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OIL CLASSIFICATION USING X-RAY SCATTERING AND PRINCIPAL COMPONENT ANALYSIS

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ABSTRACT

X-ray scattering techniques have been considered promising for the classification and characterization of many types of samples. This study employed this technique combined with chemical analysis and multivariate analysis to characterize 54 vegetable oil samples (being 25 olive oils) with different properties obtained in commercial establishments in Rio de Janeiro City. The samples were chemically analyzed using the following indexes: iodine, acidity, saponification and peroxide. In order to obtain the X-ray scattering spectrum, an X-ray tube with a silver anode operating at 40 kV and 50 μA was used. The results showed that oils can be divided in two large groups: olive oils and non-olive oils. Additionally, in a multivariate analysis (Principal Component Analysis - PCA), two components were obtained and accounted for more than 80% of the variance. One component was associated with chemical parameters and the other with scattering profiles of each sample. Results showed that use of X-ray scattering spectra combined with chemical analysis and PCA can be a fast, cheap and efficient method for vegetable oil characterization.

1. INTRODUCTION

Olive and non-olive oils are characterized by physical-chemical methods. This characterization combined with the type of oil extraction and treatment allows classifying them as extra-virgin, virgin or refined [1]. Extra-virgin oil (which is cold-extracted) has better quality because it lacks hydrolysis reactions that occur when oil is exposed to higher

temperatures, allowing thermal degradation of triacylglycerides, and thereby increasing the oil acidity and free fatty acids [2]. Analytical techniques are employed in order to separate and classify vegetable oils for its composition. BORTOLETO [3] proposed the use of Compton and Rayleigh scattering spectra for characterization of various samples composed essentially of light elements, including olive and non-olive oils, using Principal Component Analysis (PCA) as the statistical tool.

The increasing use of analytical techniques by X-ray spectroscopy for characterization of biological samples and materials is due to the low operating costs as, generally, there is no need for special sample preparation. They are based on detection and measurement of X-ray after its interaction with the sample. Compton scattering and Rayleigh scattering have a direct relationship with elementary composition of the spreader material. Thus, the ratio between the Rayleigh and Compton scattering can be useful in evaluating the atomic number of the material.

Combined with X-ray spectroscopy, multivariate analysis has been described in the literature as a good statistical tool for spectra analyses. PCA is described as a technique to group the studied elements according to their characteristics obtained from the studies [4].

This work aims to characterize samples of olive and non-olive oils normally found in market, using a low-power (4.0 W) X-ray Portable System, through scattered radiation, chemical parameters and multivariate analysis.

2. MATERIALS AND METHODS

2.1. Samples

Here the X-ray scattering profile of 54 samples of edible vegetable oils (25 olive oils and 29 non-olive oils) obtained from commercial establishments in Rio de Janeiro City were analyzed. In the chemical analysis, the following indexes were analyzed: acidity, iodine, peroxide and saponification. In Brazil, for food purposes, vegetable oils must comply with ANVISA [5] which requires vegetable oils, including olive oils and vegetable fats, comply with the compositional requirements set out in the Codex Alimentarius standards - FAO/OMS [6].

All olive and non-olive oils were protected from ambient light and kept under refrigeration (≈ 4.0 °C) in a refrigerator as soon as arrived in laboratory. All analyses were performed at room temperature (≈ 22 °C) with no need of additional prior preparation.

2.2. Experimental Setup and Scattering Profile Collection

The experimental setup is constituted by a Si-PIN semiconductor detector Amptek model XR-100CR and a silver (Ag) anode X-ray tube Amptek model MINI-X (4.0 W). The X-ray tube is fixed on an aluminum holder which keeps a fixed geometry while the detector is fixed on a movable platform so that the angle between the incident and scattered beams can vary (Figure 1).

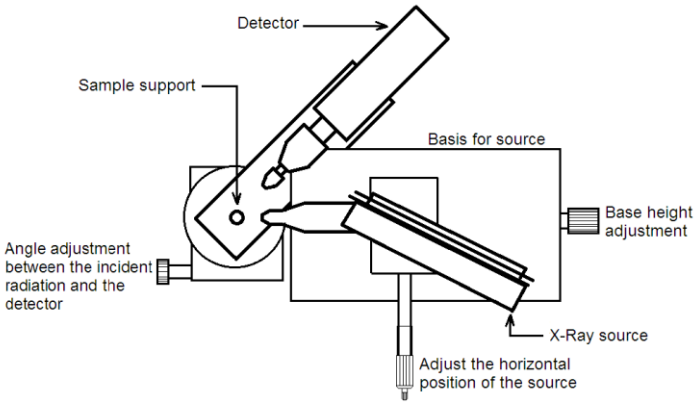

Figure 1: Schematic representation of the experimental setup.

For spectra acquisition, a voltage of 40 kV and a current of 50 μA are applied to X-ray tube during 1000 s. Aluminum collimators of 3.0 and 6.3 mm of diameter are used in X-ray tube and detector, respectively. A TiO_2 filter and a 15 μm thickness aluminum sheet are also used in the X-ray tube.

Geometry with an angle of 118º for the Compton scattering peak allows Rayleigh and Compton scattering peaks to be clearly defined and separated as shown in Figure 2.

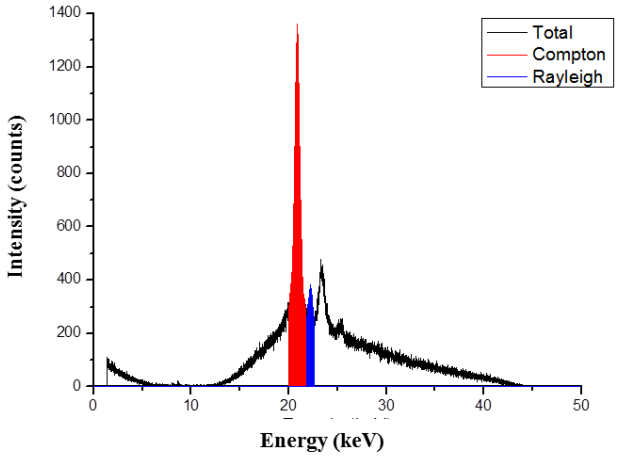

Figure 2: Representation of Rayleigh and Compton scattering peaks.

Analysis of X-ray Spectra by Interactive Least Square Fitting (AXIL) software was used for the calculation of net areas under the fotopicos generated by Compton and Rayleigh scattering.

2.3. Oil and Fat Analyses

Food analysis protocols from the Institute Adolfo Lutz [7] were used to determine acidity, iodine, peroxide and saponification indexes.

2.3.1. Determination of the acid index

The acidity index determination is based on a hot dissolution of fat in a previously neutralized solvent, followed by titration with a standard NaOH solution in the presence of phenolphthalein as an indicator.

To obtain the acidity index, an alcohol-ether solution (25 mL) and a phenolphthalein indicator are added to 2 g of the sample. Titration is then performed with a NaOH solution. Finally, the end point is observed when there is the appearance of a 30 s-persistent rosette color.

2.3.2. Determination of iodine index

This index is based on the fact that iodine and other halogens are added in a double bond of the unsaturated fatty acids chain. The iodine index by Wijis method is obtained according to the following procedure.

Iodine index is obtained by adding the Wijs solution (25 mL) and cyclohexane (10 mL) to 0.25 g of sample protected from light for 30 min. then, water (100 mL) and potassium iodine solution (KI) (10 mL) are added. Titration is carried out with addition of sodium thiosulfate until the endpoint is observed, when there is the appearance of yellowish color. The starch indicator solution is added and a dark coloration appears. Titration continues until total disappearance of the color due to addition of thiosulphate.

2.3.4. Determination of peroxide index

Peroxide index of fat is determined by dissolving a mass of fat in a chloroform-acetic acid solution, adding potassium iodide and titrating liberated iodine with a standard solution of sodium thiosulfate using starch as indicator.

To obtain the peroxide index, chloroform-acetic acid (30 mL) and KI solutions (0.5 mL) are added to 5 g of sample protected from light for 5 min. Then water (30 mL) is added and titration is conducted with sodium thiosulfate until the endpoint is observed, when there is appearance of a clear yellow color. Then, the starch indicator solution (0.5 mL) is added and titration is carried out until total disappearance of the color due to the addition of sodium thiosulfate.

2.3.5. Determination of the saponification index

Saponification index is an indicative of the relative amount of fatty acids of high and low molecular weight. Thus, saponification index of an oil or fat is defined as the number of milligrams of potassium hydroxide required to neutralize the fatty acids from the complete hydrolysis of 1 g of sample.

Sample (4.5 g) in potassium hydroxide alcoholic solution (50 mL) is heated until total saponification. Then, after cooling, phenolphthalein (1 mL) is added, and titration with hydrochloric acid is carried out until total disappearance of the color.

2.3.6. Density determination

Liquid density can be determined by mass measurements of the liquid that occupy a known volume and by flotation methods based on the Archimedes principles. Sample is placed in a vessel on an analytical balance. Then, the balance is reset and a sphere of known volume trapped in a support on the balance, is immersed in the sample. Using the Archimedes principles, the density of the sample is obtained from the ratio between the oil mass and the displaced volume, which is equal to the sphere volume.

3. RESULTS AND DISCUSSION

3.1. Acid Index

Acidity limits established by Brazilian law are 0.6 mg KOH/g, 4.0 mg KOH/g and 6.0 mg KOH/g to refined, extra-virgin and olive oils, respectively.

All oils, olive and non-olive, except the non-olive oil 61, showed values within the limits set by the Codex Alimentarius standards [6].

The acid index is an indicator of oil decomposition and free fatty acid formation. This type of decomposition is accelerated by light and heat. As acquired oils were stored in vessels wrapped with aluminum foil, protecting them from light, and stored in refrigerator, -4 °C, the effects of degradation by light and heat were reduced.

The oil 61 is rapeseed refined oil which was subjected to a frying process (180-190 °C). The acid index was very high, 2.57 ± 0.04 mg KOH/g, approximately four times above limit established by standard (0.6 mg KOH/g). This result shows that there was degradation of the oil due to thermal impact.

3.2. Iodine Index

The iodine indexes experimentally determined for all rapeseed and olive oils presented values above the limits set by the standard. Furthermore, in the case of soy oil samples, only one sample (sample 60) showed an index within limits. Values obtained for corn oils (six samples) are half within the limits (samples 7, 15 and 20) and half above limit (samples 1, 13 and 30). The iodine index for all sunflower oil samples remained within the established limits. Coconut oil (sample 34) showed an iodine index (10.9 g I_2/100 g) very close to the upper limit in the standard (6.3 - 10.6 g I_2/100 g).

3.3. Peroxide Index

The peroxide indexes for olive and non-olive oils have limits set by the Codex Alimentarius standards. Thus, for refined oils, the limit is 10 meq O_2/kg, and for extra-virgin oils the limit is 15 meq O_2/kg, and for olive oil the limit is 20 meq O_2/kg.

All samples experimentally analyzed presented values below those established by law except the samples 58 and 61. It is interesting to note that the value found for rapeseed oil that was subjected to a frying process (sample 61) was 10.52 ± 0.60 meq O_2/kg while the other

rapeseed oils samples used in this study provided values from 1.25 ± 0.10 meq O_2/kg to 2.76 ± 0.06 meq O_2/kg, which clearly shows the oil oxidation with peroxide formation when it is subjected to thermal stress.

3.4. Saponification Index

The saponification indexes of olive and non-olive oils showed values below the lower limits set by the standard except the oils 39 and 61 whose values are in accordance with standard.

According to our results, olive and non-olive oil samples showed saponification indexes very close to each other, but below the range set in the standard. These results show an indication that the analyzed olive and non-olive oils contain a predominant composition of fatty acids with high molecular weight. Saponification indexes lower than the lower limit set in standard were also obtained by CARDOSO et al. and MELLO et al. [8, 9].

3.5 - Density

Refined and extra-virgin oils have a maximum density of 0.926 g/cm³ while the olive oils have a maximum density of 0.916 g/cm³ [6]
All rapeseed oil samples showed values above the established limits. From six corn oils, four (13, 15, 20 and 30) showed values within the set by the standard. From eight soybean oils studied, half of them (17, 21, 22 and 60) were within the limits set by the standard. From five sunflower oils, only two samples (14 and 19) were within limits.

The densities experimentally obtained in this work have been measured at an average temperature of 24 °C. The density of oils and fats, in liquid state, varies with different physical parameters. The density varies inversely with temperature and varies directly with the average molecular weight of fatty acids and the degree of unsaturation of these fatty acids.

3.6. Principal Component Analysis (PCA)

To perform the PCA 11 regions with information regarding the radiation scattering profiles in each sample were selected. PCA analyses were performed using the SPSS Statistic 2.0 software for Windows.

3.6.1 Analysis of components of oils

The multivariate analysis was used to check how the samples are related. The method of multivariate analysis used was the principal component analysis (PCA). In addition, to use PCA, some factors must be analyzed. The first procedure is to analyze the suitability of the database. Suitability determines whether data is suitable for factor analysis. For this analysis, we performed the Kaiser-Meyer-Olklin (KMO) test and the Bartlett's Test of Sphericity. From analyses of KMO and Bartelett tests, data is suitable for factor analysis (KMO> 0.6 and Bartelett <0.05). It is necessary to determine the quantities of factors that best represent the pattern of correlation between the observed variables.
So, the R/C ratio, the acid index and the saponification index showed values below the acceptable value (0.500), which indicates that they presented a quite low linear correlation and therefore these variables were excluded from PCA analysis [10].

Figure 3 shows the relationship between the loads of components 1 and 2. We observed the formation of three groups: one group compound by the scattering region, another group compound by the peroxide index and type of oil (olive and non-olive oil) and a third group compound only by density. The density is represented by the positive coordinate of the component 2 and the peroxide index and type of oil in the negative coordinate of the component 2. The peroxide index is related to deterioration and presents different values in olive and non-olive oils. Formation of peroxides occurs due to oxygen reaction in double bonds. Thus, in the peroxidation reaction, there is formation of fatty acids, reducing the oil molecular weight. On the other hand, the density is directly related to molecular weight of olive and non-olive oils. Thus, the density is inversely related to peroxide index.

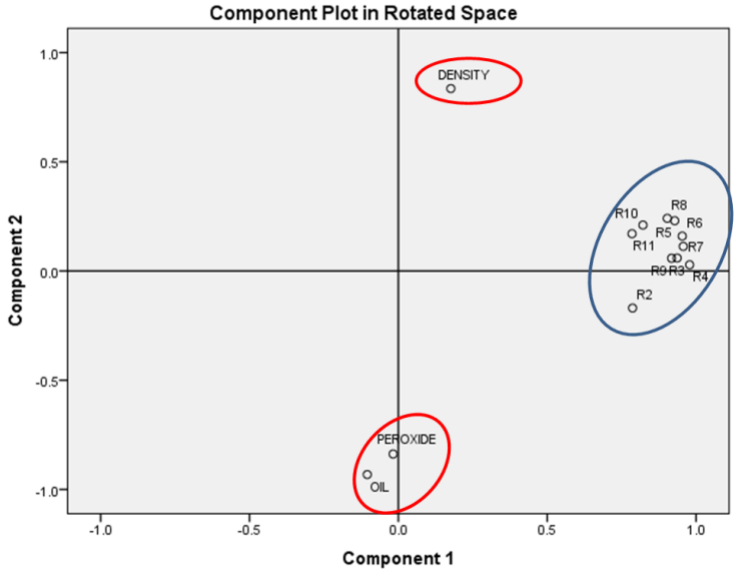

Figure 3: Principal component analysis.

Figure 4 shows the "score" graphic where the formation of two groups is observed: one blue-circled group, where the non-olive oils concentrate (48%) and another red-circled group, where the olive oils concentrate (39%). Olive and non-olive oils are distributed symmetrically in relation to the component 1 (scattering regions). In contrast, they are separated by the component 2 which is related to physical-chemical parameters. The group formed by the non-olive oils present contribution on the positive coordinates of the component 2, while olive oils present contribution on the negative coordinates of the component 2. Thus, it was possible to observe the separation of olive and non-olive oils.

In the region where the olive oils are grouped, the green points represent the refined olive oils. There is a tendency of these types of olive oils to experience only the contribution of the positive part of the component 1. The extra-virgin olive oils present a certain symmetry in relation to component 1. It was not possible to observe a clear separation between extra-virgin and refined ones. Oils marked in Figure 4 are considered "outliers" and did not show similar behavior as other olive oils in chemical and physical parameters.

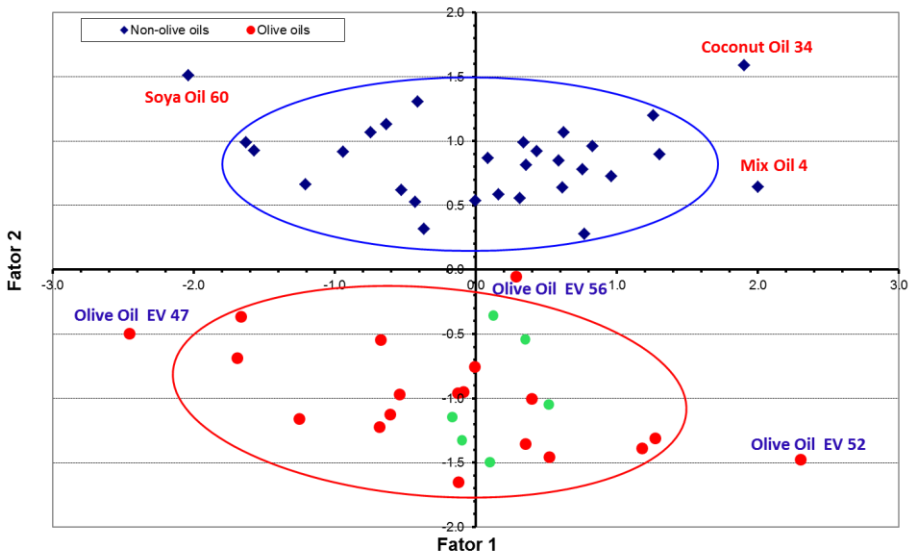

Figure 4: "Scores" graphic.

In Figure 4, most of the oils concentrate in the selected region in blue (48%) and a with a greater contribution of the component 1 (scattering regions). A certain symmetry is observed in relation to origin of the cartesian axes for the component 1. On the other hand, three oils did not show similar behavior to other oils, however, they cannot be considered "outliers" as these oils are different from other oils studied.

The mix oil (sample 4) differs from the others oils because, it has in its composition rapeseed, corn and sunflower samples. The soybean oil (sample 60) is differentiated from the others in type of extraction because it is an extra virgin oil which is cold-extracted and only by mechanical process. Besides that, it is an organic oil, i.e., its raw material is grown without chemical additives and pesticides. The coconut oil (sample 34) shows significant differences in physical-chemical parameters that characterize olive oils and non-olive oils. Thus, the coconut oil was used as a parameter to check whether there would be differentiation in the factors analyses. So, it was possible to observe the separation of these oils from the other ones (points inside the selected region in blue in Figure 4) in the factors analysis using multivariate analysis.

4. CONCLUSIONS

It was possible to separate olive from the non-olive oils through scattered radiation, chemical parameters and multivariate analysis. Two factors appear to be responsible for this separation: Factor 1 associated with radiation scattering regions and Factor 2 associated with physical-chemical parameters. In Factor 1, grouping of variables related to the X-ray scattering profile in the process of interaction with oil samples (selected regions in the scattering spectra). On the other hand, grouping of variables related to Factor 2 was associated with the peroxide index and density of the olive and non-olive oils.

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