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Advanced microwave technology for biodiesel feedstock processing

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ADVANCED MICROWAVE TECHNOLOGY FOR BIODIESEL FEEDSTOCK PROCESSING

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
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Biological and Agricultural
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in

The Department of Biological and Agricultural Engineering

by
Beatrice G. Terigar
B.S., Aurel Vlaicu University of Arad, Romania, 2007
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LIST OF SYMBOLS AND ABBREVIATIONS

a, b, m_1, m_2, m_3 – regression coefficients for logarithmic equations

m – mass of oil; g

TT – Chinese Tallow Tree

V – volume of solvent-oil mixture; ml

R – solvent to flour ratio

P – power absorbed per unit volume; W/m^3

FA – free fatty acid

AV – titratable acid value; % oleic acid

IV – iodine value; cgI_2/g

CE – conventional extraction

MAE – microwave assisted-extraction

CMAE – continuous flow microwave assisted-extraction

CFMAE – continuous flow focused microwave assisted-extraction

SB – soybean

RB – rice bran

B100 – 100% pure biodiesel

OSI – oxidative stability index; h

PV – peroxide value; meq/kg

T – absolute temperature; K

E – electric field intensity; V/m

Cp – specific heat; KJ/kgK

f – frequency; Hz

ε – relative complex permittivity or relative complex dielectric constant

ε_0 – dielectric constant of the vacuum = 8.854 10^{-12} Far/m

ε' – relative dielectric constant or storage factor

ε'' – relative dielectric loss or loss factor

j – $(-1)^{1/2}$

$\dot{m}$ - mass flow rate for a continuous system; kg/s

σ – conductivity; Siemens/m

φ - density; g/cm^3

ABSTRACT

The goal of this work was to assist in overcoming the most important bottlenecks in the biodiesel production chain: to improve the biodiesel production process (oil extraction and esterification) using advanced microwave technologies from traditional and alternative feedstock; and identify the important characteristics of an alternative, high oil yield crop (TT).

Dielectric properties of two feedstocks, soybean and rice bran were measured in presence of several solvents at different ratios, temperature and frequency. Results indicated that dielectric properties are ratio, temperature, frequency and solvent type dependent. Quasi-linear relationship using second order logarithmic transformation of the data provided a reliable estimator for the behavior of dielectric properties of the feedstocks used. Results assisted in further selection of appropriate solvent, mixing ratio, temperature and frequency for the design and develop of a continuous microwave assisted extraction (CMAE) system for oil (laboratory and pilot scale). Yield of oil extracted increased with extraction temperature and time for both feedstocks. Oil yield high as 15% for soybean and 16% for rice bran was extracted at laboratory scale. At pilot scale, 18.6% oil for soybean and 19.5% for rice bran respectively was extracted, with oil quality meeting the ASTM requirements for vegetable oil. The continuous microwave system was further used for biodiesel transesterification reaction. High conversion rates (96.7-99.4%) were achieved at the reaction times (1, 5 and 10 min) and temperature (50 and 73°C) studied, with the required specifications for biodiesel quality.

Quality analysis on oil extracted from TT kernel, provided evidence that after 12 weeks of storage in controlled atmosphere no differences in quality was observed compared to low cost air storage. Moreover, late harvested seeds did not show major degradation in oil quality necessary for biodiesel production when compared with early harvested seeds.

CHAPTER 1

INTRODUCTION

Alternative fuels for diesel engines are becoming increasingly important due to diminishing petroleum reserves and the environmental consequences of exhaust gases from petroleum-fueled engines. Today, most of the energy we use comes from fossil fuels: petroleum, coal, and natural gas. While fossil fuels are still being created today by underground heat and pressure, they are being consumed more rapidly than they are being created. For that reason, fossil fuels are considered non-renewable; that is, they are not replaced as soon as we use them (Demirbas, 2007).

Renewable resources (biomass, wind, hydroelectric, wave, solar) are more evenly distributed than fossil and nuclear resources around the world, and total energy potential from renewable resources are more than three orders of magnitude higher than current global energy use (Demirbas, 2008a). Today’s global energy system is unsustainable due to equity issues as well as environmental, economic, and geopolitical concerns that have implications far into the future (UNDP, 2000).

As the world energy demand continues to rise, the most feasible way to meet this growing demand, especially for transportation purposes, is by using alternative fuels, such as biodiesel and bioethanol (biofuels). According to the International Energy Agency (IEA), scenarios developed for the USA and the EU indicate that near-term targets of up to 6% displacement of petroleum fuels with biofuels appear feasible using conventional biofuels, given available cropland. The term biofuel can refer to liquid or gaseous fuels for the transportation sector that are predominantly produced from biomass (Demirbas, 2008b). The biofuels advantages are related to energy security, environmental concerns, savings from foreign exchange rates, and socioeconomic issues related to the rural sector (Reijnders, 2006).

Biodiesel, a biomass derived diesel substitute comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils, has received worldwide attention as a renewable transportation fuel and blending agent. Biodiesel has a high potential to displace petroleum products, lower net global warming gas emissions such as CO_2 from transportation sector, and reduce emissions of particulate matter with carcinogenetic compounds (Duffield, 2007). The advantages of biodiesel as diesel fuel are its portability, being readily available and made from renewable resources, higher combustion efficiency, lower sulfur and aromatic content (Ma and Hanna, 1999; Kulkarni et al., 2006; Knothe, 2008), higher cetane number, and higher biodegradability (Zhang et al., 2003; Van Gerpen, 2005). The main advantages of biodiesel given in the literature include its domestic origin, which would help reduce a country's dependency on imported petroleum, its biodegradability, high flash point, and inherent lubricity in the neat form (Knothe et al., 2005).

Of the more than 350 known oil bearing crops, those with the greatest production potential are sunflower, safflower, soybean, cottonseed, rapeseed, canola, corn, and peanuts. Modifying these oils to produce the methyl or ethyl esters has been shown to be essential for successful engine operation over the long term, as their direct utilization, while possible, leads to poor engine performance and clogging, especially at low temperatures.

Commercialization of biodiesel is affected by several factors, including its high cost of manufacturing, feedstock cost, plant size and value of glycerin (a byproduct of the production process). Additional acreage, improved varieties, use of idle crop land for alternative oil crops, could all increase total vegetable oil production. Each of these methods for improving production has specific challenges to overcome. For additional acreage, the price must be competitive with the displaced crop. Availability of feedstock and processing costs are currently the most limiting factor for the growth of biodiesel industry. Improved varieties require time and money for research, and idle crop land must be made available for crop production.

The goal of this work was to assist in overcoming the most important bottlenecks in the biodiesel production chain: to improve the biodiesel production process (oil extraction and esterification) using advanced microwave technologies from traditional and alternative feedstock; and identify the important characteristics of an alternative, high oil yield crop, Chinese Tallow Tree (TT).

The specific objectives related to these goals are: 1. Determine the dielectric properties of the different biodiesel feedstocks in the presence of various solvents; 2. Design, develop and test a continuous microwave system for oil extraction from these feedstocks (and an alternative one) at laboratory and pilot plant scale; 3. Develop and test a continuous microwave system at laboratory scale for production of ethyl esters from vegetable oils; and 4. Determine the storage behavior of an alternative biodiesel feedstock (TT) that does not displace food crops.

In characterizing the materials interaction with the electromagnetic field is essential to be aware of the dielectric properties of the materials. The results from the dielectric properties measurements assist in further selection of appropriate solvent, mixing ratio, temperature and frequency for the design and develop of a microwave assisted extraction system for oil and other valuable products, which can be further processed into biodiesel.

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CHAPTER 2

AN ANALYSIS OF THE MICROWAVE DIELECTRIC PROPERTIES OF OIL-SOLVENT MIXTURES AT 300 TO 3000 MHZ

Introduction

Dielectric properties are essential in characterizing materials interaction with the electromagnetic fields, inclusive in the microwave region. Whereas other variables such as microwave power level, frequency and initial temperature can influence the microwave heating process, and can be selected for a particular processing application, dielectric properties are intrinsic to the material that require empirical measurements, especially for complex materials. The practical significance of knowledge of dielectric properties is that they form the basis for design calculations for high frequency and microwave heating (Ryynanen, 1995).

Fundamentally, all dielectric properties are determined by measuring the voltage and current between a pair of electrodes in order to determine the conductance and capacitance between those electrodes (Venkatesh and Raghavan, 2004). Conductance is a measure of the material's dissipation of energy, while capacitance is a measure of the material's storage of energy (Icier and Baysal, 2004). In the microwave and radio frequency region of the electromagnetic spectrum, the materials dissipate the energy of the oscillating electric field in two major ways: conduction and dipolar rotation (Bows et al., 1999). Both mechanisms create inter- and intra-molecular friction which generates heat throughout the volume of the material (Metaxas and Meredith, 1983).

This mechanism of energy transfer can be therefore used to generate the heat normally required in solvent extraction of oils (in order to enhance the mass transfer rate). A potential additional advantage is the enhanced extraction of bioactive compounds with the oil that can be separated and purified, especially if the solvent used is relatively inert in relationship to microwave (Jocelyn and Bélanger, 1994). For processing of value-added products, as well as for improved efficiency in lipid extraction for biodiesel production, an understanding of the frequency and/or temperature-dependent dielectric properties of mixtures is important both in fundamental studies of microwave processing applications and in assessing their economic and environmental implications (Raghavan et al., 2005).

Thus, dielectric properties are important both in the construction of heating ovens and in choosing the appropriate materials for the extraction process. Information about dielectric properties of a majority of foods and particulate materials such as agricultural commodities are available in published literature (Nelson and You, 1989; Nelson, 1994; Boldor et al., 2004; Ahmed et al., 2007) whereas data on dielectric properties of cereal flours and their mixtures with different organic solvents for microwave heating is nonexistent to our knowledge. Complex equations to determine the dielectric constant and dielectric loss have been developed for heterogeneous mixtures of particulates (pulverized or sporular materials) with a continuous phase (van Beek, 1967), but were mostly applied for air and might not necessarily reflect the behavior of organic solvents as a continuous phase. In addition, the true dielectric properties of the solid particulates required in these formulae might not be readily available and therefore experimental measurements present a better alternative. Similar equations have been developed for heterogeneous mixtures of agricultural commodities with air as a function of bulk density (Nelson et al., 1991; Nelson, 1992) or of volume fractions of air and solid phase, but these approaches also suffer from similar drawbacks as they are applicable primarily for air as continuous phase. Different factors, such as frequency, moisture content, ionic nature, temperature and composition of the materials, can influence the dielectric properties of these complex mixtures.

Dielectric properties of materials are defined in terms of their relative complex permittivity (ε) (Nelson, 1978):

$$\varepsilon = \varepsilon' - j\,\varepsilon'' \qquad (1)$$

where the real part, ε', is the dielectric constant, the imaginary part, ε'', is the dielectric loss factor. Dielectric constant is associated with the potential for electrical energy storage in the material, while dielectric loss is related to the electrical energy dissipation in the material.
For microwave-assisted oil extraction, one of the parameters that significantly affect the yield is temperature, which can be determined from the amount of energy absorbed as heat by the product (Nelson, 1992; Singh, 2003):

$$P_{abs} = \sigma E^2 = 2\,\pi\, f\, \varepsilon_0\, \varepsilon''\, E^2 \qquad (2)$$

Where: P_{abs} – absorbed power (W/m^3), σ – conductivity (Siemens/m), f – frequency (Hz), ε_0 – dielectric constant of the vacuum, ε″ – relative dielectric loss, E – electric field intensity (V/m).

For a continuous flow process, the energy absorbed will translate into a temperature change according to the following equation:

$$P = \dot{m} Cp \Delta T \qquad (3)$$

Where: P – total power (W), m – mass flow rate (Kg/s) for a continuous system, C_p – specific heat of the material (J/Kg K), and ΔT – temperature change from initial conditions.

This data, presented here for the first time to our knowledge, focused on determining the dielectric properties and behavior of solvent-oil feedstock mixtures at different temperatures and solvent-feedstock mixture ratios in the frequency range of 300 to 3000 MHz using the open-ended coaxial probe technique. The model feedstocks used in the study were soybean flour and rice bran.

Materials and Methods

The rice bran used in the study was obtained from Cocodrie rice variety provided by Rice Research Station (Crowley, LA). Rice was milled using a Satake pilot scale rice mill (Satake Engineering, Co., Tokyo, Japan) located within the Biological and Agricultural Engineering Department at the LSU AgCenter. The mill was equipped with two operational units: a shelling unit (Model GPS300A, Satake Engineering, Co) and a whitening unit (Model VAF10AM, Satake Engineering, Co.). After milling, the rice bran was stabilized in an oven at 120°C and then kept in a freezer at - 10ºC. Soybeans were collected from LSU AgCenter's Central Research Station in Baton Rouge, LA and milled with a kitchen miller then stored at -10°C. Rice bran and soybean flour samples were shipped overnight in cold storage to the Food Science Department at North Carolina State University, Raleigh, NC for dielectric measurements.

Samples of rice bran and soybean flour were mixed using four different solvents: methanol, ethanol, hexane and isopropanol, at different solvent to sample ratios (1:2, 1:1 and 2:1 (w/w)). For ethanol, the experiments were performed at 23, 30, 40 and 50°C, while for all other solvents the experiments were performed at room temperature (23°C). All alcohols were commercially obtained, were analytical grade (anhydrous) and were used without further treatment.

Dielectric properties of the mixtures were determined using an HP 8753C Network Analyzer and HP 85070B dielectric probe kit (Hewlett- Packard, Palo Alto, CA) using the open-ended coaxial probe method (Engelder and Buffler, 1991; Nelson and Bartley, 2000) in a 201-point frequency sweep from 300 MHz to 3 GHz. The network analyzer was controlled by

Hewlett-Packard 85070B dielectric kit software (Hewlett-Packard, Palo Alto, CA) and calibrated using the 3-point method (short-circuit, air and water at 25°C). Dielectric properties were measured at room temperature (23°C) for all samples and ratios. After comparison it was determined that ethanol is the most suitable for microwave extraction and therefore further experiments at higher temperatures were carried out only with ethanol. When using ethanol, the samples were placed in sealed 30 mL stainless steel sample holders and heated to 50°C in an oil bath. The dielectric properties of the mixtures containing ethanol were measured as the samples reached the various target temperatures. The same procedure was used for all other solvents, with measurements performed only at room temperature. Each measurement was repeated three times per sample, with all samples being duplicated.

The measured data collected from the network analyzer were imported into Microsoft Excel® 2007 (Microsoft Corp., Redmond, WA). Data for each sample was averaged across each replicate measurement and replicate sample, and standard deviations of each sample and standard error across all samples were calculated. The results were imported into SigmaPlot® 10 (Systat Software Inc., San Jose, CA) for plotting graphs and conducting linear regression analysis. The linear regression analyses were performed using the least-square method (Holman, 2001) using a logarithmic transform of the data for dielectric constant. A multiple linear regression was also performed for dielectric loss of the two feedstocks with methanol (normal) and with isopropanol (logarithmic).The multiple linear regression of soybean and rice bran with ethanol was performed on the logarithmic transform of the data for both ε' and ε'' as a function of frequency and solvent content. Hexane mixtures, due to the extreme non-polar nature of the solvent, were not fitted with any kind of regression with reasonable coefficients of determination.

Results and Discussion

The results were analyzed for all ratios, temperatures and frequencies. A special emphasis was placed on the results at 433, 915 and 2450 MHz which are the frequencies allotted by the FCC for industrial applications of microwaves.

Dielectric Constant

The dielectric constant of all samples decreased with increasing frequency at individual temperatures (Figures 2.1 and 2.2).

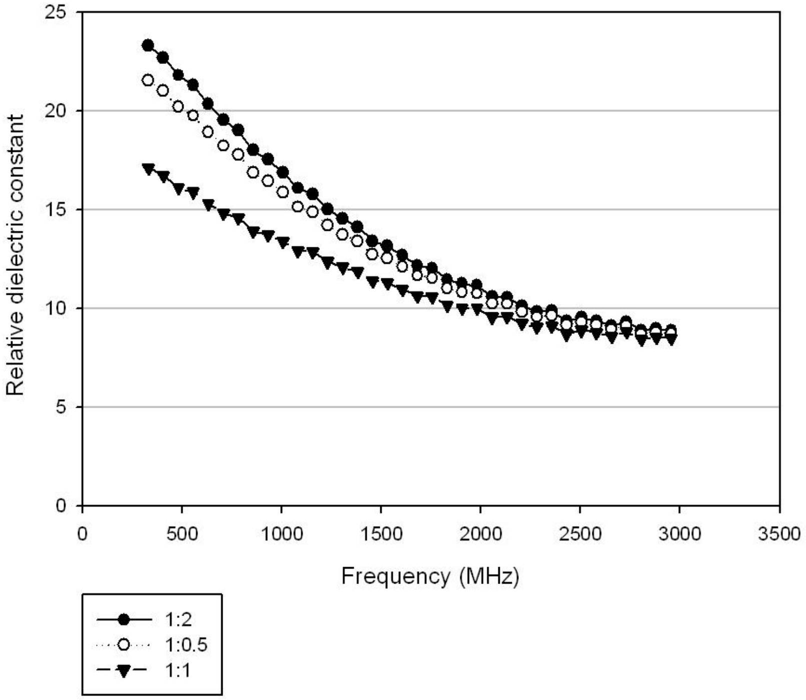


Figure 2.1. Dielectric constant of soy flour:ethanol at 23°C.

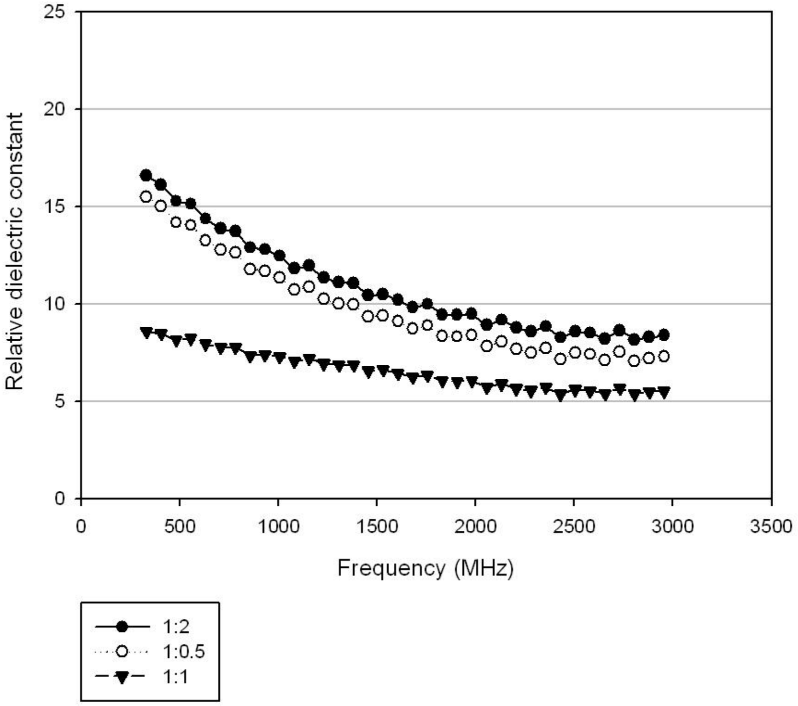


Figure 2.2. Dielectric constant of rice bran:ethanol at 23°C.

This behaviour was typical for all temperatures, all samples and solvents except the ones with hexane. Samples with hexane increased till a maximum at approximately 1500 MHz and then decreased with frequency (Figure 2.3 and 2.4). Values for ε' with methanol and hexane were lower than the ones for ethanol and isopropanol (Table 2.1 and 2.2). In general this follows the same order as the dielectric constants of pure solvents, as ethanol and isopropanol have higher values (24.3 and 18.3, respectively) than methanol and hexane (0.8 and 1.9, respectively).

Dielectric properties were also determined to be solvent ratio dependent with ethanol, the lowest value of dielectric constant was at 1:1 ratio, and the higher values at 1:2 ratios, for both soy flour and rice bran. Lower ε' values for rice bran with this solvent were probably due to the larger particle sizes in rice bran, as other investigators reported lower dielectric constant, for larger particle size agricultural commodities (Nelson, 1992, Griffiths, 1999). Hexane and isopropanol were observed to have same behavior, the 1:1 ratio having the highest dielectric constant, while 1:0.5 was the lowest one for both soy flour and rice bran (Table 2.3 and 2.4). Dielectric constant with methanol had a maximum at 1:2 with soy flour (Table 2.3) and 1:0.5 with rice bran (Table 2.4).

In the case of ethanol at higher frequencies (915 and 2450 MHz) the dielectric constant was influenced by temperature, increasing with temperature for all ethanol ratios. At lower frequencies (433 MHz) temperature did not show any major influence on dielectric constant, for neither soy flour or rice bran.

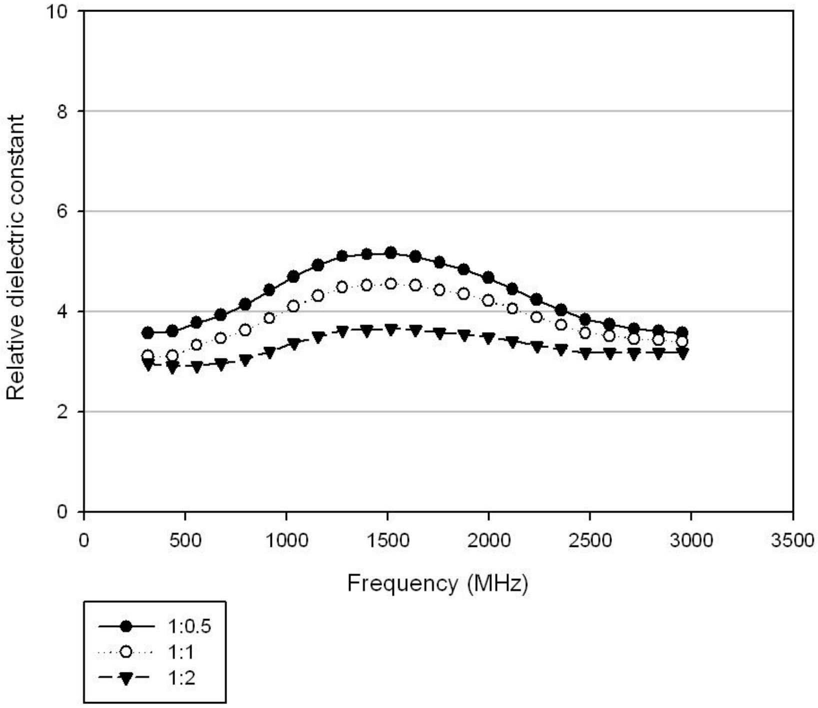


Figure 2.3. Dielectric constant of soy flour:hexane at 23°C.

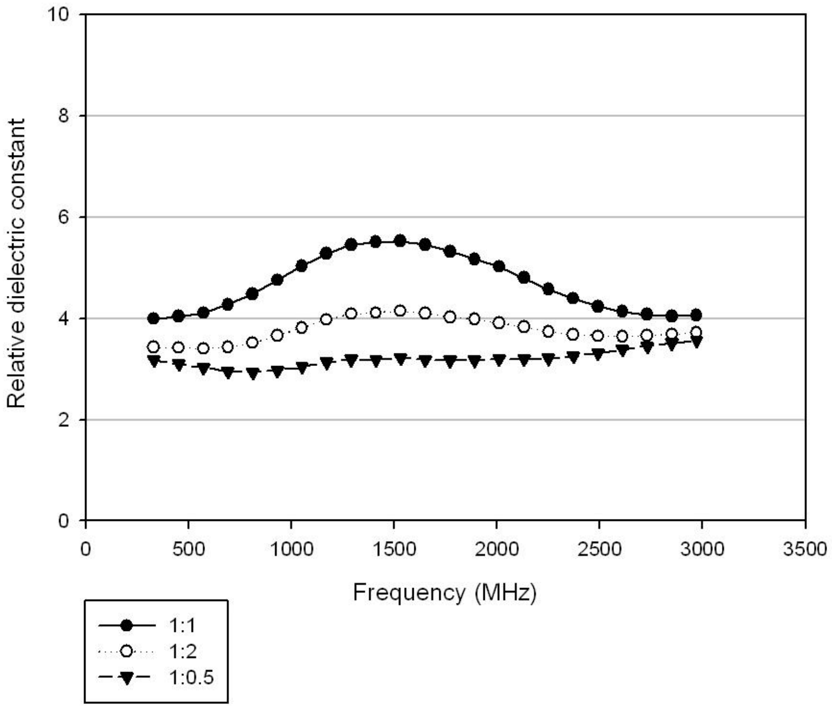


Figure 2.4. Dielectric constant of rice bran:hexane at 23°C.

Table 2.1. Values for dielectric constant of soy flour mixed ethanol.

Ratio Temp	433 MHz			915 MHz			2450 MHz		
	1:0.5	1:1	1:2	1:0.5	1:1	1:2	1:0.5	1:1	1:2
23°C	20.7±0.08	16.5±0.05	22.4±0.05	16.6±0.05	13.8±0.01	17.7±0.42	9.2±0.20	8.7±0.01	9.4±0.10
30°C	22.7±0.00	16.4±0.03	23.2±0.01	18.3±0.02	13.8±0.02	18.4±0.01	8.2±0.02	8.7±0.01	9.7±0.08
40°C	22.5±0.03	16.2±0.04	23.2±0.06	18.8±0.02	14.0±0.00	19.2±0.02	10.4±0.01	9.0±0.02	10.5±0.04
50°C	19.7±0.11	15.8±0.01	22.7±0.02	17.4±0.06	14.1±0.01	19.8±0.02	10.8±0.01	9.6±0.04	11.8±0.06

Table 2.2. Values for dielectric constant of rice bran and ethanol.

Ratio Temp	433 MHz			915 MHz			2450 MHz		
	1:0.5	1:1	1:2	1:0.5	1:1	1:2	1:0.5	1:1	1:2
23°C	14.8±0.06	8.4±0.08	15.7±0.03	11.8±0.07	7.4±0.01	14.8±0.02	7.2±0.02	5.4±0.01	8.1±0.04
30°C	14.4±0.01	8.6±0.06	16.7±0.05	11.5±0.01	7.7±0.02	14.9±0.03	7.3±0.01	5.3±0.00	9.2±0.02
40°C	14.6±0.03	8.5±0.03	17.1±0.02	12.3±0.03	7.7±0.01	15.4±0.03	7.7±0.01	5.3±0.00	9.8±0.02
50°C	11.6±0.03	8.0±0.05	17.9±0.03	10.3±0.02	7.8±0.04	16.3±0.07	7.2±0.02	5.4±0.01	10.5±0.03

Table 2.3. Dielectric constant and loss of soy flour mixed with methanol, isopropanol, and hexane.

Soy flour - Solvent	Ratio	433 MHz		915 MHz		2450 MHz	
		ε'	ε''	ε'	ε''	ε'	ε''
Methanol	1:0.5	8.39±0.12	4.77±0.20	5.63±0.04	4.30±0.39	4.00±0.04	2.06±0.03
	1:1	10.56±0.17	6.35±0.13	6.89±0.03	5.37±0.09	4.66±0.04	2.67±0.03
	1:2	12.38±0.06	7.53±0.05	8.40±0.09	5.92±0.12	5.47±0.03	2.91±0.03
Isopropanol	1:0.5	23.42±0.32	10.57±0.37	17.22±0.07	7.91±0.26	11.11±0.04	4.96±0.10
	1:1	24.74±0.14	13.54±0.06	17.16±0.03	9.84±0.08	10.60±0.02	5.42±0.03
	1:2	23.63±0.67	12.45±0.33	16.20±0.84	8.97±0.23	10.23±0.47	5.21±0.04
Hexane	1:0.5	3.60±0.11	0.20±0.07	4.42±0.10	0.18±0.11	3.61±0.07	0.07±0.02
	1:1	3.11±0.07	0.07±0.07	3.86±0.05	0.06±0.11	3.35±0.04	0.02±0.02
	1:2	2.91±0.04	0.18±0.03	3.20±0.04	0.11±0.01	2.92±0.01	0.03±0.01

Table 2.4. Dielectric constant and loss of rice bran mixed with methanol, isopropanol, and hexane.

Rice bran – Solvent	Ratio	433 MHz		915 MHz		2450 MHz	
		ε'	ε''	ε'	ε''	ε'	ε''
Methanol	1:0.5	12.79±0.04	0.04±0.08	11.43±0.07	3.67±0.16	8.47±0.03	3.98±0.02
	1:1	12.59±0.27	5.09±0.28	9.27±0.19	0.19±0.06	5.67±0.03	3.49±0.06
	1:2	3.86±0.18	0.18±0.05	5.05±0.13	0.42±0.12	3.73±0.03	0.43±0.03
Isopropanol	1:0.5	16.39±0.05	5.63±0.04	13.08±0.04	3.66±0.04	9.26±0.01	2.76±0.01
	1:1	23.69±0.07	12.25±0.04	16.95±0.02	8.51±0.03	11.05±0.01	4.90±0.02
	1:2	18.83±0.02	9.05±0.02	12.50±0.01	6.17±0.01	8.66±0.01	4.03±0.01
Hexane	1:0.5	3.16±0.03	0.18±0.07	3.04±0.03	0.08±0.02	2.95±0.01	0.12±0.02
	1:1	4.04±0.01	0.02±0.01	4.80±0.02	-0.23±001	3.49±0.00	0.00±0.00
	1:2	3.45±0.04	0.15±0.04	3.71±0.03	-0.01±0.01	3.32±0.02	0.05±0.01

Dielectric Loss

The dielectric loss of all mixtures except those with hexane (which were virtually zero) varied with frequency, temperature, solvent type, and mix ratio. For ethanol, dielectric loss increased to a maximum around 1000 MHz but then slightly decreased as frequency increased to 3000 MHz (Figure 2.5 and 2.6). In addition, the dielectric loss value at 915 and 2450 MHz were very similar, raising the possibility of a relatively smooth transition with no major changes in behavior between these two frequencies. This is especially important for the eventual scale up of a microwave processing of these mixtures from low penetration 2450 MHz commonly used in laboratory settings to 915 MHz (higher penetration depth and larger generators used in industrial settings).

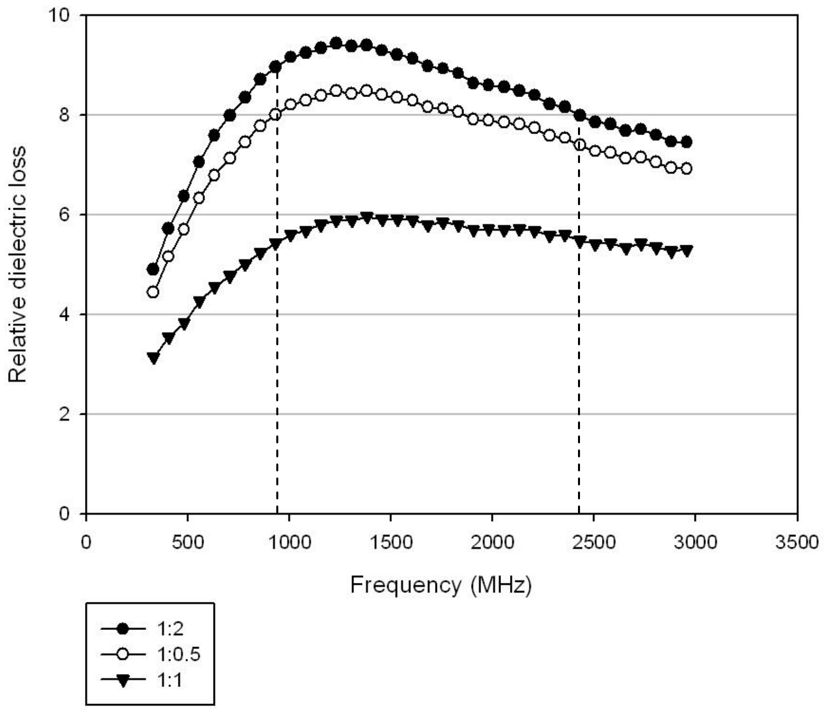


Figure 2.5. Dielectric loss of soy flour:ethanol at 23°C**.**

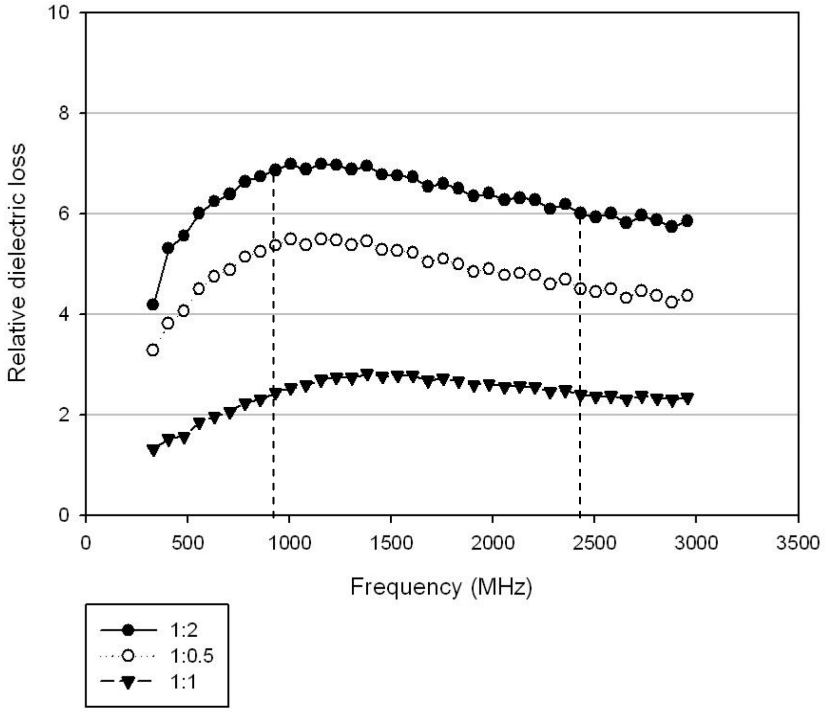


Figure 2.6. Dielectric loss of rice bran:ethanol at 23°C.

A shift in frequency of maximum values of dielectric loss as temperature increased was also observed for the ethanol mixtures. This phenomenon was observed by other researchers in pure solvents (Tang et al., 2002), and it is probably due to an increase in relaxation time of solvent molecules as viscosity decreases with temperature. In this case, dipolar rotation becomes more pronounced, with a direct consequence of shifting the peak dielectric loss toward higher frequencies, a characteristic of more polar materials. For the other solvents mixed with soy flour, the maximum value was around 433 MHz, decreasing thereafter with increasing frequency (Table 2.3). Dielectric loss of ethanol mixtures were also ratio dependent, with 1:2 ratios having the highest values, and 1:1 ratio the lowest for both soy flour and rice bran. The dielectric loss values should be even higher at ratios commonly used in solvent extraction (1:4 ÷ 1:10 ratio) leading to a more rapid temperature increase during microwave processing. Similar conclusion can be withdrawn for methanol, that also had the highest ε'' at 1:2 ratio with soy flour, but in the case of this solvent and feedstock the lowest value was noted at 1:0.5 ratio. These very low values might have been due to insufficient molecular freedom of the rice bran components during measurement at these low solvent concentrations. For methanol, ε'' were also the highest at 1:2 but the lowest values were at 1:0.5 ratios. Isopropanol had the highest value at 1:1 ratio with soy flour. For rice bran, the highest values were obtained at 1:0.5 ratios with methanol and at 1:1 when using isopropanol. These non-uniformity in ratio dependency of dielectric loss for the different solvents might be attributed to their particular physical properties especially the freedom for molecular rotation or adsorption onto the surface of the feedstock particles.

In general, dielectric loss was inversely dependent on temperature. The absorbed energy is dependent on ε'', therefore the maximum absorbance of soy flour with ethanol was at 1:2 ratios at 30°C and 915 MHz (Figure 2.7) whereas for rice bran it occurred at 30°C at 433 MHz

(Figure 2.8). At 2450 MHz, no major difference were observed for the soy flour sample at 1:1 mix ratio throughout the temperature range (Table 2.5).

Table 2.5. Values of ε'' for soy flour and ethanol.

Ratio / Temp	433 MHz			915 MHz			2450 MHz		
	1:0.5	1:1	1:2	1:0.5	1:1	1:2	1:0.5	1:1	1:2
23°C	5.4±0.30	3.7±0.02	6.0±0.27	7.9±0.42	5.4±0.03	8.9±0.38	7.3±0.29	5.4±0.01	7.9±0.26
30°C	6.1±0.01	3.7±0.02	6.2±0.03	9.1±0.01	5.5±0.02	9.2±0.03	8.2±0.01	5.5±0.01	8.3±0.03
40°C	5.4±0.04	3.4±0.01	5.5±0.06	8.5±0.03	5.2±0.02	8.6±0.04	8.4±0.01	5.5±0.00	8.6±0.00
50°C	3.8±0.06	3.0±0.03	4.4±0.04	6.3±0.09	4.5±0.04	7.3±0.06	7.3±0.05	5.4±0.01	8.8±0.00

Table 2.6. Values of ε'' for rice bran and ethanol.

Ratio / Temp	433 MHz			915 MHz			2450 MHz		
	1:0.5	1:1	1:2	1:0.5	1:1	1:2	1:0.5	1:1	1:2
23°C	3.9±0.06	1.5±0.19	3.9±0.03	5.3±0.13	2.4±0.42	2.6±0.02	4.5±0.01	2.4±0.07	3.3±0.04
30°C	3.8±0.03	1.7±0.01	3.1±0.05	5.3±0.01	2.7±0.02	1.9±0.03	4.5±0.01	2.5±0.00	1.7±0.02
40°C	3.2±0.03	1.6±0.01	1.1±0.02	4.8±0.03	2.7±0.01	1.6±0.03	4.8±0.01	2.5±0.00	1.6±0.02
50°C	1.9±0.02	1.3±0.02	0.9±0.03	3.0±0.02	2.2±0.02	1.3±0.07	3.7±0.0	2.3±0.01	1.5±0.03

For the rice bran mixed with ethanol (Table 2.6) there is a considerable decrease in ε'' as temperature increases (Figure 2.8). The lower value of ε'' for the rice bran could indicate a weaker interaction between the electromagnetic radiation and the components of the rice bran (lower protein content than soy flour which means lower conductance) responsible for dissipation of the electrical energy into heat.

Dielectric loss also showed to be dependent on solvent type. Among the solvents investigated in this study, ethanol had the highest values of ε'' but also the highest values of ε', indicating a good medium for high energy conversion into heat. Figures 2.9 and 2.10 illustrate the differences between the solvents at the ratios analyzed for soy flour and rice bran at room temperature.

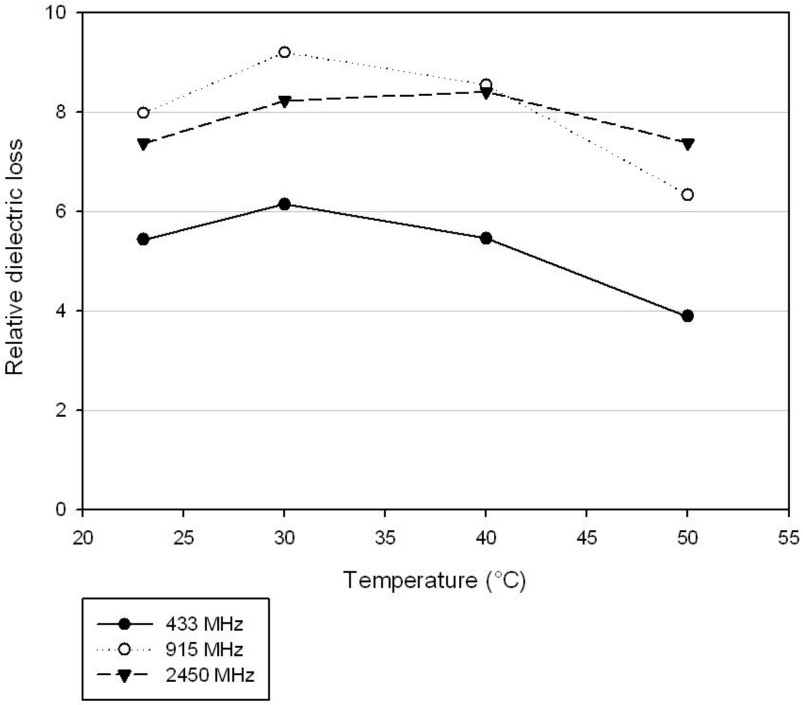


Figure 2.7. Dielectric loss of soy flour:ethanol 1:2.

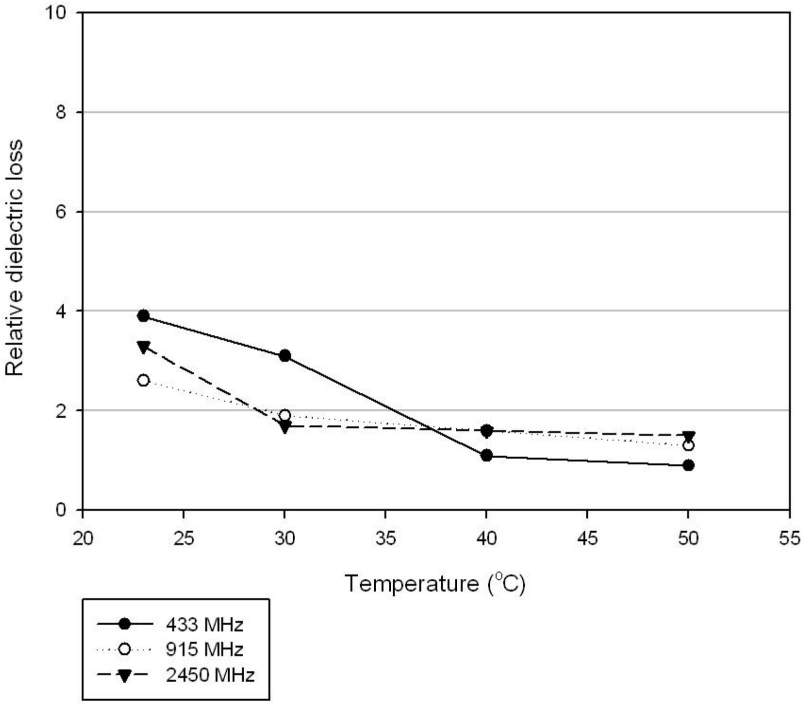


Figure 2.8. Dielectric loss of rice bran:ethanol 1:2.

From these figures it is clear that ethanol would allow for the most rapid increase in temperature during microwave heating, while hexane is virtually inert with respect to microwave energy absorption. Therefore, the ethanol presents itself as the most suitable solvent candidate for extraction of oil in a microwave system at higher temperatures, whereas hexane might be more suitable for selective extraction of temperature-labile bioactive compounds which could be extracted at lower temperature with this solvent.

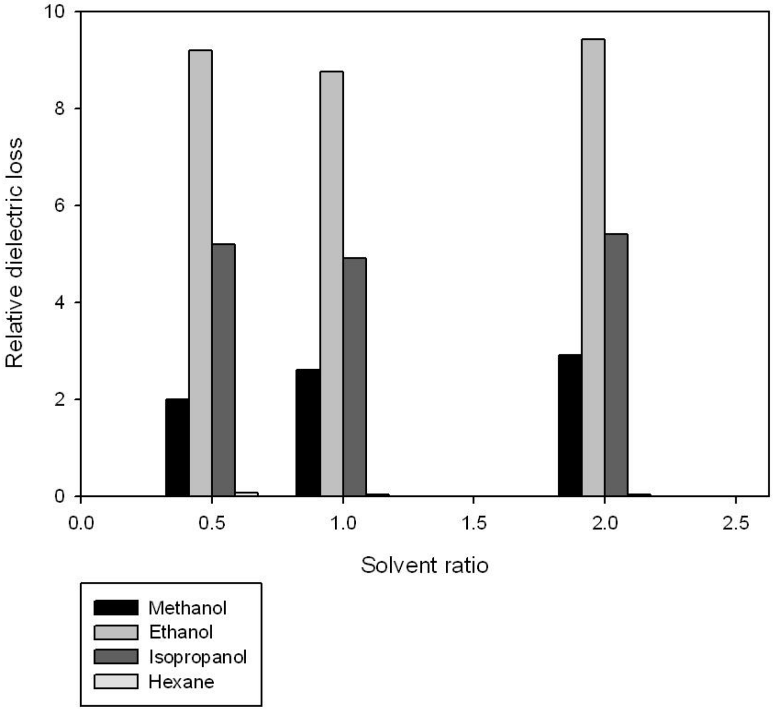


Figure 2.9. Dielectric loss of soy flour with different solvents.

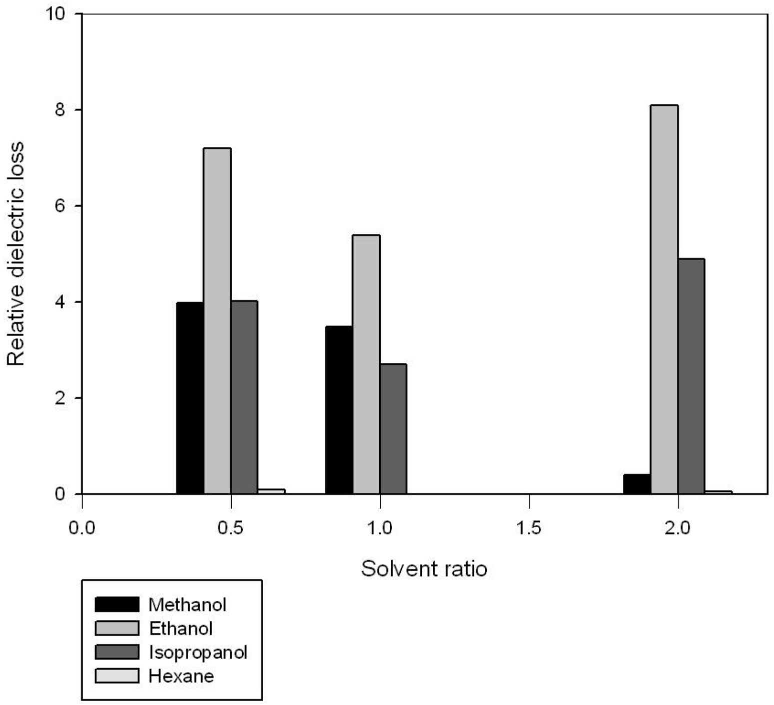


Figure 2.10. Dielectric loss of rice bran with different solvents.

Numerical Analysis

For pure polar or non-polar substances, Debye's or Cole-Cole equations are usually used to describe the behavior of dielectric properties with frequency (Nelson, 1973; von Hippel, 1954). As previously described, the complexity of the studied systems do not allow such simplifications, and a quasi-linear relationship using a second order logarithmic transformation of the data provided a reliable estimator for dielectric constant as a function of frequency for all solvents, feedstock, and solvent ratio (with the exception of hexane).

This relationship (Eq. 3) fitted well for all solvents, ratios and temperatures used in this study. The coefficient values and r^2 for the equation are presented in Table 2.7.

$$\varepsilon' = y_0 + a \ln x + b(\ln x)^2 \tag{3}$$

were: x-frequency (MHz), a, b - parameters.

Table 2.7. Coefficient values for dielectric constant fitted curve as frequency (Eq. 3).

		Soy flour			Rice bran		
		1:0.5	1:1	2:1	1:0.5	1:1	2:1
Methanol	y_0	-291.421	-316.193	-138.626	-1052.60	-309.162	-155.252
	a	30.618	32.358	16.472	102.920	29.328	15.071
	b	-0.786	-0.817	-0.459	-2.500	-0.686	-0.364
	r^2	0.990	0.988	0.981	0.979	0.970	0.956
Isopropanol	y_0	519.751	458.543	442.315	351.086	644.039	633.355
	a	-45.138	-40.239	-37.115	-59.278	-60.060	-56.086
	b	0.986	0.890	0.781	1.357	1.405	1.249
	r^2	0.968	0.955	0.974	0.915	0.855	0.968
Ethanol 23°C	y_0	-1133.44	-554.823	-961.223	-592.036	-314.965	-265.598
	a	108.995	52.968	92.301	57.240	30.084	29.365
	b	-2.599	-1.251	-2.196	-1.371	-0.712	-1.036
	r^2	0.973	0.968	0.974	0.960	0.958	0.965
Ethanol 30°C	y_0	-1223.83	-621.959	-1167.87	-638.746	-441.702	-398.459
	a	117.613	59.446	112.222	61.646	42.298	37.896
	b	-2.803	-1.407	-2.674	-1.475	-1.005	-1.698
	r^2	0.964	0.979	0.969	0.965	0.861	0.925
Ethanol 40°C	y_0	-1093.65	-557.159	-1056.38	-567.189	-472.962	-402.398
	a	104.518	52.984	100.863	54.203	45.231	40.862
	b	-2.476	-1.246	-2.386	-1.283	-1.074	-0.985
	r^2	0.958	0.958	0.965	0.9523	0.847	0.939
Ethanol 50°C	y_0	-663.127	-379.900	-741.922	-265.174	-331.072	-465.298
	a	62.489	35.592	69.697	24.792	31.481	35.437
	b	-1.455	-0.821	-1.617	-0.571	-0.742	-0.983
	r^2	0.963	0.968	0.970	0.956	0.866	0.982

Regression analysis was performed to relate the dielectric loss responses of soy flour and rice bran and different solvents to moisture content and frequency. Dielectric loss factor for methanol (Eq. 4) and isopropanol (Eq. 5), respectively, were found to be in the form of:

$$\varepsilon''=b+m_1x_1+m_2x_2+m_3x_1x_2 \tag{4}$$

$$\varepsilon''=b\cdot m_1^{x_1}\cdot m_2^{x_2}\cdot m_3^{x_1x_2} \tag{5}$$

were, x_1- frequency, x_2- moisture content, and b, m_1, m_2, m_3- parameters.

For ethanol, both the dielectric constant (Eq. 6) and loss (Eq. 7) were found to follow the logarithmic transformed linear regression:

$$\varepsilon'=b\cdot m_1^{x_1}\cdot m_2^{x_2}\cdot m_3^{x_1x_2} \tag{6}$$

$$\varepsilon''=b\cdot m_1^{x_1}\cdot m_2^{x_2}\cdot m_3^{x_1x_2} \tag{7}$$

were, x_1- frequency, x_2- moisture content, and b, m_1, m_2, m_3- parameters.

The coefficients for correlations determined using Eq. (4) through (7) are presented in Tables 2.8 and 2.9. The high values of coefficients of determination (r^2) show that these equations can be reliably used for predicting the dielectric properties of these solvent-feedstock mixtures over a range of temperatures and solvent ratios.

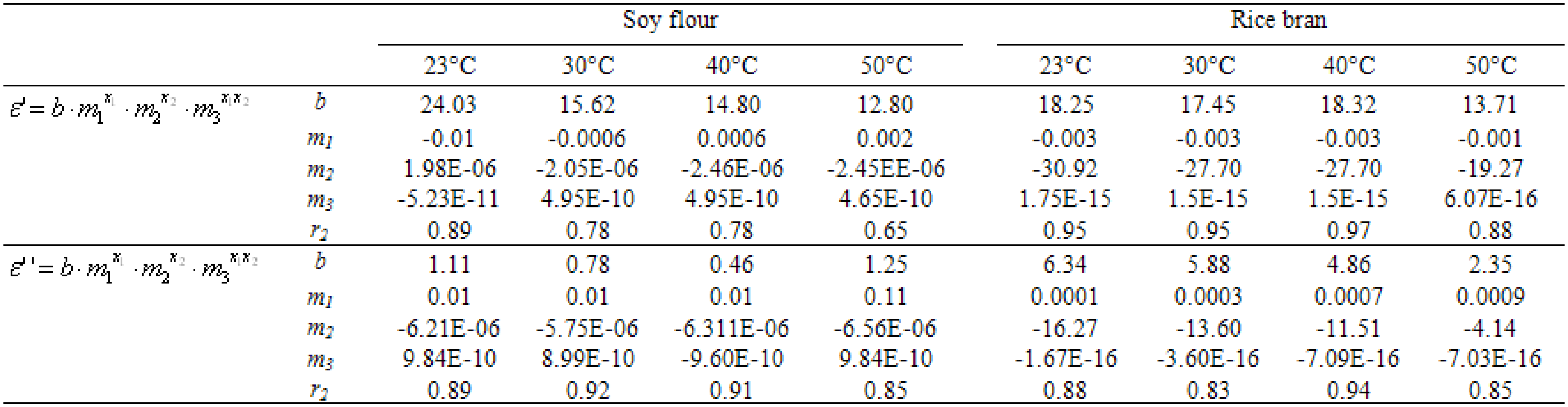
Table 2.8. Coefficients values and r^2 for the multiple linear regressions (frequency and solvent content) for methanol and isopropanol with the two feedstocks (Eq. 4 and 5).

Methanol		Soy flour	Rice bran
$\varepsilon'' = b + m_1 x_1 + m_2 x_2 + m_3 x_1 x_2$	b	8.121	13.321
	m_1	0.003	-0.002
	m_2	-4.605	-19.751
	m_3	1.13528E-06	3.47297E-05
	r^2	0.944	0.830
Isopropanol		**Soy flour**	**Rice bran**
$\varepsilon'' = b \cdot m_1^{x_1} \cdot m_2^{x_2} \cdot m_3^{x_1 x_2}$	b	16.325	14.447
	m_1	-0.011	-1.012
	m_2	3.17E-06	4.23E-06
	m_3	-2.2814E-10	-4.4922E-10
	$r2$	0.921	0.833

Conclusions

Dielectric properties of oil feedstock mixed with different solvents were measured and analyzed. Results indicated that dielectric constants were relatively unchanged throughout the frequency range, but were strongly dependent on temperature, solvent ratio and type. The dielectric constant of mixtures of both rice bran and soy bean flour with all solvents except hexane decreased with temperature, while the ones with hexane increased to a maximum and then decreased. Dielectric loss of the mixtures with isopropanol and methanol decreased with frequency, while hexane had virtually zero dielectric loss. Multiple linear regression analysis was successfully used to develop relationships between the dielectric properties and frequency and solvent-feedstock ratio.

Table 2.9. Coefficients values and r^2 for the multiple linear regressions (frequency and solvent content) for ethanol with the two feedstocks (Eq. 6 and 7).



		Soy flour				Rice bran			
		23°C	30°C	40°C	50°C	23°C	30°C	40°C	50°C
$\varepsilon' = b \cdot m_1^{x_1} \cdot m_2^{x_2} \cdot m_3^{x_1 x_2}$	b	24.03	15.62	14.80	12.80	18.25	17.45	18.32	13.71
	m_1	-0.01	-0.0006	0.0006	0.002	-0.003	-0.003	-0.003	-0.001
	m_2	1.98E-06	-2.05E-06	-2.46E-06	-2.45EE-06	-30.92	-27.70	-27.70	-19.27
	m_3	-5.23E-11	4.95E-10	4.95E-10	4.65E-10	1.75E-15	1.5E-15	1.5E-15	6.07E-16
	r_2	0.89	0.78	0.78	0.65	0.95	0.95	0.97	0.88
$\varepsilon'' = b \cdot m_1^{x_1} \cdot m_2^{x_2} \cdot m_3^{x_1 x_2}$	b	1.11	0.78	0.46	1.25	6.34	5.88	4.86	2.35
	m_1	0.01	0.01	0.01	0.11	0.0001	0.0003	0.0007	0.0009
	m_2	-6.21E-06	-5.75E-06	-6.311E-06	-6.56E-06	-16.27	-13.60	-11.51	-4.14
	m_3	9.84E-10	8.99E-10	-9.60E-10	9.84E-10	-1.67E-16	-3.60E-16	-7.09E-16	-7.03E-16
	r_2	0.89	0.92	0.91	0.85	0.88	0.83	0.94	0.85

The results of the study will assist scientists and engineers in selection of appropriate solvent, mixing ratio and frequency for designing microwave assisted extraction systems for oil human consumption and other valuable products, which can be further processed into biodiesel.

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CHAPTER 3

SOYBEAN AND RICE BRAN OIL EXTRACTION EFFICIENCY IN A CONTINUOUS MICROWAVE SYSTEM (LAB AND PILOT SCALE)

Introduction

Oil is a valuable product with universal demand that can be extracted from a number of fruits, nuts and seeds for traditional use in cooking, soap making, pharmaceutical and cosmetic industry. Recently, it has become an important primary feedstock for alternative fuels such as biodiesel. Soybean oil is the dominant edible oil in the United States (78% - Bureau of Census, USDA-ERS) and probably will continue to dominate U.S. and world vegetable protein and oil markets in spite of competition from other oilseeds. Soybeans, with an oil content ranging from 16 to 20% by weight (Bailey, 2005), are a rich source of proteins, lecithin and antioxidants. The oil from soybean is low in saturated fat, contains no trans fat, and is high in poly- and monounsaturated fats. It is also the principal source of omega-3 fatty acids in the U.S. diet, and the primary commercial source of vitamin E (Choi et al., 2006). The relatively large amount of undesirable phosphatides present in the oil (about 4%) which are removed during processing is a valuable source for commercial lecithin (Erickson et al., 1984).

Rice bran, a byproduct from rice processing, contains about 16-22% of oil by weight (Narayana et al., 2002). The rice bran oil is considered superior due to its balanced fatty acid profile and the presence of minor constituents with proven nutritional benefits such as gamma oryzanol, tocotrienols and tocopherols (Hamm and Hamilton, 2000). The oil extraction process from rice bran is challenging mainly because of unusually high content of undesirable elements - waxes (3-8%), free fatty acids, unsaponificable constituents and polar lipids such as glycolipids

(Narayana et al., 2002). Therefore, a series of refining processes (degumming, deacidification, bleaching, deodorization, winterization) are required to obtain high quality edible rice bran oil.

Various methods are reported for oil extraction but basically two main processes are available for defattening and deoiling plant products: extraction and pressing. Pressing, from an economic point of view, is only advantageous when the fat and oil content of the material is relatively high (>25% by weight). Utilizing this process, a residual fat content of at least 4 to 5% by weight remains in the processed residue. In contrast, extraction processes with organic solvents are more suitable for products with low fat content, with the residual oil content being reduced to less than 1% by weight (Erickson et al., 1984; Hamm and Hamilton, 2000).

Most extraction applications from plant materials are performed in the presence an extracting agent in liquid phase (water or an organic solvent). Solvent assisted extraction is performed at elevated temperatures, taking advantage of the enhanced solubilities, decreased viscosity (allowing better penetration of the sample in the matrix) and increased analyte mass diffusion rate.

Absolute n-hexane, a petroleum-derived product, has been extensively used as a solvent because it satisfies many of the requirements of an ideal oil solvent (Johnson and Lusas, 1983) (non polar, easily miscible with oil, low viscosity, low boiling point for subsequent separation, low cost). However, other solvents or solvent mixtures have been considered recently, due to environmental considerations and safety of n-hexane (hazardous air pollutant, volatile, ignites easily, and may affect health through inhalation) (Lusas et al., 1991; Gandhi et al., 2003). In a study on alternative solvents that can be used for oil extraction (Gandhi et al., 2003), ethanol was equally effective in the extraction of soybean oil when compared with n-hexane.

Some of the methods reported for oil extraction include ohmic heating (Lakkakula et al., 2004) where an alternating current is used for product heating; Soxhlet extraction – considered the gold standard, where oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent under reflux in a special glassware (Nur, 1976; Perez-Serradilla et al., 2007); supercritical fluid extraction (SFE) (Liu et al., 2003; Salgin et al., 2006) which resembles Soxhlet extraction except that the solvent used is a supercritical fluid substance above its critical temperature and pressure; high-intensity, high-frequency sound waves (ultrasound) (Luque-Garcia and de Castro, 2003) and ultra sonic extraction (Li et al., 2004; Luque-Garcia and de Castro, 2004). Some of the disadvantages of these known processes include oil degradation due to high thermal and pressure stress, in critical and supercritical state condition, or reduced mass transfer coefficient requiring longer extraction periods and larger amount of extracting agents required.

Another potential technique that can improve extraction of oil and other functional components from plant raw material is microwave processing, which may be used either as a thermal pretreatment for plant material prior to extraction or as a treatment process enhancement during extraction (Giese, 1992). Microwave extraction has advantages over conventional and other extraction methods, as intact organic compounds can be extracted more selectively and more rapidly (Pare et al., 1994), with the added advantage of lower energy consumption, reduced byproduct formation, and a minimal solvent usage (Letellier and Budzinski, 1999). In conventional extraction the extractability of different components depends mainly on the solubility of the compound in the solvent, mass transfer kinetics of the product and matrix interactions (Spigno and de Faveri, 2009), whereas under microwave-assisted extraction (MAE) heating rate plays an important role in extraction efficiency. This heating rate is influenced by factors such as microwave power level, frequency, initial temperature and design of microwave applicator, and can be selected for a particular processing application. For extraction two basic designs of microwave exists. The firs one is a scientific/industrial/laboratory level multimode cavity which in principle is similar with home microwave unit. In multimode, microwaves reflect of the walls and generate a standing wave pattern in which waves intersect at specific points in the cavity. The second mode use a focusing concentrated at one waveguide in which waves are reflected at specific location (Boldor et al., 2007). However a major role is played by dielectric properties, an intrinsic property of the material that requires empirical measurement but is mostly influenced by the moisture liquid/solid mixture content and spatial distribution of the water and other polar/ionic compound in the matrix. The dielectric properties of materials are defined in terms of their relative complex permittivity. For a solvent/matrix to heat up rapidly under the microwave radiation, it has to have a high dielectric constant, associated with the potential for electrical energy storage in the material, and a high dielectric loss which is related to the electrical energy dissipation in the material (Nelson, 1994). The heating of a dielectric material in the presence of an electromagnetic field is based on intermolecular friction that arises via ionic conduction and dipolar rotation (Nuchter et al., 2004).

Fats and oils extraction is currently performed in industry with n-hexane, known to be a non-polar agent, using conventional heating methods. Studies have been reported using n-hexane as an extraction agent with microwaves (Virot et al., 2007) , but other polar solvents such as isopropanol, methanol, ethanol, acetone and water are commonly employed for the extraction of plant bioactive compounds (Duvernay et al., 2005; Pan et al., 2003; Spigno and de Faveri, 2009). Since high dielectric properties solvents are more likely to be used in a microwave extraction system, ethanol was used for the extraction of oils in this study.

Disruptions of the oilseed cells is a crucial step in the extraction process, as the protoplasm of plant cells renders, becoming more permeable, and enhance the process (Baxendale et al., 2007). Temperature of water molecule inside the cells reaches the boiling point leading to high pressure gradients and rupture of cell walls, causing migration of selected compounds from sample matrix into the extraction solvent (Bhattacharya and Basak, 2006). This particularity makes the technology appealing for biodiesel, as biodiesel is produced from vegetable oil. The microwave thermal effects (localized microscopic superheating) naturally match the requirements for the disruption process of tissues and could be used to induce rupture of cells for efficient extraction of oils and other components from plants.

Until now microwave assisted extraction (MAE) has been successfully applied for the extraction of natural compounds from foodstuffs like flavonoids and polyphenols compounds from tea (Pan et al., 2003) and grape seeds (Hong et al., 2001), constituents from herbals (Guo et al., 2001), pigments from paprika (Kiss et al., 2000), antioxidants from rice bran (Zigoneanu et al., 2008), isoflavones from soybeans (Rostagno et al., 2007; Terigar et al., 2009) and also for trace analysis of organic compounds in solid and liquid samples (Eskilsson and Bjorklund, 2000).

Continuous flow microwave assisted extraction (CMAE) is a modification of the MAE process in which the mixture is continuously pumped and heated in a microwave cavity. This process is more complex due to the addition of momentum transfer to the heat generation from microwave heat transfer in the solvent/solid matrix, mass transfer through the solid/solvent (Bhattacharya and Basak, 2006).

When evaluating an extraction process it is important to consider the various factors affecting it during scale up to commercial operations. In microwave processing this usually means a change in frequency from 2450 MHz to 915 MHz. Microwaves at 915 MHz (used industrially) have much higher penetration depths into the material as compared to the higher frequency of 2450 MHz commonly used in laboratory sized equipment. The higher penetration depths allow for much larger diameter tubes and processing flow rates, and microwave generators can be built for significantly higher power and efficiencies when compared to smaller generators. In comparison, the design with a single mode applicator (as appeared to multimode commonly used in household microwaves) focuses the microwaves in the center of the applicator, where the material flows in a processing tube. This resonance mode allows for very high electric field values which increase the heating rate according to equation 2 (chapter 1).

This focusing creates an electrical field distribution with the highest values in the center of the applicator tube and decreasing as it nears the walls of the tube. Therefore, if the flow in the tube is laminar, the fluid with highest velocity in the center receives the highest amount of microwave energy. The fluid with the lowest velocity near the wall receives lower amounts of energy, therefore creating a more uniform temperature distribution when exiting the microwave applicator (Baxendale et al., 2007; Salvi, 2007). While this difference in electric field distribution may not play a significant role in small diameter tubes, when scaling up to higher flow rates and consequently larger diameter tubes, temperature uniformity becomes more important. Continuous processes using a 5kW, 915 MHz microwave have been successfully applied so far for beverage and vegetable purees sterilizations (Sabliov et al., 2008; Kumar et al., 2008), for aseptic processing (Coronel et al., 2008) and for ballast water treatments (Boldor et al., 2008).

The primary objective of this study is to investigate the viability of continuous microwave processing for soybean and rice bran oil extraction. The designed microwave system was optimized to reduce the extraction time while maximizing the quantity of oil extracted. Its performance was compared to the oil yields from conventional solvent extraction methods. Factors affecting the extraction, such as temperature and time, were analyzed for better extraction performance.

The processing parameters (microwave exposure time, temperature and holding times) obtained from the small scale continuous microwave extraction system were used to establish preliminary processing parameters at the pilot scale. Due to the amount of products required to operate this system, it is cost prohibitive to test the scaled-up process using all the parameters tested with the smaller system. Extraction temperature and times, were optimized for better extraction performance.

Materials and Methods

Feedstock for Oil Extraction

The rice bran used in the extraction studies was obtained from milling Cocodrie rice provided by Louisiana State University Agricultural Center's (LSU AgCenter) Rice Research Station (Crowley, LA). Rice was milled as described in Chapter. After milling, rice bran was kept in the freezer at -20ºC for about a week prior to use. The soybeans used were harvested and collected from LSU AgCenter's Central Research Station in Baton Rouge, LA and stored at -4°C. Soybeans were ground with a Kitchen mill (Blendtech, Utah) in order to obtain soy flour, and stored in a freezer at -20°C.

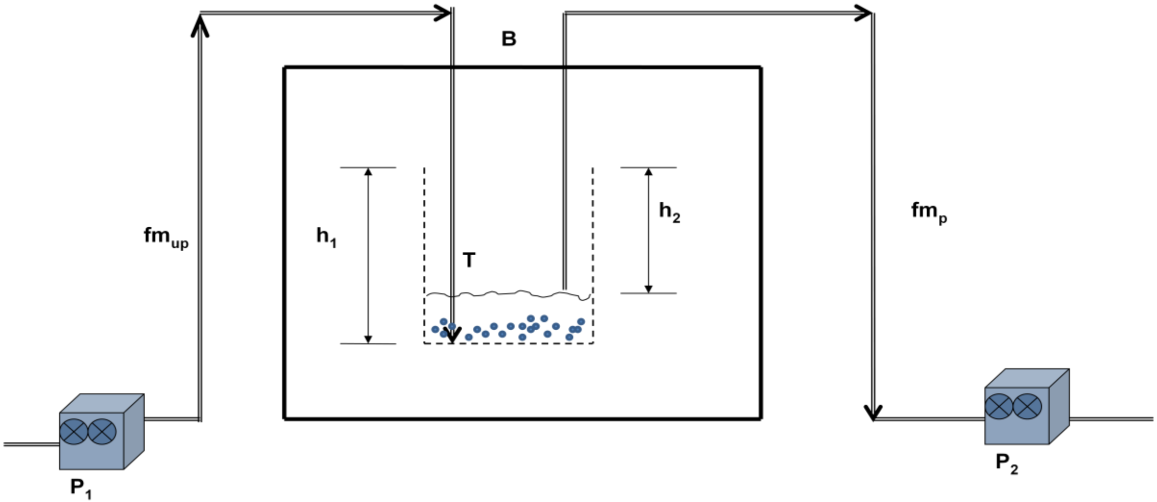
Laboratory-Scale Microwave Assisted Oil Extraction

Figure 3.1 presents a schematic of the continuous system. A commercially available, fully instrumented, batch type microwave system operating in multimode (ETHOS E Microwave Extraction System, Milestone Inc., Monroe, CT) was modified for continuous operation. The set-up included a 2 L cylindrical Teflon® beaker (SPI Supplies, West Chester, PA) of outer diameter 12 cm and height 21 cm acting as the reaction chamber in the center of the microwave application chamber. Two Teflon® (poly fluorotetraethylene) tubes of inner diameter of 0.953 cm (3/8”) were inserted through the top wall into the beaker to serve as inflow and outflow conduits. These tubes were connected to flexible PET tubing using quick disconnect fittings to facilitate dismantling and cleaning. A 3:1 solvent (Absolute Proof Ethanol) to flour ratio reaction mixture was pumped into the microwave chamber (into the beaker) at a constant flow rate of 100 mL/min using a peristaltic pump (model 7518, Cole-Parmer Instr. Co, Barrington, IL) and was pumped out using another peristaltic pump at a higher flow rate (150 mL/min). This ratio was selected to prevent settling of the mixturewhile allowing pumping through these tubes (lower solvent concentration were tested initially but resulted in operational problems). The different inflow and outflow flow rates helped ensure a continuous operation of the system while maintaining a constant sample level in the beaker. While the outlet of the Teflon® tube connected to the inflow pump was placed at the bottom of the beaker, the inlet of the second Teflon® tube (connected to the outflow pump) could be adjusted so that it was placed at various heights (measured from the bottom of the beaker) depending on the residence time required. The product to be processed was microwave heated to 50, 60 and 73°C (monitored with a fiber optic probe and automatically controlled by the system) and maintained for various residence times (4 to 20 min, at 4 min intervals). After processing, the mixture were filtered from the cake through a Whatman filter paper (Φ=47 mm) using a vacuum pump (Model SR10/50, Thomas Compressors and Vacuum Pumps, Skokie, IL) and the solvent was evaporated in a vacuum centrifuge evaporator (CentriVap Console Labconco, Kansas City, Missouri) running for approx 14-16 hours. The residual oil for each sample was weighed in order to determine the extraction yield.

Soy oil extraction yield is usually expressed as percent of total oil in the sample according to oil in product (g)/total oil in feed (g). However, the agricultural literature related to oil extraction (Bailey, 2005) expresses oil yields as %oil in initial mass of feedstock. As such, variations will appear among different crops and even among different harvest of the same variety depending on the conditions during the growth of the crop. In this work, it is assumed that the "true total oil content" does not change among the different samples, and its value is the one obtained with Soxlet method. The formula used for calculating the oil yield was:

$$\%\,yield = \frac{m}{V} \times R \times \rho^{-1}$$ (Terigar et al., 2009)

where: m - mass of oil after evaporation (g); V - volume of solvent-oil mixture (ml); R – solvent to flour ratio (3:1); ρ - density of ethanol (0.789 g/cm³).



B - Batch microwave chamber
T - Teflon beaker
P_1 - Inflow peristaltic pump
P_2 - Outflow peristaltic pump

h_1 - Fixed height of inlet tube
h_2 - Adjustable height of outlet tube
fm_{up} - Unprocessed feed mixture
fm_p - Processed feed mixture

Figure 3.1. Schematic representation of the laboratory-scale continuous microwave assisted extraction system

Figure 3.2. Laboratory-scale microwave assisted extraction system:
left – batch; right – continuous.

Conventional Solvent Extraction

The extractions were carried out in a round bottom flask equipped with a water condenser on a plate heater with a magnetic base, in a single stage extraction. The mixture (3:1, solvent:soy flour mix) was heated to the desired extraction temperatures for several minutes (4.05 min for 50°C, 5.25 min for 60°C and 6.35 min for 73°C) prior to being held at constant temperature in the oil bath to carry the extractions at different times (0, 4, 8, 12, 16 and 20 min). The mixtures were stirred constantly using a magnetic stirrer. The extraction temperature was set equal to that used in the microwave extraction process (i.e., 50, 60 and 73^{o}C). After extraction the oil yield were calculated as described above.

Soxhlet Solvent Extraction

To determine the theoretical maximum yield Soxhlet extraction was used, with 20 g of soy flour and rice bran was weighed into a cellulose thimble (30 mm x 77 mm, Whatman, Maidstone, UK), and the thimble was placed in a Soxhlet device. Two hundred milliliters of hexane and ethanol respectively was used as the extraction solvent and the extraction was performed for 10 hours. The temperature in the extraction chamber was approximately 63-65 °C when using hexane and 73-75°C when using ethanol. After the solvent was removed with a rotary evaporator, the weight of crude rice bran oil (RB) and soybean oil (SB) was measured.

Pilot-scale continuous microwave-assisted extraction

The process optimum extraction parameters obtained from the laboratory scale process were utilized in a pilot-scale continuous focused microwave system for soybean and rice bran oil extraction. The essential components of the design include a feed tank equipped with an industrial stirrer, a Seepex® progressive cavity pump (BCSB 05-12, Seepex GmbH, Bottrop, Germany) coupled with a NEMA 1 HP motor (Baldor Electric Company, Fort Smith, AR) and controlled by a AC Tech MC Series controller to provide flow rates between 0.6 l/min and 6.0 l/min of the process mixture, a 5 kW, 915 MHz continuous microwave system (Industrial Microwave Systems, Morrisville, NC) and an insulated stainless steel holding tank with sampling ports (Figure 3.3 and 3.4). The oil extraction efficiency of the system was tested for a feedstock:solvent ratio of 1:3 flowing at a rate of 1 and 0.6 l/min. The input microwave power was adjusted so that the process temperature of the mixture leaving the microwave chamber was 73^{o}C (approximately 4.2 KW for 1 l/min, and 3 KW for 0.6 l/min). The treated feedstock-solvent mixture once leaving the process chamber of the microwave is allowed to flow into the insulated holding tank and was constantly stirred at uniform velocity using a stainless steel stirrer. Samples of the treated mixture was removed at different holding times (6, 10 and 60 min), filtered and evaporated for analysis of the oil content, acidity, iodine value, phospholipids and waxes content, and fatty acid methyl ester (FAME) composition. Samples were collected in triplicate for each process condition and feedstock mixture (rice bran or soybean flour in ethanol). After each experiment, the entire process line (pumps, feed tank, holding tank, conveying tubes) was thoroughly cleaned with water and was prepared for the next experimental run.

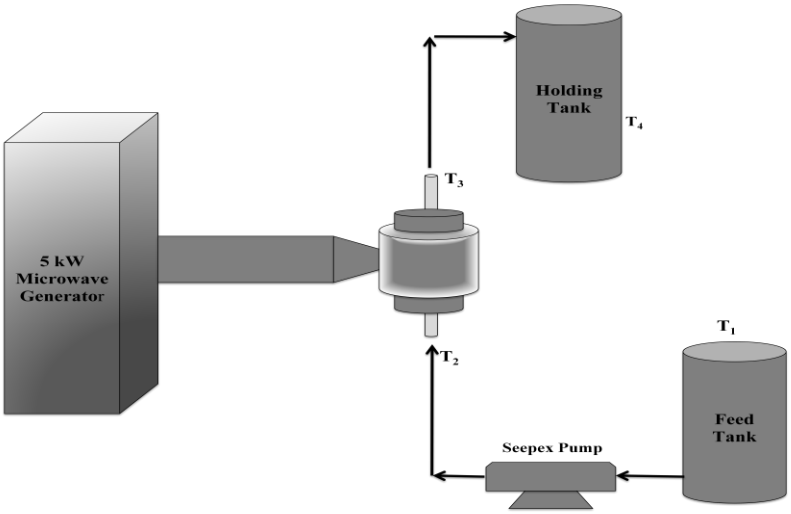


Figure 3.3. Schematic design of the pilot scale continuous microwave assisted extraction.

Figure 3.4. Design of the pilot scale continuous microwave assisted extraction.

Using the system in the current configuration limits the temperature to below the boiling point of the solvent. Higher temperatures, enhancing the process, can be accomplished by applying higher pressure, which maintains the solvent in its liquid state beyond its atmospheric boiling point.

Wax and Phospholipids Removal

The phospholipids were removed from soybean oil by hydration with water. Crude soybean oil was mixed with 3% water and the mixture was agitated for 30 min at 73°C, with care being taken to avoid air entrainment. Phospholipids and gums were afterwards separated from the oil by centrifugation and quantified. For rice bran, a winterization-dewaxing method was used to remove waxes that would normally cause cloudiness in oil when refrigerated. In general, winterization is a less sophisticated form of dry fractionation (Hamm and Hamilton, 2000). The oil was cooled slowly and kept at a low temp (5-10°C) for a minimum of 6 h to allow the wakes to crystallize. After stabilization, the samples were centrifuged for 20 min to help settling the crystals. The top oil portion was separated and the waxes were calculated by subtraction from the initial weight.

Determination of Oil Quality

The oil quality was analyzed based on acid value (AV), iodine value (IV) and fatty acid methyl ester (FAME) composition. For AV, one gram of oil was titrated with 0.1 N NaOH solution, while for IV 0.22 g of oil was titrated with 0.1 sodium thiosulfate solution in presence of carbon tetrachloride and Wij's solutions (iodine monochloride in glacial acetic acid), according to IUPAC standard methods for analysis of oil and fats (IUPAC, 1987b) and ASTM D5768-02(2006).

The fatty acids were esterified and the FAME compositions were determined by gas chromatography (Varian 450-GC) coupled with a Varian 240-MS Ion Trap Mass Spectrometer (Varian Inc. USA). A Varian FactorFour Capillary column WAXms (30m x 0.25mm i.d., 0.25 µm) was used at 245°C with helium at 1 ml/min as carrier gas, a split injector at 270°C with a split ratio of 1:20, and a detector temperature of 270°C. Components were identified by comparison to a standard FAME mix (Supelco, Bellefonte, PA).

Statistical Analysis

The experiments were performed in triplicates and the average values of oil extracted and standard deviations were calculated. Statistical analysis was performed in order to test significant differences among yields at different temperatures and holding times. A two-way ANOVA using Proc Mixed 2 x 5 factorial (version 9.1, SAS Institute, Inc., Cary, N.C.) was used to test significant differences among temperatures (50, 60 and 73°C) and different extraction times (0, 4, 8, 12, 16, 20 min) for the laboratory scale extraction and between different flow rates (0.6 l/min and 1 l/min) and holding times (6, 10 and 60 min) for the pilot scale extraction. Two-way ANOVA using Proc Multiple comparison tests were performed by using Tukey adjustment to determine the significant difference between treatments. All significant values were expressed as $p \leq 0.05$.

Results and Discussion

Effect of Temperature on Extracted Oil Yields

Extraction temperatures showed to have a statistically significant ($p \leq 0.05$) impact on the oil yield extracted from soybeans and rice bran. For soybean flour, the oil yield increased with increasing extraction temperature (Figure 3.5A). For each method of extraction used, the maximum oil amount extracted was at the highest temperature (73°C). At the highest temperature (73°C) and longest time (20 min) investigated, CMAE extracted up to 38% more oil then CE. Considering only CMAE yields were 17% at 60°C and 30% at 73°C greater than those at 50°C.

For rice bran (Figure 3.5B), increasing the temperature in the CMAE from 50°C to 60°C and 73°C resulted in an improvement in oil yield extracted of 14% and 22% respectively. Also, a significant 8% improvement in oil yield was achieved by increasing the extraction temperature from 60°C to 73°C.

When using CE, an increase of 16% for soybeans and 22% for rice bran in oil yield occurred when temperature increased from 50°C to 73°C. The increase in oil yield with the increase in temperature of extraction is a consequence of decrease solvent viscosity (Bhattacharya and Basak, 2006) and increased mass diffusion rates according to Fick's law (Geankoplis, 2003). At the same time, higher extraction temperatures require higher electric fields and rates of temperature increases. This in turn leads to higher disruption of cell walls in the matrix, facilitating extraction of oil molecules and diffusion from the sample matrix into the solvent. Similarly, Choi et al. (Choi et al., 2006) showed that the increase of soluble protein extracted from soybean was due to an increase in damage to the cells with prolonged microwave treatment.

Effect of Extraction Time on Oil Yields

The effect of time of extraction on the oil quantity during the microwave and conventional methods are presented in Figure 3.5. Extracted oil yields increased with increasing the microwave extraction time, although, as described above, the temperature used had significantly affected the oil yield extracted. For soybean (Figure 3.5A), the oil increased with temperature and time of extraction, however no significant changes in oil extracted took place between 12 and 16 min of extraction for 50°C and 60°C, but significantly improved at 20 min for both temperatures.

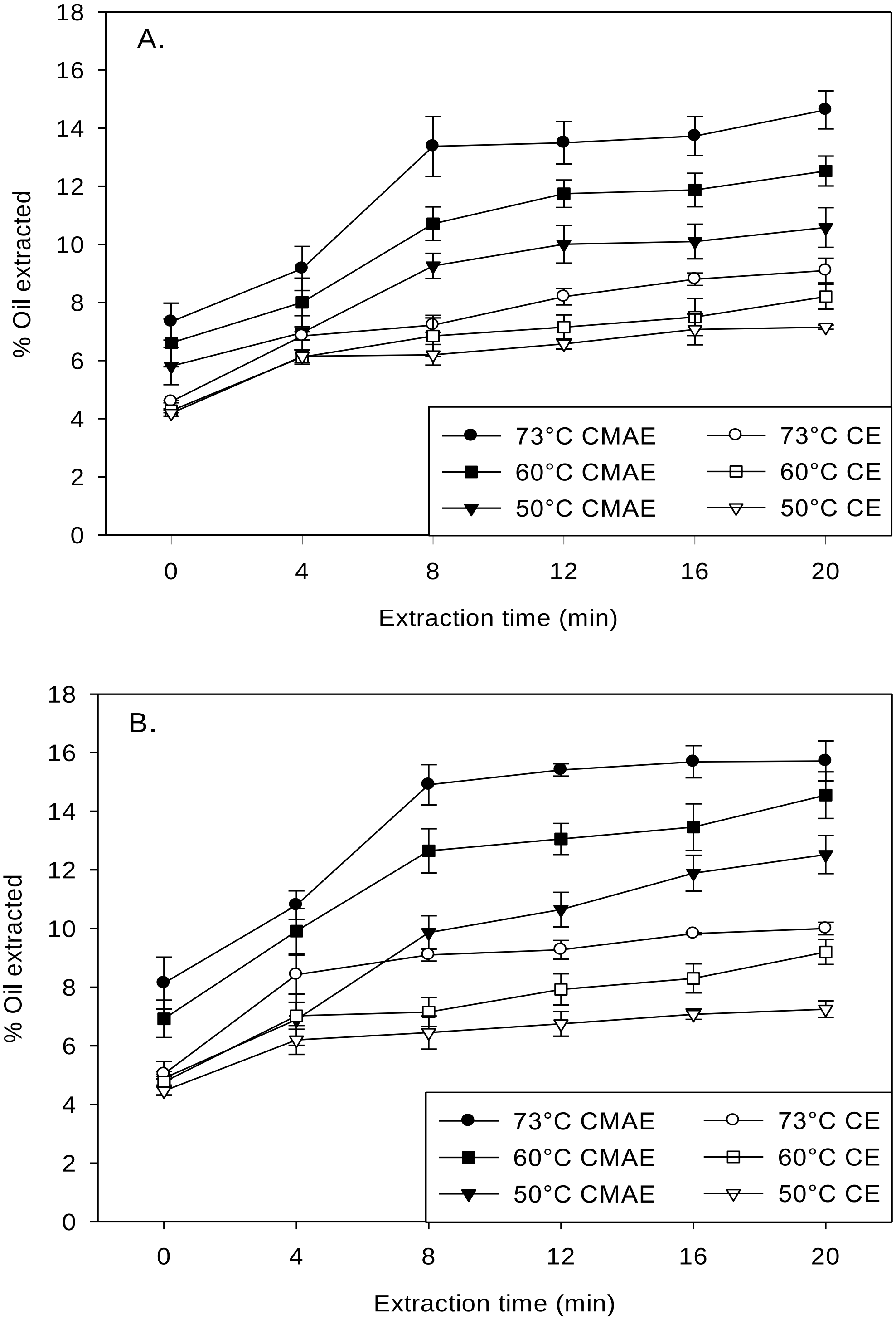


Figure 3.5. Oil extracted at different temperatures and extraction times from A. Soybean and B. Rice bran.

At 73°C, no difference in oil yield was found between 8, 12 and 16 min, but a marginal 1.3% increase (yet statistically significant) in oil yield took place by increasing the extraction time to 20 min. For all extraction temperatures, by increasing the extraction time from 0 min to 20 min, the yield of oil extracted doubled, when using CMAE.

For rice bran, the amount of oil extracted also increased with time for all temperatures (Figure 3.5B). Significant differences in the amount of oil extracted were accomplished by increasing the extraction time; however, no further improvement was observed at 73°C after 16 min of extraction.

A comparison between the methods showed that there were statistically similar oil yield results when oil was extracted with CE at 20 min and extracted with CMAE at 0 min, and CMAE giving better results at 4 min of extraction compared with 20 min CE extraction. These were expected results as a consequence of the increase of contact time between the solid phase and the liquid phase, allowing for a longer diffusion of the oil content in the solid phase into the liquid phase. Similar increase in oil yield extracted was reported by other researchers (Li et al., 2004) who studied the effect of ultrasound and microwave pretreatments in enhancing oil extraction from soy beans in a batch system indicating a linear increase in oil extraction yield with increasing microwave pretreatment time (1 and 2 min), while a 0.5 min pretreatment did not enhanced the yield extracted from the control at zero min. However these reported times are much shorter than those used in this study.

Oil Quality Analysis

Phospholipids and Waxes

Along with oil, compounds like phospholipids and waxes are also extracted from the matrix. The amount of phospholipids extracted with oil from soybeans (Figure 3.6), ranged from

1.5% to 2.8% in the feedstock, varying with increasing the temperature and time of extraction and with the amount of oil extracted. Results indicated that the amount of phospholipids are directly proportional to the amount of oil extracted, a maximum amount of phospholipids 2.8% being extracted at 73°C and 20 min of extraction. This amount was not significantly higher than the one extracted at 73°C for 16 min and 60°C for 20 min.

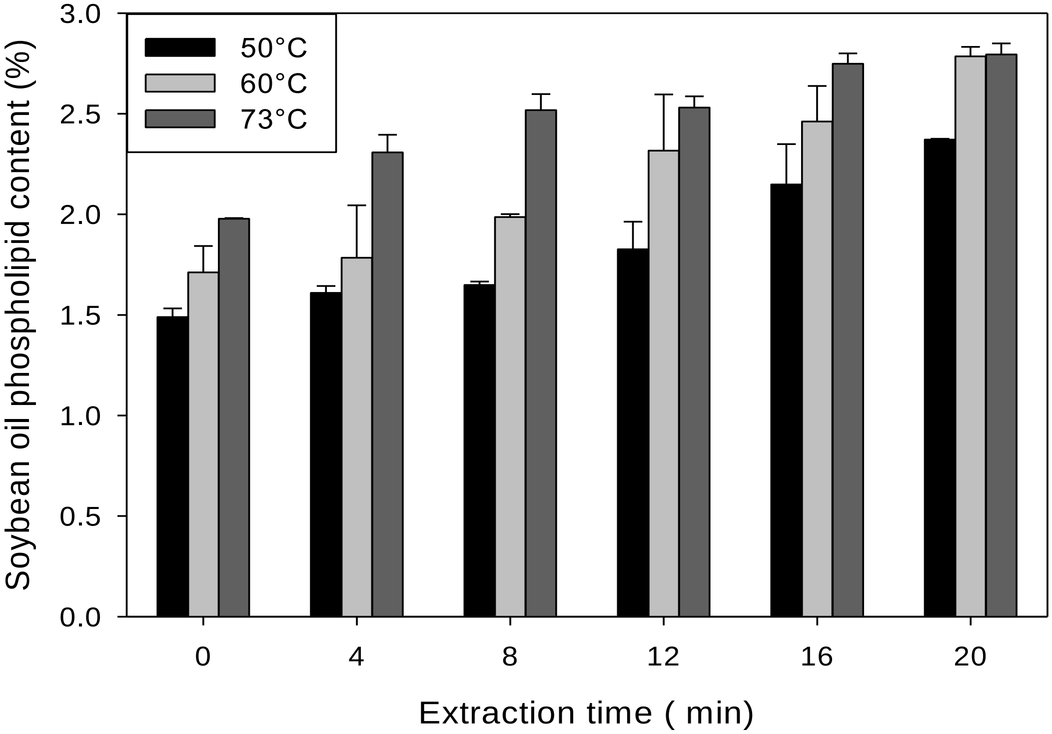


Figure 3.6. Soybean oil phospholipids extracted at different temperatures and extraction times.

Rice bran oil had an amount of waxes ranging from 1.5 to 6.6%, with the higher value for 73°C at 20 min of extraction (Figure 3.7). Similar to soybean oil, no significant differences were noticed between 16 and 20 min extraction time at 73°C, and between 12 and 16 min of extraction at 50°C.

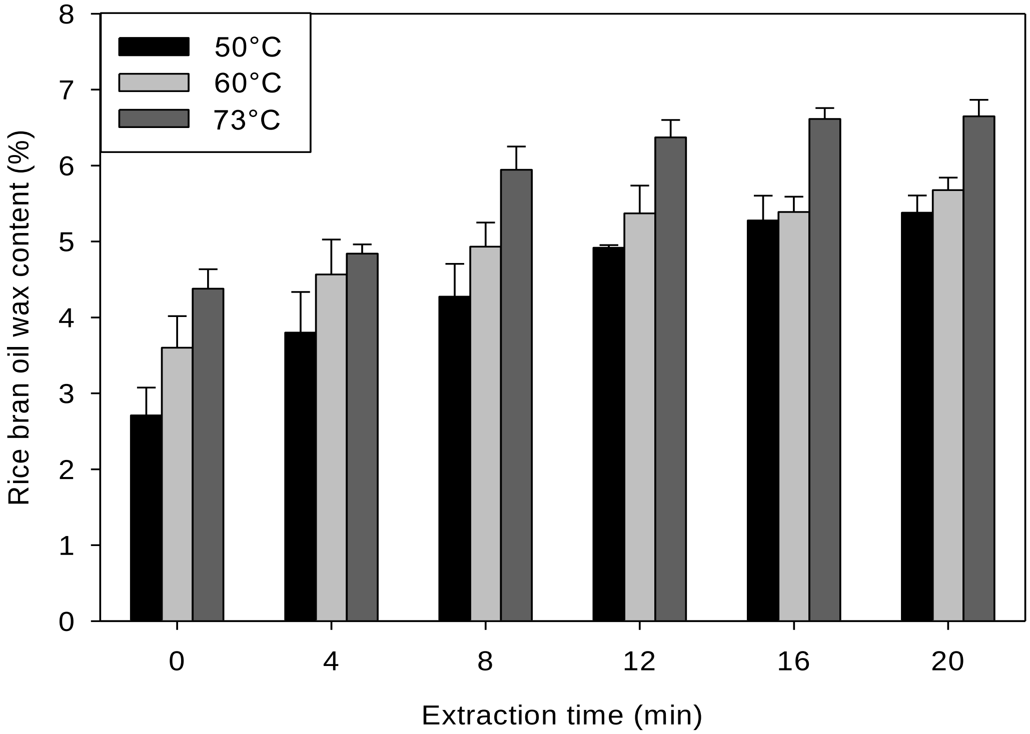


Figure 3.7. Rice bran oil waxes extracted at different temperatures and extraction times.

Effect of Extraction Method on Oil Yield

The efficiency of CMAE was compared to the efficiency obtained by conventional extraction carried out with ethanol, and standard Soxhlet extraction carried out with both n-hexane and ethanol. The results (Figure 3.8), showing the maximum percentage of oil extracted by each method, indicated that the methods of extraction have significant influence on the oil yield. In case of soybean oil, a maximum of 18.9% oil was extracted. With Soxhlet method, no significant difference ($p \geq 0.05$) was observed when compared with Soxhlet using n-hexane as an extraction agent. The oil yield extracted by Soxhlet method was 25% higher than CMAE and 50% more than conventional extraction (CE), while the amount of oil extracted with CMAE was 38% higher that CE. Similar for rice bran oil extraction (Figure 3.8), Soxhlet method using ethanol extracted 20% of oil, compared to Soxhlet method using n-hexane and CMAE were the extraction yields were no significantly different between them, extracting up to 16% oil.

Although Soxhlet extraction has higher oil yields compared to the other methods, its disadvantages (solvent:flour/bran ratios, long operation time) make it unsuitable for industrial scale extraction. From this point of view, CMAE could be a more convenient method for oil extraction, with significantly higher amounts of yields compared with CE. In the conventional extraction, heat is transferred through convection and conduction from the surface, with corresponding limitations on heat and mass diffusion rates. On the contrary, microwave causes direct generation of heat within the body, with important consequences on chemical reaction and heating kinetics, and cell wall membrane structure. As a result, the solutes within the raw material move or partition into the solvent phase and diffuse out faster of the solid matrix and out of the particulate bulk.

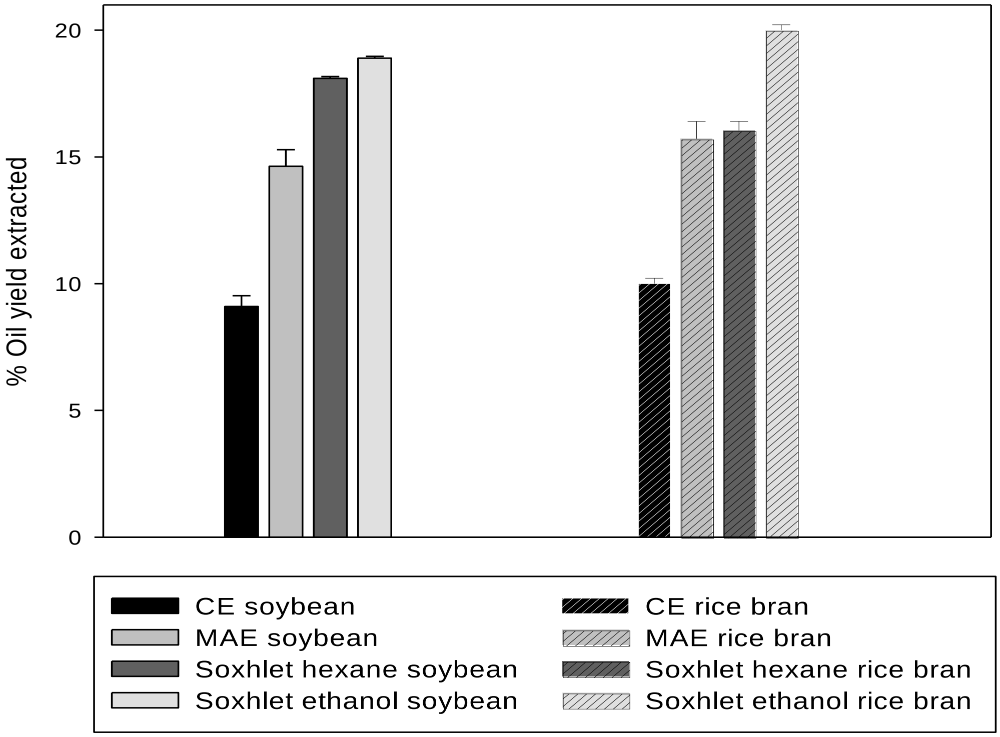


Figure 3.8. Maximum yields in different extraction methods.

Acid and Iodine Value

The acid and iodine value of soybean oil are presented in Figure 3.9. The AV increased from 0.03 to 0.05, with increasing temperature and extraction time. The values are significantly different between the oil extracted at 50°C and 73°C. However, no difference resulted in the AV of oil extracted between 0 and 16 min at 60°C and 73°C, with the AV slightly increasing when extraction time of 20 min was used. Extracted oil from rice bran showed similar increase in AV between the extraction temperatures and time (Figure 3.10). No significant change was observed between the values extracted after 4 min at 50°C and 60°C, however changes resulted with increasing the temperature at 73°C, and also with extraction time. The maximum value of 0.11% oleic acid was recorded at the highest temperature and time of extraction, value that exceeds the maximum value accepted for rice bran oil by ASTM (0.06%). The explanation for this high value could be attributed to the fact that the rice bran used for this study had not been stabilized after milling, and even a short period (one week) of storage in the freezer affected the acidity since the lipase present in the rice bran was not inactivated. Studies reflecting this issue are available in literature (Narayana et al., 2002; Ramezanzadeh et al., 1999).

The degree of saturation present in the oil is given by the IV (Bailey, 2005). Soybean oil did not undergo noticeable changes in IV when increasing the temperature of extraction from 60°C to 73°C at any time of extraction, but showed a significantly higher value in comparison with extraction at 50°C, increasing from 100 to 140 cl I_2/g (Figure 3.9). Iodine value for rice bran oil (Figure 3.10), increased with increasing temperature and time of extraction. At 50°C the IV linearly increased with time of extraction, at 60°C no change was observed after 12 min of extraction, while at 73°C no changes in IV occurred after 8 min extraction. As the final purpose of this work is to produce biodiesel, these results indicate that this extraction method produces oil suitable for base-catalyzed transesterification (the standard method used in industry).

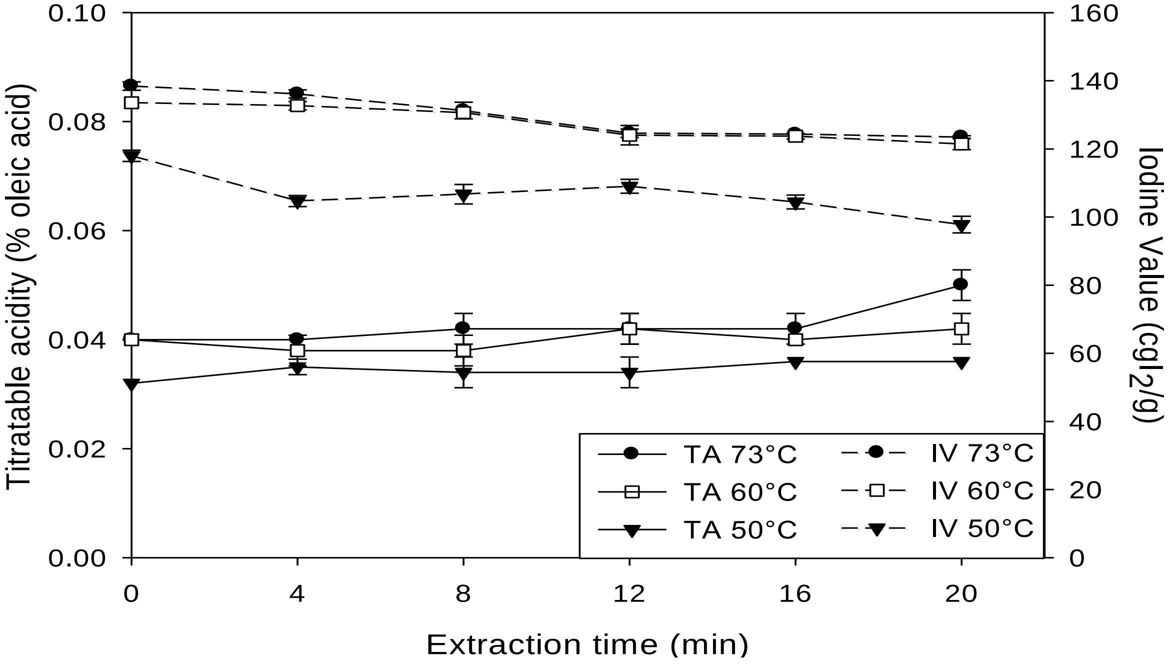


Figure 3.9. Soybean oil Acid and Iodine value at different temperatures and extraction times.

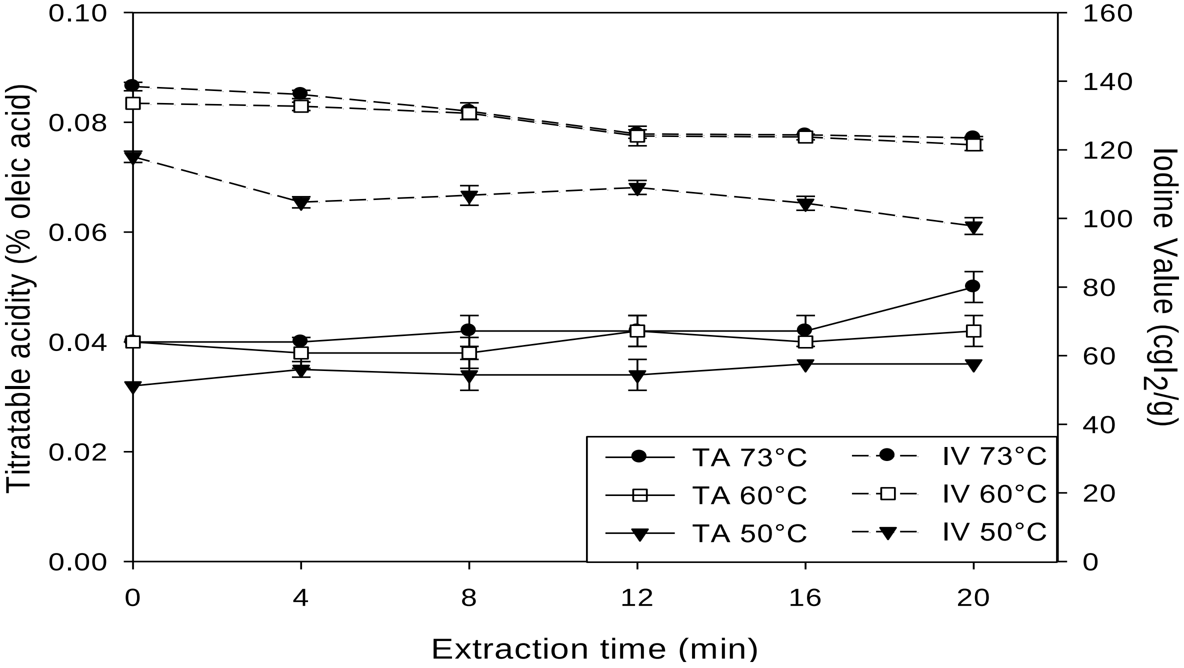


Figure 3.10. Rice bran oil Acid and Iodine value at different temperatures and extraction times.

Fatty Acid Composition

The fatty acid composition of all samples was found to be typical for the type of oil declared (Table 3.1). Within each type of vegetable oil, only minor variations in fatty acid composition were found. Since no significant differences in composition were observed between the extractions times at all temperatures, the average FFA values for each temperature are presented. Although no major changes took place between the samples extracted at different times within the same temperatures, changes in composition were observed between the different extraction temperatures.

The most significant changes in FFA in soybean oil took place for palmitic, linolenic and arachidic acid. The amount of palmitic acid increased from 7 to 12% when increasing temperature of extraction from 50°C to 73°C. One of the reasons for this increase with temperature could be the melting point of the palmitic acid, which is above 60°C (63°C), an extraction at 73°C facilitating the state condition of the acid in the matrix, making it less viscous in the matrix and therefore easier to be extracted. Correspondingly linolenic and arachidic acids decreased their concentration as the extraction temperature was increased. The saturation level of the soybean oil increased with the temperature about 24%, reducing the unsaturation level of the oil, mainly because of the changes in palmitic acid.

FFA composition of rice bran oil (Table 3.1) had a significant change in oleic and linoleic acid concentration, which decreased approximately 3% when temperature changed from 50 to 73°C. Other components of the oil did not have a significant change in values.

Table 3.1. Free fatty acid composition of soybean and rice bran oil (% of oil extracted).

	Soy bean oil			Rice bran oil		
	50°C	60°C	73°C	50°C	60°C	73°C
Palmitic (16:0)	7.18±1.66	11.21±0.83	12.04±1.54	12.87±0.30	12.70±0.61	13.00±0.48
Stearic (18:0)	1.57±0.05	1.57±0.03	1.53±0.19	1.29±0.30	1.39±0.29	1.20±0.13
Oleic (18:1)	14.90±0.68	14.88±0.64	15.06±1.33	39.37±0.81	36.97±0.75	36.02±1.82
Linoleic (18:2)	59.22±1.26	60±0.88	61.17±0.62	43.59±0.70	45.36±0.85	46.27±2.28
Linolenic (18:3)	15.07±0.32	10.99±0.48	9.57±0.70	1.53±0.21	1.93±0.16	1.74±0.33
Arachidic (20:0)	2.08±1.23	1.08±0.26	0.81±0.25	1.36±0.23	1.57±0.14	1.65±0.88
Behenic (22:0)	Tr	Tr	Tr	0.29±0.26	0.30±0.10	0.20±0.24
Total saturated	10.82±1.72	13.86±0.98	14.37±1.09	15.81±0.50	15.96±0.79	16.06±1.01
Total unsaturated	89.20±1.69	86.15±0.98	85.81±1.16	84.49±0.80	84.27±0.81	84.03±1.08

Tr – Trace (≤0.2%)

Energy Balance

In the case of materials containing a large number of polar molecules and ions, such as the ones used in these extraction processes, the efficiency of conversion form electrical energy into heat is extremely high, approaching unity. The absorbed energy will translate into a temperature change according to the following equation:

$$P = \dot{m} \times Cp \times \Delta T$$

Where: P – power (W); m – mass flow rate (Kg/s) for a continuous system;

C_p – specific heat of the material (KJ/Kg·K);

ΔT – temperature change from initial conditions.

The calculations approximating the energy consumption per gram of oil extracted are detailed as follows:

$$\dot{m} = 100\,ml\ /\ \min\ = 80\,g\ /\ \min\ = 0.0013\,Kg\ /\ s$$

$$Cp_{soybeans+EtOH} = (0.25 \times 2.25\,KJ\ /\ Kg \cdot K) + (0.75 \times 2.47\,KJ\ /\ Kg \cdot K) = 2.41\,KJ\ /\ Kg \cdot K$$

$$Cp_{ricebran+EtOH} = (0.25 \times 2.17\,KJ\ /\ Kg \cdot K) + (0.75 \times 2.47\,KJ\ /\ Kg \cdot K) = 2.39\,KJ\ /\ Kg \cdot K$$

$$P_{soybeans+EtOH} = 0.0013\,Kg\ /\ s \times 2.41\,KJ\ /\ Kg \cdot K \times (346 - 296)K = (0.156\,KJ\ /\ s)/(0.33\,g\ /\ s) =$$
$$= 0.468\,KJ\ /\ g \times 0.16 = 2.92\,KJ\ /\ g \cdot oil$$

$$P_{ricebran+EtOH} = 0.0013\,Kg\ /\ s \times 2.39\,KJ\ /\ Kg \cdot K \times (346 - 296)K = (0.155\,KJ\ /\ s)/(0.33\,g\ /\ s) =$$
$$0.465\,KJ\ /\ g \times 0.16 = 2.90\,KJ\ /\ g \cdot oil$$

We note here that specific energy consumption can be reduced by increasing the concentration of the solids into the mixture.

Scale-up Studies

Effect of Flow Rate on Oil Yields

Extraction efficiency at the pilot scale level was measured at 0.6 and 1 l/min flow rate. An average of 18.6% of soybean oil was extracted (Fig. 3.11 A). At higher flow rate (1 l/min), lesser oil was extracted compared with the yields at 0.6 l/min, although no statistically significant difference ($p>0.05$) was found between the yields. For rice bran extraction, increasing the flow rate from 0.6 to 1 l/min decreased the yields in small, but significant quantities ($p<0.05$) (Figure 3.11B); yield as high as 19.5% were extracted with this system.

Since the extraction temperature did not change (73°C) it is likely to have small differences between the extracted yields at the different flow rates used. At a higher flow rate (1 l/min) the power level used to reach the extraction temperature is higher (4 – 4.5 kW) than the one used for 0.6 l/min (3 – 3.3 kW). In this way, more energy is sent to the sample and the effect of the flow rate is diminished.

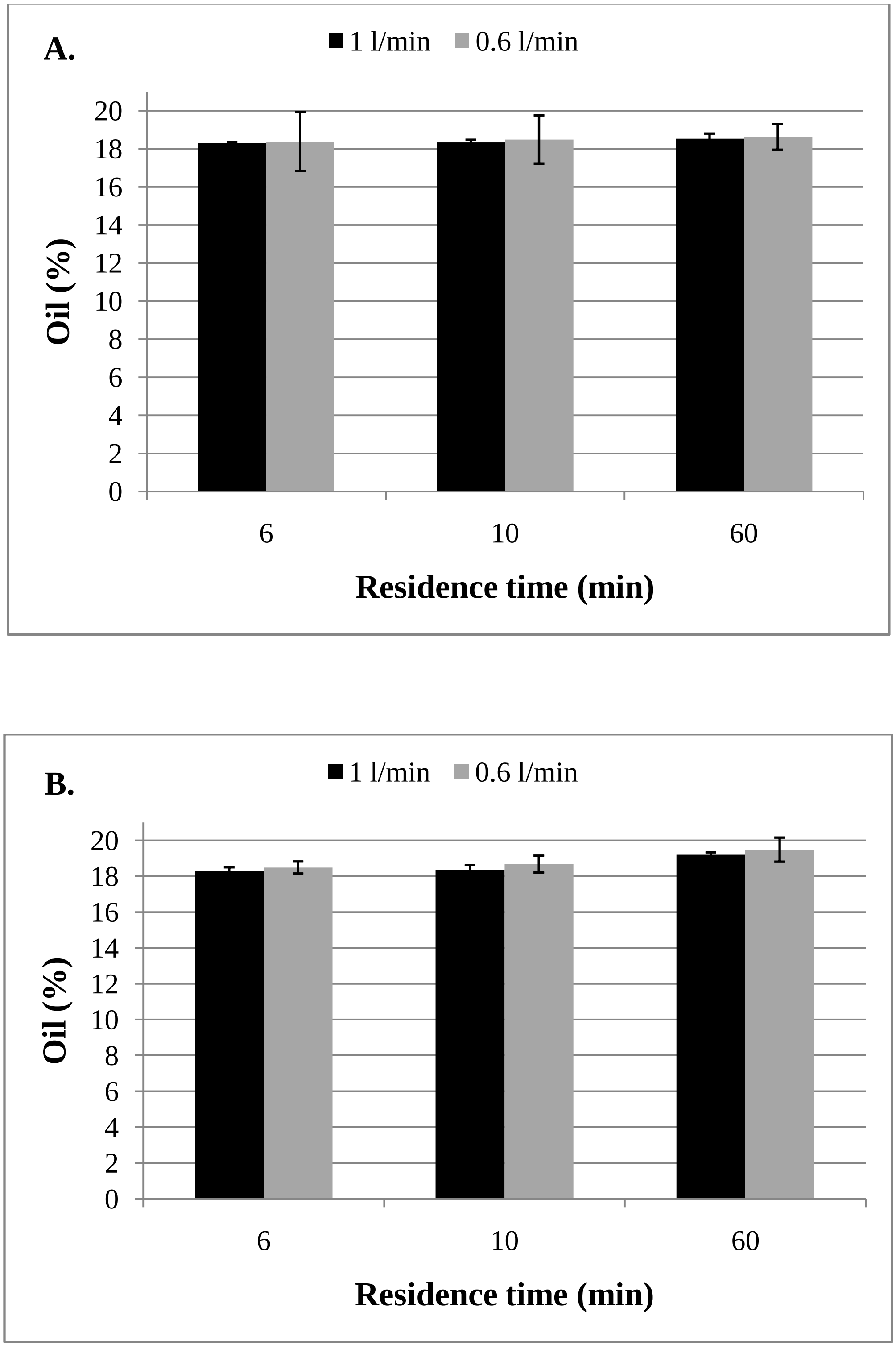


Figure 3.11. Oil extracted at different flow rates and extraction times from A. Soybean and B. Rice bran.

Effect of Extraction Time on Extracted Oil Yields

The effect of the extraction time on the oil yield depends on the feed materials. For soybeans, a linear increase in oil yield was observed as the extraction time increased from 6 to 10 and 60 min (Figure 3.11 A). Although there was a 0.1% increase in yields between the extraction times, the yields were found not to be statistically significant.

Yields of oil extracted from rice bran increased as extraction time increased (Figure 3.11 B). A statistical significant difference was found between 10 and 60 min of extraction, with 1% increase in oil yield when using 0.6 l/min and 0.9% when using 1 l/min. No difference was found between 6 and 10 min of extraction.

Scanning electron microscopy (SEM) was used to observe the damage at the micro structural level in soybean and rice bran cells. The microphotographs are presented for the three extraction times studied and compared to the untreated material (control) in Figure 3.12. At the cellular level, a difference between the structural damage can be observed between the control and the treated samples for both soybean and rice bran, at all extraction treatments studied. No major difference in microstructure can be observed between the extraction treatments. Only small differences at the cell structure level can be observed as the treatment increases from 6 to 10 and 60 min extraction, which would explain the small differences in the oil yields extracted.

Since no major differences in cell disruption occurs between the treatments, it is shown that the only influence in the yields extracted could be the extraction time, although, as we presented earlier, this differences could be insignificant in some cases. The influence of diffusivity is usually direct proportional with the cell disruption, as the liquid is more easily drawn through the cells, increasing the extraction rate.

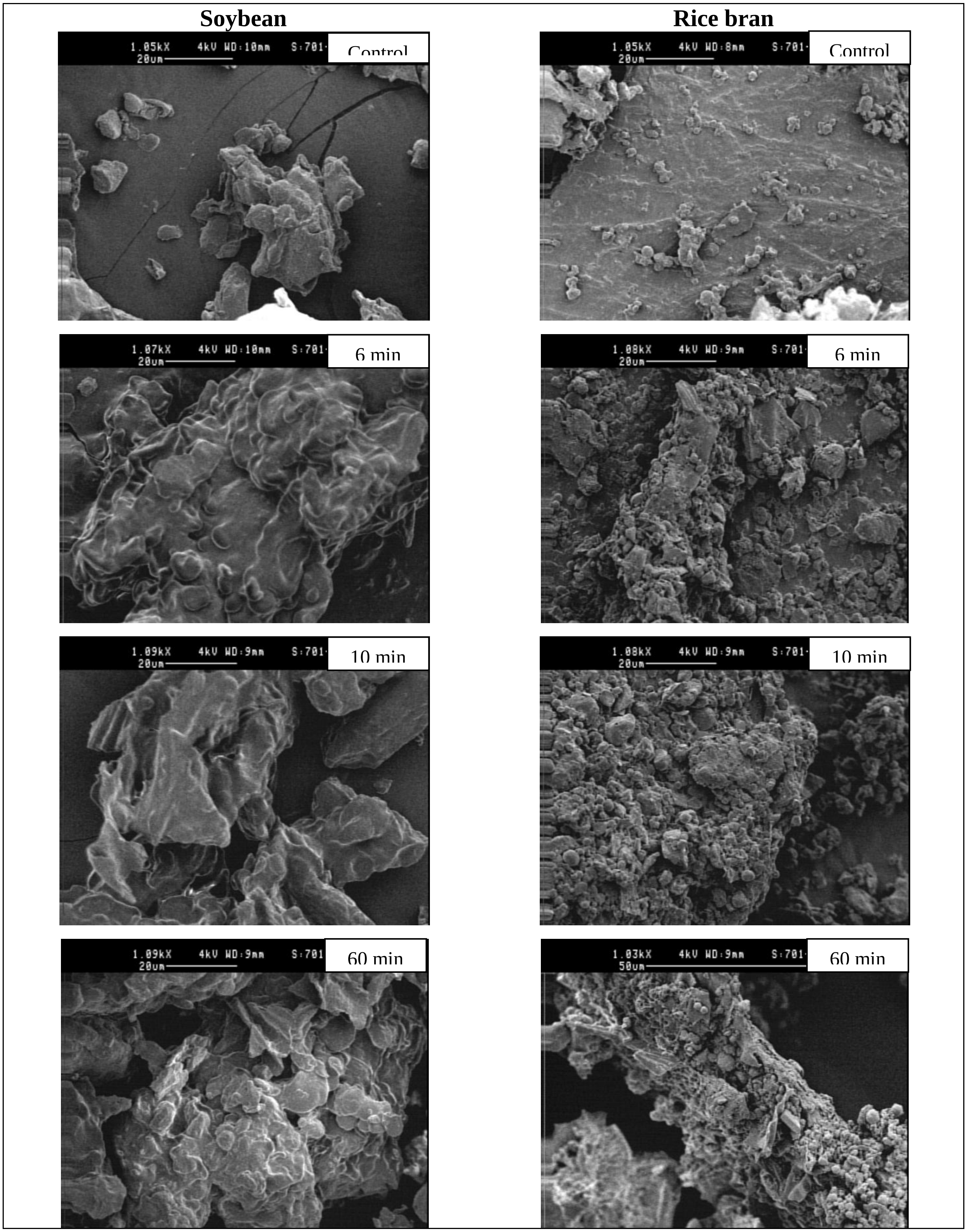


Figure 3.12.Scanning Electron Micrograph of microwave treated soybean (left column) and rice bran (right column) for 6, 10 and 60 min.

In our case, since the cell disruptions are show to be similar, the diffusivities between the different extraction times tend to be constant, with a result of only small changes in extracted oil yields.

Oil Quality Analysis

Phospholipids and waxes

The amount of phospholipids in soybean oil varied between 1.9% and 2.2% at 1 l/min, and between 2.1% and 2.3% for 0.6 l/min respectively (Figure 3.13). Significant differences ($p<0.05$) in phospholipids content were found among the exposures times performed at 1 l/min. No significant differences were found between the phospholipids extracted at different flow rates at the same extraction times.

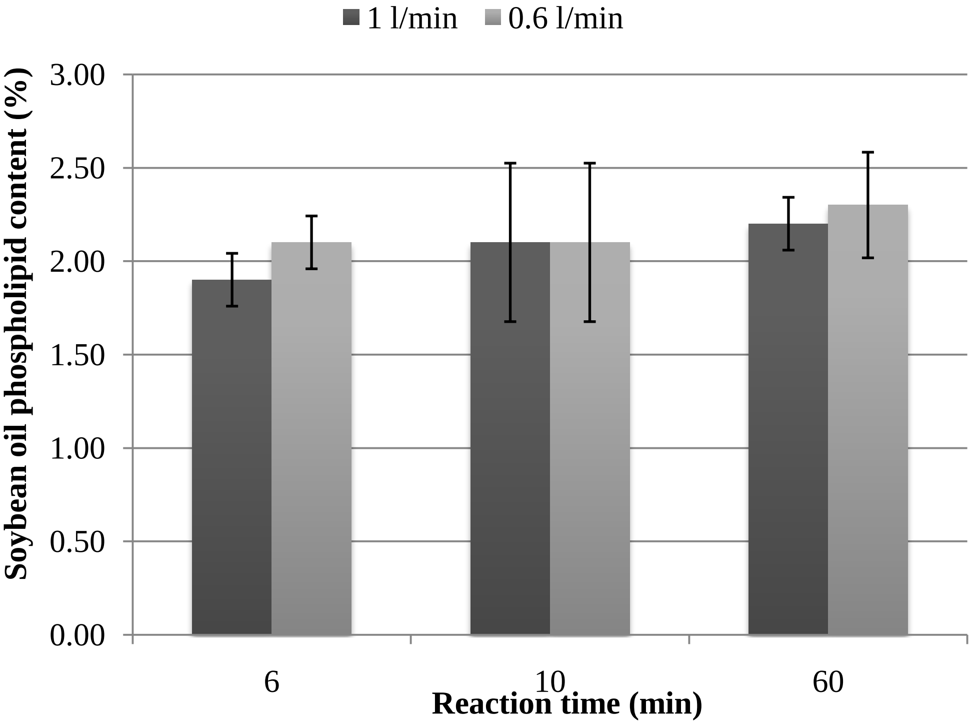


Figure 3.13. Soybean oil phospholipids extracted at different flow rates and extraction times.

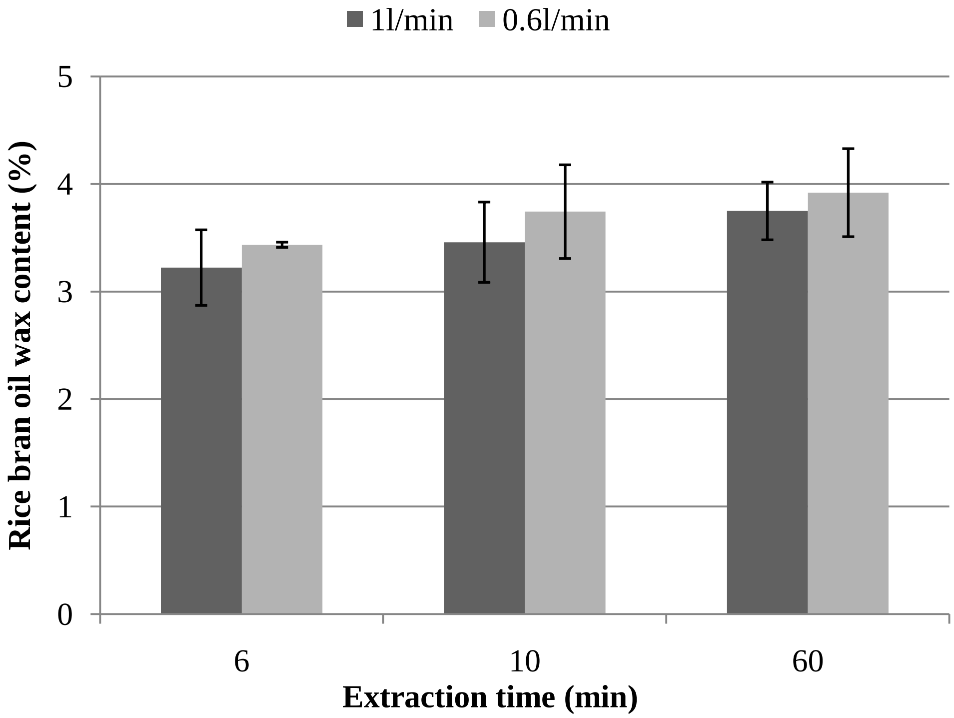


Figure 3.14. Rice bran oil waxes extracted at different flow rates and extraction times.

Concentrations of waxes from rice bran oil showed to be directly proportional to the amount of oil extracted. The highest amount of waxes were extracted at 60 min extraction and 0.6 l/min flow rate. Significant differences were found at various extraction times at both flow rates studied (Figure 3.14). Both soybean and rice bran oil meet the ASTM specifications for phospholipid and wax yields content.

Acid and Iodine Value

Both soybean and rice bran oil showed good quality properties regarding AV and IV. Both oils did not have significant changes in AV within the range of the parameters used (Table 3.2). Iodine value showed a slightly increasing trend among extraction times with significant differences between the values.These results indicate that this scale up extraction method also produces oil suitable for base-catalyzed transesterification into biodiesel without any pretreatment needed.

Table 3.2. Acid and iodine value of soybean and rice bran for different flow rates and extraction times (%).

Flow rate	Extraction time (min)	Soy bean oil		Rice bran oil	
		AV	IV	AV	IV
0.6 l/min	5	0.03±0.00	122±1.35	0.03±0.00	116±2.56
	10	0.03±0.00	126±2.33	0.03±0.01	117±1.23
	60	0.04±0.01	131±1.45	0.04±0.00	122±1.09
1 l/min	5	0.03±0.01	119±2.85	0.03±0.01	115±1.93
	10	0.03±0.00	123±1.46	0.03±0.01	117±0.56
	60	0.04±0.00	125±2.67	0.04±0.00	120±1.78

Fatty Acid Composition

Similarly to the laboratory scale results, the fatty acid composition of all samples was found to be typical for the type of oil extracted. Within each type of vegetable oil, only minor variations in fatty acid composition were found. No significant differences were found for soybean oil between the extractions times studied at both flow rates used (Table 3.3). Similar for rice bran oil, minor and insignificant (P>0.05) changes occurred at the composition level between the parameters studied (Table 3.4). Both oil meet the ASTM fatty composition requirements for each type of oil extracted.

Table 3.3. Free fatty acid composition of soybean oil (%).

	0.6 l/min			1 l/min		
	6 min	10 min	60 min	6 min	10 min	60 min
Palmitic (16:0)	4.60±1.27	5.32±0.25	5.55±0.16	5.22±0.16	4.31±0.67	5.24±0.02
Stearic (18:0)	2.57±0.27	2.62±0.08	2.93±0.09	2.64±0.11	2.31±0.07	2.64±0.13
Oleic (18:1)	12.11±0.10	12.79±0.17	12.07±0.46	13.07±0.12	12.29±0.92	13.03±0.69
Linoleic (18:2)	60.06±1.50	59.68±0.22	59.92±0.78	59.17±0.48	60.65±0.67	59.10±2.12
Linolenic (18:3)	17.64±0.61	16.61±0.40	17.07±0.12	16.92±0.49	17.11±0.40	16.85±0.48
Arachidic (20:0)	3.12±0.36	3.03±0.95	3.05±0.57	3.01±0.16	3.34±0.45	3.15±3.40
Behenic (22:0)	Tr	Tr	Tr	Tr	Tr	Tr

Tr – Trace (≤0.2%).

Table 3.4. Free fatty acid composition of rice bran oil (%).

	0.6 l/min			1 l/min		
	6 min	10 min	60 min	6 min	10 min	60 min
Palmitic (16:0)	10.88±0.37	10.89±0.31	12.05±0.72	10.54±0.69	11.13±0.99	11.16±0.79
Stearic (18:0)	3.04±0.67	3.27±0.84	3.67±0.29	1.51±0.06	2.14±0.50	2.57±0.24
Oleic (18:1)	33.95±1.06	33.85±0.13	33.23±0.52	35.52±0.33	33.06±0.72	32.86±0.20
Linoleic (18:2)	43.88±1.10	44.01±1.38	42.78±0.66	45.87±0.77	45.84±0.31	45.96±0.29
Linolenic (18:3)	3.29±0.23	2.83±0.48	4.13±0.92	2.28±0.31	3.23±0.47	3.14±1.10
Arachidic (20:0)	4.97±0.89	4.01±0.47	4.14±0.35	4.27±0.57	4.12±0.47	4.29±0.39
Behenic (22:0)	Tr	Tr	Tr	Tr	Tr	Tr

Tr – Trace (≤0.2%).

Conclusions

A continuous microwave assisted extraction method was developed in order to investigate soybean and rice bran oil extraction. A batch type microwave was modified to allow continuous processing of a 3:1 ratio solvent:feedstock mixture at several reaction times. Oil extraction yields and quality parameters were measured. Results indicated that oil extraction was temperature and time dependent. Extraction of soybean oil was found to reach a maximum value at the highest temperature and longest time exposure, while for rice bran oil maximum oil extracted took place at 73°C and 16 min of exposure with no improvement at higher exposure times. Analysis of the oils showed a high quality extraction, with characteristics suitable for commercialization and biodiesel production. Results obtained indicated that the system performance was better than traditional oil bath extraction process at the same residence times with the advantage of speed.

A continuous method was developed for pilot scale microwave-assisted extraction of oil from soybeans and rice bran. Parameters studied were extraction time and flow rate. As the extraction time and temperature increased so did the extraction effect, resulting in higher yields of oil, however with no significant improvement for the quality of the oil. In the case of CFMAE, the parameters affected only the rice bran oil yield, with no significant difference in the amount of soybean oil extracted. Higher yields were extracted (3.8% increase for soybean and 3.7% increase for rice bran) compared with the laboratory scale continuous microwave assisted solvent extraction, without affecting the oil quality, which meet the ASTM oil quality requirements.

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CHAPTER 4

TRANSESTERIFICATION OF SOYBEAN AND RICE BRAN OIL WITH ETHANOL IN A CONTINUOUS-FLOW MICROWAVE-ASSISTED SYSTEM

Introduction

Alternative fuels for diesel engines are becoming increasingly important due to diminishing petroleum reserves and the environmental consequences of exhaust gases from petroleum-fueled engines. Biodiesel, a renewable fuel comprised of mono-alkyl (methyl or ethyl) esters of long chain fatty acids from plant oils or animal fats, plays a very important role as an alternative to conventional petroleum diesel (Ma and Hanna, 1999). Worldwide, there is an increasing interest in biodiesel as a renewable transportation fuel and blending agent, with high potential to displace petroleum diesel, to lower net global warming gas emissions from transportation sector, and to reduce carcinogenic particulate matter emissions (Van Gerpen, 2005). Seeds of high oil content, such as sunflower, rapeseed and soybean seeds, have gained much attention lately as renewable energy sources both because of their relatively high yield and widespread production.

The interest in the use of renewable fuel started with the direct use of vegetable oils as a substitute for diesel. Using oils as a fuel has some advantages as (1) it is portable, (2) has high heat content (>80% of diesel fuel), (3) it is readily available and (4) it is renewable. However, their direct use in compression ignition engines was restricted due to high viscosity which resulted in poor fuel atomization, incomplete combustion and carbon deposition on the injector and the valve seats causing serious engine fouling (Altin et al., 2001). Other constraints of the direct application of vegetable oil were its low volatility, content of polyunsaturated character and oil deterioration.

To overcome these constrains, processes like pyrolysis (thermal cracking of the vegetable oil) (Demirbas, 2008c), micro-emulsification in diesel fuel (Lin and Lin, 2007), and dilution (Awang and May, 2008) have been developed. A fourth one, transesterification, is doubtless the most commonly employed (Van Gerpen, 2005). With pyrolysis, resulting products had positive characteristics such as low viscosity, high cetane number, acceptable amounts of sulphur, water and sediments and acceptable copper corrosion values, but unacceptable in the terms of their ash contents, carbon residue, and pour points. Similarly, micro-emulsion lowered the oil's viscosity but resulted in irregular injector needle sticking, heavy carbon deposits, and incomplete combustion during the tests.

Transesterification (also called alcoholysis) is the chemical reaction of a fat or oil (triglycerides) with an alcohol in the presence of a catalyst, to form esters and glycerol (Figure 4.1). Because the reaction is reversible, excess alcohol is used to shift the equilibrium to the products side. Alcohols are primary and secondary monohydric aliphatic alcohols having 1-8 carbon atoms (Fukuda et al., 2001). Among the suitable alcohols that can be used in the transesterification process, methanol and ethanol are utilized most frequently, especially methanol because of its low cost and its physical and chemical advantages (polar and shortest chain alcohol). Ethanol, also used as an extraction solvent, is preferable to methanol because of its much superior solubility for oil, resulting ethyl esters with increased heat content and cetane number, lower cloud point, and the characteristics of an entirely renewable, agricultural based feedstock for biodiesel (Demirbas, 2008b).

There are four basic routes to produce biodiesel by transesterification of vegetable oil and fats, namely, (i) based-catalyzed transesterification, (ii) acid-catalyzed transesterification, (iii) enzymatic transesterification, and (iv) non-catalytic transesterification using methanol or methanol as co-solvent (Demirbas, 2009).

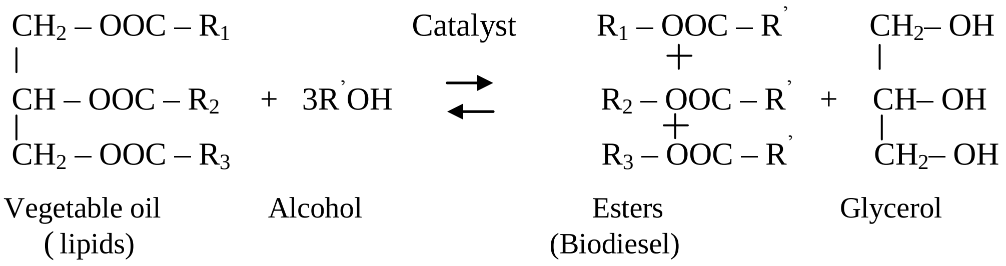

Figure. 4.1. Transesterification of triglycerides with alcohol

These methods depend mainly upon the quality of oil used. The based-catalyzed transesterification (with KOH or NaOH) is the more commercially used process mainly because the reaction can take place at relatively low temperatures (60°C or 70°C) and pressure (1 atm), high conversion yields can be obtain (98%), and involves direct conversion with no intermediate steps. Also transesterification occurs at faster rate in the presence of alkaline catalyst than in the presence of the same amount of acid catalyst (Srivastava and Prasad, 2000).

Stoichiometrically, three moles of alcohol are required for each mole of triglyceride to complete the transesterification process, but in general a higher molar ratio is needed for maximum ester production and reaction stability, depending upon the type of feedstock, amount of catalyst and temperature (Fukuda et al., 2001). It consists of a number of consecutive, reversible reactions (Schwab et al., 1987) (Figure. 4.2), triglycerides being converted in glycerol in a three step reaction. In each step an ester is formed, thus three esters are obtained from one tryglicerides molecule.

$$\text{Triglyceride (TG)} + R'OH \underset{k}{\overset{k}{\rightleftarrows}} \text{Diglyceride (DG)} + R'COOR_1$$

$$\text{Diglyceride (DG)} + R'OH \underset{k}{\overset{k}{\rightleftarrows}} \text{Monoglyceride (MG)} + R'COOR_2$$

$$\text{Monoglyceride (MG)} + R'OH \underset{k}{\overset{k}{\leftrightarrows}} \text{Glycerol (GL)} + R'COOR_3$$

Figure. 4.2. The transesterification reactions of vegetable oil with alcohol to esters and glycerol.

A major obstacle in the commercialization of biodiesel, in comparison to petroleum-based diesel fuel, is its high cost of manufacturing, feedstock cost, plant size, and low value of glycerine byproduct (Duffield, 2007). Invariably, on all aspects of feedstock, research has been performed on a wide range of biomass in order to decrease the biodiesel costs. During the last years there have been few attempts to produce biodiesel by different methods from soy bean oil (Zhou and Boocock, 2006), rapeseed oil (Jeong and Park, 2006), canola oil (Kulkarni et al., 2006), rice bran oil (Einloft et al., 2008), sunflower oil (Georgogianni et al., 2008), pumpkin oil (Schinas et al., 2009), coconut and palm oil (Kansedo et al., 2009), castor oil (Da Silva et al., 2006), waste cooking oil (Al-Widyan et al., 2002), animal fat (Demirbas, 2008c), and algae oil (Chisti, 2007). Of the non-edible oils mostly used in an attempt to reduce the cost of biodiesel we mention those with higher free fatty acids (FFA) contents such as rubber, jatropha, karanja, tobacco, etc. (Patil and Deng, 2009).

A relatively new research area investigates the production of biodiesel using controlled microwave heating for accelerating synthetic organic transformations. Using a microwave apparatus, it is possible to perform reactions more efficiently, with short separation and reaction times, reducing the quantity of by-products, all with reduced energy consumption (Hernando et al., 2007).

Several microwave assisted transesterification methods from different oil based crops have been published in the literature (Azcan and Danisman, 2008; Breccia et al., 1999; Hernando et al., 2007; Mazzocchia et al., 2004; Perin et al., 2008; Zu et al., 2009). The results showed not only the efficiency of microwave treatment but also the benefit of using base catalyzed transesterification instead of acid catalyst transesterification. Using the same temperatures used in conventional transesterification, the reactions took place in some cases in less then 5 minutes using microwave heating when compared to traditional heating method.

While many publications focus on the use of sealed vessels using microwave heating, it is also possible to use an open-vessel system with the same microwave unit (Leadbeater et al., 2008). This gives the advantage to perform the reaction continuously and study the process at higher scale. Using this procedure, a 98 % conversion to biodiesel was achieved in 1 minute (Barnard et al., 2007), again showing the efficiency of using microwave heating for the transesterification reaction.

The efficiency of the microwave-assisted transesterification stems from the unique dielectric properties of the mixtures of polar and ionic components of vegetable oil, solvent, and the catalysts. Rapid and efficient heating observed upon microwave irradiation, mostly because the microwaves waves interact with the sample on a molecular level, generating inter molecular mixing and agitation which increases the chances of an alcohol molecule to encounter an oil molecule.

The objective of this study was to investigate the continuous transesterification process with ethanol assisted by microwave heating of soybean and rice bran oils at several target temperatures (50 and 73°C) in a fully instrumented and controlled continuous batch microwave reactor at several reaction times (1, 5 and 10 min).

Materials and Methods

Materials

The vegetable oils used in this study were soybean (SB) and rice bran (RB) oils. Soybean oil was purchased from a local grocery store, while rice bran oil was purchased from Honest Foods (San Bruno, CA). The solvent, 200 proof, ACS/US Grade ethanol was purchased from Pharmco-AAPER, while all chemicals - sodium hydroxide flakes, potassium hydroxide and Hydrochloric Acid were supplied from Fisher Scientific (US). Standards of fatty acids ethyl esters (FAEE) were purchased from Sigma Aldrich.

Transesterification Reaction

Before the reaction, NaOH catalyst 0.6% (by weight of oil), was dissolved in ethanol by stirring in a small reactor. The oil was transferred into the biodiesel feed tank reactor and then the catalyst/alcohol mixture was added into the oil. The final mixture was vigorously stirred for approximately ½ h.

The microwave reactions were conducted using a commercially available, fully instrumented, batch type microwave system (ETHOS E Microwave Extraction System, Milestone Inc., Monroe, CT) which was modified for continuous operation. The detailed reaction description of the system is described in Chapter 3. A 5:1 (stoichiometric ratio) solvent (Absolute Proof Ethanol) to oil ratio reaction mixture was pumped into the microwave chamber (into the beaker) at a constant flow rate of 100 mL/min and was heated at 50 and 73°C, with residence times of 1, 5 and 10 min, monitored with a fiber optic probe and automatically controlled by the system, using the generated microwaves. A magnetic stirrer maintained the agitation.

After completion, the biodiesel mixture was quenched with 1 M Hydrochloric Acid in order to stop the reaction, and the crude glycerin was separated gravitationally for 20 h (phase separation was observed within 10 min). After settling was complete, the biodiesel was separated from the glycerin, and carefully washed with water (6% v/v) in three steps until the ester layer was clear. The samples were than centrifuged in a vacuum centrifuge evaporator (CentriVap Console Labconco, Kansas City, Missouri) running for approx 2-4 hours in order to evaporate the excess ethanol.

Quality Analysis

The products were analyzed in terms of cloud point (ASTM D 2500), flash point (ASTM D 93), kinematic viscosity at 40°C (ASTM D 445), acid number (ASTM D 974), oxidation stability index (OSI) (EN 14112), free and total glycerin (ASTM D 6584) and free fatty acid composition, using their respective ASTM and EN standard procedures.

The fatty acid ethyl ester compositions were determined by gas chromatography (Varian 450-GC) coupled with a Varian 240-MS Ion Trap Mass Spectrometer (Varian Inc. USA). A Varian FactorFour Capillary column WAXms (30m x 0.25mm i.d., 0.25 µm) was used at 255°C with helium at 1 ml/min as carrier gas, a split injector at 270°C with a split ratio of 1:20, and a detector temperature of 270°C, with a total running time of 22 min. Components were identified using a standard FAEE mix (Supelco, Bellefonte, PA).

Statistical Analysis

The experiments reported were performed in triplicates and the average values of oil extracted and standard deviations were calculated. Statistical analysis was performed in order to test significant differences among yields at different temperatures and holding times. A two-way ANOVA using Proc Mixed 2 x 5 factorial (version 9.1, SAS Institute, Inc., Cary, N.C.) was used to test significant differences among temperatures (50 and 73°C) and different reaction times (1, 5 and 10 min). Two-way ANOVA using Proc Multiple comparison tests were performed by using Tukey adjustment to determine the significant difference between treatments. All significant values were expressed as $p \leq 0.05$.

Results and Discussion

Total and Free Glycerin

Glycerin is a byproduct of biodiesel production, and, if not removed from the final product, it can result in fuel separation, material incompatibility, engine deposits and engine durability concerns. For this purpose it is important to analyze the concentration of total glycerin in the final product, free and bound glycerin.

Total and free glycerins were shown to be dependent on the reaction temperature and exposure time to microwave irradiation (Table 4.1). For SB significant differences were found only between the results at 50°C and 1 min (0.03%) and those at 10 min irrespective of the temperature (0.01%). The other values for the free glycerin did not significantly change within the process parameters. For RB biodiesel the free glycerin analysis did not show any significant changes between the treatments, the values falling in the intervals between 0.02 and 0.01 % (Table 4.1).

In an ideal reaction, the amount of total residual glycerin should not exceed 0.25% (w/w) (ASTM D6751). In this study, total glycerin in SB biodiesel was below this level with a maximum of 0.23% for 50°C and 1 min reaction time. No significant difference was found between the total glycerins for the reaction times carried out at 73°C, the lowest value was 0.16% at 10 min reaction time. Similar for RB biodiesel, significant differences were found between the

1 and 5 min reaction time at 50°C and compared with the 10 min reaction time the ones at 73°C. No major changes were found between the reactions carried out at 73°C.

The transesterification conversion rates increased with increasing temperature and time of exposure. No significant differences among conversion rates for SB biodiesel were found between different times at the same temperature, although changes were found between the reactions at 50 and 73°C at all reaction times. The highest conversion rate was achieved at 73°C and 10 min treatment (99.25%), while the lowest conversion was at 50°C for 1 min reaction (96.67%).

For RB biodiesel, the only significant difference was found between the lowest and the highest temperature and time treatment (Table 4.1). Conversion as high as 99.34% was achieved, while the lowest conversion rate was 98.35%.

Similar result were found in the literature (Leadbeater and Stencel, 2006) where a study on biodiesel conversion showed a 98% conversion rate after 5 minutes when using microwave assisted reaction with methanol at a 1:6 oil to solvent ratio and using NaOH as a catalyst.

Table 4.1. Free and total glycerin of SB and RB biodiesel at studied temperatures and reaction times**.**

Temperature	Time (min)	SB biodiesel		RB biodiesel		Conversion (%)	
		Free glycerin % mass	Total glycerin % mass	Free glycerin % mass	Total glycerin % mass	SB	RB
50°C	1	$0.03^{ab}\pm0.01$	$0.23^{a}\pm0.01$	$0.02^{b}\pm0.01$	$0.21^{a}\pm0.01$	96.67^{a}	98.35^{ab}
	5	$0.02^{b}\pm0.01$	$0.19^{ab}\pm0.02$	$0.01^{b}\pm0.01$	$0.19^{ab}\pm0.01$	97.46^{a}	98.59^{b}
	10	$0.01^{ab}\pm0.01$	$0.18^{ab}\pm0.01$	$0.02^{b}\pm0.01$	$0.18^{ab}\pm0.02$	97.79^{a}	98.88^{b}
73°C	1	$0.02^{b}\pm0.01$	$0.17^{ab}\pm0.02$	$0.01^{b}\pm0.01$	$0.17^{ab}\pm0.02$	98.87^{ab}	99.18^{b}
	5	$0.02^{b}\pm0.01$	$0.17^{ab}\pm0.01$	$0.01^{b}\pm0.01$	$0.16^{ab}\pm0.01$	98.89^{ab}	99.22^{b}
	10	$0.01^{ab}\pm0.00$	$0.16^{a}\pm0.01$	$0.01^{b}\pm0.00$	$0.16^{ab}\pm0.01$	99.25^{ab}	99.34^{ab}
ASTM #		Max 0.03	Max 0.25	Max 0.03	Max 0.25		

[a] Numbers from the same column, significantly different from each other.

[b] Numbers from same the column, not significantly different from each other.

Fatty Acid Ethyl Esters Composition

FAEE's showed to be typical to the type of oil used for biodiesel. Between the reaction parameters no major changes were found between the biodiesel compositions. For SB no significant changes were detected between the reactions at 50°C and 73°C (Table 4.2). In the same time, reaction times did not influence the change in FAEE. Similar for RB no significant changes were found in FAEE among the parameters studied (Table 4.3).

Table 4.2. FAEE of SB biodiesel at studied temperatures and reaction times.

	50°C			73°C		
	1 min	5 min	10 min	1 min	5 min	10 min
Myristic (14:0)	0.04±0.03	0.02±0.01	0.07±0.02	0.05±0.01	0.06±0.01	0.05±0.01
Palmitic (16:0)	8.39±0.19	7.32±0.01	8.19±0.57	8.03±0.02	8.42±0.24	8.45±0.37
Stearic (18:0)	3.51±0.44	3.65±0.20	3.59±0.15	3.55±0.18	3.76±0.09	3.43±0.01
Oleic (18:1)	25.08±0.45	22.99±1.18	23.64±2.54	25.29±0.13	25.26±0.24	25.42±0.07
Linoleic (18:2)	55.70±0.05	57.64±1.19	56.79±2.31	55.42±0.33	55.41±0.36	55.09±0.36
Linolenic (18:3)	6.89±0.16	8.00±0.11	7.21±0.13	7.36±0.30	6.59±0.26	7.33±0.05
Eicosanoic (20:0)	0.03±0.03	0.05±0.01	0.04±0.06	0.02±0.02	0.07±0.03	0.02±0.01
Eicosenoic (20:1)	0.14±0.10	0.15±0.04	0.27±0.09	0.03±0.01	0.17±0.23	0.02±0.03
Docosanoic (22:0)	0.20±0.11	0.13±0.04	0.16±0.04	0.20±0.02	0.20±0.02	0.16±0.01
Docosenoic (22:1)	0.06±0.02	0.04±0.02	0.04±0.01	0.05±0.02	0.06±0.01	0.04±0.01

Table 4.3. FAEE of RB biodiesel at studied temperatures and reaction times.

	50°C			73°C		
	1 min	5 min	10 min	1 min	5 min	10 min
Myristic (14:0)	0.23±0.01	0.22±0.03	0.23±0.02	0.24±0.01	0.20±0.02	0.22±0.02
Palmitic (16:0)	14.53±0.28	16.09±1.51	13.79±0.81	14.26±0.10	14.05±0.45	14.55±0.20
Stearic (18:0)	2.03±0.23	1.94±0.23	1.98±0.15	1.98±0.30	1.82±0.04	1.93±0.07
Oleic (18:1)	40.82±0.53	40.25±0.99	42.41±0.25	41.39±1.34	42.87±0.17	42.05±0.04
Linoleic (18:2)	40.26±0.45	39.76±0.01	39.42±1.11	40.02±0.28	39.11±0.20	39.35±0.37
Linolenic (18:3)	1.62±0.06	1.42±0.13	1.61±0.05	1.57±0.14	1.42±0.06	1.43±0.08
Eicosanoic (20:0)	0.07±0.01	0.05±0.01	0.05±0.01	0.07±0.01	0.04±0.01	0.09±0.03
Eicosenoic (20:1)	0.16±0.08	0.04±0.06	0.21±0.07	0.23±0.02	0.24±0.08	0.11±0.09
Docosanoic (22:0)	0.16±0.02	0.17±0.01	0.18±0.02	0.13±0.01	0.15±0.04	0.16±0.02
Docosenoic (22:1)	0.12±0.01	0.06±0.09	0.13±0.02	0.11±0.01	0.10±0.01	0.10±0.02

Cloud Point

Cloud point of biodiesel is temperature and time dependent (Figure 4.3). For both SB and RB biodiesel, cloud point values decreased with increasing the temperature and time of reaction. Significant differences in the values were found for SB biodiesel between the two temperatures at all exposure times, however significant differences were not detected at 73°C among the exposure times studied. For RB biodiesel significant differences were observed between the two temperatures (50°C and 73°C) and between the reaction times, higher values being noticed at lower temperatures and shorter times. Acceptable values for cloud point should not be higher than 10°C, according to ASTM D 2500.

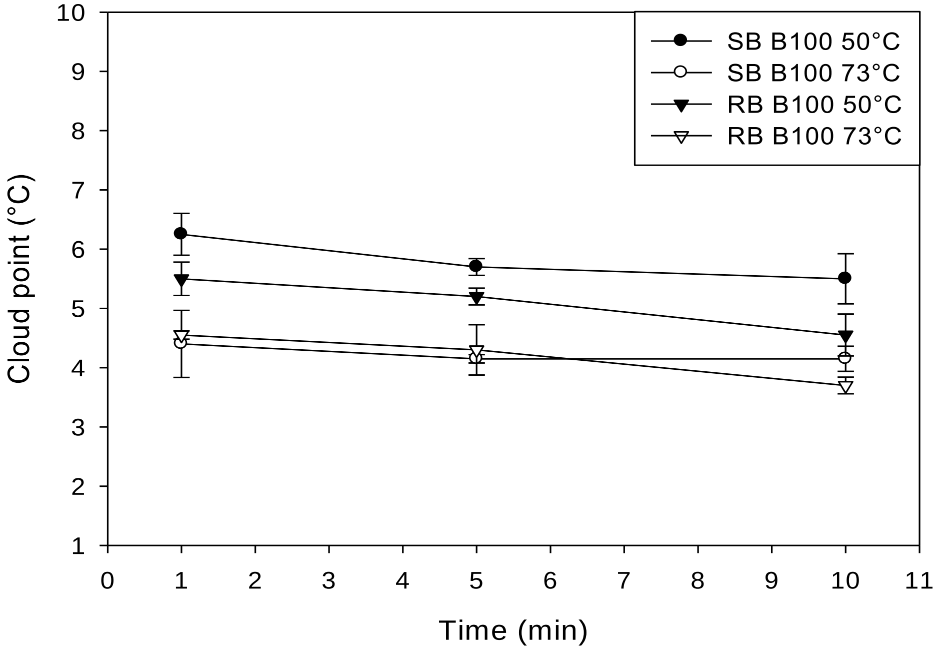

Figure 4.3. Cloud point results for soybean and rice bran biodiesel at different temperature and reaction times.

Flash Point

The flash point temperatures, the minimum value at which the fuel will ignite (flash) under specified conditions, are plotted in Figure 4.4. A higher value indicates lower volatility and therefore better safety in storage. For both biodiesel oil origins, this parameter was influenced by the temperature and reaction times. Higher flash point values were observed at the higher temperature (73°C), and increasing with exposure time. For SB biodiesel, flash point measured at 50°C was significantly lower than the one measured at 73°C at all reaction times studied. Also lower exposure times (1 and 5 min) gave significantly lower flash point values than the ones at 10 min of exposure. Similar trends were observed for RB biodiesel. All values for this parameter meet ASTM standard specifications (minimum 130°C), even at the lower temperature and shorter times.

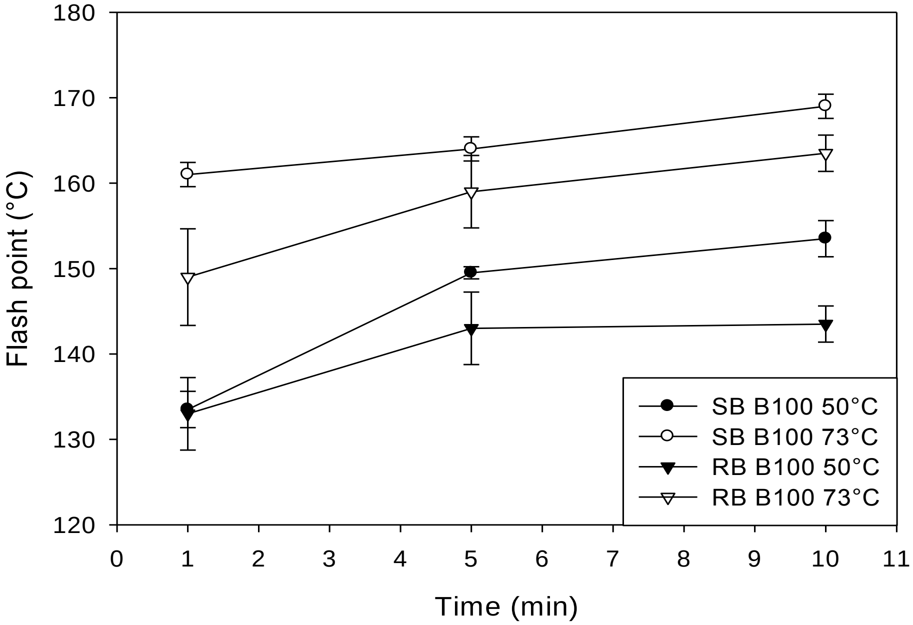

Figure 4.4. Flash point results for soybean and rice bran biodiesel at different temperature and reaction times.

Viscosity

Kinematic viscosity affects injector lubrication and fuel atomization. Biodiesel fuels generally have improved lubricity (Demirbas, 2007); however, their higher viscosity levels tend to form larger droplets on injection, which can cause poor combustion and increased exhaust smoke. The viscosity showed to decrease significantly with higher temperature and increased time exposure (Figure 4.5).

The higher viscosity of biodiesel is usually based on the presence of unreacted fatty acid chains after transesterification, influencing the conversion rate and ultimately the viscosity of the final product. Also viscosity tends to increase with chain length and with increasing the degree of saturation (Falk and Meyer-Pittroff, 2004). Free fatty acids have higher molecular weight and viscosity than the corresponding ethyl esters, though the viscosity is being influenced by the presence of higher double bound configuration, which could also indicate the presence of some unbroken chains (unreacted fatty acids) during the transesterification reaction. In our study the biodiesel from the two different sources did not exceed viscosity specifications, although the ethyl esters formed at 50°C were at the limit of the values specified in the ASTM standards (1.9-6.0 cSt).

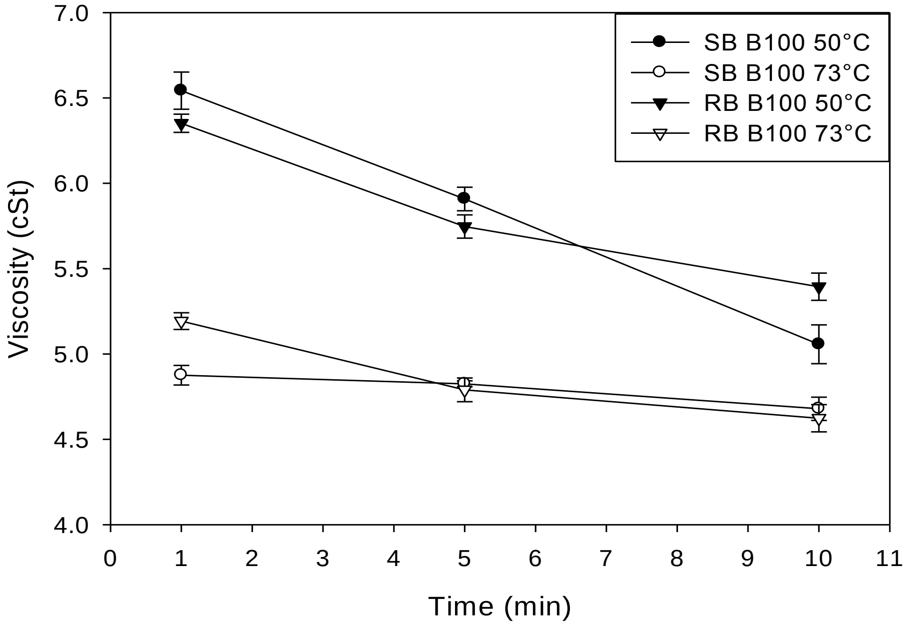

Figure 4.5. Kinematic viscosity results for soybean and rice bran biodiesel at different temperature and reaction times.

Acid Number

Acid values for both SB and RB biodiesel generally decreased with increasing reaction temperature and exposure time (Figure 4.6). This decrease was significant for SB at 50°, but not at 73°C. At the same time, no significant difference was found between acid values at the two temperatures at 10 min exposure time. For RB biodiesel the only significant difference was found as at 50°C between 1 and 5 min exposure time. Similarly with SB, at 10 min no difference was observed between the two temperatures.

Acid number, which measures the acids in the fuel, could emanate from two sources: (i) catalysts utilized in the production of the biodiesel that are not completely removed in the production process; and (ii) degradation of oil by oxidation. Since the samples were analyzed immediately after washing, it is unlikely that the samples had time to oxidize. Therefore the higher acid values are probably the consequence of some small-unreacted quantities of catalyst, or some leftover acid from the quenching step. The acid values for these samples were within ASTM standard specifications (maximum of 0.7 mg KOH/g).

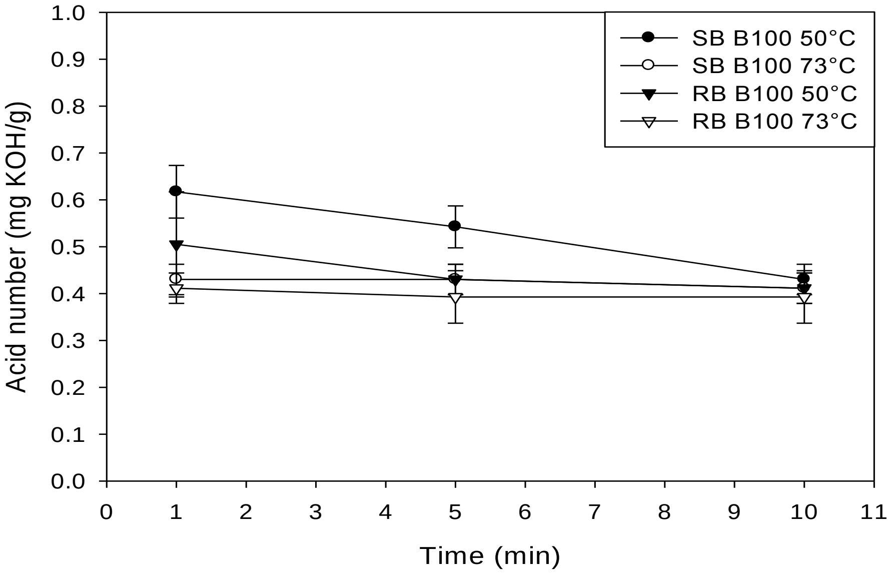

Figure 4.6. Acid number for SB and RB biodiesel at different temperature and reaction times.

Oxidative Stability Index (OSI)

The OSI results are showed in Figure 4.7. Temperature reactions showed to influence the oxidation rate, significantly higher stability being registered at higher temperatures for both biodiesel sources. For RB biodiesel a linear increase with exposure time was observed at both studied temperatures, with significant differences between the values. However for SB biodiesel the only noticeable difference were between the 1 and 5 min of reaction time. No significant differences were found between the 5 and 10 min reaction times, for both temperatures used for SB transesterification reaction.

Besides the presence of air, various other factors influence the oxidation process of biodiesel including the presence of light, elevated temperatures, the presence of some extraneous materials, peroxides and the fatty acid profile, which are generally the consequence of inadequate storage. Although lower stability indexes were noticed at lower temperatures and reaction times, the values presented in Figure 4.7 are within ASTM specifications (minimum 3h).

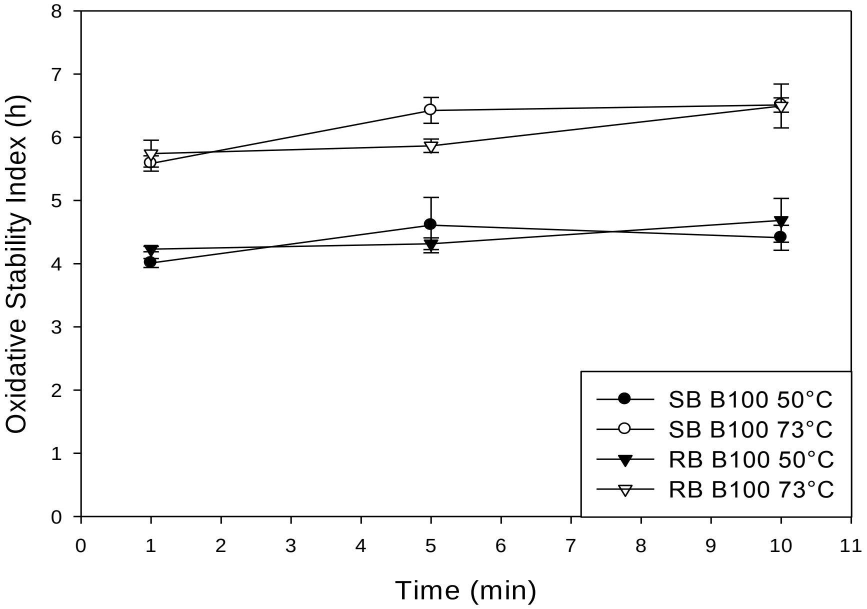

Figure 4.7. Oxidative stability indexes for soybean and rice bran biodiesel at different temperature and reaction times.

Conclusions

Overall, our continuous-flow microwave-heating compares very favorably over conventional methods mentioned in the literature (Encinar et al., 2002; Cervero et al., 2008; Van Gerpen, 2005), where heating can be relatively slow and inefficient because transferring energy into a sample depends upon convection currents and the thermal conductivity of the reaction mixture.

The conversion of biodiesel at 50°C and 73°C showed to meet the ASTM requirements, at all the reaction times used in the study (1, 5 and 10 min). Conversion rates from 96.67-99.25% for biodiesel derived from soy bean oil and 98.35-99.34% from rice bran oil was achieved in the process, higher yields and using lower alcohol/oil ratio(5:1 molecular ratio) in shorter time compared to conventional methods (Rashid and Anwar, 2008). In other studies on microwave-assisted transesterification reaction similar yield of biodiesel were achieved by using other alcohol reagents (methanol, butandiol) or other types of catalyst systems, supporting our results and concluding the benefits of usage of microwave irradiation for biodiesel production.

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CHAPTER 5

CONTINUOUS MICROWAVE EXTRACTION OF OIL FROM CHINESE TALLOW TREE, AN ALTERNATIVE BIODIEESEL FEEDSTOCK. EFFECT OF STORAGE CONDITIONS ON OIL QUALITY

Introduction

Chinese tallow tree (TT) - *Sapium sebiferum* is an ancient and valuable oil seed-producing tree with a long history of large scale commercial production in China and other parts of Asia. It is a deciduous tree, and it has been used in the US as an ornamental tree due to its exceptional foliage in the fall and flowering in the spring (Fig. 5.1 a, b). Although it is known as an invasive tree in the US, TT has been regarded as a promising biomass candidate in the Gulf Coast regions, due to its ability to re-sprout, its rapid growth rate, and its drought and salt tolerance (Scheld and Cowles, 1981). It is one of nature's most prolific producers of renewable hydrocarbons, yielding the equivalent of 500 gallons (12 barrels) of fats and oils per acre per year (4,700 liters per hectare per year), far exceeding other traditional oil seed crops (Scheld, 1984). The seeds contain approximately 40%-50% lipid (Duke, 1997), almost equally distributed in the external vegetable tallow coating (21%), which has higher wax content, and in the kernel (19%) as a drying (Stillingia) oil (Fig. 5.1c) , suitable for conversion into biodiesel (Shupe and Catallo, 2006; Christie, 1969). Chinese tallow can be grown over large areas by conventional agricultural methods and can provide woody biomass for direct burning or conversion to charcoal, ethanol, and methanol (Scheld HW, 1980). In addition, other value-added components have been identified in the leaves and bark of this tree (Lee et al., 2004; Shupe and Catallo, 2006; Liu et al., 1988). The oil has also been reported to have been used in Chinese medicine but overdoses might cause violent sickness and perhaps death (Duke, 1997).

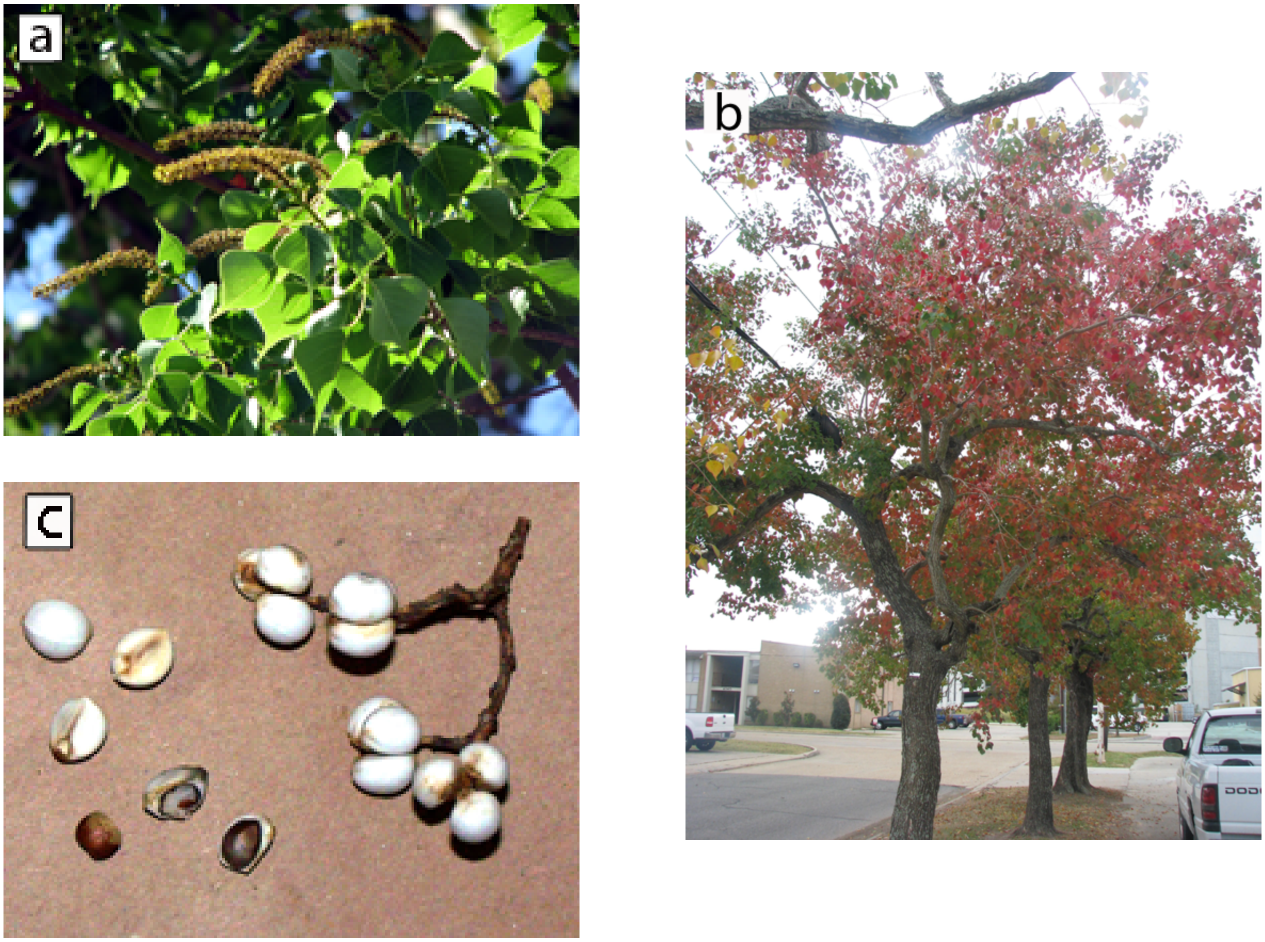

Figure 5.1. Chinese Tallow Tree: a) Green tree with flowers; b) in the fall with multiple colors; c) seeds with coating and kernel.

There is little information available on this species with regards to its oil composition , yields and behavior in storage. Due to the seasonal nature of the TT and due to its potentially enormous worldwide economic importance, it is critical to recognize the various factors contributing to the deterioration of TT seeds. In general, once the seeds have reached their full maturity they are at the peak of their germinability and vigor. From that point, their lifespan diminishes due to aging, although the rate of aging depends on moisture content of the seed and storage conditions (Doijode, 2001). Deterioration in seed quality can occur during handling and storage after harvest until it reaches its end-user. Oil quality is directly related to the physiological conditions of the seeds from which oil is extracted. The aim of storage is to preserve properties of products and maintain the quality of extractable compounds. If suitable storage conditions are not employed for specific product varieties, qualitative and quantitative losses increase. Appropriate storage conditions and management preserves seed viability and vigor for relative extended periods by reducing the rate of seed deterioration. Optimal seed storage is achieved by preserving seeds in favorable climatic conditions and/or by modifying the environment around the seeds so that it is suitable for different storage periods of time (Hoveland, 1980). In turn, post-storage losses may be also influenced by conditions during storage. For example, the storability of grains is affected mostly by their temperature, moisture content and environmental conditions (Hall, 1980). Respiratory activity may accelerate some deterioration of the seeds and cause breakdown of the cell structure. Ultimately, oil extracted from deteriorated and damaged seeds can develop greater amounts of volatile acids and can be high in acidity and low in stability (Priestley, 1986).

Loss may be considered in terms of either quantity or quality. Qualitative loss is more difficult to assess and is perhaps best identified through comparison with well defined standards. Nutritional loss and loss of seed are both aspects of quality losses. Lipid foods can be stored for only limited time periods because of their susceptibility to oxidation in air (Kazantzis et al., 2003). Natural oils are normally a mixture of triglycerides of saturated and unsaturated fatty acids. Oxygen can oxidize fatty acids in a multi-step reaction that occurs in principle according to a radical chain mechanism. The oxidation products formed by this reaction (mainly formic acid) are volatile (Fennema, 1996), and oxidative stability of natural oils is therefore a standard quality control method in the food industry (Zambiazi et al., 2007).

Numerous handling, transportation and storage systems have evolved over the years for postharvest preservation of fresh fruits, vegetables and crop seeds. Depending upon the commodity and the specific preservation objective, there is a wide selection of techniques and systems to choose from. They vary in complexity from common storage involving little or no control of the postharvest environment to highly sophisticated systems such as hypobaric storage (Burg and Burg, 1966; Lougheed et al., 1978), controlling within very narrow limits the temperature and humidity (Roberts, 1981; Guo et al., 2008), concentration of oxygen (Roberts, 1981), carbon dioxide (Chope et al., 2007; Shmulevich et al., 2003) and other gases (Singh and Pal, 2008). There are numerous studies that report about storage conditions affecting the quality of oil obtained from various feedstocks like olives, rice and sunflower (Agar et al., 1999; Clodoveo et al., 2007; Ramezani, 2004; Besbes et al., 2004). Extensive literature is also available on the influence of different storage conditions on vegetable seeds and perishable materials (Arabhosseini et al., 2007; Besbes et al., 2004; Chope et al., 2007; Gamli and Hayoglu, 2007; Gomez and Artes, 2004; Lougheed et al., 1978).

The aim of this study is two fold: 1. To evaluate the long-term effect of various storage conditions on oil yield and composition in TT seeds during the storage period that follows the harvest; 2. To investigate the yields and composition obtained in a continuous microwave extraction system to be further used for biodiesel production. Results of this research will be used for optimum storage of TT seeds to preserve their quality.

Materials and Methods

Seeds Collection

Seeds were manually harvested from tallow trees in and around Baton Rouge area between October-November 2007. Another harvest of seeds left on other trees took place in March the following year, for comparison purposes. After harvest, the seeds were air dried in the open for 2-3 days and separated from twigs and leaves by a combination of threshing, blowing and hand picking. Later, the cleaned seeds were stored in a freezer at - 4°C until further use.

Storage Parameters

For the storage study 50 g of TT seeds were individually packaged using a Multivac system (Model C200, MultiVac Inc., Kansas City, MO) under different environmental conditions (3% CO_2, 6% CO_2, normal atmosphere and vacuum of 0.05 atm). The packaged samples were stored under two temperatures, one under refrigeration (4^oC) and the other at controlled room temperature (24^oC) for three months. The storage temperatures were monitored and recorded daily. Parameters studied were based on literature information and typical storage conditions applied for other crops (i.e. soybeans, sunflower, and grains).

Oil Extraction

Every week, two packages from each storage temperature and at each storage condition were removed (total of eight packages), and the seeds were cracked manually in order to separate the endocarp from the seed shell. A batch microwave system (model Ethos E, Milestone Inc., Monroe, CT) was used to extract the oil from the seeds kernel using ethanol (ACS/UPS grade, 200 proof) as a solvent in a ratio of 1 to 3 (seed : ethanol, w/w). The extraction was performed in two steps: a gradual temperature increased to extraction temperature for 5 min, followed by holding at the extraction temperature of 130°C for 15 min. The temperature of the sample inside the vessels was monitored with a fiber optic probe connected to the control system. After a ventilation period of 20 min in which the samples were cooled down, the vessels were unsealed and the oil and solvent were filtered from the cake through a Whatman® filter paper (Φ=47mm, 1.2 μm) using a vacuum pump (Model SR 10/50, Thomas Compressors and Vacuum Pumps, Skokie, IL). Solvent was evaporated in a vacuum centrifuge evaporator (CentriVap Console

Labconco, Kansas City, Missouri) running for approx 14-16 h. The residual oil for each sample was weighed in order to determine the extraction yield. Oil extracted from the entire seeds (coat and kernel), from seeds harvested in fall and spring were also extracted for comparison purposes to estimate changes in yield and composition if the seeds are left on the trees past the seeds maturity date.

For the oil extraction yields whole seeds were milled using a coffee grinder. The oil extraction was performed with ethanol in a 3:1 ratio similarly with the procedure described in Chapter 2 for soybeans and rice bran.

Determination of Oil Quality

The oil quality indices - titratable acidity, peroxide value, oxidative stability and fatty acid composition, were determined according to the IUPAC standard methods for analysis of oil and fats (IUPAC, 1987a). Titratable acidity was measured by titrating 1 g of oil with 0.1N KOH solution, and 3 g of oil were titrated with $Na_2S_2O_3$ for peroxide value analysis. The oxidative stability index (OSI) of the oil samples was evaluated using a Rancimat oxidative stability instrument (model 743, Metrohm USA, Inc., Riverview, FL) operating at 110°C on three grams samples exposed to air flow at 0.01 m^3/h. The volatile products formed as a result of the oxidation reaction were absorbed by distilled water, and the change in electrical conductivity of the water was measured as an indication of the oil's oxidative stability.

The fatty acid compositions were determined by quantifying the methyl esters through gas chromatography (Varian 450-GC, located at the Callegari Environmental Center, LSU Ag Center, Baton Rouge) coupled with a Varian 240-MS Ion Trap Mass Spectrometer (Varian Inc. USA). A Varian FactorFour Capillary column WAXms (30m x 0.25mm i.d., 0.25 µm) was used at 245°C with helium at 1 ml/min as carrier gas, a split injector at 270°C with a split ratio of

1:20, and a detector temperature of 270°C. Components were identified using a standard FAME mix (Supelco, Bellefonte, PA).

Statistical Analysis

All measurements were carried out in duplicates and expressed as mean values. Statistical analysis was performed in order to test significant differences among the different storage conditions in time. A two-way ANOVA using Proc Mixed 2x5 factorial (SAS system, SAS Institute Inc., Cary, NC) was used to test significant differences among temperatures (4 and 24°C) and environmental atmospheric conditions. Two-way ANOVA using Proc Multiple comparison tests were performed by using Tukey adjustment to determine the significant difference between treatments. All significances were tested at $\alpha = 0.05$. For oil extraction yield the statistical analysis was performed as described in Chapter 2.

Results and Discussion

Chemical Properties of Stillingia Oil

Chemical properties of oil can be used to ascertain its quality. Acid value (AV) is an important indicator of vegetable oil quality and is expressed as the amount of KOH (in mg) necessary to neutralize free fatty acids contained in 1 g of oil (D. Firestone, 1996). However, the acid value of the oil must not be too high as this denotes an excessively high content of free fatty acids, which causes the oil to turn sour. The AV will give important information on the type of catalyst needed for an eventual biodiesel conversion. Acidity index was measured across the 12 weeks of storage and are presented in Table 5.1. Compared to the value at harvest, neither air nor controlled atmosphere modified AV. Although the AV shows a linear increase during the storage period, the differences was not significant in value within the storage conditions. Moreover, temperature of storage did not seem to have an influence on the AV.

The peroxide value (PV) of the oil is used as a measurement of the extent to which rancidity reactions have occurred during storage, peroxides being known as intermediates in the auto oxidation reaction. The PV of the oil from the stored seeds for the weeks studied ranged from 0.72 to 0.82 meq/kg (Table 5.1). Within expected range values for this particular oil, the stillingia oil underwent a slow, insignificantly increase in PV, concluding that the different storage conditions and storage temperatures do not affect the oil quality from this parameters perspective. Although the PV of the oil was not different during the storage period, there is a possibility of the oil being biologically different enough to potentially have some effect on oxidative stability index values which are discussed further in this article. Peroxide value was shown to be inversely related to the α-tocopherol content in shelled and roasted almonds stored over 9 months (Garcia-Pascual et al., 2003), and the rate of increase of peroxide value in stored pistachio has been shown to be greater when stored in air than when stored in a CO_2 rich environment (Maskan and Karatas, 1999). A study conducted by Kaul et al. (Kaul et al., 2009) on the effect of aging on jojoba (*Simmondsia chinensis*) oil quality reveals that the acid value, peroxide value and iodine value of solvent extracted oil increased with storage time (over 18 months storage). Our results show marginal increase (though not significant) in AV and PV values over time for the 3 month storage period. Similar increase in peroxide value over time (but for 12 months) has been reported by Mexis et al. (Mexis et al., 2009) in their study on the storage condition of oil from shelled walnuts. The peroxide value of the oil from the stored walnuts was found to increase at a faster rate at higher storage temperatures (20^oC) when compared at a storage temperature of 4^oC. Temperature influences the rate of rancidity of fats and oils and also has an influence in gaseous diffusion rate (through the packaging material). Even though our study agrees with other research describing the positive increase in peroxide value with storage temperature (Maskan and Karatas, 1999) an increase in the storage period and multiple temperatures could provide a better picture on the long term quality of tallow tree seeds.

Table 5.1. Average titratable acidity (TA) (mg KOH/g sample) and peroxide values (PV) (meq/kg) of the stored TT seeds.

			Storage time (weeks)												
			0	1	2	3	4	5	6	7	8	9	10	11	12
Air	4°C	TA	1.62	1.64	1.65	1.65	1.67	1.65	1.67	1.65	1.65	1.69	1.67	1.70	1.71
		PV	0.72	0.72	0.73	0.73	0.74	0.73	0.75	0.78	0.74	0.76	0.80	0.79	0.79
	24°C	TA	1.62	1.63	1.66	1.66	1.68	1.68	1.67	1.66	1.69	1.72	1.70	1.70	1.71
		PV	0.72	0.72	0.72	0.74	0.74	0.75	0.76	0.75	0.78	0.76	0.80	0.83	0.82
Vacuum	4°C	TA	1.62	1.62	1.64	1.64	1.64	1.63	1.65	1.68	1.66	1.68	1.72	1.69	1.70
		PV	0.72	0.72	0.72	0.72	0.72	0.75	0.74	0.77	0.80	0.83	0.79	0.80	0.81
	24°C	TA	1.62	1.63	1.65	1.65	1.67	1.68	1.67	1.70	1.68	1.69	1.72	1.70	1.71
		PV	0.72	0.72	0.73	0.73	0.75	0.74	0.77	0.80	0.79	0.77	0.82	0.80	0.81
3 % CO$_2$	4°C	TA	1.62	1.63	1.63	1.65	1.67	1.67	1.65	1.65	1.64	1.65	1.69	1.67	1.67
		PV	0.72	0.72	0.72	0.74	0.74	0.73	0.75	0.73	0.76	0.76	0.78	0.76	0.77
	24°C	TA	1.62	1.64	1.64	1.65	1.64	1.66	1.66	1.65	1.66	1.68	1.70	1.67	1.69
		PV	0.72	0.72	0.73	0.74	0.74	0.72	0.76	0.74	0.75	0.79	0.79	0.77	0.79
6% CO$_2$	4°C	TA	1.62	1.62	1.63	1.63	1.62	1.64	1.64	1.67	1.68	1.72	1.69	1.70	1.71
		PV	0.72	0.72	0.72	0.74	0.75	0.75	0.73	0.76	0.78	0.77	0.80	0.79	0.80
	24°C	TA	1.62	1.64	1.64	1.66	1.66	1.68	1.66	1.69	1.67	1.71	1.70	1.69	1.70
		PV	0.72	0.72	0.74	0.74	0.74	0.76	0.76	0.78	0.75	0.76	0.79	0.81	0.81

Fatty Acids

The FFA major components were selected to examine any changes in oil composition as a function of time and storage parameters. Although stillingia oil is composed from more than seven kinds of fatty acids, five major fatty acids (palmitic, stearic, oleic, linoleic and linolenic acids) comprise about 95% of the total fatty acid content. Fatty acid composition of the extracted stillingia oil was 0.5% miristic, 4.29% palmitic, 1.18% stearic, 11.07% oleic, 18.30% linoleic, 63.21% linolenic, 0.5% arachidic and 1.13% “japanic acid” (eicosanedionic and docosanedioic acids) using values from freshly harvested kernel seeds. Similar values for fatty acid composition for this crop have been reported earlier (Crossley and Hilditch, 1953; Chen et al., 1987).

Storage of TT seeds for 3 months had no significant effect on FFA composition of kernel oil between the studied controlled atmospheres. Since no significant difference was observed between the storage times at the same storage conditions, average values ± standard deviations for the 12 weeks of study are presented in Table 5.2. The FFA contents detected in the stillingia oil stored under modified atmosphere packaging at ambient temperature or 4°C remained stable for up to 3 months. Similar behavior was found for FA contents of in-shell or shelled and peeled almonds under different atmosphere storage, where the percentages of oleic and linoleic acid remained unchanged even after 12 months of study at lower temperature storage (Kazantzis et al., 2003).

Although there were no significant changes in the oil composition between the different storage conditions, the amount of miristic and “japanic acid” could only be detected in trace amounts following the first week of study. The presence of japanic acid is believed to be an important factor in the dryness of stillingia oil when used as a coating material. One good explanation for its disappearance could be that japanic acid is a highly volatile compound, the presence of air oxygen causing its deterioration. As this compound was not detected even in vacuum packaging, we can infer that its degradation occurs mostly in the several days between harvesting time and packaging. From Table 5.2, it can be observed that the TT kernel oil contains a high degree of unsaturated fatty acids (oleic, linoleic and linolenic) comprising more than 93% of total fatty acids. This makes this oil highly susceptible to oxidation resulting in the development of rancidity and off-flavors, but it is suitable for transesterification into biodiesel. TT oil has a higher percentage of linolenic acid (> 63%) than oleic acid (between 10-11%). This could lead to faster rate of rancidity in the oil during extended storage and exposure to higher storage temperature.

Table 5.2. Fatty acid composition of stillingia oil (%).

Fatty acid	Air		Vacuum		3% CO$_2$		6% CO$_2$	
	4°C	24°C	4°C	24°C	4°C	24°C	4°C	24°C
Myristic, C 14:0	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]
Palmitic, C 16:0	4.18[b]±0.38	4.35[b]±0.38	4.30[b]±0.58	4.40[b]±0.37	4.19[b]±0.58	4.11[b]±0.65	4.02[b]±0.18	4.20[b]±0.40
Stearic, C 18:0	1.14[b]±0.07	1.19[b]±0.13	1.22[b]±0.12	1.23[b]±0.07	1.14[b]±0.11	1.11[b]±0.17	1.11[b]±0.07	1.13[b]±0.11
Oleic, C 18:1	10.88[b]±0.85	10.79[b]±1.07	11.13[b]±0.67	10.88[b]±0.56	10.74[b]±0.58	10.84[b]±1.03	10.61[b]±0.61	11.04[b]±0.61
Linoleic, C 18:2	19.39[b]±0.65	19.68[b]±0.70	19.59[b]±1.07	19.67[b]±0.73	20.25[b]±0.90	19.95[b]±0.82	19.70[b]±0.74	19.84[b]±0.62
Linolenic, C 18:3	64.26[b]±1.15	63.72[b]±1.85	63.67[b]±1.68	63.54[b]±0.99	63.64[b]±1.66	63.92[b]±1.56	64.54[b]±.082	63.63[b]±0.91
Arachidic, C 20:0	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]	Tr.[a]

[a] Trace ≤ 0.3%.

[b] Value in the same row for each treatment , i.e., storage condition and temperature, are not significantly different (p≥0.05).

Oxidative stability index (OSI)

During long term storage, oxidation due to contact with air (autoxidation) represents a legitimate concern with respect to maintaining oil quality. Figure 5.2 shows the changes in oxidation stability of the oils obtained from seed kernels stored at different temperatures and under different storage conditions. Although no major differences were detected in the FFA compositions of the oils, there was a significant change in the OSI. The stability of the oils was shown to be influenced by time, temperature and atmosphere used for storage. Significant differences could not be found between the first week and the last week of storage; however the OSI for all the storage conditions increased at a maximum after the seventh week of storage. The conditions of storage also influenced the OSI. The oils from the seeds stored in 6% CO_2 had the highest OSI of 16 h, followed by the ones stored at 3% CO_2 and vacuum (Fig. 5.2). As it was expected based on existent literature data, the oils from the seeds stored in normal headspace had the lowest OSI, most commonly resulted from a higher autoxidation reaction that took place in contact with air, although the lower values reported (approx 4h) are similar to the other values reported in the literature for other vegetable oils with a minimum of 3h (Bailey, 2005).

Storage temperatures also had an impact on OSI. Oils from seeds stored at 4°C showed to have a higher stability over the ones stored at ambient temperature, with exception for 3% CO_2 environment were room temperature condition showed to have a slightly higher value (Fig. 5.2). Studies on olive oil storage (Clodoveo et al., 2007) also showed a high decrease in OSI for olive oil stored at ambient temperature compared to the one stored at 5°C at different conditions and an increase in oxidized triglycerides and diglycerides with storage time.

One of the most important parameters that influence lipid oxidation and its stability is the degree of unsaturation of its fatty acids. Although the unsaturated level in our study did not have a significant change between the storage conditions and storage time, there could be other changes in oil composition that could influence the OSI. The increase in OSI may also be an effect of the presence of natural compounds having different chemical structures that exhibit antioxidant activity that may also affect the oxidative rate.

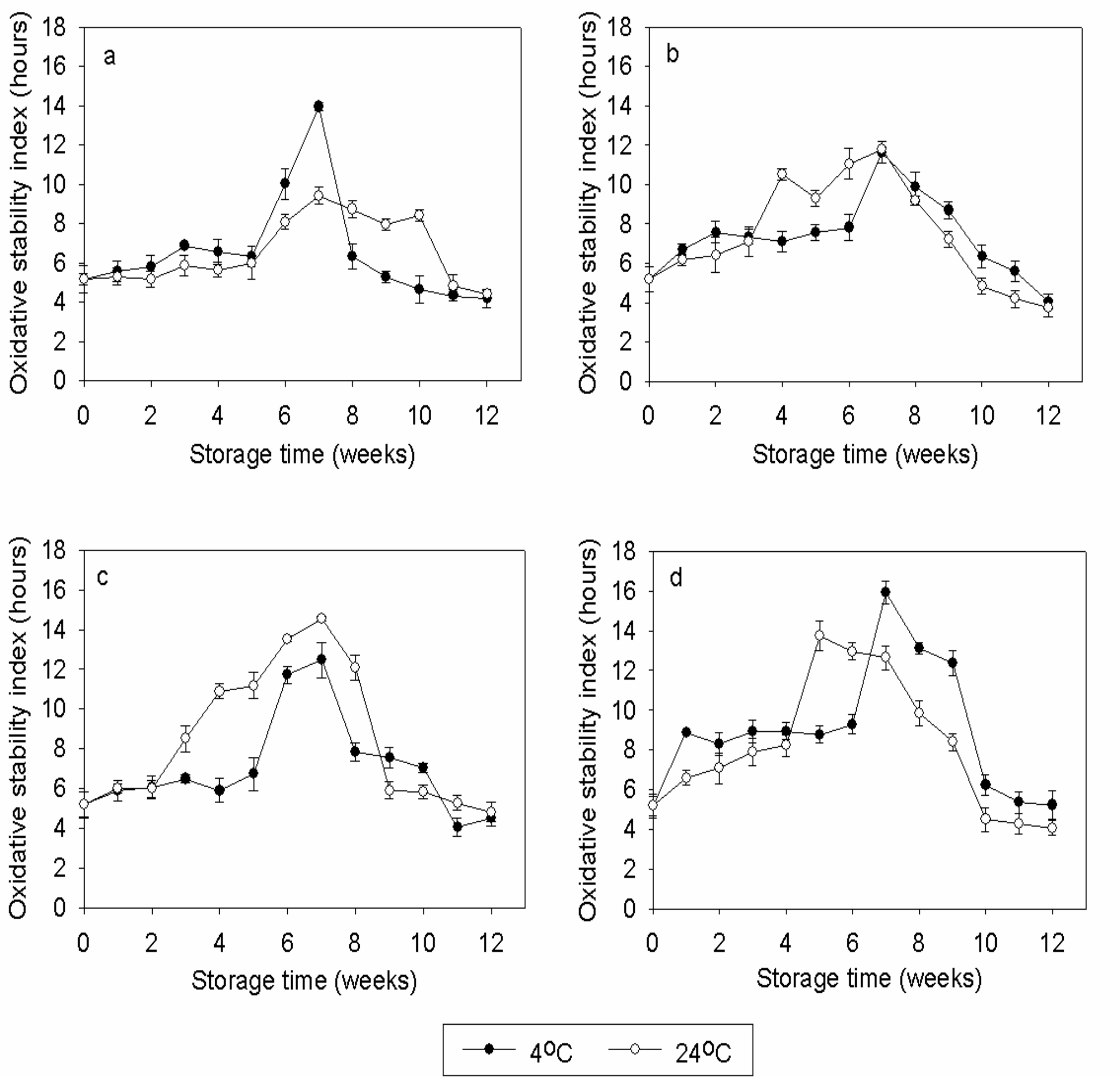

Figure 5.2. Changes in the stability to oxidation (hours) stored under (a) air atmosphere; (b) vacuum; (c) 3% CO_2; (d) 6% CO_2.

Another possible alteration mechanism is lipid hydrolysis, with consequent free fatty acid (FFA) generation, by chemical or enzymatic action. Although the original causes and consequences of oxidative and hydrolytic degradation processes are quite different, they might interact with each other and contribute to the observed changes in the oil stability. In fact, several papers have been published on the pro oxidant action of FF, which seems to be exerted by the carboxylic molecular group, accelerating the rate of decomposition of hydroperoxides (Hyun Jung et al., 2007; Mistry and Min, 1988; Miyashita and Takagi, 1986). Base on this literature data and the results of our experiments, it could be hypothesized that, after reacting with FFA, these amino or imino groups liberate the phenolic groups that could further bond to hydrogen, and displaying higher antioxidant activity.

The longevity and no appreciable change in oil properties of the in-shelled materials could be attributed to the role of the shell which can behave as a barrier to moisture and atmospheric exchange, increasing the quality aspects of the kernel. Prior research (Barton, 1960) reported that cardinal flower seeds sealed in oxygen lost viability rapidly and that storage in carbon dioxide, nitrogen, or partial vacuum extended storage life. In contrast, another study on sorghum and crimson seeds (Bass, 1963) showed no significant advantages in using partial vacuum, carbon dioxide or nitrogen, argon or helium instead of air.

Early and Late Harvested Seeds

Early and late harvested TT seeds were collected and the whole seeds oil compositions and chemical properties are presented in Table 5.3. Whereas the oil yield did not change among the two harvesting times, the oil composition showed to be significantly affected by the time of harvest. Percentages of myristic, palmitic, stearic and oleic acids significantly decreased in the oil from late harvested seeds. The change in seed coating could be visually observed on the late harvested samples, becoming darker than the early harvested seeds, which could be the direct cause for this decreased value, especially for myristic and palmitic acids that are present in the outer coat of the seed. The japanic acid, similar to the phenomenon observed in kernel oil, did not appeared in the late harvested samples due to higher degree of oxidation, while stearic acid could only be found in traces.

The increase in linolenic and arachidic acids is a good evaluation of the oxidation between the harvesting times. The increase of these compounds is the direct consequence of the decrease in oxidation stability, also observed from the OSI (Table 5.3), were the stability for oxidation decreased from 24 to11.6 h for the two harvested periods. Chemical properties of the seeds also showed to change between the two harvesting periods. TA increased from 4.2 to 5.1 (mg KOH/g) of oil, due to the prolonged oxidation processes. An increase in value was also observed for the PV, where change from 2.66 to 3.35 (meg/kg) was measured between the two harvesting times, also indicating a higher level of rancidity of the oil.

Table 5.3. Properties and composition of the oil from early and late harvested seeds (values reported as %, unless otherwise noted).

	Early	Late
Myristic, C 14:0	13.63±1.33	3.83±0.96
Palmitic, C 16:0	14.61±1.75	3.86±0.37
Stearic, C 18:0	2.42±0.11	Tr.[a]
Oleic, C 18:1	8.96±0.14	3.94±1.14
Linoleic, C 18:2	26.01±0.73	25.01±0.17
Linolenic, C 18:3	30.89±0.98	36.17±2.13
Arachidic, C 20:0	2.44±0.28	21.36±0.95
Eicosanedioic, $C_{20}H_{38}O_4$	0.51±0.06	-
Docosanedioic, $C_{22}H_{42}O_4$	0.59±0.04	-
Total unsaturated	65.85±1.85	70.97±1.85
Total saturated	33.10±3.48	29.06±1.42
TA (mg KOH/g)	4.2±0.61	5.1±0.39
PV (meg/kg)	2.66±0.36	3.35±0.48
OSI (h)	24.18±1.5	11.64±1.35
Oil yield (%)	34.55±0.32	34.33±0.65

[a] Trace <0.3%.

These results, comparing the quality of the oil from seeds harvested at different times, are giving important information about the behavior of the material depending on the intended end use (transesterification into biodiesel or other uses). First of all, the percentage of oil extracted did not significantly change between the seeds harvested at the two different times (Table 5.3). Moreover, the change in FFA composition does not affect the oil quality requirements for the biodiesel reaction; it is especially the content of unsaturated fatty acids in relation to the saturated fatty acids that is important for the product. The TA of the oil is also an important factor that can affect the transesterification reaction into biodiesel. Although early harvested seeds had a TA value (Table 5.3) that would permit a base catalyst biodiesel reaction, the TA value of 5 for the later harvested seeds would probably necessitate a preliminary acid-based catalysis to successfully complete the reaction, which is a common case of other pure and waste vegetable oils. Further increase in storage period is warranted to obtain a better understanding on the effect of storage environment on the TT oil quality.

Continuous Microwave Assisted Oil Extraction

Oil extraction was performed at 50°C, 60°C and 73°C in a continuous microwave system, the results being compared with conventional extraction performed at the same temperatures as for the microwave extraction. As the temperature increased, the oil yield extracted increased for both methods of extraction in a linear way, the highest amount extracted been measured at 73°C (Figure 5.3). Statistical differences were found between all temperatures for both extraction methods, and with statistical significant differences between the methods at the same extraction temperature. At 73°C extraction temperature, 9.3% more oil was extracted when using CMAE in comparison with conventional extraction method.

Similarly, oil yields increased with extraction time. For CMAE significant differences were found between the oil extracted at the different extraction times. The highest amount of oil extracted was after 20 min extraction at a temperature of 73°C (35.3%) (Figure 5.3). At 60°C, the amount of oil extracted after 12 min did not significantly differ with the yield extracted after 16 min. Increasing the extraction time from 16 to 20 min, yield of oil extracted significantly increased with 1.3% at 60°C and with 2.2% at 73°C extraction temperature.

These results highlight the fact that the CMAE system optimized for the use of soybean and rice bran oil extraction can be applied for other type of oil feedstocks, with high percentages of oil extracted.

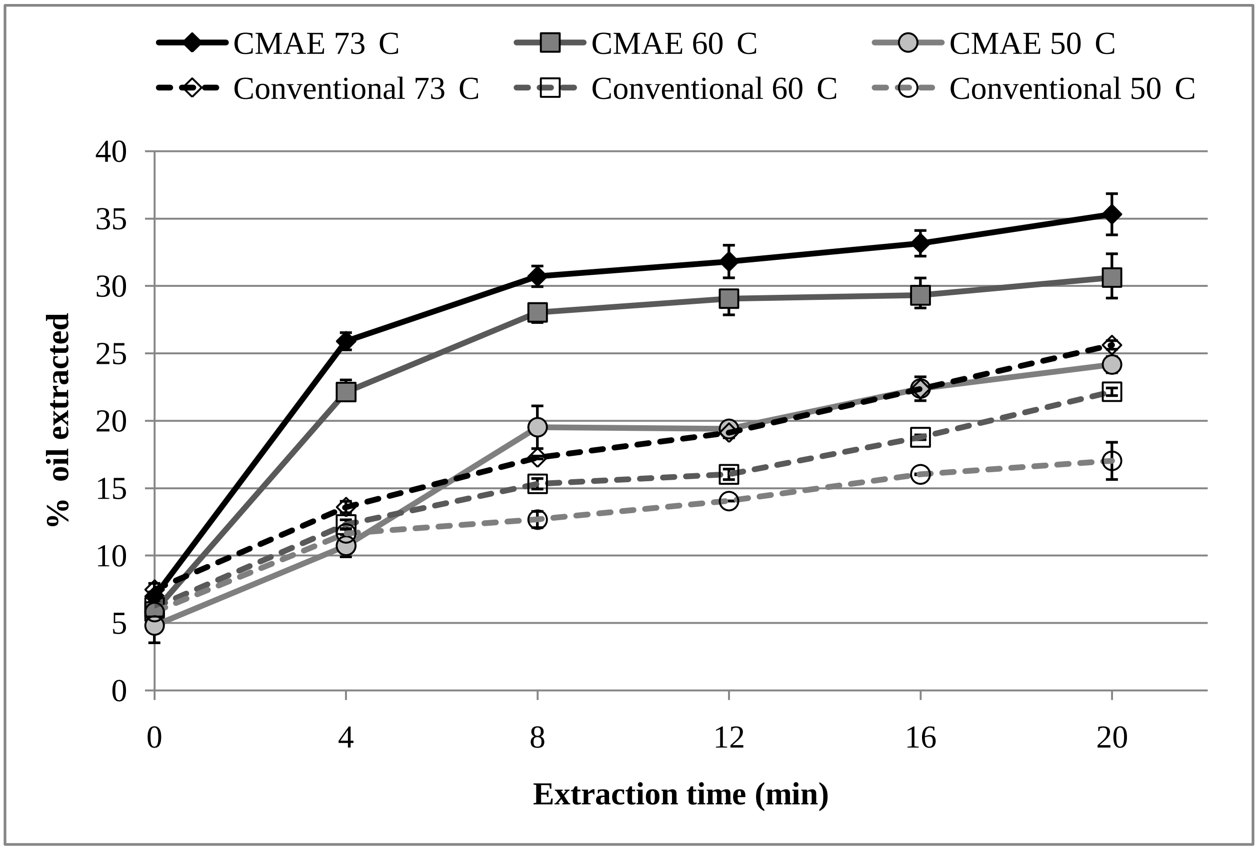

Figure 5.3. Oil extracted at different temperature and time for CMAE and conventional extraction.

Conclusions

The purpose of this study was to evaluate different environmental factors and their effects on the TT oil quality during storage, as to date there is no study investigating these effects. Oil quality analysis provided evidence that, after 12 weeks of storage in a controlled atmosphere, no differences were observed compared to low cost air storage. The results support the belief that no elaborate storage conditions are required to store TT seeds for further use in biodiesel conversion. Further increase in storage period is needed to obtain a better understanding on the effect of storage environment on the TT oil quality. The extraction yields in a laboratory scale continuous microwave extraction system showed a high yield of oil compared to soybean and rice bran.

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CHAPTER 6

CONCLUSIONS AND FUTURE WORK

Dielectric properties of two feedstocks, soybean and rice bran were measured in presence of several solvents (methanol , ethanol, isopropanol and hexane) at different ratios (1:0.5, 1:1, 1:2), temperature (23, 30, 40, 50°C) and frequency (300-3000MHz). Results indicated that dielectric constant decreased with frequency and was strongly dependent on temperature, solvent ratio and solvent type. Similar, dielectric loss increased to a maximum of 1000 MHz for feedstock with ethanol, and to a maximum of 433 MHz for other solvents while for hexane showed to be virtually 0. Quasi-linear relationship using second order logarithmic transformation of the data provided a reliable estimator for the behavior of dielectric properties of the feedstocks used. These findings were significantly helpful in indicating the appropriate parameters and materials to be used for CMAE. Results indicated that ethanol is more suitable solvent for high energy conversion into heat for both feedstocks, while a ratio of 3:1 solvent to feedstock was necessary for operating the system. CMAE was successfully applied for oil extraction. Yield of oil extracted increased with extraction temperature and time for both feedstocks. Oil yield high as 15% for soybean and 16% for rice bran was extracted at laboratory scale at 73°C and 20 min extraction time, whit quality meeting the ASTM requirements for vegetable oil consumption and also quality oil for biodiesel production.

The optimum parameters from CMAE were further applied on a 5KW, 915 MHZ focused microwave system. At pilot scale, 18.6% oil for soybean and 19.5% for rice bran respectively was extracted at 73°C after only 6 min of residence time with no significant difference in oil extracted after 10 and 60 min residence time. With the advantage of higher oil yields extracted in less time when compared with laboratory scale and conventional extraction methods, CFMAE has the ability for higher material process which can be applied at an eventual industrial commercial scale oil extraction with oil at the required quality specifications.

The CMAE system was further used for biodiesel transesterification reaction were high conversion rates (96.7-99.4%) were achieved at the reaction times (1, 5 and 10 min) and temperature (50 and 73°C) studied. The advantage in using a continuous microwave system for biodiesel production is significantly less needed time for transesterification reaction (1 min compared with 2 hours conventional reaction), lesser amount of solvent and input energy.

Quality analysis on oil extracted from TT kernel, provided evidence that after 12 weeks of storage in controlled atmosphere no differences in quality was observed compared to low cost air storage. Moreover, late harvested seeds did not show major degradation in oil quality necessary for biodiesel production when compared with early harvested seeds, concluding that no elaborate storage conditions are needed for biodiesel production from this feedstock.

Future Directions

The main target of the thesis research was to design and develop a continuous microwave system for oil extraction from several feedstocks and a further biodiesel conversion using microwave heating. Although significant progress was made toward designing and understanding the system developed, other areas of research should be addressed. The following research directions are found necessary such that they deserve further considerations:

- Test the CMAE system at higher temperatures with higher flow rates, by applying a back up pressure, which would maintain the solvent in its liquid state.
- Investigate the CMAE system with higher available oil yield feedstocks (i.e. algae).
- Test and optimize the 5KW, 915 MHz pilot system for biodiesel conversion.

- An elaborated study on energy efficiency would be necessary to support the result from this work, especially in the case of eventual industrial purposes of the process.
- An eventual further increase in storage period would be needed to obtain a better understanding on the effect of storage environment on TT oil quality.
- A study regarding TT oil conversion to biodiesel should follow up the extraction procedure. Parameters like reaction temperature, reaction time and appropriate catalyst should be researched for a quality TT oil conversion.

APPENDIX A. OIL EXTRACTION DATA

Table A1. CMAE rice bran oil extraction data

Temp (ºC)	Time (min)	Oil Extracted+tube (g)					Oil extracted (g)					Oil extracted (%)					Average	SD
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5		
73	0	14.06	14.05	14.24	14.12	14.10	0.68	0.73	0.65	0.6	0.55	8.62	9.25	8.24	7.60	6.97	8.14	0.88
	4	14.22	14.17	14.24	14.27	14.26	0.81	0.82	0.85	0.9	0.88	10.27	10.39	10.77	11.41	11.15	10.80	0.49
	8	14.41	14.55	14.45	14.54	14.49	1.1	1.25	1.16	1.19	1.18	13.94	15.84	14.70	15.08	14.96	14.90	0.69
	12	14.55	14.59	14.54	14.55	14.60	1.19	1.23	1.23	1.22	1.21	15.08	15.59	15.59	15.46	15.34	15.41	0.21
	16	14.61	14.58	14.56	14.59	14.65	1.2	1.19	1.24	1.27	1.29	15.21	15.08	15.72	16.10	16.35	15.69	0.55
	20	14.67	14.67	14.63	14.60	14.59	1.32	1.25	1.19	1.25	1.19	16.73	15.84	15.08	15.84	15.08	15.72	0.68
60	0	14.35	14.24	14.35	14.28	14.39	0.59	0.49	0.6	0.55	0.5	7.48	6.21	7.60	6.97	6.34	6.92	0.64
	4	14.17	14.15	14.24	14.20	14.18	0.81	0.74	0.85	0.81	0.7	10.27	9.38	10.77	10.27	8.87	9.91	0.77
	8	14.45	14.34	14.35	14.34	14.39	1.08	1.02	0.95	0.93	1.01	13.69	12.93	12.04	11.79	12.80	12.65	0.76
	12	14.41	14.45	14.39	14.45	14.48	1.01	1.07	1.08	1	0.99	12.80	13.56	13.69	12.67	12.55	13.05	0.53
	16	14.40	14.42	14.48	14.39	14.48	1.09	0.97	1.09	1.03	1.13	13.81	12.29	13.81	13.05	14.32	13.46	0.79
	20	14.52	14.50	14.58	14.46	14.48	1.23	1.09	1.2	1.11	1.11	15.59	13.81	15.21	14.07	14.07	14.55	0.79
50	0	14.45	14.34	14.35	14.34	14.39	0.39	0.32	0.41	0.44	0.37	4.94	4.06	5.20	5.58	4.69	4.89	0.57
	4	14.03	13.83	13.87	13.91	14.01	0.61	0.46	0.53	0.5	0.62	7.73	5.83	6.72	6.34	7.86	6.89	0.88
	8	14.15	14.17	14.20	14.14	14.13	0.73	0.76	0.84	0.81	0.75	9.25	9.63	10.65	10.27	9.51	9.86	0.58
	12	14.21	14.22	14.18	14.22	14.30	0.81	0.81	0.82	0.84	0.92	10.27	10.27	10.39	10.65	11.66	10.65	0.59
	16	14.34	14.36	14.33	14.30	14.31	1.02	0.91	0.92	0.9	0.94	12.93	11.53	11.66	11.41	11.91	11.89	0.61
	20	14.36	14.36	14.34	14.37	14.34	1.07	0.97	0.93	0.98	0.99	13.56	12.29	11.79	12.42	12.55	12.52	0.65

Table A2. CMAE soybean oil extraction data

Temp (ºC)	**Time (min)**	**Oil Extracted+tube (g)**					**Oil extracted (g)**					**Oil extracted (%)**					**Average**	**SD**
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5		
73	0	13.78	13.85	13.46	13.85	13.25	0.6	0.64	0.6	0.54	0.5	7.60	8.11	7.60	6.84	6.34	7.34	0.64
	4	14.05	14.07	14.14	14.17	14.16	0.65	0.66	0.78	0.79	0.75	8.24	8.37	9.89	10.01	9.51	9.17	0.76
	8	14.39	14.4	14.43	14.45	14.39	1.08	1.1	1.14	1.1	0.98	13.69	13.94	14.45	13.94	12.42	13.37	1.03
	12	14.51	14.39	14.31	14.45	14.5	1.15	1.03	1.00	1.12	1.05	14.58	13.05	12.67	14.20	13.31	13.50	0.73
	16	14.49	14.42	14.38	14.49	14.51	1.12	1.1	0.98	1.08	1.12	14.20	13.94	12.42	13.69	14.20	13.73	0.67
	20	14.55	14.51	14.46	14.53	14.6	1.13	1.1	1.1	1.2	1.22	14.32	13.94	13.94	15.21	15.46	14.63	0.65
60	0	13.98	13.65	13.49	13.65	13.85	0.56	0.43	0.5	0.47	0.58	7.10	5.45	6.34	5.96	7.35	6.61	0.82
	4	13.99	13.96	14.02	14.05	14.03	0.63	0.58	0.63	0.66	0.55	7.98	7.35	7.98	8.37	6.97	8.00	0.84
	8	14.15	14.2	14.28	14.23	14.28	0.78	0.82	0.88	0.82	0.9	9.89	10.39	11.15	10.39	11.41	10.71	0.58
	12	14.33	14.32	14.24	14.34	14.37	0.93	0.95	0.93	0.89	0.88	11.79	12.04	11.79	11.28	11.15	11.74	0.47
	16	14.31	14.2	14.38	14.28	14.37	0.96	0.85	0.94	0.93	0.97	12.17	10.77	11.91	11.79	12.29	11.87	0.58
	20	14.31	14.37	14.41	14.32	14.39	1.02	0.96	1.03	0.97	1.02	12.93	12.17	13.05	12.29	12.93	12.53	0.52
50	0	13.29	13.54	13.61	13.25	13.68	0.4	0.42	0.47	0.5	0.53	5.07	5.32	5.96	6.34	6.72	5.81	0.64
	4	13.9	13.97	13.91	13.92	13.85	0.61	0.58	0.5	0.53	0.5	7.73	7.35	6.34	6.72	6.34	6.96	0.58
	8	14.09	14.04	14.07	14.08	14.09	0.73	0.68	0.76	0.75	0.7	9.25	8.62	9.63	9.51	8.87	9.26	0.43
	12	14.11	14.09	14.1	14.16	14.14	0.8	0.7	0.78	0.84	0.78	10.14	8.87	9.89	10.65	9.89	10.00	0.65
	16	14.18	14.2	14.17	14.18	14.15	0.86	0.75	0.76	0.78	0.78	10.90	9.51	9.63	9.89	9.89	10.10	0.60
	20	14.2	14.2	14.18	14.21	14.18	0.83	0.88	0.78	0.8	0.8	10.52	11.15	9.89	10.14	10.14	10.58	0.69

Table A3. CFMAE oil extraction data

Temperature (°C)	**Time (min)**	**Oil + tube (g)**		**Tube (g)**		**Oil (g)**		**Oil%**		**Average**	**SD**
Rice Bran 1Liter/min											
		1	**2**	**1**	**2**	**1**	**2**	**1**	**2**		
73	6	15.31	15.33	13.4	13.39	1.91	1.94	18.15	18.44	18.29	0.20
	10	15.33	15.26	13.38	13.35	1.95	1.91	18.53	18.15	18.34	0.26
	60	15.32	15.42	13.31	13.39	2.01	2.03	19.10	19.29	19.20	0.13
Rice Bran 0.6 Liter/min											
73	6	15.21	15.32	13.29	13.35	1.92	1.97	18.25	18.72	18.48	0.33
	10	15.32	15.37	13.39	13.37	1.93	2	18.34	19.01	18.67	0.47
	60	15.51	15.41	13.41	13.41	2.1	2	19.96	19.01	19.48	0.67
Soybean 1Liter/min											
73	6	15.23	15.29	13.31	13.36	1.92	1.93	18.25	18.34	18.29	0.06
	10	15.37	15.3	13.45	13.36	1.92	1.94	18.25	18.44	18.34	0.13
	60	15.32	15.32	13.39	13.35	1.93	1.97	18.34	18.72	18.53	0.26
Soybean 0.6 Liter/min											
73	6	15.34	15.24	13.29	13.42	2.05	1.82	19.49	17.30	18.39	1.54
	10	15.26	15.41	13.41	13.37	1.85	2.04	17.51	19.39	18.48	1.27
	60	15.29	15.35	13.38	13.34	1.91	2.01	18.15	19.10	18.63	0.67

APPENDIX B. OIL AND BIODIESEL COMPOSITION DATA

Table B1. FA composition of rice bran oil (%).

Sample	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**C20:0**	**C22:0**
R 50.0	12.87	1.13	39.32	43.20	1.56	1.59	0.33
R 50.4	12.34	1.27	39.87	43.78	1.45	1.32	0.00
R 50.8	12.85	1.26	40.35	43.75	1.70	1.16	0.27
R 50.12	12.98	1.88	38.69	43.52	1.80	1.16	0.00
R 50.16	13.25	1.20	39.84	42.59	1.21	1.22	0.55
R 50.20	12.92	1.04	38.18	44.69	1.48	1.68	0.59
Average	12.87	1.29	39.37	43.59	1.53	1.36	0.29
SD	0.30	0.30	0.81	0.70	0.21	0.23	0.26
R 60.0	13.27	1.38	37.18	44.81	1.81	1.49	0.59
R 60.4	12.42	1.09	36.23	45.52	2.23	1.51	0.00
R 60.8	12.37	1.86	36.58	45.29	1.83	1.78	0.14
R 60.12	13.61	1.25	38.15	45.41	1.99	1.52	0.37
R 60.16	11.98	1.59	36.26	46.84	1.90	1.42	0.00
R 60.20	12.56	1.16	37.39	44.32	1.85	1.72	0.69
Average	12.70	1.39	36.97	45.36	1.93	1.57	0.30
SD	0.61	0.29	0.75	0.85	0.16	0.14	0.30
R 73.0	12.97	0.95	36.46	46.44	1.90	1.55	0.35
R 73.4	13.27	1.33	37.53	44.70	2.00	1.11	0.00
R 73.8	12.06	1.21	33.84	50.46	1.10	0.76	0.56
R 73.12	13.11	1.23	34.54	46.51	1.78	2.83	0.00
R 73.16	13.42	1.22	35.19	45.57	1.91	2.64	0.00
R 73.20	13.18	1.26	38.56	43.94	1.74	1.03	0.30
Average	13.00	1.20	36.02	46.27	1.74	1.65	0.20
SD	0.48	0.13	1.82	2.28	0.33	0.88	0.24

Table B2. FA composition of soybean oil (%)

Sample	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0
S 50.0	8.92	1.56	14.23	58.93	14.92	1.65	<0.2
S 50.4	9.64	1.48	15.30	57.28	14.57	1.72	<0.2
S 50.8	6.02	1.62	15.83	60.27	15.15	1.11	<0.2
S 50.12	6.24	1.61	14.49	58.48	15.54	3.64	<0.2
S 50.16	6.38	1.59	15.33	60.70	15.19	0.77	<0.2
S 50.20	5.85	1.55	14.22	59.68	15.07	3.57	<0.2
Average	7.18	1.57	14.90	59.22	15.07	2.08	<0.2
SD	1.66	0.05	0.68	1.26	0.32	1.23	<0.2
S 60.0	12.35	1.55	15.52	59.61	10.15	0.82	<0.2
S 60.4	11.53	1.53	14.05	61.67	11.21	0.00	<0.2
S 60.8	11.33	1.61	14.83	61.05	11.04	0.14	<0.2
S 60.12	11.58	1.57	15.61	60.12	11.11	0.00	<0.2
S 60.16	10.23	1.61	15.01	59.71	11.58	1.92	<0.2
S 60.20	10.23	1.55	14.26	59.53	10.83	3.59	<0.2
Average	11.21	1.57	14.88	60.28	10.99	1.08	<0.2
SD	0.83	0.03	0.64	0.88	0.48	1.43	<0.2
S 73.0	13.36	1.63	15.35	60.92	8.74	0.60	<0.2
S 73.4	12.54	1.51	12.37	62.22	9.91	1.45	<0.2
S 73.8	9.03	1.14	15.38	61.64	10.71	2.23	<0.2
S 73.12	12.99	1.61	15.85	60.81	9.74	0.00	<0.2
S 73.16	12.18	1.66	15.55	60.54	9.16	0.91	<0.2
S 73.20	12.12	1.61	15.88	60.90	9.18	0.25	<0.2
Average	12.04	1.53	15.06	61.17	9.57	0.81	<0.2
SD	1.549502	0.19588	1.336806	0.629062	0.7017	0.900882	0.00

Table B3. Input SAS file and data for FA TT oil composition

Environment	Week	replicate	C16:0	C18:0	C18:1	C18:2	C18:3
1	0	1	4.52	1.12	11.01	19.23	63.23
1	0	2	4.46	1.23	11.12	19.36	63.59
1	1	1	4.69	1.26	11.41	18.91	63.73
1	1	2	4.78	1.32	11.36	19.23	63.63
1	2	1	4.46	1.28	10.60	21.42	62.01
1	2	2	4.56	1.36	10.65	21.21	62.99
1	3	1	3.99	1.11	11.35	19.76	63.79
1	3	2	4.23	1.23	11.21	19.62	63.88
1	4	1	3.92	1.13	11.78	19.67	63.51
1	4	2	4.26	1.21	11.52	19.87	63.59
1	5	1	4.14	1.25	11.37	20.32	62.37
1	5	2	4.25	1.19	11.42	20.56	62.56
1	6	1	4.05	1.25	10.08	19.00	65.52
1	6	2	4.12	1.23	10.23	19.65	65.69
1	7	1	4.27	1.28	10.54	18.96	64.16
1	7	2	4.45	1.30	11.20	19.32	64.25
1	8	1	4.19	1.42	11.72	21.27	59.94
1	8	2	4.25	1.35	11.56	20.95	60.25
1	9	1	5.81	1.33	10.87	19.14	62.85
1	9	2	4.45	1.39	10.99	19.25	62.99
1	10	1	5.17	0.95	9.80	17.62	66.46
1	10	2	4.98	1.25	10.23	18.65	65.26
1	11	1	4.29	1.27	10.96	19.56	63.76
1	11	2	4.26	1.32	11.02	19.74	63.23
1	12	1	3.84	1.21	10.05	20.46	64.44
1	12	2	3.74	1.23	10.65	20.32	64.20
2	0	1	4.52	1.12	11.01	19.23	63.23
2	0	2	4.46	1.23	11.12	19.36	63.59
2	1	1	4.42	1.35	11.24	19.24	63.75
2	1	2	4.36	1.25	11.32	19.36	63.23
2	2	1	4.70	1.30	11.32	19.40	62.91
2	2	2	4.98	1.26	11.11	19.32	62.30
2	3	1	3.57	1.18	10.79	19.91	64.55
2	3	2	3.84	1.21	11.12	19.00	62.99
2	4	1	3.82	1.13	10.07	19.81	65.17
2	4	2	4.29	1.36	10.69	19.72	65.26
2	5	1	4.90	1.30	10.91	18.99	63.89
2	5	2	4.56	1.23	11.23	19.32	62.85
2	6	1	4.30	1.29	10.63	21.00	62.51
2	6	2	4.26	1.25	10.36	20.56	62.56
2	7	1	4.14	1.21	10.77	20.19	63.28
2	7	2	4.23	1.32	10.69	20.36	62.99
2	8	1	4.09	1.14	11.77	20.35	62.56
2	8	2	4.25	1.19	10.96	20.69	62.65
2	9	1	4.65	1.24	11.62	19.56	62.92

2	9	2	4.76	1.13	11.23	20.21	62.00
2	10	1	4.29	1.20	10.87	18.43	65.21
2	10	2	4.15	1.13	10.45	18.25	66.02
2	11	1	4.27	1.14	11.49	18.61	64.50
2	11	2	4.36	1.23	11.23	19.25	64.23
2	12	1	4.45	1.21	12.06	19.53	62.75
2	12	2	4.39	1.25	11.85	20.63	63.12
3	0	1	4.52	1.12	11.01	19.23	63.23
3	0	2	4.46	1.23	11.12	19.36	63.59
3	1	1	4.23	1.06	9.66	18.96	66.09
3	1	2	3.74	1.12	9.77	18.45	65.32
3	2	1	4.01	1.18	10.37	19.79	64.65
3	2	2	4.42	1.23	10.23	19.65	64.56
3	3	1	4.73	1.22	11.28	19.03	63.57
3	3	2	4.52	1.19	11.36	19.25	63.75
3	4	1	4.30	1.03	10.68	19.52	64.47
3	4	2	4.46	1.06	10.54	19.65	64.25
3	5	1	4.10	1.27	10.45	20.20	63.49
3	5	2	4.56	1.21	10.65	20.03	63.60
3	6	1	4.34	1.20	10.52	18.39	64.45
3	6	2	4.70	1.31	10.74	18.96	64.41
3	7	1	4.07	1.23	9.98	19.15	65.23
3	7	2	3.84	1.15	10.25	19.29	65.14
3	8	1	4.30	1.15	11.16	19.82	63.42
3	8	2	4.36	1.56	11.29	19.63	63.16
3	9	1	5.40	1.48	13.79	20.80	58.54
3	9	2	4.98	1.45	13.02	20.34	59.65
3	10	1	4.15	1.11	10.32	20.24	64.18
3	10	2	4.29	1.32	10.16	20.32	64.06
3	11	1	4.37	1.26	10.11	19.91	63.25
3	11	2	4.90	1.36	10.37	19.65	63.21
3	12	1	4.15	1.04	11.14	20.41	63.27
3	12	2	4.29	1.15	11.05	20.13	63.13
4	0	1	4.52	1.12	11.01	19.23	63.23
4	0	2	4.46	1.23	11.12	19.36	63.59
4	1	1	4.75	1.14	12.93	18.42	62.65
4	1	2	4.15	1.21	11.32	19.36	63.23
4	2	1	4.23	1.23	10.93	20.64	62.64
4	2	2	4.70	1.35	11.11	19.36	62.99
4	3	1	3.49	1.11	10.18	19.85	65.37
4	3	2	4.10	1.46	10.56	19.40	65.17
4	4	1	4.18	1.17	10.76	19.96	63.92
4	4	2	4.07	1.29	11.24	19.81	63.59
4	5	1	4.16	1.08	10.74	19.98	64.05
4	5	2	4.34	1.23	11.12	19.23	63.75
4	6	1	4.44	1.21	10.14	18.71	64.79
4	6	2	4.37	1.12	10.53	19.32	64.20
4	7	1	4.01	1.19	10.97	19.74	63.73

4	7	2	4.56	1.26	10.65	20.32	63.23
4	8	1	3.84	1.07	10.08	19.34	65.66
4	8	2	4.36	1.25	10.79	19.24	64.26
4	9	1	4.54	1.29	10.68	18.98	64.32
4	9	2	4.30	1.18	11.32	19.00	62.30
4	10	1	4.50	1.12	12.14	19.39	62.85
4	10	2	4.98	1.23	11.12	19.91	62.91
4	11	1	3.64	1.03	10.66	18.96	65.71
4	11	2	4.29	1.13	11.01	19.02	64.55
4	12	1	4.37	1.12	10.32	18.77	65.42
4	12	2	4.56	1.30	10.07	19.13	65.45
5	0	1	4.52	1.12	11.80	19.23	63.33
5	0	2	4.46	1.23	11.12	19.36	63.59
5	1	1	4.87	1.25	12.15	19.24	62.50
5	1	2	4.34	1.21	10.36	18.99	63.23
5	2	1	3.89	1.09	10.24	19.72	65.06
5	2	2	4.56	1.23	10.07	19.24	63.23
5	3	1	4.37	1.08	10.34	20.05	64.15
5	3	2	4.07	1.30	11.11	20.56	63.28
5	4	1	4.19	1.11	10.55	19.61	64.54
5	4	2	4.30	1.30	11.23	19.36	63.80
5	5	1	4.43	1.25	9.95	20.66	63.33
5	5	2	4.98	1.35	11.32	19.00	63.75
5	6	1	4.27	1.17	10.20	20.02	64.08
5	6	2	4.36	1.29	11.12	20.19	64.55
5	7	1	4.48	1.13	12.33	21.48	60.50
5	7	2	5.40	1.26	10.63	19.32	62.99
5	8	1	3.42	1.02	11.12	19.82	64.62
5	8	2	4.15	1.12	11.32	19.72	65.17
5	9	1	4.27	1.31	12.84	18.31	63.27
5	9	2	4.10	1.18	11.12	19.32	62.56
5	10	1	3.74	1.00	10.20	19.35	65.65
5	10	2	4.29	1.25	10.79	21.00	63.89
5	11	1	4.87	1.28	10.37	20.55	62.93
5	11	2	4.70	1.21	10.77	19.40	62.51
5	12	1	2.53	0.69	9.79	20.57	66.41
5	12	2	3.84	1.25	10.91	19.81	64.32
6	0	1	4.52	1.12	11.01	19.23	63.23
6	0	2	4.46	1.23	11.12	19.36	63.59
6	1	1	3.94	1.12	11.68	19.95	63.31
6	1	2	3.63	1.36	11.51	20.41	62.36
6	2	1	4.43	1.09	10.96	19.30	64.21
6	2	2	4.84	1.15	11.23	19.89	63.28
6	3	1	3.68	1.10	10.02	20.64	64.57
6	3	2	3.99	1.56	10.29	20.29	64.05
6	4	1	4.46	1.20	10.39	19.81	64.15
6	4	2	4.65	1.54	10.65	20.21	64.56
6	5	1	3.82	1.02	9.95	19.60	65.61

6	5	2	4.05	1.23	10.56	20.62	64.68
6	6	1	4.51	1.24	10.50	20.00	63.74
6	6	2	4.21	1.12	10.96	20.56	64.26
6	7	1	4.21	1.28	11.28	22.53	60.31
6	7	2	4.56	1.06	11.69	21.56	63.80
6	8	1	5.31	1.30	11.31	19.87	62.21
6	8	2	4.65	1.12	11.54	20.58	63.17
6	9	1	3.81	0.96	10.87	20.16	64.20
6	9	2	3.98	1.06	11.27	20.78	64.09
6	10	1	4.66	1.16	11.35	21.33	61.51
6	10	2	4.41	1.25	11.37	21.65	62.59
6	11	1	4.36	1.21	10.34	20.39	63.61
6	11	2	4.85	1.14	10.95	20.86	62.87
6	12	1	3.05	1.00	10.19	19.47	66.29
6	12	2	3.69	1.13	11.56	20.24	62.19
7	0	1	4.52	1.12	11.01	19.23	63.23
7	0	2	4.46	1.23	11.12	19.36	63.59
7	1	1	3.65	0.99	10.94	19.83	64.59
7	1	2	4.05	1.13	10.76	20.32	65.71
7	2	1	4.06	1.08	9.91	20.53	64.42
7	2	2	4.65	1.29	10.32	19.74	62.30
7	3	1	4.37	1.20	10.47	20.14	63.82
7	3	2	4.65	1.98	11.01	19.13	63.23
7	4	1	3.88	0.97	10.83	18.95	65.38
7	4	2	4.21	1.26	11.24	19.81	64.79
7	5	1	3.91	1.11	11.53	18.93	63.93
7	5	2	3.82	1.12	10.07	19.98	64.32
7	6	1	5.22	1.41	10.61	19.10	63.01
7	6	2	4.56	1.17	10.79	18.71	64.55
7	7	1	3.95	1.12	12.38	20.10	62.36
7	7	2	4.21	1.46	10.74	18.77	62.91
7	8	1	4.14	1.15	11.08	19.99	63.46
7	8	2	4.29	1.29	10.65	18.98	65.17
7	9	1	4.38	1.18	11.22	20.30	62.93
7	9	2	4.51	1.08	10.53	19.00	63.73
7	10	1	4.34	1.06	11.44	20.82	62.33
7	10	2	4.98	1.23	12.14	19.36	64.20
7	11	1	4.10	1.09	10.87	20.05	63.42
7	11	2	4.46	1.68	11.32	19.39	63.59
7	12	1	4.36	1.20	11.18	19.36	63.91
7	12	2	5.31	1.30	10.56	19.24	63.23
8	0	1	4.52	1.12	11.01	19.23	63.23
8	0	2	4.46	1.23	11.12	19.36	63.59
8	1	1	3.9	1.0	9.8	19.3	66.0
8	1	2	4.3	1.1	11.1	19.7	64.6
8	2	1	4.4	1.1	10.6	18.8	65.1
8	2	2	3.6	1.1	11.5	19.6	63.3
8	3	1	3.8	1.1	10.1	21.0	64.0

8	3	2	4.6	1.3	11.2	17.6	63.8
8	4	1	4.0	1.1	10.3	20.2	64.5
8	4	2	4.2	1.0	10.7	19.3	63.9
8	5	1	4.1	1.2	10.2	20.1	64.2
8	5	2	4.4	1.0	11.0	19.2	62.6
8	6	1	3.9	1.1	11.7	19.8	63.5
8	6	2	4.5	1.2	10.6	19.3	62.3
8	7	1	4.0	1.1	11.7	19.1	64.0
8	7	2	4.5	1.2	9.7	21.3	63.0
8	8	1	4.2	1.1	10.5	19.5	64.6
8	8	2	4.3	1.3	11.3	19.0	63.2
8	9	1	4.0	1.2	10.1	20.8	63.8
8	9	2	4.1	1.1	9.8	19.4	62.9
8	10	1	4.1	1.3	10.6	19.7	64.4
8	10	2	4.4	2.0	10.9	21.0	65.3
8	11	1	4.0	1.1	11.1	19.5	64.3
8	11	2	3.9	1.0	12.1	19.1	62.9
8	12	1	3.7	1.0	10.6	18.6	66.1
8	12	2	4.6	1.1	11.4	20.3	65.2

Were: Environment 1=vaccum 24°C; 2=vaccum 4°C; 3=air 24C; 4=air 4°C;
5=3% 24°C; 6=3% 4°C; 7=6% 24°C; 8=6% 4°C.

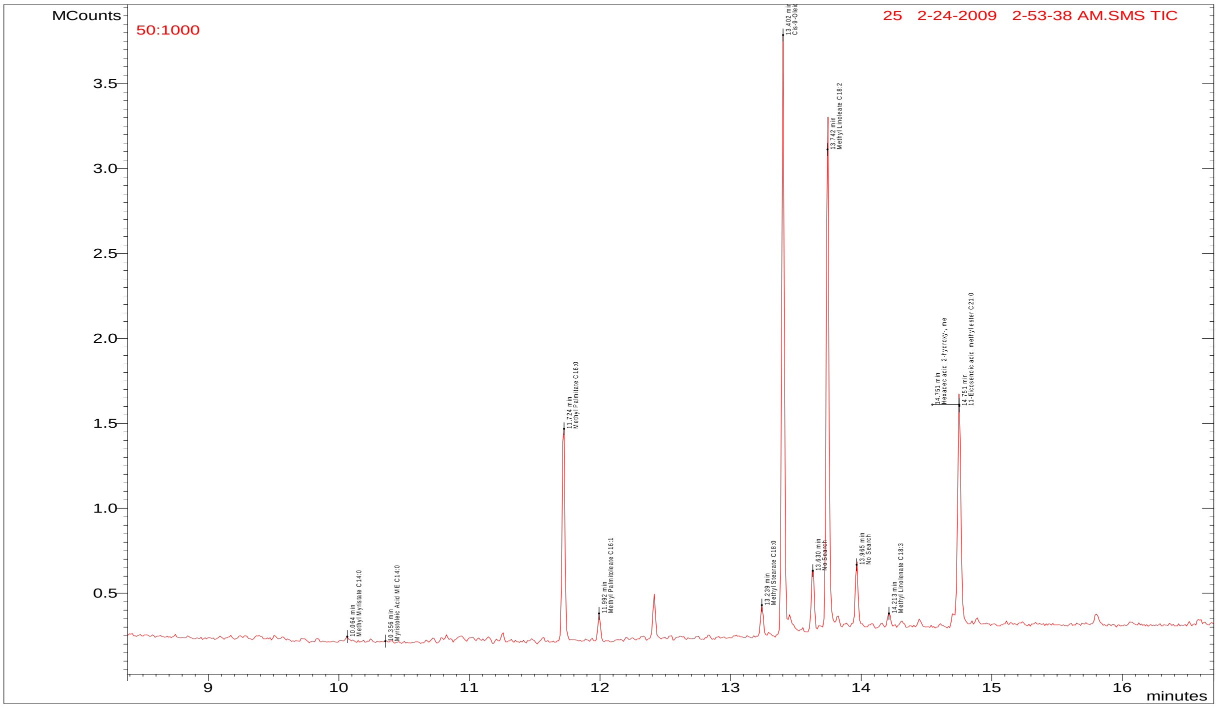

Figure B1. Oil FAME composition by GC-MS sample.

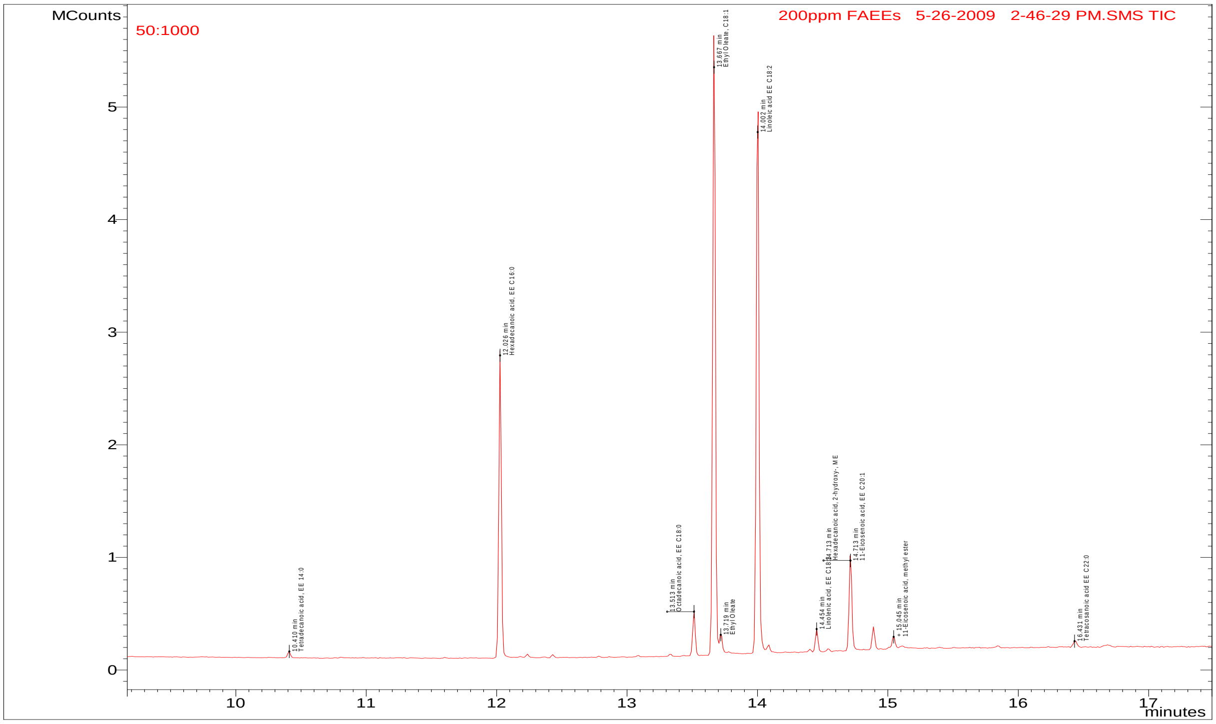

Figure B2. Biodiesel FAEE composition by GC-MS sample.

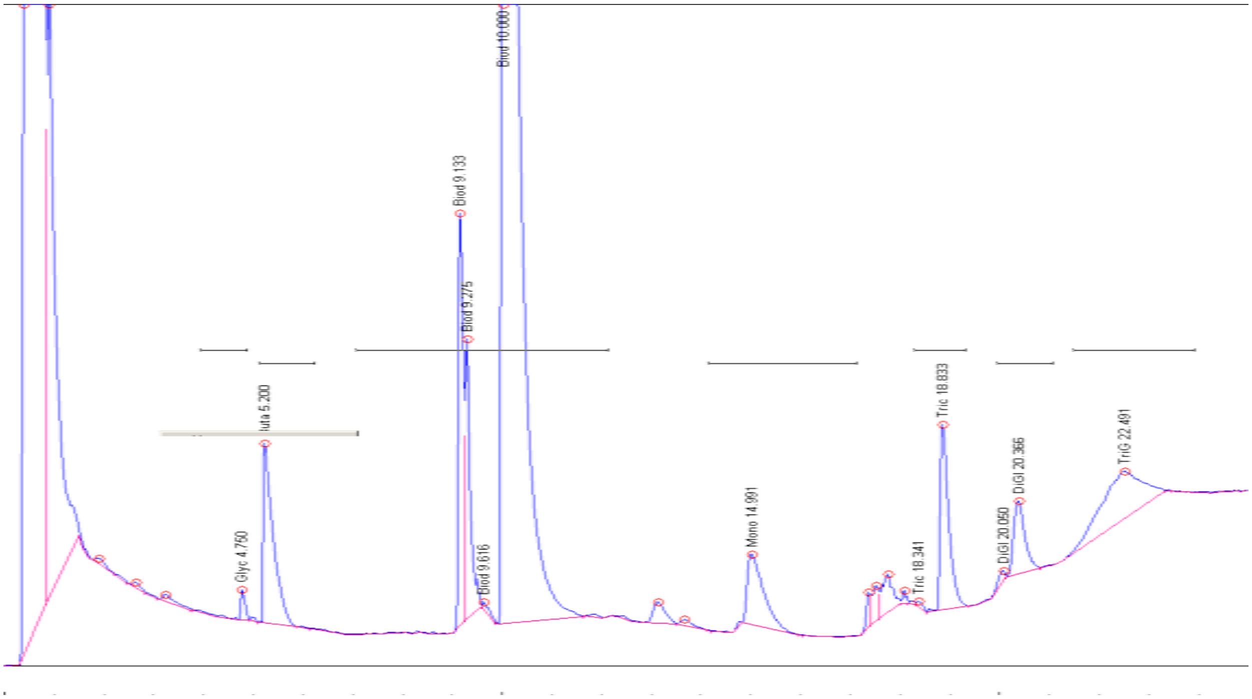

Figure B3.Total and Free Glycerin by GC-FID sample.

APPENDIX C. STATISTICAL DATA

C1. Oil extraction SAS input data code (used for all oil extraction comparisons)

```
dm "output;clear;log;clear";
ODS RTF File="\\Exst-server2\cleona6$\Stat. Consulting\Boldor\Beatrice\Soy.rtf";

proc import out = work.stat
datafile = "H:\LSU\Stat. Consulting\Boldor\Beatrice\Beatrice_SoySAS2.xls"
dbms = excel replace;
SHEET="Sheet1$";
    GETNAMES=YES;
    MIXED=YES;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;

RUN;

data two; set stat;
          temp=temperature;
          run;
*proc print data=two;*run;

title1 'Soy No significant 3-way interaction';
proc mixed data=two;
          class method temp time;
          model value =  method|temp|time/ ddfm=kr outp=mix1;
          lsmeans method temp method*temp time time*method time*temp/ pdiff adj=tukey;
          ods output diffs=ppp16;
          ods output lsmeans=mmm16;
run;

          %include '\\Exst-server2\cleona6$\Stat. Consulting\Gressley\Saxton\pdmix800.sas';
          %pdmix800 (ppp16,mmm16,alpha=.05,sort=no);
          run;
title1 'Residuals plot';
proc gplot data=mix1;
          plot resid*pred;
run;
proc univariate data=mix1 plot normal;
          var resid;
run;

quit;
ods rtf close;
```

C2. Fatty Acid composition TT study SAS input data code (used for al FA composition comparisons)

```
dm "output;clear;log;clear";
ODS RTF File="\\Exst-server2\cleona6$\Stat. Consulting\Boldor\Beatrice\FA.rtf";

proc import out = work.stat
datafile = "\\Exst-server2\cleona6$\Stat. Consulting\Boldor\Beatrice\CTT_GC.xls"
dbms = excel replace;
SHEET="Sheet1$";
    GETNAMES=YES;
    MIXED=YES;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;

RUN;

data two; set stat;
          env=Environment_;
          drop Environment_;
          if env=1 then temp=24;if env=3 then temp=24;if env=5 then temp=24;if env=7 then
temp=24;
          if env=2 then temp=4;if env=4 then temp=4;if env=6 then temp=4;if env=8 then temp=4;
          if env=1 then type='vac';if env=2 then type='vac';
          if env=3 then type='air';if env=4 then type='air';
          if env=5 then type='3%';if env=6 then type='3%';
          if env=7 then type='6%';if env=8 then type='6%';
          if env=2 and week=10 and replicate=1 then C18_3=65.21;
          tot=c16_0+c18_0+C18_1+c18_2+C18_3;

          run;

title1 'Analysis with fixed categorical effects C16:0';
proc mixed data=two;
          class type temp week;
          model c16_0 =  type|temp|week/ ddfm=kr;
          lsmeans type|temp|week/ pdiff adj=tukey;
          ods output diffs=ppp16;
          ods output lsmeans=mmm16;
run;

          %include '\\Exst-server2\cleona6$\Stat. Consulting\Gressley\Saxton\pdmix800.sas';
          %pdmix800 (ppp16,mmm16,alpha=.05,sort=no);
          run;
```

```
title1 'Analysis with fixed categorical effects C18:0';
proc mixed data=two;
        class type temp week;
        model c18_0 =  type|temp|week/ ddfm=kr;
        lsmeans type|temp|week/ pdiff adj=tukey;
        ods output diffs=ppp180;
        ods output lsmeans=mmm180;
run;

        %include '\\Exst-server2\cleona6$\Stat. Consulting\Gressley\Saxton\pdmix800.sas';

%pdmix800 (ppp180,mmm180,alpha=.05,sort=no);

title1 'Analysis with fixed categorical effects C18:1';
proc mixed data=two;
        class type temp week;
        model c18_1 =  type|temp|week/ ddfm=kr;
        lsmeans type|temp|week/ pdiff adj=tukey;
        ods output diffs=ppp181;
        ods output lsmeans=mmm181;
run;

        %include '\\Exst-server2\cleona6$\Stat. Consulting\Gressley\Saxton\pdmix800.sas';
        %pdmix800 (ppp181,mmm181,alpha=.05,sort=no);

title1 'Analysis with fixed categorical effects C18:2';
proc mixed data=two;
        class type temp week;
        model c18_2 =  type|temp|week/ ddfm=kr;
        lsmeans type|temp|week/ pdiff adj=tukey;
        ods output diffs=ppp182;
        ods output lsmeans=mmm182;
run;

        %include '\\Exst-server2\cleona6$\Stat. Consulting\Gressley\Saxton\pdmix800.sas';
        %pdmix800 (ppp182,mmm182,alpha=.05,sort=no);


title1 'Analysis with fixed categorical effects C18:3';
proc mixed data=two;
        class type temp week;
        model c18_3 =  type|temp|week/ ddfm=kr outp=mix1;
        lsmeans type|temp|week/ pdiff adj=tukey;
        ods output diffs=ppp183;
        ods output lsmeans=mmm183;
run;
```

```
        %include '\\Exst-server2\cleona6$\Stat. Consulting\Gressley\Saxton\pdmix800.sas';
        %pdmix800 (ppp183,mmm183,alpha=.05,sort=no);
/*proc gplot data=mix1;
        plot resid*pred;
run;
proc univariate data=mix1 plot normal;
var resid;
run;*/

ods rtf close;
quit;
```

C3. CMAE of Soybean oil statistical data output

Effect=temp   Method=Tukey(P<.05)   Set=2

	Method	temp	Time	Estimate	Standard Error	Letter Group
1		50	_	7.4138	0.1011	C
2		60	_	8.4389	0.1011	B
3		73	_	9.6356	0.1011	A

Effect=Time   Method=Tukey(P<.05)   Set=4

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
4		_	0	5.1716	0.1430	E
5		_	4	7.2102	0.1430	D
6		_	8	8.8555	0.1430	C
7		_	12	9.5285	0.1430	B
8		_	16	9.8462	0.1430	AB
9		_	20	10.3646	0.1430	A

Effect=temp*Time   Method=Tukey(P<.05)   Set=6

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
10		50	0	4.4545	0.2476	M

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
11		50	4	6.5562	0.2476	JK
12		50	8	7.7301	0.2476	HIJ
13		50	12	8.2891	0.2476	GHI
14		50	16	8.5875	0.2476	FGH
15		50	20	8.8656	0.2476	EFGH
16		60	0	5.2909	0.2476	LM
17		60	4	7.0647	0.2476	IJ
18		60	8	8.7810	0.2476	FGH
19		60	12	9.4474	0.2476	DEFG
20		60	16	9.6858	0.2476	CDEF
21		60	20	10.3634	0.2476	BCD
22		73	0	5.7693	0.2476	KL
23		73	4	8.0098	0.2476	HI
24		73	8	10.0555	0.2476	BCDE
25		73	12	10.8490	0.2476	ABC
26		73	16	11.2652	0.2476	AB
27		73	20	11.8648	0.2476	A

Effect=Method   Method=Tukey-Kramer(P<.05)   Set=1

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
28	conv	_	_	6.6897	0.1011	B
29	mw	_	_	10.3025	0.05836	A

Effect=Method*temp   Method=Tukey-Kramer(P<.05)   Set=3

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
30	conv	50	_	6.0417	0.1751	E
31	conv	60	_	6.6325	0.1751	E
32	conv	73	_	7.3950	0.1751	D

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
33	mw	50	_	8.7860	0.1011	C
34	mw	60	_	10.2453	0.1011	B
35	mw	73	_	11.8762	0.1011	A

Effect=Method*Time   Method=Tukey-Kramer(P<.05)   Set=5

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
36	conv	_	0	3.7550	0.2476	G
37	conv	_	4	6.3750	0.2476	F
38	conv	_	8	6.7583	0.2476	EF
39	conv	_	12	7.3083	0.2476	DEF
40	conv	_	16	7.7917	0.2476	DE
41	conv	_	20	8.1500	0.2476	D
42	mw	_	0	6.5881	0.1430	F
43	mw	_	4	8.0455	0.1430	D
44	mw	_	8	10.9527	0.1430	C
45	mw	_	12	11.7487	0.1430	B
46	mw	_	16	11.9007	0.1430	B
47	mw	_	20	12.5792	0.1430	A

C4. CMAE of Rice bran oil statistical data output

Effect=temp   Method=Tukey(P<.05)   Set=2

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
1		50	_	7.8929	0.1061	C

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
2		60	_	9.5404	0.1061	B
3		73	_	10.9983	0.1061	A

Effect=Time Method=Tukey(P<.05) Set=4

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
4		_	0	5.5449	0.1500	E
5		_	4	8.2091	0.1500	D
6		_	8	10.0191	0.1500	C
7		_	12	10.5105	0.1500	BC
8		_	16	11.0399	0.1500	AB
9		_	20	11.5397	0.1500	A

Effect=temp*Time Method=Tukey(P<.05) Set=6

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
10		50	0	4.5886	0.2598	I
11		50	4	6.5474	0.2598	H
12		50	8	8.1553	0.2598	G
13		50	12	8.6982	0.2598	EFG
14		50	16	9.4817	0.2598	DEF
15		50	20	9.8861	0.2598	CDE
16		60	0	5.6301	0.2598	HI
17		60	4	8.4681	0.2598	FG
18		60	8	9.8995	0.2598	CDE
19		60	12	10.4897	0.2598	CD
20		60	16	10.8800	0.2598	BC
21		60	20	11.8750	0.2598	AB
22		73	0	6.4159	0.2598	H
23		73	4	9.6117	0.2598	CDEF

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
24		73	8	12.0025	0.2598	AB
25		73	12	12.3435	0.2598	A
26		73	16	12.7579	0.2598	A
27		73	20	12.8580	0.2598	A

Effect=Method Method=Tukey-Kramer(P<.05) Set=1

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
28	conv	_	_	7.4039	0.1035	B
29	mw	_	_	11.5505	0.06547	A

Effect=Method*temp Method=Tukey-Kramer(P<.05) Set=3

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
30	conv	50	_	6.3350	0.1793	F
31	conv	60	_	7.3233	0.1793	E
32	conv	73	_	8.5533	0.1793	D
33	mw	50	_	9.4508	0.1134	C
34	mw	60	_	11.7575	0.1134	B
35	mw	73	_	13.4432	0.1134	A

Effect=Method*Time Method=Tukey-Kramer(P<.05) Set=5

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
36	conv	_	0	4.4400	0.2535	H
37	conv	_	4	7.2167	0.2535	FG
38	conv	_	8	7.5667	0.2535	FG
39	conv	_	12	7.9833	0.2535	EF
40	conv	_	16	8.4000	0.2535	DEF

Obs	Method	temp	Time	Estimate	Standard Error	Letter Group
41	conv	_	20	8.8167	0.2535	DE
42	mw	_	0	6.6498	0.1604	G
43	mw	_	4	9.2015	0.1604	D
44	mw	_	8	12.4715	0.1604	C
45	mw	_	12	13.0376	0.1604	BC
46	mw	_	16	13.6798	0.1604	AB
47	mw	_	20	14.2628	0.1604	A

C5. CTT storage study FA composition SAS output

For C16:0

Effect=type Method=Tukey(P<.05) Set=1

Obs	type	temp	week	Estimate	Standard Error	Letter Group
1	3%	_	_	4.2721	0.04110	A
2	6%	_	_	4.2598	0.04110	A
3	air	_	_	4.3483	0.04110	A
4	vac	_	_	4.3716	0.04110	A

Effect=temp Method=Tukey(P<.05) Set=2

Obs	type	temp	week	Estimate	Standard Error	Letter Group
5		4	_	4.2724	0.02906	A
6		24	_	4.3535	0.02906	A

Effect=type*temp Method=Tukey(P<.05) Set=3

Obs	type	temp	week	Estimate	Standard Error	Letter Group
7	3%	4	_	4.2592	0.05812	A
8	3%	24	_	4.2851	0.05812	A
9	6%	4	_	4.1720	0.05812	A
10	6%	24	_	4.3475	0.05812	A
11	air	4	_	4.3047	0.05812	A
12	air	24	_	4.3918	0.05812	A
13	vac	4	_	4.3537	0.05812	A
14	vac	24	_	4.3896	0.05812	A

Effect=week Method=Tukey(P<.05) Set=4

Obs	type	temp	week	Estimate	Standard Error	Letter Group
15		_	0	4.4900	0.07409	A
16		_	1	4.2339	0.07409	ABC
17		_	2	4.4090	0.07409	ABC
18		_	3	4.1237	0.07409	BC
19		_	4	4.1972	0.07409	ABC
20		_	5	4.2819	0.07409	ABC
21		_	6	4.3843	0.07409	ABC
22		_	7	4.3057	0.07409	ABC
23		_	8	4.2581	0.07409	ABC
24		_	9	4.5013	0.07409	A
25		_	10	4.4755	0.07409	AB
26		_	11	4.3531	0.07409	ABC
27		_	12	4.0548	0.07409	C

Effect=type*week Method=Tukey(P<.05) Set=5

Obs	type	temp	week	Estimate	Standard Error	Letter Group
28	3%	_	0	4.4900	0.1482	ABC
29	3%	_	1	4.1947	0.1482	ABC
30	3%	_	2	4.4316	0.1482	ABC
31	3%	_	3	4.0284	0.1482	BCD
32	3%	_	4	4.3999	0.1482	ABC
33	3%	_	5	4.3191	0.1482	ABC
34	3%	_	6	4.3377	0.1482	ABC
35	3%	_	7	4.6633	0.1482	ABC
36	3%	_	8	4.3842	0.1482	ABC
37	3%	_	9	4.0404	0.1482	BCD
38	3%	_	10	4.2757	0.1482	ABC
39	3%	_	11	4.6949	0.1482	ABC
40	3%	_	12	3.2781	0.1482	D
41	6%	_	0	4.4900	0.1482	ABC
42	6%	_	1	3.9595	0.1482	BCD
43	6%	_	2	4.1889	0.1482	ABC
44	6%	_	3	4.3494	0.1482	ABC
45	6%	_	4	4.0613	0.1482	ABCD
46	6%	_	5	4.0569	0.1482	ABCD
47	6%	_	6	4.5537	0.1482	ABC
48	6%	_	7	4.1660	0.1482	ABC
49	6%	_	8	4.2373	0.1482	ABC
50	6%	_	9	4.2421	0.1482	ABC
51	6%	_	10	4.4553	0.1482	ABC
52	6%	_	11	4.1232	0.1482	ABCD
53	6%	_	12	4.4932	0.1482	ABC
54	air	_	0	4.4900	0.1482	ABC

Obs	type	temp	week	Estimate	Standard Error	Letter Group
55	air	_	1	4.2193	0.1482	ABC
56	air	_	2	4.3393	0.1482	ABC
57	air	_	3	4.2094	0.1482	ABC
58	air	_	4	4.2541	0.1482	ABC
59	air	_	5	4.2894	0.1482	ABC
60	air	_	6	4.4624	0.1482	ABC
61	air	_	7	4.1209	0.1482	ABCD
62	air	_	8	4.2154	0.1482	ABC
63	air	_	9	4.8042	0.1482	AB
64	air	_	10	4.4811	0.1482	ABC
65	air	_	11	4.3000	0.1482	ABC
66	air	_	12	4.3421	0.1482	ABC
67	vac	_	0	4.4900	0.1482	ABC
68	vac	_	1	4.5620	0.1482	ABC
69	vac	_	2	4.6762	0.1482	ABC
70	vac	_	3	3.9075	0.1482	CD
71	vac	_	4	4.0734	0.1482	ABCD
72	vac	_	5	4.4621	0.1482	ABC
73	vac	_	6	4.1833	0.1482	ABC
74	vac	_	7	4.2727	0.1482	ABC
75	vac	_	8	4.1955	0.1482	ABC
76	vac	_	9	4.9187	0.1482	A
77	vac	_	10	4.6901	0.1482	ABC
78	vac	_	11	4.2941	0.1482	ABC
79	vac	_	12	4.1057	0.1482	ABCD

Effect=temp*week Method=Tukey(P<.05) Set=6

Obs	type	temp	week	Estimate	Standard Error	Letter Group
80		4	0	4.4900	0.1048	AB
81		4	1	4.1738	0.1048	BC
82		4	2	4.4920	0.1048	AB
83		4	3	3.8807	0.1048	C
84		4	4	4.2040	0.1048	ABC
85		4	5	4.2905	0.1048	ABC
86		4	6	4.3166	0.1048	ABC
87		4	7	4.2778	0.1048	ABC
88		4	8	4.3784	0.1048	ABC
89		4	9	4.2654	0.1048	ABC
90		4	10	4.4575	0.1048	AB
91		4	11	4.2129	0.1048	ABC
92		4	12	4.1016	0.1048	BC
93		24	0	4.4900	0.1048	AB
94		24	1	4.2939	0.1048	ABC
95		24	2	4.3259	0.1048	ABC
96		24	3	4.3666	0.1048	ABC
97		24	4	4.1904	0.1048	ABC
98		24	5	4.2733	0.1048	ABC
99		24	6	4.4520	0.1048	AB
100		24	7	4.3336	0.1048	ABC
101		24	8	4.1378	0.1048	BC
102		24	9	4.7373	0.1048	A
103		24	10	4.4935	0.1048	AB
104		24	11	4.4932	0.1048	AB
105		24	12	4.0079	0.1048	BC

Effect=type*temp*week Method=Tukey(P<.05) Set=7

Obs	type	temp	week	Estimate	Standard Error	Letter Group
106	3%	4	0	4.4900	0.2096	ABCDEF
107	3%	4	1	3.7836	0.2096	CDEF
108	3%	4	2	4.6370	0.2096	ABCDE
109	3%	4	3	3.8355	0.2096	BCDEF
110	3%	4	4	4.5540	0.2096	ABCDE
111	3%	4	5	3.9345	0.2096	ABCDEF
112	3%	4	6	4.3621	0.2096	ABCDEF
113	3%	4	7	4.3872	0.2096	ABCDEF
114	3%	4	8	4.9803	0.2096	ABCD
115	3%	4	9	3.8966	0.2096	ABCDEF
116	3%	4	10	4.5354	0.2096	ABCDE
117	3%	4	11	4.6033	0.2096	ABCDE
118	3%	4	12	3.3695	0.2096	EF
119	3%	24	0	4.4900	0.2096	ABCDEF
120	3%	24	1	4.6058	0.2096	ABCDE
121	3%	24	2	4.2262	0.2096	ABCDEF
122	3%	24	3	4.2213	0.2096	ABCDEF
123	3%	24	4	4.2458	0.2096	ABCDEF
124	3%	24	5	4.7036	0.2096	ABCD
125	3%	24	6	4.3133	0.2096	ABCDEF
126	3%	24	7	4.9394	0.2096	ABCD
127	3%	24	8	3.7880	0.2096	CDEF
128	3%	24	9	4.1841	0.2096	ABCDEF
129	3%	24	10	4.0160	0.2096	ABCDEF
130	3%	24	11	4.7865	0.2096	ABCD
131	3%	24	12	3.1867	0.2096	F
132	6%	4	0	4.4900	0.2096	ABCDEF

Obs	type	temp	week	Estimate	Standard Error	Letter Group
133	6%	4	1	4.0703	0.2096	ABCDEF
134	6%	4	2	4.0235	0.2096	ABCDEF
135	6%	4	3	4.1868	0.2096	ABCDEF
136	6%	4	4	4.0779	0.2096	ABCDEF
137	6%	4	5	4.2487	0.2096	ABCDEF
138	6%	4	6	4.2200	0.2096	ABCDEF
139	6%	4	7	4.2516	0.2096	ABCDEF
140	6%	4	8	4.2598	0.2096	ABCDEF
141	6%	4	9	4.0384	0.2096	ABCDEF
142	6%	4	10	4.2498	0.2096	ABCDEF
143	6%	4	11	3.9678	0.2096	ABCDEF
144	6%	4	12	4.1519	0.2096	ABCDEF
145	6%	24	0	4.4900	0.2096	ABCDEF
146	6%	24	1	3.8487	0.2096	BCDEF
147	6%	24	2	4.3543	0.2096	ABCDEF
148	6%	24	3	4.5119	0.2096	ABCDEF
149	6%	24	4	4.0447	0.2096	ABCDEF
150	6%	24	5	3.8652	0.2096	ABCDEF
151	6%	24	6	4.8875	0.2096	ABCD
152	6%	24	7	4.0804	0.2096	ABCDEF
153	6%	24	8	4.2147	0.2096	ABCDEF
154	6%	24	9	4.4458	0.2096	ABCDEF
155	6%	24	10	4.6608	0.2096	ABCDE
156	6%	24	11	4.2787	0.2096	ABCDEF
157	6%	24	12	4.8346	0.2096	ABCD
158	air	4	0	4.4900	0.2096	ABCDEF
159	air	4	1	4.4524	0.2096	ABCDEF
160	air	4	2	4.4653	0.2096	ABCDEF

Obs	type	temp	week	Estimate	Standard Error	Letter Group
161	air	4	3	3.7955	0.2096	CDEF
162	air	4	4	4.1272	0.2096	ABCDEF
163	air	4	5	4.2495	0.2096	ABCDEF
164	air	4	6	4.4025	0.2096	ABCDEF
165	air	4	7	4.2869	0.2096	ABCDEF
166	air	4	8	4.1023	0.2096	ABCDEF
167	air	4	9	4.4192	0.2096	ABCDEF
168	air	4	10	4.7398	0.2096	ABCD
169	air	4	11	3.9672	0.2096	ABCDEF
170	air	4	12	4.4639	0.2096	ABCDEF
171	air	24	0	4.4900	0.2096	ABCDEF
172	air	24	1	3.9862	0.2096	ABCDEF
173	air	24	2	4.2133	0.2096	ABCDEF
174	air	24	3	4.6232	0.2096	ABCDE
175	air	24	4	4.3811	0.2096	ABCDEF
176	air	24	5	4.3293	0.2096	ABCDEF
177	air	24	6	4.5224	0.2096	ABCDE
178	air	24	7	3.9549	0.2096	ABCDEF
179	air	24	8	4.3285	0.2096	ABCDEF
180	air	24	9	5.1893	0.2096	A
181	air	24	10	4.2224	0.2096	ABCDEF
182	air	24	11	4.6327	0.2096	ABCDE
183	air	24	12	4.2204	0.2096	ABCDEF
184	vac	4	0	4.4900	0.2096	ABCDEF
185	vac	4	1	4.3890	0.2096	ABCDEF
186	vac	4	2	4.8423	0.2096	ABCD
187	vac	4	3	3.7049	0.2096	DEF
188	vac	4	4	4.0568	0.2096	ABCDEF

Obs	type	temp	week	Estimate	Standard Error	Letter Group
189	vac	4	5	4.7293	0.2096	ABCD
190	vac	4	6	4.2817	0.2096	ABCDEF
191	vac	4	7	4.1854	0.2096	ABCDEF
192	vac	4	8	4.1710	0.2096	ABCDEF
193	vac	4	9	4.7074	0.2096	ABCD
194	vac	4	10	4.3051	0.2096	ABCDEF
195	vac	4	11	4.3133	0.2096	ABCDEF
196	vac	4	12	4.4214	0.2096	ABCDEF
197	vac	24	0	4.4900	0.2096	ABCDEF
198	vac	24	1	4.7350	0.2096	ABCD
199	vac	24	2	4.5100	0.2096	ABCDEF
200	vac	24	3	4.1100	0.2096	ABCDEF
201	vac	24	4	4.0900	0.2096	ABCDEF
202	vac	24	5	4.1950	0.2096	ABCDEF
203	vac	24	6	4.0850	0.2096	ABCDEF
204	vac	24	7	4.3600	0.2096	ABCDEF
205	vac	24	8	4.2200	0.2096	ABCDEF
206	vac	24	9	5.1300	0.2096	AB
207	vac	24	10	5.0750	0.2096	ABC
208	vac	24	11	4.2750	0.2096	ABCDEF
209	vac	24	12	3.7900	0.2096	CDEF

For C18:0

Effect=type Method=Tukey(P<.05) Set=1

Obs	type	temp	week	Estimate	Standard Error	Letter Group
1	3%	_	_	1.1812	0.01948	A
2	6%	_	_	1.1915	0.01948	A

Obs	type	temp	week	Estimate	Standard Error	Letter Group
3	air	_	_	1.2072	0.01948	A
4	vac	_	_	1.2403	0.01948	A

Effect=temp Method=Tukey(P<.05) Set=2

Obs	type	temp	week	Estimate	Standard Error	Letter Group
5		4	_	1.1918	0.01377	A
6		24	_	1.2184	0.01377	A

Effect=type*temp Method=Tukey(P<.05) Set=3

Obs	type	temp	week	Estimate	Standard Error	Letter Group
7	3%	4	_	1.1825	0.02754	A
8	3%	24	_	1.1799	0.02754	A
9	6%	4	_	1.1569	0.02754	A
10	6%	24	_	1.2262	0.02754	A
11	air	4	_	1.1958	0.02754	A
12	air	24	_	1.2186	0.02754	A
13	vac	4	_	1.2318	0.02754	A
14	vac	24	_	1.2488	0.02754	A

Effect=week Method=Tukey(P<.05) Set=4

Obs	type	temp	week	Estimate	Standard Error	Letter Group
15		_	0	1.1750	0.03511	A
16		_	1	1.1829	0.03511	A
17		_	2	1.2069	0.03511	A
18		_	3	1.2695	0.03511	A
19		_	4	1.1774	0.03511	A
20		_	5	1.1886	0.03511	A

Obs	type	temp	week	Estimate	Standard Error	Letter Group
21		_	6	1.2192	0.03511	A
22		_	7	1.2237	0.03511	A
23		_	8	1.2189	0.03511	A
24		_	9	1.2203	0.03511	A
25		_	10	1.2294	0.03511	A
26		_	11	1.2166	0.03511	A
27		_	12	1.1376	0.03511	A

Effect=type*week Method=Tukey(P<.05) Set=5

Obs	type	temp	week	Estimate	Standard Error	Letter Group
28	3%	_	0	1.1750	0.07022	A
29	3%	_	1	1.2350	0.07022	A
30	3%	_	2	1.1391	0.07022	A
31	3%	_	3	1.2606	0.07022	A
32	3%	_	4	1.2874	0.07022	A
33	3%	_	5	1.2111	0.07022	A
34	3%	_	6	1.2051	0.07022	A
35	3%	_	7	1.1826	0.07022	A
36	3%	_	8	1.1396	0.07022	A
37	3%	_	9	1.1268	0.07022	A
38	3%	_	10	1.1651	0.07022	A
39	3%	_	11	1.2112	0.07022	A
40	3%	_	12	1.0175	0.07022	A
41	6%	_	0	1.1750	0.07022	A
42	6%	_	1	1.0709	0.07022	A
43	6%	_	2	1.1418	0.07022	A
44	6%	_	3	1.3905	0.07022	A
45	6%	_	4	1.0790	0.07022	A

Obs	type	temp	week	Estimate	Standard Error	Letter Group
46	6%	_	5	1.1024	0.07022	A
47	6%	_	6	1.2067	0.07022	A
48	6%	_	7	1.2262	0.07022	A
49	6%	_	8	1.2033	0.07022	A
50	6%	_	9	1.1333	0.07022	A
51	6%	_	10	1.3816	0.07022	A
52	6%	_	11	1.2202	0.07022	A
53	6%	_	12	1.1593	0.07022	A
54	air	_	0	1.1750	0.07022	A
55	air	_	1	1.1309	0.07022	A
56	air	_	2	1.2472	0.07022	A
57	air	_	3	1.2453	0.07022	A
58	air	_	4	1.1361	0.07022	A
59	air	_	5	1.1979	0.07022	A
60	air	_	6	1.2101	0.07022	A
61	air	_	7	1.2073	0.07022	A
62	air	_	8	1.2571	0.07022	A
63	air	_	9	1.3473	0.07022	A
64	air	_	10	1.1953	0.07022	A
65	air	_	11	1.1951	0.07022	A
66	air	_	12	1.1497	0.07022	A
67	vac	_	0	1.1750	0.07022	A
68	vac	_	1	1.2948	0.07022	A
69	vac	_	2	1.2995	0.07022	A
70	vac	_	3	1.1817	0.07022	A
71	vac	_	4	1.2073	0.07022	A
72	vac	_	5	1.2430	0.07022	A
73	vac	_	6	1.2550	0.07022	A

Obs	type	temp	week	Estimate	Standard Error	Letter Group
74	vac	_	7	1.2787	0.07022	A
75	vac	_	8	1.2758	0.07022	A
76	vac	_	9	1.2737	0.07022	A
77	vac	_	10	1.1756	0.07022	A
78	vac	_	11	1.2399	0.07022	A
79	vac	_	12	1.2241	0.07022	A

Effect=temp*week   Method=Tukey(P<.05)   Set=6

Obs	type	temp	week	Estimate	Standard Error	Letter Group
80		4	0	1.1750	0.04966	A
81		4	1	1.1987	0.04966	A
82		4	2	1.1972	0.04966	A
83		4	3	1.2507	0.04966	A
84		4	4	1.2206	0.04966	A
85		4	5	1.1583	0.04966	A
86		4	6	1.1851	0.04966	A
87		4	7	1.2069	0.04966	A
88		4	8	1.1812	0.04966	A
89		4	9	1.1420	0.04966	A
90		4	10	1.3118	0.04966	A
91		4	11	1.1231	0.04966	A
92		4	12	1.1422	0.04966	A
93		24	0	1.1750	0.04966	A
94		24	1	1.1671	0.04966	A
95		24	2	1.2167	0.04966	A
96		24	3	1.2883	0.04966	A
97		24	4	1.1342	0.04966	A

Obs	type	temp	week	Estimate	Standard Error	Letter Group
98		24	5	1.2189	0.04966	A
99		24	6	1.2533	0.04966	A
100		24	7	1.2405	0.04966	A
101		24	8	1.2566	0.04966	A
102		24	9	1.2985	0.04966	A
103		24	10	1.1470	0.04966	A
104		24	11	1.3101	0.04966	A
105		24	12	1.1330	0.04966	A

Effect=type*temp*week Method=Tukey(P<.05) Set=7

Obs	type	temp	week	Estimate	Standard Error	Letter Group
106	3%	4	0	1.1750	0.09931	AB
107	3%	4	1	1.2417	0.09931	AB
108	3%	4	2	1.1207	0.09931	AB
109	3%	4	3	1.3312	0.09931	AB
110	3%	4	4	1.3680	0.09931	AB
111	3%	4	5	1.1245	0.09931	AB
112	3%	4	6	1.1812	0.09931	AB
113	3%	4	7	1.1716	0.09931	AB
114	3%	4	8	1.2078	0.09931	AB
115	3%	4	9	1.0108	0.09931	AB
116	3%	4	10	1.2040	0.09931	AB
117	3%	4	11	1.1735	0.09931	AB
118	3%	4	12	1.0631	0.09931	AB
119	3%	24	0	1.1750	0.09931	AB
120	3%	24	1	1.2283	0.09931	AB
121	3%	24	2	1.1575	0.09931	AB

Obs	type	temp	week	Estimate	Standard Error	Letter Group
122	3%	24	3	1.1899	0.09931	AB
123	3%	24	4	1.2067	0.09931	AB
124	3%	24	5	1.2978	0.09931	AB
125	3%	24	6	1.2290	0.09931	AB
126	3%	24	7	1.1936	0.09931	AB
127	3%	24	8	1.0714	0.09931	AB
128	3%	24	9	1.2428	0.09931	AB
129	3%	24	10	1.1263	0.09931	AB
130	3%	24	11	1.2489	0.09931	AB
131	3%	24	12	0.9718	0.09931	B
132	6%	4	0	1.1750	0.09931	AB
133	6%	4	1	1.0804	0.09931	AB
134	6%	4	2	1.1010	0.09931	AB
135	6%	4	3	1.1924	0.09931	AB
136	6%	4	4	1.0409	0.09931	AB
137	6%	4	5	1.0892	0.09931	AB
138	6%	4	6	1.1237	0.09931	AB
139	6%	4	7	1.1636	0.09931	AB
140	6%	4	8	1.1904	0.09931	AB
141	6%	4	9	1.1380	0.09931	AB
142	6%	4	10	1.6181	0.09931	A
143	6%	4	11	1.0556	0.09931	AB
144	6%	4	12	1.0710	0.09931	AB
145	6%	24	0	1.1750	0.09931	AB
146	6%	24	1	1.0615	0.09931	AB
147	6%	24	2	1.1826	0.09931	AB
148	6%	24	3	1.5885	0.09931	AB
149	6%	24	4	1.1172	0.09931	AB

Obs	type	temp	week	Estimate	Standard Error	Letter Group
150	6%	24	5	1.1155	0.09931	AB
151	6%	24	6	1.2896	0.09931	AB
152	6%	24	7	1.2887	0.09931	AB
153	6%	24	8	1.2162	0.09931	AB
154	6%	24	9	1.1285	0.09931	AB
155	6%	24	10	1.1451	0.09931	AB
156	6%	24	11	1.3847	0.09931	AB
157	6%	24	12	1.2476	0.09931	AB
158	air	4	0	1.1750	0.09931	AB
159	air	4	1	1.1732	0.09931	AB
160	air	4	2	1.2879	0.09931	AB
161	air	4	3	1.2857	0.09931	AB
162	air	4	4	1.2290	0.09931	AB
163	air	4	5	1.1535	0.09931	AB
164	air	4	6	1.1655	0.09931	AB
165	air	4	7	1.2249	0.09931	AB
166	air	4	8	1.1603	0.09931	AB
167	air	4	9	1.2318	0.09931	AB
168	air	4	10	1.1739	0.09931	AB
169	air	4	11	1.0785	0.09931	AB
170	air	4	12	1.2067	0.09931	AB
171	air	24	0	1.1750	0.09931	AB
172	air	24	1	1.0885	0.09931	AB
173	air	24	2	1.2065	0.09931	AB
174	air	24	3	1.2048	0.09931	AB
175	air	24	4	1.0431	0.09931	AB
176	air	24	5	1.2423	0.09931	AB
177	air	24	6	1.2546	0.09931	AB

Obs	type	temp	week	Estimate	Standard Error	Letter Group
178	air	24	7	1.1897	0.09931	AB
179	air	24	8	1.3539	0.09931	AB
180	air	24	9	1.4627	0.09931	AB
181	air	24	10	1.2167	0.09931	AB
182	air	24	11	1.3117	0.09931	AB
183	air	24	12	1.0926	0.09931	AB
184	vac	4	0	1.1750	0.09931	AB
185	vac	4	1	1.2996	0.09931	AB
186	vac	4	2	1.2791	0.09931	AB
187	vac	4	3	1.1933	0.09931	AB
188	vac	4	4	1.2445	0.09931	AB
189	vac	4	5	1.2659	0.09931	AB
190	vac	4	6	1.2699	0.09931	AB
191	vac	4	7	1.2673	0.09931	AB
192	vac	4	8	1.1665	0.09931	AB
193	vac	4	9	1.1875	0.09931	AB
194	vac	4	10	1.2513	0.09931	AB
195	vac	4	11	1.1849	0.09931	AB
196	vac	4	12	1.2282	0.09931	AB
197	vac	24	0	1.1750	0.09931	AB
198	vac	24	1	1.2900	0.09931	AB
199	vac	24	2	1.3200	0.09931	AB
200	vac	24	3	1.1700	0.09931	AB
201	vac	24	4	1.1700	0.09931	AB
202	vac	24	5	1.2200	0.09931	AB
203	vac	24	6	1.2400	0.09931	AB
204	vac	24	7	1.2900	0.09931	AB
205	vac	24	8	1.3850	0.09931	AB

Obs	type	temp	week	Estimate	Standard Error	Letter Group
206	vac	24	9	1.3600	0.09931	AB
207	vac	24	10	1.1000	0.09931	AB
208	vac	24	11	1.2950	0.09931	AB
209	vac	24	12	1.2200	0.09931	AB

For C18:1

Effect=type Method=Tukey(P<.05) Set=1

Obs	type	temp	week	Estimate	Standard Error	Letter Group
1	3%	_	_	10.9139	0.06604	A
2	6%	_	_	10.8700	0.06604	A
3	air	_	_	10.8560	0.06604	A
4	vac	_	_	11.0137	0.06604	A

Effect=temp Method=Tukey(P<.05) Set=2

Obs	type	temp	week	Estimate	Standard Error	Letter Group
5		4	_	10.9286	0.04670	A
6		24	_	10.8982	0.04670	A

Effect=type*temp Method=Tukey(P<.05) Set=3

Obs	type	temp	week	Estimate	Standard Error	Letter Group
7	3%	4	_	10.9441	0.09339	A
8	3%	24	_	10.8837	0.09339	A
9	6%	4	_	10.7897	0.09339	A
10	6%	24	_	10.9504	0.09339	A
11	air	4	_	10.9034	0.09339	A
12	air	24	_	10.8086	0.09339	A

Obs	type	temp	week	Estimate	Standard Error	Letter Group
13	vac	4	_	11.0773	0.09339	A
14	vac	24	_	10.9502	0.09339	A

Effect=week Method=Tukey(P<.05) Set=4

Obs	type	temp	week	Estimate	Standard Error	Letter Group
15		_	0	11.0650	0.1191	AB
16		_	1	11.0822	0.1191	AB
17		_	2	10.6993	0.1191	B
18		_	3	10.7793	0.1191	AB
19		_	4	10.8209	0.1191	AB
20		_	5	10.7772	0.1191	AB
21		_	6	10.6019	0.1191	B
22		_	7	10.9685	0.1191	AB
23		_	8	11.1322	0.1191	AB
24		_	9	11.3301	0.1191	A
25		_	10	10.8708	0.1191	AB
26		_	11	10.9151	0.1191	AB
27		_	12	10.8318	0.1191	AB

Effect=type*week Method=Tukey(P<.05) Set=5

Obs	type	temp	week	Estimate	Standard Error	Letter Group
28	3%	_	0	11.0650	0.2381	AB
29	3%	_	1	11.4230	0.2381	AB
30	3%	_	2	10.6253	0.2381	B
31	3%	_	3	10.4399	0.2381	B
32	3%	_	4	10.7042	0.2381	B
33	3%	_	5	10.4452	0.2381	B
34	3%	_	6	10.6958	0.2381	B

Obs	type	temp	week	Estimate	Standard Error	Letter Group
35	3%	_	7	11.4826	0.2381	AB
36	3%	_	8	11.3240	0.2381	AB
37	3%	_	9	11.5270	0.2381	AB
38	3%	_	10	10.9275	0.2381	AB
39	3%	_	11	10.6070	0.2381	B
40	3%	_	12	10.6143	0.2381	B
41	6%	_	0	11.0650	0.2381	AB
42	6%	_	1	10.6546	0.2381	B
43	6%	_	2	10.5909	0.2381	B
44	6%	_	3	10.7148	0.2381	B
45	6%	_	4	10.7594	0.2381	B
46	6%	_	5	10.6895	0.2381	B
47	6%	_	6	10.9032	0.2381	AB
48	6%	_	7	11.1284	0.2381	AB
49	6%	_	8	10.8721	0.2381	AB
50	6%	_	9	10.4127	0.2381	B
51	6%	_	10	11.2594	0.2381	AB
52	6%	_	11	11.3442	0.2381	AB
53	6%	_	12	10.9162	0.2381	AB
54	air	_	0	11.0650	0.2381	AB
55	air	_	1	10.9194	0.2381	AB
56	air	_	2	10.6602	0.2381	B
57	air	_	3	10.8443	0.2381	AB
58	air	_	4	10.8061	0.2381	B
59	air	_	5	10.7399	0.2381	B
60	air	_	6	10.4839	0.2381	B
61	air	_	7	10.4629	0.2381	B
62	air	_	8	10.8301	0.2381	AB

Obs	type	temp	week	Estimate	Standard Error	Letter Group
63	air	_	9	12.2029	0.2381	A
64	air	_	10	10.9343	0.2381	AB
65	air	_	11	10.5359	0.2381	B
66	air	_	12	10.6433	0.2381	B
67	vac	_	0	11.0650	0.2381	AB
68	vac	_	1	11.3317	0.2381	AB
69	vac	_	2	10.9209	0.2381	AB
70	vac	_	3	11.1183	0.2381	AB
71	vac	_	4	11.0140	0.2381	AB
72	vac	_	5	11.2343	0.2381	AB
73	vac	_	6	10.3249	0.2381	B
74	vac	_	7	10.8001	0.2381	B
75	vac	_	8	11.5028	0.2381	AB
76	vac	_	9	11.1780	0.2381	AB
77	vac	_	10	10.3621	0.2381	B
78	vac	_	11	11.1733	0.2381	AB
79	vac	_	12	11.1534	0.2381	AB

Effect=temp*week   Method=Tukey(P<.05)   Set=6

Obs	type	temp	week	Estimate	Standard Error	Letter Group
80		4	0	11.0650	0.1684	ABC
81		4	1	11.3635	0.1684	AB
82		4	2	11.0995	0.1684	ABC
83		4	3	10.5417	0.1684	BC
84		4	4	10.5963	0.1684	BC
85		4	5	10.7092	0.1684	BC
86		4	6	10.6675	0.1684	BC
87		4	7	10.9298	0.1684	ABC

Obs	type	temp	week	Estimate	Standard Error	Letter Group
88		4	8	11.0275	0.1684	ABC
89		4	9	10.8618	0.1684	BC
90		4	10	11.1064	0.1684	ABC
91		4	11	11.1070	0.1684	ABC
92		4	12	10.9969	0.1684	ABC
93		24	0	11.0650	0.1684	ABC
94		24	1	10.8008	0.1684	BC
95		24	2	10.2992	0.1684	C
96		24	3	11.0170	0.1684	ABC
97		24	4	11.0455	0.1684	ABC
98		24	5	10.8452	0.1684	BC
99		24	6	10.5363	0.1684	BC
100		24	7	11.0071	0.1684	ABC
101		24	8	11.2370	0.1684	AB
102		24	9	11.7985	0.1684	A
103		24	10	10.6353	0.1684	BC
104		24	11	10.7232	0.1684	BC
105		24	12	10.6667	0.1684	BC

Effect=type*temp*week Method=Tukey(P<.05) Set=7

Obs	type	temp	week	Estimate	Standard Error	Letter Group
106	3%	4	0	11.0650	0.3367	BCD
107	3%	4	1	11.5932	0.3367	ABCD
108	3%	4	2	11.0973	0.3367	BCD
109	3%	4	3	10.1530	0.3367	BCD
110	3%	4	4	10.5190	0.3367	BCD
111	3%	4	5	10.2546	0.3367	BCD
112	3%	4	6	10.7309	0.3367	BCD

Obs	type	temp	week	Estimate	Standard Error	Letter Group
113	3%	4	7	11.4832	0.3367	ABCD
114	3%	4	8	11.4273	0.3367	ABCD
115	3%	4	9	11.0720	0.3367	BCD
116	3%	4	10	11.3586	0.3367	ABCD
117	3%	4	11	10.6441	0.3367	BCD
118	3%	4	12	10.8753	0.3367	BCD
119	3%	24	0	11.0650	0.3367	BCD
120	3%	24	1	11.2528	0.3367	BCD
121	3%	24	2	10.1534	0.3367	BCD
122	3%	24	3	10.7268	0.3367	BCD
123	3%	24	4	10.8894	0.3367	BCD
124	3%	24	5	10.6358	0.3367	BCD
125	3%	24	6	10.6606	0.3367	BCD
126	3%	24	7	11.4819	0.3367	ABCD
127	3%	24	8	11.2207	0.3367	BCD
128	3%	24	9	11.9821	0.3367	ABC
129	3%	24	10	10.4964	0.3367	BCD
130	3%	24	11	10.5699	0.3367	BCD
131	3%	24	12	10.3533	0.3367	BCD
132	6%	4	0	11.0650	0.3367	BCD
133	6%	4	1	10.4582	0.3367	BCD
134	6%	4	2	11.0654	0.3367	BCD
135	6%	4	3	10.6895	0.3367	BCD
136	6%	4	4	10.4865	0.3367	BCD
137	6%	4	5	10.5822	0.3367	BCD
138	6%	4	6	11.1076	0.3367	BCD
139	6%	4	7	10.6966	0.3367	BCD
140	6%	4	8	10.8806	0.3367	BCD

Obs	type	temp	week	Estimate	Standard Error	Letter Group
141	6%	4	9	9.9499	0.3367	CD
142	6%	4	10	10.7277	0.3367	BCD
143	6%	4	11	11.5922	0.3367	ABCD
144	6%	4	12	10.9641	0.3367	BCD
145	6%	24	0	11.0650	0.3367	BCD
146	6%	24	1	10.8511	0.3367	BCD
147	6%	24	2	10.1163	0.3367	BCD
148	6%	24	3	10.7401	0.3367	BCD
149	6%	24	4	11.0323	0.3367	BCD
150	6%	24	5	10.7967	0.3367	BCD
151	6%	24	6	10.6989	0.3367	BCD
152	6%	24	7	11.5602	0.3367	ABCD
153	6%	24	8	10.8636	0.3367	BCD
154	6%	24	9	10.8754	0.3367	BCD
155	6%	24	10	11.7912	0.3367	ABCD
156	6%	24	11	11.0961	0.3367	BCD
157	6%	24	12	10.8684	0.3367	BCD
158	air	4	0	11.0650	0.3367	BCD
159	air	4	1	12.1236	0.3367	AB
160	air	4	2	11.0194	0.3367	BCD
161	air	4	3	10.3698	0.3367	BCD
162	air	4	4	11.0011	0.3367	BCD
163	air	4	5	10.9288	0.3367	BCD
164	air	4	6	10.3356	0.3367	BCD
165	air	4	7	10.8086	0.3367	BCD
166	air	4	8	10.4360	0.3367	BCD
167	air	4	9	10.9988	0.3367	BCD
168	air	4	10	11.6305	0.3367	ABCD

Obs	type	temp	week	Estimate	Standard Error	Letter Group
169	air	4	11	10.8335	0.3367	BCD
170	air	4	12	10.1938	0.3367	BCD
171	air	24	0	11.0650	0.3367	BCD
172	air	24	1	9.7152	0.3367	D
173	air	24	2	10.3010	0.3367	BCD
174	air	24	3	11.3189	0.3367	ABCD
175	air	24	4	10.6111	0.3367	BCD
176	air	24	5	10.5509	0.3367	BCD
177	air	24	6	10.6321	0.3367	BCD
178	air	24	7	10.1171	0.3367	BCD
179	air	24	8	11.2242	0.3367	BCD
180	air	24	9	13.4069	0.3367	A
181	air	24	10	10.2381	0.3367	BCD
182	air	24	11	10.2384	0.3367	BCD
183	air	24	12	11.0927	0.3367	BCD
184	vac	4	0	11.0650	0.3367	BCD
185	vac	4	1	11.2791	0.3367	ABCD
186	vac	4	2	11.2159	0.3367	BCD
187	vac	4	3	10.9543	0.3367	BCD
188	vac	4	4	10.3786	0.3367	BCD
189	vac	4	5	11.0711	0.3367	BCD
190	vac	4	6	10.4961	0.3367	BCD
191	vac	4	7	10.7309	0.3367	BCD
192	vac	4	8	11.3662	0.3367	ABCD
193	vac	4	9	11.4263	0.3367	ABCD
194	vac	4	10	10.7087	0.3367	BCD
195	vac	4	11	11.3581	0.3367	ABCD
196	vac	4	12	11.9543	0.3367	ABC

Obs	type	temp	week	Estimate	Standard Error	Letter Group
197	vac	24	0	11.0650	0.3367	BCD
198	vac	24	1	11.3843	0.3367	ABCD
199	vac	24	2	10.6259	0.3367	BCD
200	vac	24	3	11.2824	0.3367	ABCD
201	vac	24	4	11.6493	0.3367	ABCD
202	vac	24	5	11.3974	0.3367	ABCD
203	vac	24	6	10.1536	0.3367	BCD
204	vac	24	7	10.8694	0.3367	BCD
205	vac	24	8	11.6394	0.3367	ABCD
206	vac	24	9	10.9296	0.3367	BCD
207	vac	24	10	10.0154	0.3367	BCD
208	vac	24	11	10.9885	0.3367	BCD
209	vac	24	12	10.3525	0.3367	BCD

For C18:2

Effect=type Method=Tukey(P<.05) Set=1

Obs	type	temp	week	Estimate	Standard Error	Letter Group
1	3%	_	_	20.0609	0.07926	A
2	6%	_	_	19.5928	0.07926	B
3	air	_	_	19.5103	0.07926	B
4	vac	_	_	19.7063	0.07926	B

Effect=temp Method=Tukey(P<.05) Set=2

Obs	type	temp	week	Estimate	Standard Error	Letter Group
5		4	_	19.7607	0.05604	A
6		24	_	19.6744	0.05604	A

Effect=type*temp   Method=Tukey(P<.05)   Set=3

Obs	type	temp	week	Estimate	Standard Error	Letter Group
7	3%	4	_	20.3572	0.1121	A
8	3%	24	_	19.7645	0.1121	B
9	6%	4	_	19.6041	0.1121	B
10	6%	24	_	19.5815	0.1121	B
11	air	4	_	19.4013	0.1121	B
12	air	24	_	19.6193	0.1121	B
13	vac	4	_	19.6802	0.1121	B
14	vac	24	_	19.7323	0.1121	B

Effect=week   Method=Tukey(P<.05)   Set=4

Obs	type	temp	week	Estimate	Standard Error	Letter Group
15		_	0	19.2950	0.1429	B
16		_	1	19.3586	0.1429	B
17		_	2	19.8462	0.1429	AB
18		_	3	19.7003	0.1429	AB
19		_	4	19.6976	0.1429	AB
20		_	5	19.7988	0.1429	AB
21		_	6	19.5823	0.1429	AB
22		_	7	20.0943	0.1429	A
23		_	8	19.9197	0.1429	AB
24		_	9	19.7087	0.1429	AB
25		_	10	19.8952	0.1429	AB
26		_	11	19.6204	0.1429	AB
27		_	12	19.8111	0.1429	AB

Effect=type*week   Method=Tukey(P<.05)   Set=5

Obs	type	temp	week	Estimate	Standard Error	Letter Group
28	3%	_	0	19.2950	0.2858	BCD
29	3%	_	1	19.6472	0.2858	ABCD
30	3%	_	2	19.5387	0.2858	BCD
31	3%	_	3	20.3848	0.2858	ABC
32	3%	_	4	19.7464	0.2858	ABCD
33	3%	_	5	19.9705	0.2858	ABCD
34	3%	_	6	20.1913	0.2858	ABCD
35	3%	_	7	21.2237	0.2858	A
36	3%	_	8	19.9957	0.2858	ABCD
37	3%	_	9	19.6409	0.2858	ABCD
38	3%	_	10	20.8321	0.2858	AB
39	3%	_	11	20.3009	0.2858	ABC
40	3%	_	12	20.0238	0.2858	ABCD
41	6%	_	0	19.2950	0.2858	BCD
42	6%	_	1	19.8031	0.2858	ABCD
43	6%	_	2	19.6500	0.2858	ABCD
44	6%	_	3	19.4619	0.2858	BCD
45	6%	_	4	19.5430	0.2858	BCD
46	6%	_	5	19.5659	0.2858	ABCD
47	6%	_	6	19.2400	0.2858	BCD
48	6%	_	7	19.8231	0.2858	ABCD
49	6%	_	8	19.3714	0.2858	BCD
50	6%	_	9	19.8738	0.2858	ABCD
51	6%	_	10	20.1968	0.2858	ABCD
52	6%	_	11	19.5070	0.2858	BCD
53	6%	_	12	19.3754	0.2858	BCD
54	air	_	0	19.2950	0.2858	BCD

Obs	type	temp	week	Estimate	Standard Error	Letter Group
55	air	_	1	18.7978	0.2858	CD
56	air	_	2	19.8585	0.2858	ABCD
57	air	_	3	19.3826	0.2858	BCD
58	air	_	4	19.7348	0.2858	ABCD
59	air	_	5	19.8602	0.2858	ABCD
60	air	_	6	18.8446	0.2858	CD
61	air	_	7	19.6237	0.2858	ABCD
62	air	_	8	19.4966	0.2858	BCD
63	air	_	9	19.7798	0.2858	ABCD
64	air	_	10	19.9650	0.2858	ABCD
65	air	_	11	19.3838	0.2858	BCD
66	air	_	12	19.6113	0.2858	ABCD
67	vac	_	0	19.2950	0.2858	BCD
68	vac	_	1	19.1863	0.2858	BCD
69	vac	_	2	20.3377	0.2858	ABC
70	vac	_	3	19.5718	0.2858	ABCD
71	vac	_	4	19.7661	0.2858	ABCD
72	vac	_	5	19.7985	0.2858	ABCD
73	vac	_	6	20.0533	0.2858	ABCD
74	vac	_	7	19.7066	0.2858	ABCD
75	vac	_	8	20.8152	0.2858	AB
76	vac	_	9	19.5404	0.2858	BCD
77	vac	_	10	18.5870	0.2858	D
78	vac	_	11	19.2897	0.2858	BCD
79	vac	_	12	20.2339	0.2858	ABCD

Effect=temp*week Method=Tukey(P<.05) Set=6

Obs	type	temp	week	Estimate	Standard Error	Letter Group
80		4	0	19.2950	0.2021	B
81		4	1	19.4752	0.2021	B
82		4	2	19.5311	0.2021	B
83		4	3	19.7081	0.2021	AB
84		4	4	19.8423	0.2021	AB
85		4	5	19.6379	0.2021	AB
86		4	6	19.9129	0.2021	AB
87		4	7	20.6393	0.2021	A
88		4	8	19.8218	0.2021	AB
89		4	9	19.8613	0.2021	AB
90		4	10	20.1203	0.2021	AB
91		4	11	19.4596	0.2021	B
92		4	12	19.5846	0.2021	AB
93		24	0	19.2950	0.2021	B
94		24	1	19.2420	0.2021	B
95		24	2	20.1614	0.2021	AB
96		24	3	19.6925	0.2021	AB
97		24	4	19.5529	0.2021	B
98		24	5	19.9597	0.2021	AB
99		24	6	19.2517	0.2021	B
100		24	7	19.5493	0.2021	B
101		24	8	20.0176	0.2021	AB
102		24	9	19.5562	0.2021	B
103		24	10	19.6701	0.2021	AB
104		24	11	19.7812	0.2021	AB
105		24	12	20.0376	0.2021	AB

Effect=type*temp*week Method=Tukey(P<.05) Set=7

Obs	type	temp	week	Estimate	Standard Error	Letter Group
106	3%	4	0	19.2950	0.4041	BCDE
107	3%	4	1	20.1793	0.4041	ABCDE
108	3%	4	2	19.5966	0.4041	ABCDE
109	3%	4	3	20.4626	0.4041	ABCDE
110	3%	4	4	20.0102	0.4041	ABCDE
111	3%	4	5	20.1113	0.4041	ABCDE
112	3%	4	6	20.2794	0.4041	ABCDE
113	3%	4	7	22.0468	0.4041	A
114	3%	4	8	20.2236	0.4041	ABCDE
115	3%	4	9	20.4680	0.4041	ABCDE
116	3%	4	10	21.4889	0.4041	AB
117	3%	4	11	20.6255	0.4041	ABCDE
118	3%	4	12	19.8569	0.4041	ABCDE
119	3%	24	0	19.2950	0.4041	BCDE
120	3%	24	1	19.1150	0.4041	BCDE
121	3%	24	2	19.4809	0.4041	BCDE
122	3%	24	3	20.3070	0.4041	ABCDE
123	3%	24	4	19.4827	0.4041	BCDE
124	3%	24	5	19.8298	0.4041	ABCDE
125	3%	24	6	20.1033	0.4041	ABCDE
126	3%	24	7	20.4006	0.4041	ABCDE
127	3%	24	8	19.7677	0.4041	ABCDE
128	3%	24	9	18.8138	0.4041	CDE
129	3%	24	10	20.1753	0.4041	ABCDE
130	3%	24	11	19.9764	0.4041	ABCDE
131	3%	24	12	20.1908	0.4041	ABCDE
132	6%	4	0	19.2950	0.4041	BCDE

Obs	type	temp	week	Estimate	Standard Error	Letter Group
133	6%	4	1	19.5316	0.4041	ABCDE
134	6%	4	2	19.1660	0.4041	BCDE
135	6%	4	3	19.2885	0.4041	BCDE
136	6%	4	4	19.7076	0.4041	ABCDE
137	6%	4	5	19.6770	0.4041	ABCDE
138	6%	4	6	19.5776	0.4041	ABCDE
139	6%	4	7	20.2079	0.4041	ABCDE
140	6%	4	8	19.2543	0.4041	BCDE
141	6%	4	9	20.0993	0.4041	ABCDE
142	6%	4	10	20.3025	0.4041	ABCDE
143	6%	4	11	19.2952	0.4041	BCDE
144	6%	4	12	19.4508	0.4041	BCDE
145	6%	24	0	19.2950	0.4041	BCDE
146	6%	24	1	20.0746	0.4041	ABCDE
147	6%	24	2	20.1340	0.4041	ABCDE
148	6%	24	3	19.6354	0.4041	ABCDE
149	6%	24	4	19.3785	0.4041	BCDE
150	6%	24	5	19.4548	0.4041	BCDE
151	6%	24	6	18.9024	0.4041	CDE
152	6%	24	7	19.4382	0.4041	BCDE
153	6%	24	8	19.4885	0.4041	BCDE
154	6%	24	9	19.6483	0.4041	ABCDE
155	6%	24	10	20.0912	0.4041	ABCDE
156	6%	24	11	19.7189	0.4041	ABCDE
157	6%	24	12	19.3000	0.4041	BCDE
158	air	4	0	19.2950	0.4041	BCDE
159	air	4	1	18.8885	0.4041	CDE
160	air	4	2	19.9994	0.4041	ABCDE

Obs	type	temp	week	Estimate	Standard Error	Letter Group
161	air	4	3	19.6261	0.4041	ABCDE
162	air	4	4	19.8869	0.4041	ABCDE
163	air	4	5	19.6057	0.4041	ABCDE
164	air	4	6	19.0138	0.4041	BCDE
165	air	4	7	20.0288	0.4041	ABCDE
166	air	4	8	19.2909	0.4041	BCDE
167	air	4	9	18.9915	0.4041	BCDE
168	air	4	10	19.6501	0.4041	ABCDE
169	air	4	11	18.9884	0.4041	BCDE
170	air	4	12	18.9517	0.4041	BCDE
171	air	24	0	19.2950	0.4041	BCDE
172	air	24	1	18.7071	0.4041	DE
173	air	24	2	19.7175	0.4041	ABCDE
174	air	24	3	19.1392	0.4041	BCDE
175	air	24	4	19.5828	0.4041	ABCDE
176	air	24	5	20.1148	0.4041	ABCDE
177	air	24	6	18.6753	0.4041	DE
178	air	24	7	19.2186	0.4041	BCDE
179	air	24	8	19.7023	0.4041	ABCDE
180	air	24	9	20.5680	0.4041	ABCDE
181	air	24	10	20.2799	0.4041	ABCDE
182	air	24	11	19.7792	0.4041	ABCDE
183	air	24	12	20.2710	0.4041	ABCDE
184	vac	4	0	19.2950	0.4041	BCDE
185	vac	4	1	19.3014	0.4041	BCDE
186	vac	4	2	19.3622	0.4041	BCDE
187	vac	4	3	19.4553	0.4041	BCDE
188	vac	4	4	19.7645	0.4041	ABCDE

Obs	type	temp	week	Estimate	Standard Error	Letter Group
189	vac	4	5	19.1575	0.4041	BCDE
190	vac	4	6	20.7808	0.4041	ABCD
191	vac	4	7	20.2735	0.4041	ABCDE
192	vac	4	8	20.5184	0.4041	ABCDE
193	vac	4	9	19.8863	0.4041	ABCDE
194	vac	4	10	19.0398	0.4041	BCDE
195	vac	4	11	18.9293	0.4041	CDE
196	vac	4	12	20.0790	0.4041	ABCDE
197	vac	24	0	19.2950	0.4041	BCDE
198	vac	24	1	19.0712	0.4041	BCDE
199	vac	24	2	21.3132	0.4041	ABC
200	vac	24	3	19.6883	0.4041	ABCDE
201	vac	24	4	19.7677	0.4041	ABCDE
202	vac	24	5	20.4395	0.4041	ABCDE
203	vac	24	6	19.3257	0.4041	BCDE
204	vac	24	7	19.1396	0.4041	BCDE
205	vac	24	8	21.1121	0.4041	ABCD
206	vac	24	9	19.1945	0.4041	BCDE
207	vac	24	10	18.1341	0.4041	E
208	vac	24	11	19.6501	0.4041	ABCDE
209	vac	24	12	20.3887	0.4041	ABCDE

For C18:3

Effect=type Method=Tukey(P<.05) Set=1

Obs	type	temp	week	Estimate	Standard Error	Letter Group
1	3%	_	_	63.6169	0.1033	A
2	6%	_	_	63.8871	0.1033	A

Obs	type	temp	week	Estimate	Standard Error	Letter Group
3	air	_	_	63.8314	0.1033	A
4	vac	_	_	63.5256	0.1033	A

Effect=temp   Method=Tukey(P<.05)   Set=2

Obs	type	temp	week	Estimate	Standard Error	Letter Group
5		4	_	63.7643	0.07301	A
6		24	_	63.6662	0.07301	A

Effect=type*temp   Method=Tukey(P<.05)   Set=3

Obs	type	temp	week	Estimate	Standard Error	Letter Group
7	3%	4	_	63.5551	0.1460	A
8	3%	24	_	63.6788	0.1460	A
9	6%	4	_	64.0006	0.1460	A
10	6%	24	_	63.7737	0.1460	A
11	air	4	_	63.9836	0.1460	A
12	air	24	_	63.6792	0.1460	A
13	vac	4	_	63.5181	0.1460	A
14	vac	24	_	63.5331	0.1460	A

Effect=week   Method=Tukey(P<.05)   Set=4

Obs	type	temp	week	Estimate	Standard Error	Letter Group
15		_	0	63.4100	0.1862	BCDE
16		_	1	63.9936	0.1862	ABCD
17		_	2	63.4968	0.1862	ABCDE
18		_	3	63.9937	0.1862	ABCD
19		_	4	64.2416	0.1862	AB
20		_	5	63.6856	0.1862	ABCD

Obs	type	temp	week	Estimate	Standard Error	Letter Group
21		_	6	64.0054	0.1862	ABCD
22		_	7	63.2417	0.1862	DE
23		_	8	63.3474	0.1862	CDE
24		_	9	62.6874	0.1862	E
25		_	10	64.1990	0.1862	ABC
26		_	11	63.6592	0.1862	ABCD
27		_	12	64.3370	0.1862	A

Effect=type*week   Method=Tukey(P<.05)   Set=5

Obs	type	temp	week	Estimate	Standard Error	Letter Group
28	3%	_	0	63.4100	0.3723	BCDEF
29	3%	_	1	62.8514	0.3723	CDEFG
30	3%	_	2	63.9453	0.3723	ABCDE
31	3%	_	3	64.0118	0.3723	ABCDE
32	3%	_	4	63.8868	0.3723	ABCDE
33	3%	_	5	64.3430	0.3723	ABCD
34	3%	_	6	64.1599	0.3723	ABCD
35	3%	_	7	61.9001	0.3723	EFG
36	3%	_	8	63.7935	0.3723	ABCDE
37	3%	_	9	63.5288	0.3723	BCDE
38	3%	_	10	63.4093	0.3723	BCDEF
39	3%	_	11	62.9788	0.3723	CDEFG
40	3%	_	12	64.8015	0.3723	ABC
41	6%	_	0	63.4100	0.3723	BCDEF
42	6%	_	1	65.2145	0.3723	AB
43	6%	_	2	63.7765	0.3723	ABCDE
44	6%	_	3	63.6959	0.3723	ABCDE
45	6%	_	4	64.6392	0.3723	ABCD

Obs	type	temp	week	Estimate	Standard Error	Letter Group
46	6%	_	5	63.7603	0.3723	ABCDE
47	6%	_	6	63.3314	0.3723	BCDEFG
48	6%	_	7	63.0633	0.3723	BCDEFG
49	6%	_	8	64.1206	0.3723	ABCD
50	6%	_	9	63.3313	0.3723	BCDEFG
51	6%	_	10	64.0421	0.3723	ABCDE
52	6%	_	11	63.5460	0.3723	BCDE
53	6%	_	12	64.6015	0.3723	ABCD
54	air	_	0	63.4100	0.3723	BCDEF
55	air	_	1	64.3227	0.3723	ABCD
56	air	_	2	63.7113	0.3723	ABCDE
57	air	_	3	64.4646	0.3723	ABCD
58	air	_	4	64.0581	0.3723	ABCDE
59	air	_	5	63.7212	0.3723	ABCDE
60	air	_	6	64.4609	0.3723	ABCD
61	air	_	7	64.3323	0.3723	ABCD
62	air	_	8	64.1262	0.3723	ABCD
63	air	_	9	61.2005	0.3723	G
64	air	_	10	63.4992	0.3723	BCDEF
65	air	_	11	64.1833	0.3723	ABCD
66	air	_	12	64.3175	0.3723	ABCD
67	vac	_	0	63.4100	0.3723	BCDEF
68	vac	_	1	63.5858	0.3723	BCDE
69	vac	_	2	62.5539	0.3723	DEFG
70	vac	_	3	63.8024	0.3723	ABCDE
71	vac	_	4	64.3825	0.3723	ABCD
72	vac	_	5	62.9180	0.3723	CDEFG
73	vac	_	6	64.0693	0.3723	ABCDE

Obs	type	temp	week	Estimate	Standard Error	Letter Group
74	vac	_	7	63.6709	0.3723	ABCDE
75	vac	_	8	61.3493	0.3723	FG
76	vac	_	9	62.6892	0.3723	CDEFG
77	vac	_	10	65.8456	0.3723	A
78	vac	_	11	63.9288	0.3723	ABCDE
79	vac	_	12	63.6274	0.3723	BCDE

Effect=temp*week   Method=Tukey(P<.05)   Set=6

Obs	type	temp	week	Estimate	Standard Error	Letter Group
80		4	0	63.4100	0.2633	ABCD
81		4	1	63.6367	0.2633	ABC
82		4	2	63.3397	0.2633	ABCD
83		4	3	64.3044	0.2633	ABC
84		4	4	64.3801	0.2633	ABC
85		4	5	63.9528	0.2633	ABC
86		4	6	63.4772	0.2633	ABC
87		4	7	63.0397	0.2633	CD
88		4	8	63.5460	0.2633	ABC
89		4	9	63.3114	0.2633	ABCD
90		4	10	63.8953	0.2633	ABC
91		4	11	64.0813	0.2633	ABC
92		4	12	64.5617	0.2633	A
93		24	0	63.4100	0.2633	ABCD
94		24	1	64.3505	0.2633	ABC
95		24	2	63.6538	0.2633	ABC
96		24	3	63.6829	0.2633	ABC
97		24	4	64.1032	0.2633	ABC
98		24	5	63.4184	0.2633	ABCD

Obs	type	temp	week	Estimate	Standard Error	Letter Group
99		24	6	64.5336	0.2633	AB
100		24	7	63.4437	0.2633	ABCD
101		24	8	63.1488	0.2633	BCD
102		24	9	62.0635	0.2633	D
103		24	10	64.5028	0.2633	AB
104		24	11	63.2372	0.2633	ABCD
105		24	12	64.1123	0.2633	ABC

Effect=type*temp*week Method=Tukey(P<.05) Set=7

Obs	type	temp	week	Estimate	Standard Error	Letter Group
106	3%	4	0	63.4100	0.5265	ABCDEF
107	3%	4	1	62.8372	0.5265	ABCDEF
108	3%	4	2	63.7435	0.5265	ABCDE
109	3%	4	3	64.3077	0.5265	ABCDE
110	3%	4	4	64.3538	0.5265	ABCDE
111	3%	4	5	65.1451	0.5265	ABCD
112	3%	4	6	64.0014	0.5265	ABCDE
113	3%	4	7	62.0568	0.5265	DEFG
114	3%	4	8	62.6911	0.5265	ABCDEF
115	3%	4	9	64.1426	0.5265	ABCDE
116	3%	4	10	62.0481	0.5265	DEFG
117	3%	4	11	63.2384	0.5265	ABCDEF
118	3%	4	12	64.2401	0.5265	ABCDE
119	3%	24	0	63.4100	0.5265	ABCDEF
120	3%	24	1	62.8656	0.5265	ABCDEF
121	3%	24	2	64.1471	0.5265	ABCDE
122	3%	24	3	63.7158	0.5265	ABCDE
123	3%	24	4	63.4199	0.5265	ABCDEF

Obs	type	temp	week	Estimate	Standard Error	Letter Group
124	3%	24	5	63.5408	0.5265	ABCDE
125	3%	24	6	64.3185	0.5265	ABCDE
126	3%	24	7	61.7434	0.5265	EFG
127	3%	24	8	64.8960	0.5265	ABCDE
128	3%	24	9	62.9149	0.5265	ABCDEF
129	3%	24	10	64.7706	0.5265	ABCDE
130	3%	24	11	62.7193	0.5265	ABCDEF
131	3%	24	12	65.3630	0.5265	ABCD
132	6%	4	0	63.4100	0.5265	ABCDEF
133	6%	4	1	65.2762	0.5265	ABCD
134	6%	4	2	64.1912	0.5265	ABCDE
135	6%	4	3	63.8678	0.5265	ABCDE
136	6%	4	4	64.1967	0.5265	ABCDE
137	6%	4	5	63.3956	0.5265	ABCDEF
138	6%	4	6	62.8806	0.5265	ABCDEF
139	6%	4	7	63.4875	0.5265	ABCDE
140	6%	4	8	63.9266	0.5265	ABCDE
141	6%	4	9	63.3375	0.5265	ABCDEF
142	6%	4	10	64.8171	0.5265	ABCDE
143	6%	4	11	63.5880	0.5265	ABCDE
144	6%	4	12	65.6328	0.5265	ABC
145	6%	24	0	63.4100	0.5265	ABCDEF
146	6%	24	1	65.1527	0.5265	ABCD
147	6%	24	2	63.3618	0.5265	ABCDEF
148	6%	24	3	63.5240	0.5265	ABCDE
149	6%	24	4	65.0816	0.5265	ABCD
150	6%	24	5	64.1250	0.5265	ABCDE
151	6%	24	6	63.7821	0.5265	ABCDE

Obs	type	temp	week	Estimate	Standard Error	Letter Group
152	6%	24	7	62.6392	0.5265	ABCDEF
153	6%	24	8	64.3145	0.5265	ABCDE
154	6%	24	9	63.3251	0.5265	ABCDEF
155	6%	24	10	63.2672	0.5265	ABCDEF
156	6%	24	11	63.5040	0.5265	ABCDE
157	6%	24	12	63.5702	0.5265	ABCDE
158	air	4	0	63.4100	0.5265	ABCDEF
159	air	4	1	62.9424	0.5265	ABCDEF
160	air	4	2	62.8171	0.5265	ABCDEF
161	air	4	3	65.2699	0.5265	ABCD
162	air	4	4	63.7542	0.5265	ABCDE
163	air	4	5	63.8985	0.5265	ABCDE
164	air	4	6	64.4936	0.5265	ABCDE
165	air	4	7	63.4775	0.5265	ABCDE
166	air	4	8	64.9612	0.5265	ABCDE
167	air	4	9	63.3080	0.5265	ABCDEF
168	air	4	10	62.8830	0.5265	ABCDEF
169	air	4	11	65.1342	0.5265	ABCD
170	air	4	12	65.4366	0.5265	ABC
171	air	24	0	63.4100	0.5265	ABCDEF
172	air	24	1	65.7030	0.5265	ABC
173	air	24	2	64.6056	0.5265	ABCDE
174	air	24	3	63.6594	0.5265	ABCDE
175	air	24	4	64.3619	0.5265	ABCDE
176	air	24	5	63.5439	0.5265	ABCDE
177	air	24	6	64.4281	0.5265	ABCDE
178	air	24	7	65.1871	0.5265	ABCD
179	air	24	8	63.2912	0.5265	ABCDEF

Obs	type	temp	week	Estimate	Standard Error	Letter Group
180	air	24	9	59.0930	0.5265	G
181	air	24	10	64.1154	0.5265	ABCDE
182	air	24	11	63.2324	0.5265	ABCDEF
183	air	24	12	63.1983	0.5265	ABCDEF
184	vac	4	0	63.4100	0.5265	ABCDEF
185	vac	4	1	63.4910	0.5265	ABCDE
186	vac	4	2	62.6069	0.5265	ABCDEF
187	vac	4	3	63.7722	0.5265	ABCDE
188	vac	4	4	65.2156	0.5265	ABCD
189	vac	4	5	63.3720	0.5265	ABCDEF
190	vac	4	6	62.5332	0.5265	ABCDEF
191	vac	4	7	63.1368	0.5265	ABCDEF
192	vac	4	8	62.6050	0.5265	ABCDEF
193	vac	4	9	62.4575	0.5265	CDEF
194	vac	4	10	65.8331	0.5265	AB
195	vac	4	11	64.3644	0.5265	ABCDE
196	vac	4	12	62.9372	0.5265	ABCDEF
197	vac	24	0	63.4100	0.5265	ABCDEF
198	vac	24	1	63.6806	0.5265	ABCDE
199	vac	24	2	62.5008	0.5265	BCDEF
200	vac	24	3	63.8326	0.5265	ABCDE
201	vac	24	4	63.5495	0.5265	ABCDE
202	vac	24	5	62.4639	0.5265	CDEF
203	vac	24	6	65.6054	0.5265	ABC
204	vac	24	7	64.2050	0.5265	ABCDE
205	vac	24	8	60.0936	0.5265	FG
206	vac	24	9	62.9208	0.5265	ABCDEF
207	vac	24	10	65.8581	0.5265	A

Obs	type	temp	week	Estimate	Standard Error	Letter Group
208	vac	24	11	63.4932	0.5265	ABCDE
209	vac	24	12	64.3176	0.5265	ABCDE

VITA

Beatrice G. Terigar was born in Arad, Romania, in April1984. She received her Bachelor of Science degree in food technology in June 2007 from Aurel Vlaicu University of Arad, Romania. Beatrice began a master's program in Fall 2007 in biological and agricultural engineering at Louisiana State University with a concentration in vegetable oil extraction and biodiesel production. She is planning to continue her education pursuing a Doctorate of Philosophy in engineering sciences at Louisiana State University.