EXTENT OF DETERIORATION AND FORMATION OF TOXIC SUBSTANCES IN COOKING OIL (COCONUT OIL) WHEN HEATED REPEATEDLY ABOVE SMOKE POINT

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ABSTRACT

Commercial cooking oil sample (Refined, Bleached and Deodorized-Coconut Oil or RBD-CNO) was subjected to heat treatments. Twelve samples of four treatments and three replicates for each treatment: namely, the untreated (UN), heated once (T1), heated twice (T2) with intermittent cooling and, heated thrice (T3) with intermittent cooling were analyzed for fatty acid profile, free fatty acid value, peroxide value and the presence of PAH. Data were statistically analyzed using one way analysis of variance (ANOVA). Significant treatment mean differences were established using DMRT. The fatty acid profile showed that only linoleic acid decreased in quantity constantly up to the third heating. Free fatty acid and peroxide values showed an increasing trend. All of the 22 PAH included in the analysis were present in all treatments in small quantities. The only PAH which showed an increasing trend in the 4 treatments was 1,6,7-trimethylnaphthalene, the quantities of the rest remained the same. The decrease in the amount of linoleic acid in the free fatty acid profile indicated that unsaturated fatty acids decompose on heating and this could be the source of peroxides, but saturated fatty acid components were stable at high temperature.

***Keywords**: fatty acid profile, free fatty acid, peroxide, poly aromatic hydrocarbons.*

INTRODUCTION

In the preparation of food cooking by frying with oil is inevitable. Most Filipino households practice the reuse of cooking oil up to the nth time until the color of the oil becomes dark brown, viscosity increased and rancid odor developed.

In the process of frying, smoke point is the temperature at which a cooking fat or oil smokes or burns, and begins to break down releasing free radicals. This is also the point when natural nutrients in oils and phytochemicals are destroyed (Good, 2012).RBD-CNO, cooking oil (Dayrit, *et al*., 2007) commonly used in the Philippines has a smoke point of 232^0C while the unrefined, the likes of VCO (Virgin Coconut oil) smokes at 170^0C (Coconut Oils Facts 2017). Every time oil is heated, smoke point is decreased and reusing it for several times lowers the smoke point to about the frying temperature (Wikipedia, 2009). As the oil is reused deterioration and chemical reactions takes place at lower temperatures.

In reusing cooking oil (RBD-CNO), the researcher would like to find out to what extent does it deteriorate? Are toxic products, peroxides and poly-aromatic hydrocarbons formed? Does the amount of peroxide increase as heating above smoke point is repeated? The study would determine the stability of RBD–CNO cooking oil and its safety of reuse in cooking of food.

Objectives of the study

This study aimed to determine the deterioration and the formation of toxic substances in cooking oil that was subjected to different heat treatments. Specifically this study aimed to determine the following:

1. changes in the fatty acid profile and free fatty acid value; and,

2. the peroxide value and presence of polyaromatic hydrocarbons (PAH), before heating and every time it was reheated above smoke point up to the third time.

Significance of the Study

This study will be beneficial to:

1. households as food is prepared for the family two to three times a day;
2. restaurants, food chains, caterers, canteens where the general public eat; and,
3. side walk food vendors who engage in deep fried food.

Awareness of the toxic substances formed when cooking oil is reused is important to these sectors as they are responsible for food preparation. This experiment would establish the health risk in reusing cooking oil and its possible connection to recurring diseases.

Scope and Limitation of the study

The analysis included in this study were fatty acid profile, free fatty acid value, peroxide value and determination of PAH determination value in cooking oil-RBD-CNO, before it was heated and every time it was reheated. The heating was above smoke point (232^0C) for 30 minutes. There were four treatments: heated once, heated twice and heated thrice and the untreated. There were three replicates for each of the treatments.

This study did not include frying of food, as components of food would add reactants to the cooking oil. For different food products, a variety of reactants maybe added to the cooking oil.

METHODOLOGY

Materials and Preparation of Samples

The sample was bought from a grocery store. Twelve 250 mL portions were measured as were measured as samples for four treatments with three replicates each. The four treatments were the UN (untreated), T1 (heated once), T2 (heated twice with intermittent cooling to room temperature), T3 (heated three times with intermittent cooling to room temperature). The temperature used for the treatment was 250^oC and maintained for 30 minutes, then the oils were stored in amber bottles and refrigerated prior to analysis.

Fatty Acid Profiling

A portion of the four samples of three replicates were brought to SEAFDEC, Tigbauan, Iloilo for fatty acid profiling. Methylation of samples to fatty acid methyl esters (FAME) was done by saponification of sample with 0.5N KOH/methanol and subsequent trans-esterification with borontrifluoride (Metcalfe, *et al*., 1966). The fatty acid profile was analyzed using Shimadzu Gas Chromatograph Model GC-17A, with Supelco Omega wax 320 capillary column, having a dimension of 30m x 0.32mm x 0.25µm. The standard used in the GC analysis was cod liver oil. The fatty acid composition was expressed as percent normalized value.

Free Fatty Acid

A portion of oils in the four treatments with three replicates was analyzed by the researcher for free fatty acid value at the laboratory of the Chemistry Department of Central Philippine University, Jaro, Iloilo City by titrimetry, based on AOAC (1990) 940.28 modified method. The free fatty acid was analyzed by titration of a measured amount of sample with a standard sodium hydroxide solution using phenolphthalein as the indicator. The results were computed and expressed as % butyric acid.

Peroxide Values

Another portion of oils in the four treatments and three replicates was analyzed for peroxide value at the laboratory of the Chemistry Department of Central Philippine University, Jaro, Iloilo City using titrimetry, AOAC (1990) Official Method 965.33. Peroxide value

was measured by the reduction of excess iodide with peroxide in the sample and subsequent oxidation of the molecular iodine by titration with standardized sodium thiosulfate to iodide. Iodine reacts with thiosulfate; thiosulfate losses one electron. One equivalent of iodine is equal to one equivalent of thiosulfate. The amount of peroxide was expressed as milli equivalent peroxide per kilogram oil.

Determination of the Presence of Polyaromatic Hydrocarbons (PAH)

Representative samples of each treatment with the three replicates were brought to the Oil Spill Response Program, University of the Philippines Visayas, Freshwater Aquaculture Station, Miag-ao, Iloilo for the analysis of poly aromatic hydrocarbons (PAH) by gas chromatography-mass spectrometry (GC-MS). The amount of poly aromatic hydrocarbons in mcg/mL was determined using the method EPA-8270-C which is the determination of semi-volatile organic compounds by GC-MS. The extraction of PAH was done using the method EPA-3540, solid phase extraction of PAHs using SPE tube DSC-Si (EPA Method 3535A). The instrument used was GC: Clarus 600 Gas Chromatograph; MS: Clarus 600T Mass Spectrometer. Analysis parameters used: Oven temperature program, initial temperature, 80^0C for 0 min. Ramp 1, 8.0^0C /min to 240^0C, hold for 20 min. Ramp 2, 8.00^0C/min to 250^0C, hold for 20 min. Injection Volume: 1.0µL; splitless; Carrier gas: He gas; transfer length: 60m; MS scan: Time 8.100 min to 61.2500 min, Mass 50.00 to 450.00El+; Solvent delay: start 0.00(min), End 8.00(min).

Statistical Analysis

Results on fatty acid profile, free fatty acid and peroxide values and amount of PAH were statistically analyzed by one way analysis of variance (ANOVA) at the 5 % level of significance.

Significant differences between or among pairs of means were determined using the Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

The sample used was a commercially-sold cooking oil and the physical appearance was clear and pale yellow in color. As the oil was heated, the color darkened and viscosity increased (Fig. 1). Thickening was the result of evaporation of the more volatile components of the oil (Choe, *et al*., 2007).

Figure 1. Color of the oil before and after heat treatments

Fatty Acid Profile

As shown in Table 1, six fatty acids were identified in the samples, and there were some which were not identified as the determination was limited to the standard being used which is cod liver oil. Four of these fatty acids namely, Lauric, Myristic, Palmitic and Stearic, were saturated, while two, Oleic acid and Linoleic, were unsaturated. Among these fatty acids present Lauric acid is the highest in percent composition, consistent with the study of Dayrit, *et al*., (2007), that coconut oil is made up of mostly medium chain fatty acids and small quantities of oleic and linoleic acid. Only linoleic acid significantly decreased in quantity on repeated heating while the rest did not show any substantial change in amount. This is because linoleic acid is unsaturated and is readily oxidized in the double bonds. Saturated fatty acids are stable to thermal decomposition as compared with unsaturated fatty acids. DMRT results showed that the amount of linoleic acid in the unheated sample was significantly higher compared to those in Treatment 1, Treatment 2, and Treatment 3 but there was no significant difference in the amount among the heated samples. The finding also shows that coconut oil is not easily deteriorated by heating.

Table 1. The fatty acid profile (expressed as % normalized value)

Fatty Acid	Abbrev. Symbol	% Fatty Acid Mean ± SD			
		UN	Treatment 1	Treatme	Treatment
Lauric	C12	31.01 ± 4.91	34.43 ± 1.64	36.83 ± 1.14	33.75 ± 5.04
Myristic	C14	21.74 ± 1.24	21.44 ± 0.30	22.82 ± 1.92	21.68 ± 0.82
Palmitic	C16	16.61 ± 2.40	15.14 ± 0.85	15.52 ± 1.59	16.00 ± 1.83
Stearic	C18	8.17 ± 1.63	7.16 ± 0.26	6.99 ± 0.42	8.11 ± 2.28
Oleic	C18:1n9	13.37 ± 1.54	11.94 ± 0.43	11.65 ± 0.93	11.75 ± 2.44
Linoleic	C182n6	4.39 ± 0.30^{a}	3.23 ± 0.32^{b}	1.81 ± 1.57^{bc}	2.14 ± 0.57^{bc}
Unidentified		4.72 ± 1.62	6.65 ± 0.94	4.38 ± 3.80	6.58 ± 1.61
Total		100.00%	100.00%	100.00%	100.00%

*Linoleic acid is significant while the rest is not significant at α=5% by one way ANOVA

^{abcd} Treatment means having the same letter superscript are not significantly different at 5% level of probability by DMRT.

Percent Free Fatty Acids

Free fatty acids are products of the breakdown of fat molecules by hydrolysis. Its presence in large quantities in the sample signifies deterioration. Hydrolysis of oil would breakdown triglycerides to free fatty acids and glycerol. The presence of free fatty acids at high levels is undesirable as it gives an unpleasant odor to the oil. Hydrolysis is brought about by reaction of water vapor in the air to the oil during the process of repeated heating and intermittent cooling. According to the study of Dayrit, *et al.,* (2007), the range for FFA in RBD in several commercially available cooking oil which included the sample that was used in the study, were within

0.008% to 0.076% and the average of 0.021%. The Philippine Coconut Authority (PCA) recommended value is 0.2% (Dayrit, *et al.,* 2007).

As shown in Table 2, the amount of free fatty acids in the samples increased from the untreated to the third reheating. There was significant difference for all the treatments. Results showed that the free fatty acid content of the untreated oil is 0.03% and increased steadily up to more than 0.1% on the third heating. Repeated heating of the oil brought about hydrolysis but maximum amount of free fatty acid did not exceed the limits set by the (PCA). When the pairs of mean of different treatments were compared by DMRT, results showed that all data were significantly different from each other. Again the result of this analysis shows that RBD-CNO is stable at high temperature due to its unsaturated nature.

Table 2. Percent Free Fatty Acids expressed as % Butyric Acid

Sample	% Free Fatty Acid (% Butyric Acid) MEAN ± SD
Untreated	0.0314 ± 0.0002^{a}
Treatment 1	0.0482 ± 0.0002^{b}
Treatment 2	0.0665 ± 0.0003^{c}
Treatment 3	0.1129 ± 0.0003^{d}

*Significant at α=5% by one way ANOVA

^{abcd} Treatment means having the same letter superscript are not significantly different at 5% level of probability by DMRT.

Peroxide Value

Peroxides (hydroxyl peroxides) are produced from the oxidation of double bonds in unsaturated fatty acids in an oil molecule. Hydroxyl peroxides are groups of compounds that are highly reactive, which could react with lipids in the cell resulting to cell damage (Trevisan, *et al*., 2001). Lipid peroxidation results to products such as malondialdehyde and 4-hydroxy nonenal that are mutagenic and carcinogenic (Marnett, 1999).

The data in Table 3 showed that the amount of peroxide in the sample had an increasing trend. The study of Dayrit, *et al*., (2007), for RBD-CNO gave an average peroxide value of 0.98 meq/kg oil and a range of 0.27 to 3.39 meq/kg for fresh RBD-CNO. Asian Pacific Coconut Community (APCC) set a limit of 3.00 meq/kg oil. Results obtained in the study showed peroxide value of 2.01 meq peroxide per kg of oil for the untreated and increased to 10.64 meq peroxide per kg oil on the third treatment. ANOVA showed significant difference between means at 5% α. Comparing pairs of mean by DMRT, the differences were significant between treatments. The results showed that toxic substances like peroxide is produced and increases on repeated heating.

Table 3. Peroxide Value *Expressed as milli equivalent of Peroxide per Kilogram Sample*

Samples	Peroxide Value (meq of peroxide per kg of oil) MEAN±SD
Untreated	2.01 ± 0.04[a]
Treatment 1	7.84 ± 0.02[b]
Treatment 2	9.66 ± 0.04[c]
Treatment 3	10.64 ± 0.03[d]

*Significant at α=5% by one way ANOVA

[abcd] Treatment means having the same letter superscript are not significantly different at 5% level of probability by DMRT.

Polycyclic Aromatic Hydrocarbons

The quantities of the 22 most commonly encountered, supposed to be most harmful PAH in micrograms per milliliter, a unit that can also be expressed in parts per million (ppm) are shown in Table 4. The only PAH that significantly increased in amount up to the third reheating was 1,6,7-trimethyl naphthalene at 5% α. No reference could be found on the allowable limit for these PAH. For the rest of the PAH analyzed, there were no increasing trend on repeated heating. Statistical test showed no significant differences on the mean for all the treatments.

According to the Official Journal of the European Union COMMISSION REGULATION (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards to the maximum levels for polycyclic aromatic hydrocarbons in foodstuffs, the

maximum amount of benzopyrene for oils and fats intended for human consumption is 2.0 microgram per kilogram of oil which is about 0.002 ppm. Benzopyrene, a proven carcinogenic and toxic PAH that is found in meat barbecue, was not detected in the analysis. The European union also stated in the same document that the sum total of benzopyrene, benzanthracene, benzofloranthene and crysene, should be at 20 µg per kg of oil which is about 0.02 ppm. The data gotten in this study showed a total average amount of 0.05 µg/mL or 0.05 ppm for benzanthracene and benzo fluoranthene only, which is higher as compared to the amount of the four PAH prescribed by the European Union.

The Agency for Toxic Substances and Disease Registry (ASTDR) in the United States released a Public Health Statement in August of 1995, Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAH) set a limit for the amount of daily PAH intake to 0.3 mg of anthracene, 0.06 mg of acenaphtene, 0.04 mg of fluoranthene, 0.04 mg of fluorene and 0.03 mg of pyrene per kilogram body weight. Data obtained by MS-GC for these PAH is below the ASTDR limits (Table 4). Benzopyrene was not included in the ASTDR limits. No other data could be found for allowable limits of daily intake in humans for the rest of the PAH included in the determination. Generally, the formation of PAH does not show an increasing trend on repeated heating but, the results showed that small quantities of this PAH is already present in coconut oil even before it was heated. These findings would further vouch that reuse of RBD-CNO for frying is not advisable.

Table 4. Polycyclic Aromatic Hydrocarbons

PAH	Polycyclic Aromatic Hydrocarbons (µg/mL) MEAN ± SD			
	Untreated	Treatment 1	Treatment 2	Treatment 3
1.) Acenaphthylene	<0.0125	<0.0125	<0.0125	<0.0125
2.) Acenaphthene	0.0078 ± 0.0001	0.0078 ± 0.0001	0.0085 ± 0.0012	0.0080 ± 0.0001
3.) Anthracene	0.0729 ± 0.0963	<0.0038	0.0055 ± 0.0098	<0.0038
4.) Benz[a]anthracene	0.0233 ± 0.0219	0.0093 ± 0.0028	0.0331 ± 0.0388	0.0350 ± 0.0219
5.) Benzo fluoranthene	0.0189 ± 0.0148	0.0081 ± 0.0037	0.0517 ± 0.0499	0.0353 ± 0.0253
6.) Benzo pyrene	0	0	0	0
7.) Fluoranthene	0.0043 ± 0.0044	0.0004 ± 0.0008	0.0063 ± 0.0038	0.0044 ± 0.0077
8.) Fluorene	0.0065 ± 0.00004	0.0064 ± 0.0001	0.0069 ± 0.0005	0.0066 ± 0.0002
9.) Naphthalene	0.0694 ± 0.0025	0.0679 ± 0.00001	0.0680 ± 0.0001	0.0686 ± 0.0012
10.) Phenanthrene	0.0192 ± 0.0207	0.0032 ± 0.0036	0.0668 ± 0.0514	0.0026 ± 0.0032
11.) Pyrene	0.0083 ± 0.0085	0.0017 ± 0.0009	0.0023 ± 0.0006	0.0044 ± 0.0046
12.) 1,4,5,9-tetramethyl Naphthalene	0.0406 ± 0.0292	0.0183 ± 0.0028	0.0610±0.0308	0.1155 ± 0.1672
13.)1,6,7-trimethylnaphthalene	0.0175 ± 0.0004	0.0173 ± 0.0007	0.0176 ± 0.0009	0.0191 ± 0.0004
14.)1-methylnaphthalene	<0.0413	<0.0413	<0.0413	<0.0413
15.)1,6-dimethyl naphthalene	0.0132 ± 0.0001	0.0133 ± 0.00001	0.0132 ± 0.0001	0.0134 ± 0.00001
16.) 2,8-dimethyldibenzothiophene	0.0399 ± 0.0219	0.0515 ± 0.0365	0.0667 ± 0.0182	0.0370 ± 0.0219
17.) 2-methylanthracene	0.0361 ± 0.0330	0.0550 ± 0.0587	0.0253 ± 0.0093	0.0262 ± 0.0133
18.) 3,6-dimethylphenanthrene	0.0190 ± 0.0017	0.0175 ± 0.0014	0.0218 ± 0.0034	0.0178 ± 0.0018
19.) 4-methyldibenzothiophene	0.0250 ± 0.0039	0.0284 ± 0.0088	0.0349 ± 0.0141	0.0317 ± 0.0143
20.) 1-methyl-9H-Fluorene	0.0575 ± 0.0615	0.0354 ± 0.0247	0.0315 ± 0.0272	0.0803 ± 0.0965
21.) 9,9-dimethyl-9-H Fluorene	0.0034 ± 0.0024	0.0033 ± 0.0025	0.0049 ± 0.0040	0.0062 ± 0.0058
22.) 1,12-dimethylbenz[a] anthracene	0.2039 ± 0.1610	0.2590 ± 0.1325	0.9063 ± 1.0029	0.8624 ± 0.4274

*1,6,7-trimethylnaphthalene is significant while the rest is not significant at α=5% by one way ANOVA

SUMMARY CONCLUSION AND RECOMMENDATIONS

Summary

This study aimed to determine the deterioration and detect the formation of toxic matter in cooking oil that was repeatedly heated to establish the stability and safety of reuse in the cooking of food, which could relate to the recurrence of some diseases associated with food preparation practices.

Cooking oil, (RBD-CNO) was used in the study. Twelve bottles of cooking oil were prepared which underwent four treatments of three replicates each. These samples were analyzed for fatty acid profile, free fatty acid, peroxides and PAH.

Among the fatty acids in the fatty acid profile, only linoleic acid had significantly decreased in quantity on the third reheating which showed that unsaturated fatty acids deteriorated more and oxidized faster than saturated fatty acids.

There were increasing trends in the amounts of free fatty acids and peroxide values which were significant at 5% α by one way ANOVA. All treatment means for both parameters were significantly different from each other at 5% level of probability by DMRT.

Except for 1, 6, 7-trimethyl naphthalene, the other PAH analyzed in this study did not show an increasing trend in the result, which means that there were no significant changes in the amount of PAH in the untreated up to the third reheating treatment.

Benzopyrene, the PAH that has been proven a carcinogen found in charcoal grilled meat (ATSDR, 1995), was not detected in the analysis.

Conclusions

The cooking oil RBD-CNO used as sample in the study exhibits minimal deterioration as the fatty acid profile showed that only linoleic acid (unsaturated fatty acid) decreased in quantity on the third heating. Free fatty acid was found to increase on repeated heating significantly but did not exceed the standard of 0.2% set by the Philippine Coconut Authority. The result revealed that there is minimal deterioration of the sample. The increase in the amount of peroxide on repeated heating, from 2.01 meq peroxide per kilogram sample to 10.64 meq peroxide per kilogram of oil on the third heating signifies that the reuse of cooking oil in the frying of food is inadvisable. This is a cause for concern as this product is carcinogenic and causes serious diseases.

The amount of PAH is also a cause for concern as some of them are carcinogenic and all of them are suspected carcinogens. Although Benzopyrene is not detected in the samples, some of the amounts of Benzanthracene and Benzo fluoranthene exceeded the amounts set by the European Union. Repeated heating of oil did not show an effect on the amount of PAH as statistical test showed no significant increase for almost all of the PAH determined except for 1, 6, 7 trimethylnaphthalene. However, it should be noted that the untreated already contained levels of PAH that are higher than the limits set by EU.

Recommendation

It is recommended that: awareness should be created among the different sectors involved in the preparation of food like the restaurants and food vendors on the ill effects of the reuse of cooking oil. For food preparation involving deep frying of food, there should be alternative ways to do the process but if unavoidable, should be used oil properly so that it does not find its way back to the consumers.
Households should also be informed about peroxides in reused cooking oil and its ill effects like cancer and heart diseases. It is further recommended that when frying foods use just enough amount of oil so there will be no excesses for reuse. There are also cookwares that make frying possible with a small amount of cooking oil

Further studies could be done on: the peroxide formation and presence of PAH on cooking oils that are reused in cooking of food and compare peroxides and PAH in unsaturated cooking oils like canola, olive oil and RBD-CNO.

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