quality for human consumption [124]. Attempts to effectively refine crude oils with 3 weak alkalis has been tried. The FFA contents of crude, degummed and refined sunflower seed oils with different alkali materials are shown in Table 5.12. Initial FFA values for crude and degummed sunflower seed oils were 0.30 % and 0.26 %, respectively. There were no significantly differences between crude and degummed sunflower oils ($P>0.05$). The FFA content of crude sunflower seed oil decreased from 0.30 to 0.16 % after neutralizing with 0.2 % NaOH. The FFA content of neutralized oil by $Ca(OH)_2$, MgO and Na_2SiO_3 under optimal parameters were 0.07, 0.15 and 0.13% respectively. There were no significantly differences among sunflower seed oils refined by NaOH, refined by MgO and refined by Na_2SiO_3 ($P>0.05$). However, FFA content of sunflower seed oil refined by $Ca(OH)_2$ significantly decreased compared to the others ($P<0.05$). While neutralizing with calcium hydroxide, it produced the lowest FFA content and neutralizing with sodium hydroxide produced the highest FFA content.

Some studies have been observed lower FFA results after neutralization stage. Rade et al. [1] found that FFA value of crude and alkali refined sunflower oil as % oleic acid are 1.60 and 0.06, respectively. Karabulut et al. [8] examined that the neutralization aimed to remove FFA as sodium soaps. FFA contents of hazelnut oil were significantly reduced during neutralization. Abitogun et al. [25] reported that free fatty acid is essential for the determination of the identity and edibility of oil. This was detected to be 1.40 %. The low value of free fatty acid indicated that the oil could be refined to sunflower oil. Kuleasan and Tekin [55] found that the free fatty acid content of the crude soybean oil decreased from 0.56 to 0.14–0.2 % in 60 min. Suliman et al. [30] stated that free fatty acid value of sunflower crude oil decreased from 0.5 to 0.1 % during neutralization step because of the alkali treatment. Kreps et al. [59] that crude sunflower and rapeseed oils contained 1.2–1.3% w/w of free fatty acids. Pal et al. [44] found that FFA value for sunflower oil decreased from 1.1 to 0.24 during neutralization. The FFA values of kenaf oil reduced from 1.94 to 0.12 %, respectively after neutralization [124].

Shah et al. [34] claimed that FFA is one of the most frequently used quality markers to estimate the oxidative stability of oil, suitability and edibility of oils. Considerable alteration in the decrease of FFA was measured in neutralization. FFA in crude sunflower oil changed from 0.56 to 0.14% (75.0%) for neutralization step. Chew et al. [89] showed that FFA values of kenaf seed oils decreased after refining process for 24 storage day. There was no significant difference in the FFA values in the crude oil during the accelerated storage. High FFA content leads to an undesirable flavor in the edible oil. Since FFA are pro-oxidants in oils as the carboxylic group enhances the rate of hydroperoxide decomposition. Another study claimed that since oil has lower FFA value it has the more stability during storage without getting rancid. FFA of crude oils (cotton, flaxseed, groundnut, soybean, mustard) varied from 0.9- 3.49 % as oleic acid, from 0.3 to 1.98 % as oleic acid for refined oils [128].

Table 5.12 Chemical Characteristics of crude (B), degummed (D) and neutralized sunflower seed oils by NaOH (T), $Ca(OH)_2$(C), MgO (M) and Na_2SiO_3(N)

Refining Step	FFA Content (oleic acid%)	α-Tocopherol Content (ppm)	TPC (mg CAE/100g oil)	p-Anisidine Value
B	0.30 ± 0.03^{C}	250.3 ± 1.2^{C}	1.60 ± 0.08^{B}	0.85 ± 0.05^{A}
D	0.26 ± 0.02^{C}	248.5 ± 10.0^{C}	1.59 ± 0.25^{B}	0.83 ± 0.02^{A}
T	0.16 ± 0.02^{B}	180.8 ± 5.0^{A}	1.35 ± 0.11^{AB}	2.21 ± 0.06^{C}
C	0.07 ± 0.01^{A}	209.1 ± 1.0^{B}	1.55 ± 0.28^{AB}	1.06 ± 0.30^{A}
M	0.15 ± 0.03^{B}	191.2 ± 4.0^{A}	1.29 ± 0.33^{AB}	1.33 ± 0.18^{B}
N	0.13 ± 0.02^{B}	205.6 ± 10.0^{B}	1.16 ± 0.14^{A}	1.42 ± 0.01^{B}

$^{A–C}$Means in the same column followed by different uppercase letters represent significant differences at a level α =0.05 level

5.3.3.2 α-Tocopherol Content

Tocopherols having health benefits for humans is one of the important bioactive compounds in vegetable oils. Tocopherols have ability of inhibition of lipid peroxidation in oils, by donating their phenolic hydrogens to lipid free-radicals. Tocopherols that are the primary antioxidants in all vegetable oils can be divided into four groups as α, β, γ, and δ forms. Alpha-tocopherol is higher antioxidant activity than other tocopherols [76, 82, 83, 129].

The α-tocopherol content of sunflower seed oils with different chemicals under optimal conditions after traditional and minimal refining processes were determined by high-performance liquid chromatography (HPLC). The α-tocopherol contents in all sunflower oils are shown in Table 5.12. There were no significantly differences between crude and degummed sunflower oils ($P>0.05$). The α-tocopherol content of crude sunflower oil (B) was significantly reduced after refining ($P<0.05$). The α-tocopherol content of the crude and degummed oil was 250.3±1.2 and 248.5±10.0 ppm, respectively then decreased till 180.8±5.0 ppm during the traditional neutralization. It was shown that sunflower oil refined by $Ca(OH)_2$ and sunflower oil refined by Na_2SiO_3 having no statistically differences ($P>0.05$) were 209.1±1.0 and 205.6±10.0, respectively (Table 5.12). There were also no significantly differences between sunflower oil refined by NaOH and sunflower oil refined by MgO ($p>0.05$). Neutralizing with $CaOH_2$ produced the highest α-tocopherol concentration (209.1±1.0), while neutralizing with NaOH produced the lowest α-tocopherol concentration (180.8±5.0). Calcium hydroxide has the least impact on the alteration of α-tocopherol concentration for sunflower seed oil. The reduction of α-tocopherols in sunflower seed oils refined with NaOH may be due to the instability of tocopherols in the presence of air for longer time. The use of strong alkali (NaOH) and heat in the traditional hot-water washing step could be another reason of bigger α-tocopherol loss.

Karabulut et al. [8] found that α-tocopherol content of crude hazelnut oil was mainly reduced from 39.89 mg/100 g to 37.13 mg/100 g during neutralization. Another study stated that tocopherol values of crude and neutralized hazelnut oils was 23.78 mg/100 g and 25.11 mg/100 g, respectively [126]. Marmesat et al. [130] found that α-tocopherol content of high oleic sunflower oil had 283 mg/kg. Naz et al. [39] claimed that individual tocopherols were significantly reduced during refining process. Suliman et al. [40] indicated that the total tocopherols content of the crude oil sample decreased from 750 ppm to 530 ppm after refining.

Kreps et al. [59] claimed that tocopherols were analyzed by normal phase HPLC, crude sunflower oil contained 332.4 mg/kg of tocopherols. After refining tocopherol content reduced to 202.7 mg/kg of tocopherol.

Durmaz and Gökmen [129] revealed that the main tocopherol is α-tocopherol with the concentration of 512.75 and 494.42 mg/kg in pressed and neutralized oils. Total tocopherol values of refined cotton, flaxseed, ground nut, soybean and mustard oil was 616, 329, 219, 829 and 627 mg/kg, respectively [46]. Gao et al. [131] investigated the effects of different extraction solvents on the quality of walnut oil such as tocopherol contents. The total tocopherol content (α-,β-,γ-,and δ-tocopherol) of the samples changed from 295.7 mg/kg to 578.0 mg/kg. Ghazani et al. [13] stated that α-tocopherol amount of crude and degummed canola oil was 154.1 and 135.0 ppm, respectively.

5.3.4.3 Free Radical Scavenging Capacity and Total Phenolics Content

Total phenolics and free radical scavenging capacity were measured to determine how different alkalis can influence antioxidants in the sunflower oil. DPPH was used to evaluate the free radical scavenging effectiveness of antioxidants in sunflower oils [132]. The effect of neutralization by different alkali materials under optimal conditions on total phenolics content and DPPH-scavenging capacities of sunflower oils was investigated. The free radical scavenging capacities of crude, degummed and traditional refined oils and minimal refined sunflower oils under optimal conditions are shown in Figure 5.19.

DPPH-scavenging capacities of crude, degummed and sunflower seed oils neutralized with $Ca(OH)_2$, MgO, Na_2SiO_3 and NaOH under optimal conditions ranged from 13.64 to 13.69 mmol TE/kg oil. Crude and degummed oil had no significant effects on the free radical scavenging activity ($P>0.05$). Free radical scavenging activity of sunflower seed oil refined by $Ca(OH)_2$ was not significantly changed ($P>0.05$) compared to the crude oil. It was determined that no statistical difference was present between sunflower oil refined by MgO and sunflower oil refined by Na_2SiO_3 with respect to radical scavenging activity ($P>0.05$). There were also no significant differences between sunflower oil refined by NaOH and sunflower oil refined by $Ca(OH)_2$ ($P>0.05$). Unlike sunflower seed oil refined by $Ca(OH)_2$, sunflower oil refined by MgO and sunflower oil refined by Na_2SiO_3 have the same effect in free radical scavenging activity and significantly decreased ($P<0.05$). Castelo-Branco et al. [94] showed that total antioxidant capacity of refined sunflower oil is 2.44 mmole TE/kg. Pellegrini et al. [110] observed the total

antioxidant capacity of refined sunflower oil (1.17 mmol TE/kg) using TEAC (trolox equivalent antioxidant capacity) assay. Karamac et al. [95] reported that ABTS scavenging activity was expressed as Trolox equivalent antioxidant capacity of sunflower seed fractions. The TEAC values varied from 0.10 mmol Trolox eq/g to 2.21 mmol Trolox eq/g. Laullo et al. [108] stated that the loss of phenolic compounds causes a decrease in radical scavenging activity. It is also indicated that the crude oil has better activity which decreases as the oil is treated.

Total phenolics content of crude, degummed and degummed-neutralized sunflower oils during refining processes with different alkali materials under optimal conditions ranged from 1.16 to 1.60 mg caffeic acid equivalent/100 g oil (Table 5.12). Total phenolics content of degummed oils was not significantly altered compared to crude oil ($P<0.05$). There were no statistically difference present in sunflower oil refined by $Ca(OH)_2$, MgO and NaOH ($P>0.05$). Significant differences were observed between sunflower seed oil refined by $Ca(OH)_2$ and sunflower seed oil refined by Na_2SiO_3 ($P<0.05$). Sunflower seed oil refined by $Ca(OH)_2$ had the highest total phenolics contents and sunflower seed oil refined by Na_2SiO_3 had the lowest total phenolics content. There was linear relationship between tocopherol and antioxidant capacity. Sunflower refined by $Ca(OH)_2$ had both highest α-tocopherol content and total phenolics content compared to others (Table 5.12). Thus, it can be said that use of $Ca(OH)_2$ in the neutralization of sunflower seed oil could provide inhibiting oxidation and produce a high quality oil. Similar TPC results have been observed elsewhere. The total phenolics content of refined sunflower seed oil using the Folin-Ciocalteu reagent method was found 1.55 mg of gallic acid equivalent/100 g of oil [94].

Siger et al. [111] reported that the total phenolics content of cold-pressed sunflower oil was 1.20 mg CAE/100 g. Janu et al. [133] studied the antioxidant potential of some vegetable oils where the oils were extracted with methanol; and these methanolic extracts were used for the antioxidant studies. The percentage TPC of sunflower oil extracts was expressed as gallic acid equivalence. It was found that percentage of TPC was 0.49 mg gallic acid equivalent /100 g oil. Unrefined oils have been found to have greater antioxidant activity than refined oils, which indicates the importance of optimizing oil processing stages to preserve the polyphenolic content in the oil.

Guzel et al. [134] reported that the prediction of TPC is a good measure of the antioxidant efficacy and the phenolic compounds can contribute directly to antioxidant action. TPC in sunflower oil was measured as 0.412 mM gallic acid equivalent /L

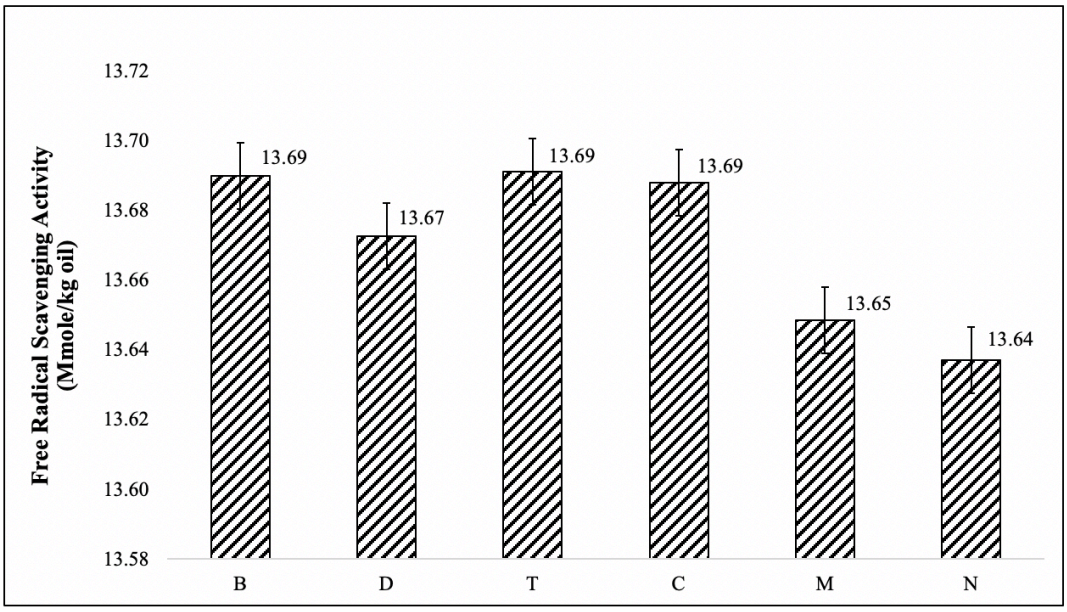

Figure 5.19 Free Radical Scavenging Capacities of crude (B), degummed (D) and neutralized sunflower seed oils by NaOH (T), $Ca(OH)_2$(C), MgO (M) and Na_2SiO_3(N)]

5.3.4.4 Lipid Oxidation Studies

There are so markers that have been reported to comprehensively estimate the level of lipid oxidation [64]. Peroxide value is used to determine the amount of total peroxides which are regarded as primary oxidation products. Anisidine value and hexanal are a reliable measurement of secondary oxidation products [75, 132]. To better understand which weak alkaline is more effective in producing high quality oil having more oxidative stability, higher α-tocopherol content and lower FFA content are desired. Therefore, this study was conducted to examine optimization of parameters such as alkaline content, process temperature and reaction time in order to provide the most suitable minimal refining conditions and the effect of refining by different weak alkalis on lipid oxidation of sunflower oil was investigated. Oil samples were withdrawn periodically from the incubator at 55°C for two oxidation tests (lipid

hydroperoxides and hexanal). Lipid oxidation markers of sunflower seed oil during refining processes with different alkali materials under optimal conditions were shown in Table 5.12 and Figure 5.20. The neutralized oils by NaOH had the shortest hydroperoxide and hexanal lag phases meaning it was the least stable oils. Unlike sodium hydroxide, there were no significantly differences among lipid oxidation markers of refined oils by $Ca(OH)_2$, MgO and Na_2SiO_3 under optimum conditions. FFA and tocopherol would be two important factors in the oxidative stability of the oil with FFA accelerating oxidation and tocopherol inhibiting oxidation. Oils refined by $Ca(OH)_2$, MgO and Na_2SiO_3 under optimum conditions exhibited lower FFA content and higher α-tocopherol content than oils neutralized by NaOH which can explain why oxidative stability is different. Therefore, weak alkalis were more oxidatively stable than NaOH. According to Table 5.12, anisidine values of sunflower oils ranged from 0.83 to 2.21. It was shown that anisidine values of sunflower seed oil refined by MgO and sunflower oil refined by Na_2SiO_3 have no statistically differences ($P>0.05$). These values were 1.33 and 1.42, respectively. There were also no significant differences between crude sunflower seed oil and degummed sunflower seed oil p-anisidine values ($P>0.05$). Neutralizing with calcium hydroxide showed the lowest anisidine value (1.06), while neutralizing with sodium hydroxide produced the highest anisidine value (2.21). As indicated in Table 5.12 and Figure 5.20, Oil neutralized by three weak alkalis had similar oxidative stability. Since the neutralized oils by $Ca(OH)_2$ had the lowest FFA, anisidine value and highest total phenolic and tocopherol content and had better oxidative stability than strong alkali NaOH suggesting that $Ca(OH)_2$ could be more effective in producing a high quality oil.

Ghazani et al. [13] also studied minimal refining for canola oil. They stated that according to PV results of refined oils, traditionally refined canola oils having the least lag phase oxidized very fast among all refined oils. These traditionally neutralized oils also had the highest anisidine value compared to other minimally neutralized sunflower seed oils.

According to Figure 5.20, it was found that increase in peroxide value in traditionally refined oil may be due to their low α-tocopherol content. Another reason may be higher amounts of free fatty acids in traditionally refined sunflower oil (Table 5.12).

These observations are in accord with Yi et al. [67]. They aimed the effect of FFA on oxidation stability in the water in stripped walnut oil system and reported that enhancing levels of oleic acid accelerated lipid oxidation. Chew et al. [124] reported that peroxide value was 2.90 meq/kg for crude kenaf seed oils 1.57 meq/kg for neutralized kenaf seed oils.

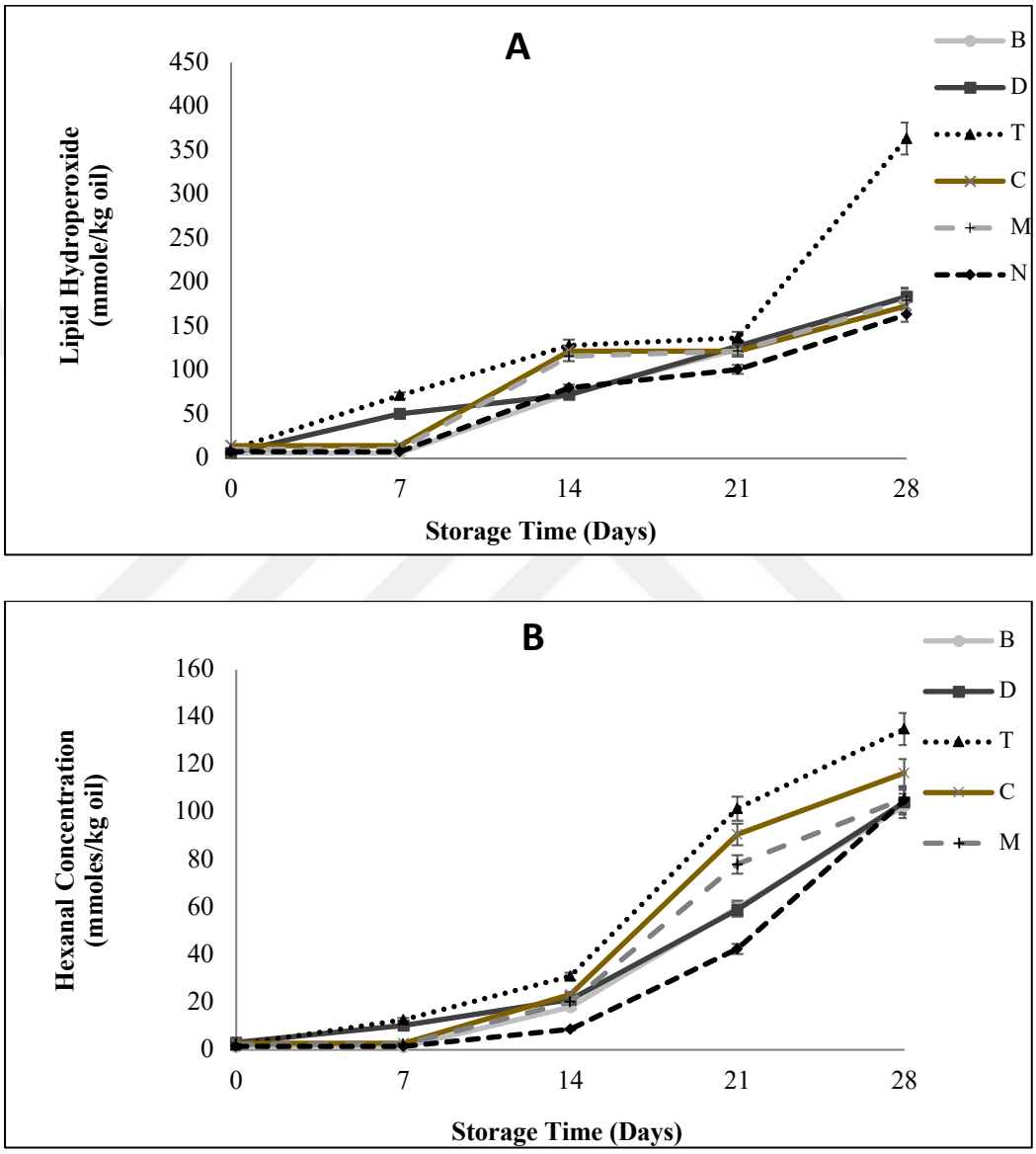

Figure 5.20 Formation of lipid hydroperoxides (**A**) and hexanal concentration (**B**) in B: Crude oil, D: Degummed oil, T: Traditional neutralized oil and sunflower oil neutralized at optimal conditions C: $Ca(OH)_2$, M: MgO, N: Na_2SiO_3 and during storage at 55 °C

5.4 Conclusion

Final part of the study focused on which weak alkaline has higher oxidation stability and minimum FFA content and maximum acceptable tocopherol content. The optimization process was performed to find the most optimal process conditions by determining independent variables such as amount of alkaline, process temperature and reaction time. The estimated optimal values for minimal neutralization of sunflower oil were $Ca(OH)_2$ at 0.30 %, 53.0 °C and 19.7 minutes; MgO at 0.38 %, 57.6 °C and 17.8 minutes; Na_2SiO_3 at 0.81 %, 55.7 °C and 19.1 minutes. These results indicated that the NaOH neutralized oils had the shortest hydroperoxide and hexanal lag phases were the least stable oils all weak alkalis had similar oxidative stability. Since the neutralized oils by $Ca(OH)_2$ had also the lowest FFA, p-anisidine value and highest α-tocopherol and total phenolic content and also had better oxidative stability than strong alkali (NaOH). It could be said that the highest quality oil may be produced by using $Ca(OH)_2$.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

In the first part of present study; new minimal neutralization approach was developed as alternatives to traditional neutralization. It was realized that use of weak alkali in the minimal neutralization has lowering effect on FFA content of sunflower seed oil compared to crude sunflower seed oil. Second part aimed to compare between minimal and traditional neutralization. After neutralizing with $Ca(OH)_2$, MgO and Na_2SiO_3, FFA content of crude oil reduced from 0.30 to 0.05, 0.07 and 0.11 % respectively. It was indicated that sunflower seed oil obtained by minimal neutralization having lower FFA content and more oxidative stable than sunflower seed oil produced by traditional neutralization.

Third part focused on differences between neutralization with traditional NaOH and weak alkalis [$Ca(OH)_2$, MgO and Na_2SiO_3] on the oxidation stability, FFA and α-tocopherol content. An optimization process was conducted using independent variable to determine the best amount of alkaline, process temperature and reaction time. The estimated optimal parameters for neutralization of sunflower seed oil were for $Ca(OH)_2$ at 0.30 %, 53.0 °C and 19.7 minutes; for MgO at 0.38 %, 57.6 °C and 17.8 minutes; for Na_2SiO_3 at 0.81 %, 55.7 °C and 19.1 minutes. Lipid oxidation rates at these optimum conditions were compared to oil neutralized with NaOH (0.20 %, 40°C, 15 min).

In conclusion; oils neutralized by NaOH had the shortest hydroperoxide and hexanal lag phases and thus were the least stable oils. Oils neutralized by $Ca(OH)_2$, MgO and Na_2SiO_3 had lower FFA (free fatty acid) and better oxidative stability than oil neutralized by NaOH. Since the neutralized oils by $Ca(OH)_2$ had the lowest FFA, anisidine value and highest total phenolic and tocopherol content and had better oxidative stability than strong alkali NaOH suggesting that $Ca(OH)_2$ could be more effective in producing a high quality oil.

Based on this study, it has been shown that minimal neutralization could be optimized successfully by response surface methodology and it can be used as an alternative to traditional neutralization. Moreover, studies are need to be done in order to provide milder conditions and prevent adverse reactions in oil refining process. Therefore, minimal refining process can be applied to all refining steps, e.g., use of alternative adsorbents instead of acidified clays used in the bleaching step or omitting deodorization step. In addition, more intensely economic, environmental, marketing and industrial scale studies should be carried out to better make clear the advantages of this new approach for oil processing industry.

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ACADEMIC/WORK EXPERIENCES

Position	Institution	Department	Year
Research Assistant	Kilis 7 Aralık University	Food Engineering	2013-…
Visiting Researcher	University of Technology and Life Sciences	Food Processing	2016-2016
Research Scholar	University of Massachusetts Amherst	Food Science	2018-2020

STUDENT SCHOLARSHIPS AND AWARDS

2018 The Council of Higher Education, YÖK-YUDAB, International Doctorate Research Scholarship, University of Massachusetts Amherst, United States of America.

2016 Erasmus Staff Mobility, University of Technology and Life Sciences, Poland.

PROFESSIONAL AFFILIATIONS

2019 American Oil Chemists Society

SCIENTIFIC PUBLICATIONS

Gumus, P., Decker, E., Maskan, M. (2020) Neutralization of sunflower seed oil by $Ca(OH)_2$, MgO, and Na_2SiO_3 as an alternative to NaOH. Fresenius Environmental Bulletin 29(9), 7913-7921.

Gumus, P., Hayaloglu, A. A. (2019). Effects of blends of camel and calf chymosin on proteolysis residual coagulant activity microstructure and sensory characteristics of Beyaz peynir. Journal of Dairy Science, 102(7), 5945–5956.

Gumus, P., Decker, E., Maskan, M. "Minimal Refining Processes of Sunflower Oil to Enhance Oxidative Stability" 9th Annual Life Sciences Graduate Research Symposium November 22, 2019, Massachusetts, USA.

Gumus, P., Decker, E., Maskan, M. "Some Quality Characteristics of The Minimal Refined Sunflower Oil" Applied Sciences Congress December 20-22, 2019.

Gumus, P., Hayaloglu, A.A. "Comparison of The Residual Coagulant Activity in Different Blends of Calf Rennet and Camel Chymosin of White-Brined Cheese" International Conference on Raw Materials to Processed Foods, 11-13 April 2018, Spice Hotel, Antalya-Turkey.

Gumus, P., Satouf, M., Karadayı, D., Fırat, F., Koçum, E., Kiraz, E. Özkaya, R. "Effect of Some Packaging Materials During Storage on Quality and Shelf-life of Olive Oil" International Conference on Raw Materials to Processed Foods, 11-13 April 2018, Spice Hotel, Antalya-Turkey.

Gumus, P., Maskan, M. "Minimal Refining Method for Sunflower Seed Oil" International Multidisciplinary Congress of Eurasia (IMCOFE'17) August 23-25, 2017, Roma-Italy.

Gumus, P., Uzun Özcan, A. "Soy Milk and Soy Milk Products" International Conference on Natural Science and Engineering (ICNASE'16) March 19-20, 2016, Kilis-Türkiye.

Uzun Ozcan, A., Gumus, P. "Stimulating the Usage of Wheat Germ Partially Replaced by Flour in Ready to Eat Cakes" International Conference on Natural Science and Engineering (ICNASE'16*)* March 19-20, 2016, Kilis-Türkiye.

Ozpalas, B., Gumus, P. "Effects of Probiotics on some Gastrointestinal Disorders" 1st International Mediterranean Science and Engineering Congress (IMSEC 2016), October 26- 28, 2016, Adana/Turkey.

Gumus, P., Ozpalas, B., "Applications of Some nanomaterials in Food Packaging" 1st International Mediterranean Science and Engineering Congress (IMSEC 2016), October 26- 28, 2016, Adana/Turkey.

Ozpalas, B., Gumus, P. "Use of Nano-Sensors in Food Packaging" 1st International Mediterranean Science and Engineering Congress (IMSEC 2016), October 26-28, 2016, Adana/Turkey.

Ucan, F., Gumus, P., The 3rd International Symposium on "Traditional Foods from Adriatic to Caucasus" 01-04 October 2015, Sarajevo – Bosnia and Herzegovina.

Gumus, P., Hayaloglu, A.A., "Comparison of The Blends of Camel Chymosin and Calf Rennet on Cheese-Making Performance and Quality Characteristics of White-Brined Cheese" 9th Cheese Symposium, 12-13 November 2014, Cork-Ireland.

PROJECTS

2014-2015	Effects of blends of camel and calf chymosin on some quality characteristics of white-brined cheese	BAPYB, Researcher
2018-2020	Minimal refining process of crude sunflower oil: process optimization, effect on minor components of oil and oxidation stability	BAPYB, Researcher