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Characterization and Glyceride Composition Approximation Analysis of Oil from *Nypa Fruticans* (Nipa) Nuts

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Abstract

Nipa oil is unknown to chemistry handbooks. Due to lack of study on nipa nut oil, it became the subject of this study. The study aimed to characterize and approximate the glyceride composition of the oil extracted from matured nipa nuts. Nipa oil was extracted using soxhlet apparatus. The physical and chemical characteristics of nipa oil as to melting points, refractive index, saponification value and iodine value were determined. Comparative analysis of the characteristics of nipa oil with the physical and chemical constants of coconut oil, cottonseed oil, olive oil, and soya oil was done. Discrepancies were observed between the theoretical and experimental results. These were resolved on the basis of the established relationships between the oils characteristics with their glyceride structural composition. Nipa oil was found to have specific gravity of 0.9153, refractive index of 1.4730, melting point of 23.70, saponification value of 195.22, and iodine value of 88.94. Glyceride composition and structure of nipa oil were theoretically approximated to be composed of a combination of saturated fatty acids and conjugated, unsaturated fatty acids, but its chain of unsaturation is not high enough to be classified as drying or semidrying oil. It follows that nipa oil has relatively higher proportions of saturated to unsaturated fatty acid contents.

Keywords

Fats and Oils, Fatty Acids, Kernel Oil, Iodine Number, Soxhlet Extraction, Conjugated Linoleic Acid (CLA)

1. Introduction

Nypa fruticans (nipa plant) is the only species of nipa found in the world [1]. They are commonly found in South East Asia and Australia. Generally, they are found in tropical areas and can be introduced to subtropical areas. Nipa has no definite blooming season, it flowers throughout the year. Nipa nuts grow in bunch. Those nuts that grow along the central portion of the cluster or bunch are likely to contain edible meat while those at the apical and basal region may remain immature throughout the fruiting phase. Nipa nuts contain an oblong shaped edible meat which in common practice is processed as preserved fruit or can be eaten raw. Young meat is soft, sweet, and translucent with mild oily tannin-like taste.The meat of its fruits is removed by either chopping into halves or removing the husk[2]. In this study, only the meat of matured nuts were used.

Many studies had been conducted on nipa plant that led to its numerous uses. However, on its fruits, only its used as preserved food has been given importance. . Due to the plants proliferation in the Philippines and lack of study on nipa nut oil, characterization of nipa oil and glyceride composition approximation analysis became the subject of the study.

A characteristics study on nipa oil had been done by Hamzah (2011), but only its viscosity, iodine value, and saponification value were determined [3] while this study includes specific gravity, refractive index, melting point, saponification value and iodine value which are necessary to approximate the glyceride contents of the oil through rational and comparative analysis

The study aimed to characterize the nipa oil and approximate its glyceride composition. Specifically, the study aimed to determine the nipa oil's physical and chemical characteristics as to specific gravity, melting point, refractive index, saponification value and iodine value. Finally, the study aimed to theoretically approximate the glyceride contents of nipa oil through relational and comparative analysis out of the characteristics of nipa oil with other known oils. This study however, recognizes the limitation of theoretical approximation as against empirical results. Thus, spectroscopical analysis is recommended as confirmatory analytical test.

2. Methodology

Samples of nipa nuts were collected and prepared for extraction. The extracted oil was subjected to chemical analysis, specifically, its physical and chemical characteristics. The physical and chemical characteristics of the nipa oil were compared with the physical and chemical constants of other known oils, namely cottonseed oil, olive oil, soya oil and coconut oil in order to approximate the glyceride composition of nipa oil. This was complimented by relational analysis based on established relationships between the physical and chemical constants of known oils with their glyceride composition.

2.1. Collection and Preparation of Sample

The sample was prepared by breaking or chopping the nuts into halves. The matured meat was taken out from the shell, hand grated, and oven dried at 105-110°C for 12 hours. The sample was ground manually into fine powder and stored in desiccator.

2.2. Extraction of the Oil

The oil was extracted from the matured meat of the nipa nuts through soxhlet method.

About two grams of the dried sample was prepared and placed in a filter paper tied with a thread to keep the sample in place in the filter paper and in the extractor tube.

The heating was controlled so that the solvent siphoned over four (4) times an hour. The solvent used was petroleum ether which was distilled from “Petron [Extra] at a temperature range of 65-75°C. Petroleum ether being a nonpolar solvent does not take up moisture. However, its flammability was carefully noted. The heating was done consistently for 18-20 hours in every extraction.

After removing the sample from the soxhlet extractor, the apparatus was heated again and the solvent was made to siphon over once in order to flush out any oil that may adhere to the tube. The set-up was heated again until the side arm was nearly full, then heating was stopped. The solvent was removed by tilting the tube to let the liquid pass out into its container.

The remaining solvent in the flask was evaporated on a steam bath. The flask and its contents were later oven dried to constant weight for 105°C at a consistent period of 25-30 hours for every determination.

The nipa oil obtained through continuous soxhlet extraction using petroleum ether as solvent was golden yellow which became lighter when solidified. It has a mild pleasant odor. It is more viscous than coconut oil as it does not easily flow.

The oil sample was then stored and properly labeled for the determination of its physical and chemical constants.

2.3. Determination of Some Physical Characteristics

2.3.1. Refractive Index

The index of refraction of the oil was measured using Abbe Refractometer.

2.3.2. Specific Gravity

A small empty flask was weighed. Two mL of the oil was measured using a pipette and put into the flask. The flask with the oil was weighed and the weight of the oil was obtained.

The temperature was maintained at 25°C. The specific gravity was taken as the weight per unit volume of oil as compared to that of pure water. Two trials were made.

2.3.3. Melting Point

The capillary tube method was used in this determination. A column of 1-2cm of oil was drawn carefully through capillarity process into the capillary tube. One end of the tube was then sealed being careful not to burn the sample in it. The capillary tube was then attached to a thermometer so that the bulb was leveled with that of the oil in the capillary tube, then it was cooled below room temperature for 10 hours for it to solidify. Then the capillary tube with the thermometer was suspended in a beaker of water and was heated very slowly. The temperature at which the fat became transparent was taken as the melting point (clear point).

2.3.4. Solubility

The solubility of the oil in different solvents was determined. Approximately 1.0-mL of oil was added to 3.0-mL of each solvent in a vial using a 1.0 mL pipette. Each mixture was shaken thoroughly just after adding the oil. The oil was found insoluble in water; slightly soluble in 95% alcohol; and soluble in hexane, chloroform, and carbon tetrachloride.

2.4. Determination of Chemical Characteristics

2.4.1. Saponification Value

Five grams of oil was weighed accurately into a 250-mL flask. Fifty milliliters of 0.5N alcoholic KOH was added from a burette and an equal volume was added into a similar flask containing no sample. Each flask was closed with a cork with a straight glass tube. The solution was allowed to reflux gently for thirty minutes until saponification was completed. The sample and the blank were cooled and 1.0 mL of phenolphthalein solution was added to each flask. The excess alkali was back titrated with 0.5N HCl solution. The volume of 0.5N HCl required for the blank represents the strength of alcoholic potash solution. The saponification value was calculated from the difference between the numbers of mL of 0.5N HCl required for the determination. The average of the two blanks was calculated

2.4.2. Iodine Number

Iodine number was determined using the method presented by Snell and Biffen [4].

A 0.50-g oil sample was weighed into 250-mL flask. The sample was dissolved in 10.0 mL chloroform. The same volume of chloroform was placed in empty flask. Twenty five milliliters of Hanus solution were run from the burette into the flask containing the sample and an equal volume into the blank. The sample and the blank were allowed to stand for exactly thirty minutes with occasional shaking. Fifty milliliters of water and 10.0 mL of 15% KI solution were added to each flask, rinsing the sides of the flask at the same time. The solution was titrated rapidly with 0.1N $Na_2S_2O_3$ solution (sodium thiosulfate being easily attacked by microorganism was noted) with thorough shaking to extract the iodine from the solvent, until a yellow color nearly disappeared. At this point, 1.0mL of starch solution was added and the solution was titrated slowly until the blue color disappeared. The iodine value was calculated.

2.5. Theoretical Approximation

The glyceride contents of nipa oil were theoretically approximated through analysis on the basis of established structural relationships with physical and chemical constants as compared to other known oils (Fig. 1)

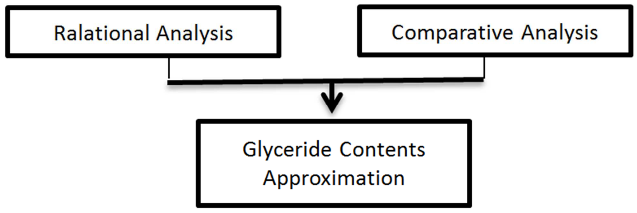

Fig. 1. *Theoretical Approximation Analysis.*

The oils and their physical and chemical constants used for comparative analysis are summarized in Table 1.

Table 1. *Physical and Chemical Constants of some known oils.*

Physical and Chemical Constants	Cottonseed oil*	Olive oil*	Soy bean oil*	Coconut oil*
Specific Gravity(15°C/13°C)	0.91825°C/25°C	0.914-0.918	0.924-0.927	0.926
Refractive Index	1.47440°C	1.46840°C	1.47340°C	1.44940°C
Melting Point/Solidification point (°C)	+12 to -13	-6	-10 to -16	14 to 22
Saponification Value	194-196	185-196	189-194	153-262

*Dean, J. A (Ed.). (1972). Lange, N. A. Handbook of Chemistry, 15th Ed. New York: McGraw Hill Book Inc. pp. 10.69-10.71 [5]

3. Results and Discussion

3.1 Results of Characterization of Nipa Oil

Lange's handbook [6]characterized fats and oils according to their physical and chemical constants: refractive index, specific gravity, melting point, viscosity, saponification value, iodine value and acid value. In this study, only those that were used in the glyceride contents approximation analysis were analyzed. Table 2 shows the results of chararacterization of Nipa Oil.

Table 2. *Physical and Chemical Charactertstics of Nipa Oil.*

Physical and Chemical Constants	Nipa Oil[1] (Average)[2]
Specific Gravity(15°C/13°C)	0.9153
Refractive Index	1.4730
Melting Point/Solidification point (°C)	23.70
Saponification Value	195.22
Iodine value	88.94

Note: Chemical Analyses were done in duplicates plus analyses of its blanks.

3.2. Molecular Weight & Melting Point

Melting points of oils are influenced much by the degree of unsaturation than their corresponding molecular weights[7]. and that the greater the degree of unsaturation, the lower the melting point[8]. Thus, saturated oils has higher melting points than unsaturated ones. Relative proportions of saturated and unsaturated fatty acid contents of oils also influence the melting points. Thus, vegetable oils generally having a higher proportion of unsaturated to saturated fatty acids than animal fats have lower melting points [9] [10].

Results showed that nipa oil has the highest melting point (23.70°C) compared to that of cottonseed oil, olive oil, soya oil, and coconut oil. The saponification value of oil falls within the range of cottonseed, olive oil and coconut oil, higher than that of soya oil. This indicates that the average molecular weight of nipa oil is approximately equivalent to both olive and cottonseed oils lower than that of soya oil.

Because molecular weight is proportional to the melting point[11], it follows that nipa oil has melting point lower than that of soya oil and approximately the same as that of olive and cottonseed oils. However, experimental data show that the melting point of nipa oil is similar to that of coconut oil and very much higher than that of soya oil, olive oil and cottonseed oil.

The disagreement in the melting points may be due to a)the difference in the proportions of unsaturated fatty acids, thus varying the relative double bonds; b) the relative proportions of fatty acids of high and low molecular weights; and c) the geometrical structure of fatty acids as to cis and trans forms. Trans fatty acid has a higher melting point than its equivalent cis form [12]. It should be noted that trans fatty acid is not common to naturally occurring oils.

Looking at the melting point of the oils, it follows that nipa oil has the highest proportion of saturated to unsaturated fatty acid contents compared to cottonseed oil, olive oil, soya oil, and coconut oil.

3.3. Iodine Value, Refractive Index & Saponification Value

Iodine value is related to the refractive index. As iodine value increases, refractive index increases in nearly linear relationship. As presented by Eckey, E. W. (1956), the average relationship between iodine value (I. V.), saponification value (S. V.), acid value (A. V.) and refractive index (N^{40}_{d}) is expressed in this equation [13]:

$$N^{40}_{d} = 1.4643 - 0.000066(\text{S. V.}) - 0.0096\ (\text{A. V./S. V.}) + 0.0001171\ (\text{I. V.})$$

Thus, nipa oil should have a refractive index nearly equal to that of olive oil, lower than that of cottonseed oil and very much lower than that of soya oil. However, experimental data show that the refractive index of nipa oil is very much closer to that of cottonseed oil, a little higher than that of olive oil, and falls within the range of that of soya oil. This disagreement may be due to the conjugation of the unsaturated oils. Conjugated oils have a higher refractive index than that of unconjugated unsaturated oil [14].

Owing to the higher melting point of oil and relatively higher refractive index, the oil may contain conjugated, trans fatty acid that could possibly be conjugated linoleic acid (CLA) [15].

It should be noted that the higher the degree of unsaturation, the greater the liability of the oil to go rancid by oxidation, thus the soya oil having higher iodine value has a greater risk of oxidative rancidity than that of nipa oil and olive oil.

3.4. Non-Drying Property

The property of oil as a drying oil may be assessed by looking at its iodine value. In the Chemical Analysis of Foods by Pearson, D. (1981), the following iodine values are presented: for non-drying oils (such as Olive oil, arachis oil, almond oil) iodine value ranges from 80-110, for semidrying oils iodine value ranges from 80-140 (such cottonseed oil, sesame oil, soya oil), and for drying oil iodine value ranges from 125 – 200 (such as linseed oil and sunflower oil) [16]. This shows that the iodine value of nipa (88.94) falls within the range of either non-drying or semidrying oils.

Looking at Table 1 and Table 2, it follows that the iodine value of nipa oil is very much close to the range of that of the non-drying olive oil (79-88) than that of semidrying soya oil (122-134) and cottonseed oils (103-111). Therefore, nipa oil can be classified as a non-drying oil. This also implies that its degree of unsaturation falls short of that of a drying oil or semidrying oil.

4. Conclusion

From the analysis based on established structural relationships of the physical and chemical constants of fats and oils complimented by comparative analysis with other known oils, nipa oil is theoretically composed of a combination of saturated fatty acids and conjugated, unsaturated fatty acids, but its chain of unsaturation is not high enough to be classified as drying or semidrying oil. It follows that nipa oil has relatively higher proportions of saturated to unsaturated fatty acid contents.

Recommendation

Like many other accepted tests, confirmatory test as to conjugation, unsaturation, and geometrical structures is recommended. Thus, spectroscopical analysis is recommended for certainty on the fatty acid structures, existence of CLA, and the lipid composition of nipa oil. Effects on melting point of other possible lipid content in nipa oil is also recommended.

On glyceride contents approximation of fats and oils may be strengthened by the following tests: thiocyanogen value to estimate the percent oleic, linoleic, and saturated fatty acids; Hehner value to estimate the total insoluble fatty acids; Polenske value to estimate the amount of caprylic, capric, and lauric acids.

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