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Peroxide Value Determination in Vegetable Oils - Comparative Analysis of Titrimetric and Spectrophotometric Methods

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Abstract: It is essential to understand the shelf life and quality of vegetable oils, as these factors are significantly influenced by the peroxide value (PV), a critical indicator of lipid oxidation [1]. Lipid oxidation not only alters the oils' sensory and nutritional properties but also leads to the formation of hydroperoxides, affecting the odor and overall quality. Traditionally, the PV is measured through iodometric titration, where iodine is released in reaction to peroxides [1]. However, the demand for quicker, more efficient, and environmentally friendly methods has led to the exploration of spectrophotometric techniques [1]. These techniques, including the International Dairy Federation (IDF) method, involve the oxidation of Fe(II) to Fe(III) ions forming colored complexes, offering a potential alternative to titrimetric methods [1]. This study evaluates and compares the effectiveness of these spectrophotometric methods against the traditional iodometric approach for determining PV in vegetable oils. It particularly focuses on the feasibility of using spectrophotometric analysis for PV quantification, aiming to provide a faster and cleaner alternative. The study also explores various solvents like isopropanol and mixtures of methanol, ethanol, and n-hexane as potential substitutes in PV measurements. Preliminary results suggest that the alternative methods, especially when paired with the right solvent mixture, can effectively determine the PV, offering a viable replacement for the standard titration procedure. This advance could meet the growing need for rapid and eco-friendly PV assessments in the food industry. The findings are significant, promising more streamlined and sustainable approaches to ensuring the quality and safety of vegetable oils.

Keywords: Vegetable Oils; Peroxide Value; Titrimetric; Spectrophotometric

1. Introduction

Lipid oxidation, which occurs during processing and storage, has significant implications for the quality and value of vegetable oils because of the unpleasant flavors that they develop [2]. During the oxidative reaction, three stages can be distinguished. The first stage involves the disappearance of oxidation substrates, such as oxygen and unsaturated lipids [3]. Then, the first compounds formed are peroxides, especially hydroperoxides, which are referred to as the primary oxidation product [4]. These products accumulate and subsequently decompose to form stable low molecular weight oxygenated constituents such as alcohols, aldehydes, free fatty acids, and ketones, resulting in rancidity [5]. Finally, the hydroperoxides usually undergo further oxidation, transforming them into secondary oxidation products. In this process, the secondary oxidation products such as aldehydes, ketones, epoxides, hydroxylated compounds, oligomers, and polymers are formed. These include volatile and non-volatile compounds, such as hexanal or malondialdehyde (MDA), respectively [3, 4].

Hydroxyperoxides can be used to assess the state of oxidation, due to the toxicity of these compounds present in oils. The peroxide value (PV) is often used as an index to measure pre-rancidity (characterized by the formation of unstable peroxides) and indicates the state of preservation of the fatty material [6]. PV is expressed in milliequivalents (meq) or millimol of hydroperoxides per kilogram (kg) of oil (meq O_2/kg of oil) [5].

For the determination of PV, various reagents can be used, including simple inorganic ions such as iodide or ferrous ion, using the traditional iodometric and spectroscopic methods, respectively [4].

Iodometry is the most conventional method, mainly due to the simplicity of the experimental procedure. Even though the lipids need to be extracted first, results are obtained quickly and clearly. In an acidic medium, hydroperoxides and other peroxides react with the iodide ion to generate iodine, which is titrated

2. Materials and methods

Peroxide values were determined by two different methods: titration and spectrophotometry.

2.1. Chemicals and materials

Acetic acid, chloroform, starch, sodium thiosulfate ($Na_2S_2O_3$), isopropanol, methanol, and n-hexane were acquired from Sigma-Aldrich (Saint Louis, USA), purity ≥99,08%. Ammonium thiocyanate (NH_4SCN), iron (II) chloride ($FeCl_2$), iron (III) chloride ($FeCl_3$), hydrochloric acid, hydrogen peroxide and nitric acid (HNO_3) were bought from Merck (Algés, Portugal).

The starch solution, 1.0 % (w/v), was prepared with deionized water and used as an indicator. The with a solution of sodium thiosulfate in the presence of starch. With this method, PV represents the amount of active oxygen (in meq) contained in 1 kg of lipid.

However, this method has disadvantages, as iodide is highly sensitive to oxidation in the presence of molecular oxygen and accelerated by light exposure [4]. In addition, overestimation can occur due to two factors, the spontaneous formation of hydroperoxides and the absorption of iodine by unsaturated fatty acids [7]. To avoid interference problems, it is necessary to use anhydrous systems, which require the extraction of lipids from oil samples, which increases in contact with oxygen [4].

In addition to the volumetric method, spectroscopic methods are quite simple and moderately sensitive, reliable, and reproducible when carried out under standardized conditions. Compared to iodometry, the ferrous oxidation method for determining peroxide content is simpler to use. This is due to the lower sensitivity of ferrous ions to spontaneous oxidation by oxygen in the air, compared to the high susceptibility to oxidation of iodide solutions. This method consists of the oxidation of Fe(II) to Fe(III), mediated by the reduction of hydroperoxides under acidic conditions and in the presence of thiocyanate or xylenol orange (FOX), where these two compounds can be measured using a UV-Vis spectrophotometer [4, 8, 9].

However, there are disadvantages. For example, the thiocyanate method requires large quantities of solvent and, as far as FOX is concerned, it detects a small range of peroxide concentrations, and the molar absorbency of the ferrilxylenol-orange complex varies according to the different dye manufacturing procedures [10].

So, besides evaluating and comparing the effectiveness of spectrophotometric methods with the traditional iodometric approach, this study also explores various solvents, such as isopropanol and mixtures of methanol, ethanol and n-hexane, as potential substitutes for measuring PV in vegetable oils.

saturated-potassium iodide solution was made by dissolving the excess potassium iodide in freshly boiled water. The sodium thiosulfate solution (0.01 M) was obtained by transferring the weighted mass of the $Na_2S_2O_3$ to a 100 mL volumetric flask and diluting it in distilled water.

Two hydrochloric acid solutions, solution I (10 mol/L) and solution II (0.2 mol/L) were also prepared.

The iron (II) chloride ($FeCl_2$) solution, 1 mg/mL, was prepared by dissolving 0.35 g of iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) in 100 mL water and 2 mL of HCl 10 mol/L.

The NH_4SCN solution was obtained by dissolving the weighed mass in 100 mL of deionized water.

For the titration method, a dissolving solvent for the oil samples was prepared by mixing acetic acid with chloroform in a 3:2 ratio.

As for the spectrophotometry analysis with quartz photometer cells, two different solvents using (i) isopropanol and (ii) a mixture of methanol/1-decanol/n-hexane in a 3:2:1 volume fraction ratio was employed.

All reagents were of analytical grade and the solutions prepared daily and stored in the dark.

2.2. Samples

Three distinct vegetable oil samples (1 refined olive oil, 1 extra-virgin olive oil and 1 sunflower oil) were used in this study.

2.3. Instruments

A BioTek, Synergy HT spectophotometer was used to perform the spectrophotometric measurements.

2.4. Peroxide value determination by titration

The peroxide value of the three oil samples was determined according to [11]. Briefly, each oil sample was weighted (~5.0 g) and dissolved in 30.0 mL of the acetic acid: chloroform solution (3:2 ratio). Then, 0.5 mL of the saturated-KI solution was added, and to the mixture was shaken for 1 min. Immediately after 30 mL of water followed by 0.5 mL of the starch solution was added to the mixture and titrated with the standard sodium thiosulfate (0.1 M).

For each sample, a separate blank analysis was employed. The peroxide value was determined using the following equation 1:

$$PV = \frac{(V - V_0) \times C}{m} \times 1000$$

where m was the mass of oil (g), C the concentration of sodium thiosulphate (meq/mL), V volume of titrant (mL) for sample and V_0 volume of titrant (mL) for blank.

2.5. Peroxide value determination by spectrophotometry

The peroxide values measured by spectrophotometry were adapted from ISO:3976, IDF 74 [12]. Briefly, 0.33 g of each sample were weighed into a test tube. Then, 9.60 mL of either isopropanol or the isopropanol mix were added to the oil samples. Afterwards, 0.05 mL of the ammonium thiocyanate solution was inserted into the sample mixture. Finally, all blank samples and oil samples were transferred to a photometer cell, which rested for 10 min to obtain equilibrium before each measurement.

3. Results and discussion

Extra-virgin olive oil obtains the highest peroxide value for the 3 methos. To determine the PV, titration and the spectrophotometric method were used. The latter using the isopropanol and a mixture of methanol, ethanol and n-hexane as solvents.After titration, equation 1 was used, and the absorbances were obtained using spectroscopic methods to obtain the PV value (Table 1).

Analyzing table 1, the lowest peroxide value was observed by titration for the olive oil sample (0.185 ± 0.021 mEq O_2/kg), whereas the highest was detected in the extra-virgin olive oil by titration (7.39 ± 0.89 mEq O_2/kg).

Overall, comparing the PV obtained from the two spectrophotometry solvents, we can observe that, in general, the mix has a better peroxide extraction capacity than isopropanol, except in the extra virgin olive oil sample, since the PV obtained did not change significantly with the difference in solvents. Comparing the results for each sample, it is evident that the results for the olive oil samples have lest difference between the two methods, followed by the sunflower vegetable oil.

The results presented in this study (Table 1) are similar to those reported by Ghohestani et al. (2023) [2], who developed a new paper-based analytical device to quantify the PV in vegetable oils, comparing it with the standard iodometric titration method. They reported PVs for sunflower oil ranging from 0.55 ± 0.03 to 5.68 ± 0.43 mEq O_2/kg and for olive oil ranging from 3.90 ± 0.28 to 22.94 ± 1.12 mEq O_2/kg using the standard iodometric titration. With the paper-based device, they observed values from 0.8 ± 0.07 to 4.48 ± 0.71 mEq O_2/kg for sunflower oil and from 4.65 ± 0.31 to 18.97 ± 2.14 mEq O_2/kg for olive oil.

Unfortunately, Ghohestani et al. (2023) [2] did not analyze an extra-virgin olive oil sample.

Table 1. Peroxide Value (mEq O2/kg) in vegetable oils: a comparison between the IDF method using two different solvents and the titration method.

Sample	Isopropanol	Mix	Titration
Olive oil	0.430 ± 0.023	0.729 ± 0.032	0.185 ± 0.021
Virgin olive oil	6.16 ± 0.16	6.02 ± 0.23	7.39 ± 0.89
Sunflower oil	0.457 ± 0.013	0.931 ± 0.056	0.370 ± 0.045

4. Conclusions

The peroxide value determination is a very important to evaluate vegetable oils. With the present work it was possible to evaluate that these two different solvents obtain the same profile. However, comparing the two solvents used for the spectrophotometric method the data obtained with isopropanol is more similar with the data obtain by the titration method.

The iodometric method has disadvantages in meeting an increasing demand for rapid, clean, and cost-effective PV measurements. To dissolve the samples and extract the fats, less toxic solvents such as isopropanol can be used instead of chloroform.

This work shows that there are alternative and greener analytical methods capable of replacing the standard titration procedure currently in use for determining PV in vegetable oils.

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Author Contributions

Santos Francisca: Methodology, Investigation, Writing - original draft. Morais Stephanie: Methodology, Validation, Investigation. Ramalhosa, Maria João: Writing – review & editing. Delerue-Matos, Cristina: Resources. Domingues Valentina F.: Conceptualization, Validation, Supervision, Writing - review & editing. Soares Cristina Conceptualization, Validation, Supervision, Writing - review & editing.

Conflicts of Interest

The authors declare no conflict of interest.

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