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Short Report

A Green Potentiometric Method for Determination of the Acid Number of Oils and Fats

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Um método potenciométrico verde é proposto para a determinação do número de acidez de óleos vegetais e de gorduras animais. A amostra é dissolvida em mistura etanol-água (1:1 v/v) e titulada, usando um eletrodo combinado de vidro, com uma solução aquosa padrão 0,02 mol L^{-1} de NaOH. Analisaram-se óleos de canola, girassol, linhaça, mamona, milho e soja além de gordura de porco, em um total de doze amostras. Os resultados obtidos com o método proposto foram estatisticamente comparados com aqueles resultantes dos métodos AOCS Cd 3d-63, ABNT NBR 14448 e de titulação verde com detecção visual, através de procedimento de regressão linear em nível de confiança de 95%, não sendo encontrada diferença sistemática. O desvio padrão relativo médio observado para o método proposto foi de 2,7%, enquanto que para os procedimentos ABNT e AOCS foi de 5,4% e para o método verde de titulação visual foi 4,8%.

A green titrimetric method using potentiometry is proposed for determination of the acid number of vegetable oils and animal fats. The sample is dissolved in a water-ethanol mixture (1:1 v/v) and potentiometrically titrated with a 0.02 mol L^{-1} aqueous NaOH standardized solution using a glass pH combined electrode. Canola, sunflower, linseed, castor, corn and soy oils as well as swine lard, a total of twelve real samples, were analyzed. The results were compared with those from the application of the procedures AOCS Cd 3d-63 and ABNT-NBR 14448 and a green visual titrimetric method, through a statistical linear regression procedure at the 95% confidence level. No evidence for systematic differences was observed. Mean relative standard deviation for the proposed procedure was 2.7%, whereas that for the AOCS and ABNT methods 5.4% and for the visual green method 4.8%.

Keywords: acid number, vegetable oil, animal fat, potentiometric method, green chemistry

Introduction

Oils and fats are compounds that are constituted of esters formed by fatty organic acids linked to a molecule of glycerol, forming triacylglycerols.[1-3] Due to their structural composition, they are susceptible to diverse degradation reactions.[4]

In the hydrolytic rancidity reactions, the carboxylic group suffers the action of enzymes of a microbial order commonly found in oleaginous seeds. It can also react with water. These reactions cause the breaking of the triacylglycerol molecule leading to the formation of free fatty acids which are responsible for the unpleasant flavor characteristic of the oxidized fats as for example, rancid butter.[5-7] The oxidative rancidity reactions are characterized by reactions of the unsaturations in the fatty acid chain and by the formation of degradation compounds such as alcohols, aldehydes, ketones, etc.[8-10] The oxidation reactions are influenced by several external factors as, for example, oxygen from the air, temperature, the presence of metallic cations, light and humidity.[11-14]

The presence of free fatty acids is undesirable in oils and fats as it reflects the nutritional quality of the product. The quantity of these acids indicates how the feedstock was treated during industrial processing and during the storage. High concentrations mean loss of money as the rating of the product decreases. Therefore, the determination of their concentrations throughout the refining process and during the storage is important for monitoring the occurrence of degradation reactions.[9,10,15,16]

During the cooking of the food submitted to frying, the triacylglycerols are degraded as a consequence of the

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high temperature, of the loss of water and of exposure to the oxygen of the air.[9,10,16-18] Besides the formation of the oxidized products and the resulting disagreeable flavor and taste, polymeric compounds can be produced which present unhealthy properties as they can promote arteriosclerosis and cancer.[9,16,17] Therefore, it is very important to know if an oil or a fat is appropriate for human intake because as part of the diet it is absorbed by the body.

The acid number is a parameter that quantifies the acid products of degradation reactions of an oil or a fat. It is expressed in mg of KOH *per* g of sample. The usual method for its determination is the AOCS Cd 3d-63 (AOCS, 1973) procedure, in which the sample is dissolved in toluene and isopropyl alcohol and a visual titration is performed with a 0.1 mol L^{-1} KOH solution prepared in this alcohol. The detection of the end point is done with phenolphthalein.

This non aqueous potentiometric titration presents as a disadvantage the use of organic solvents that, besides the toxicological and environmental problems of the solvents, also causes the dehydration of the glass membrane of the electrode. Several authors, with the aim of overcoming these aspects have described methods using infrared detection,[19-21] flow injection with detection in the visible region of the spectrum[22,23] and an absorbance/fluorescence procedure.[24]

Recently a green visual titrimetric method was presented for the determination of the acid number of oils and fats, using as titrant an aqueous 0.02 mol L^{-1} NaOH solution, and a mixture of water and aqueous ethanol as solvent.[25] Despite the good results and the simplicity of this method, an instrumental option was desired. Therefore, the present work has as objective to apply potentiometric detection to the titrimetric method aiming at low cost, operational simplicity and, especially, as environmentally friendly procedure. Knowledge related to this subject, acquired in developing earlier methods, was applied in the present work.[25-27]

Experimental

Materials and methods

Instruments

A Metrohm model 691 pH meter and a Metrohm 808 Titrando titrator were used to perform, respectively, point-by-point and automatic titration. A Metrohm Solvotrode, (3.0 mol L^{-1} in ethanol) electrode was used for the ABNT-NBR 14448 method and a 3.0 mol L^{-1} aqueous solution was used for the green method proposed herein. The electrodes were calibrated as usual with aqueous buffer solutions of pH 7.00 ± 0.05 (25 ºC, phosphate buffer) and pH 4.00 ± 0.05 (25 ºC, acetate buffer) before the analytical measurements. The potentiometric curves obtained with point-by-point titrations were constructed using Origin® 7.0 software and the bisectrix method was applied to obtain equivalence points. The automatic titrator Metrohm Tiamo® software was used to determine the equivalence points through the first derivative method.

An ultrasonic bath was used to degasify solvents and solutions. A Biohit 50 mL Digitall Burette and a Brand 50 mL Digital Burette III were used for the point-by-point titrations.

Reagents, solutions and solvents

All the reagents and solvents were of analytical grade. Distilled water was obtained from a glass distiller. Solutions of sodium hydroxide and of potassium hydroxide were prepared by dissolving adequate masses of the bases in water. The obtained concentrations were standardized through automatic potentiometric titration using potassium biphthalate as primary standard.

Soy, corn, canola, sunflower, linseed and castor oils and swine lard used in the present study were purchased at local markets.

Analytical procedures

Green potentiometric method

Weight with at least 0.01 g precision, 20 g of the sample directly into a 200 mL tall form beaker. Using a graduated cylinder, add 75 mL of the 1:1 v/v water/ethanol mixture, and gently mix with a stirring bar on a magnetic stirrer for 1 min. Potentiometrically titrate with 0.02 mol L^{-1} aqueous sodium hydroxide standardized solution. After each titration, carefully rinse the electrode with small portions of ethanol (total of 15 mL) to remove traces of oil or fat, followed by washing with distilled water, and immerse the electrode in distilled water for 1 min to rehydrate the glass membrane. The acid number of the 75 mL of solvent (blank value) must be obtained by a similar titration. The acid number is calculated using equation 1 where A is the titrant solution volume (mL) used in the titration of the sample, B is the titrant solution volume (mL) used in titration of the blank. C is the concentration of the titrant solution (mol L^{-1}), and m is the mass of the sample (g).

$$\text{Acid number} = \frac{(A - B) \times C \times 56.1}{m} \ (\text{mg KOH g}^{-1} \text{ sample}) \quad (1)$$

AOCS Cd 3d-63 official method[28]

The acid number of oils and fats is determined through the titration of the sample with a standardized 0.1 mol L^{-1}

solution of KOH in isopropyl alcohol. According to this method, a quantity of the sample, which varies with its acidity, is dissolved in 125 mL of a solvent that is a 1:1 v/v mixture of toluene with isopropyl alcohol. This solvent mixture is previously neutralized with the standard KOH solution using phenolphthalein until the appearance of a slight pink color. The sample is then added and the obtained solution is titrated under vigorous shaking throughout until the moment a pink color that persists for 30 s is observed. The acid number of oils and fats is calculated through equation 1.

ABNT-NBR 14448 method[29]

Weigh the sample directly into a 200 mL tall form beaker. Add 125 mL of the toluene/isopropyl alcohol/water (1.0:0.95:0.5 v/v/v) mixture and mix gently with a magnetic stirrer for 1 min. Potentiometrically titrate with 0.1 mol L^{-1} potassium hydroxide in isopropyl alcohol standardized against potassium biphthalate. The electrode (3.0 mol L^{-1} LiCl in ethanol) is rinsed with isopropyl alcohol and also with the solvent mixture. Then, it is placed in distilled water for 5 min to rehydrate the glass membrane. It was observed in the present work, that after about 10 titrations, it is advisable to keep the electrode immersed in a 0.1 mol L^{-1} HCl solution for at least 24 h in order to re-activate the glass membrane. The acid number is calculated using equation 1. It should be noted that this method was originally proposed for the determination of the acid number of mineral oils and of biodiesel.

Statistical treatment

Comparison of the analytical results of the proposed method with those of the AOCS Cd 3d-63, of the ABNT-NBR 14448 and with the visual green titration[25] procedures was performed through weighted linear regression procedure at 95 and at 99% confidence level.[30,31]

Results and Discussion

In order to verify the stability of the basic titrant solutions, 0.1 mol L^{-1} KOH in isopropyl alcohol (AOCS and ABNT) and 0.02 mol L^{-1} NaOH in water (potentiometric green method), standardization against potassium biphthalate using an automatic potentiometric procedure was repeated throughout 20 days. The solutions of the bases were stored in alkaline resistant bottles. Both 0.1 mol L^{-1} KOH in isopropyl alcohol (relative standard deviation (RSD) of 2.2%) and aqueous 0.02 mol L^{-1} NaOH (RSD of 1.5%) solutions were stable during this period within the precision of the standardization method.

Soy, corn, canola, sunflower, linseed and castor oils and swine lard were used in the present study. The potentiometric method herein proposed was performed point-by-point and also using an automatic titrator in order to obtain the acid number of the solvents. Figure 1 shows titrations curves obtained for the ethanol-water solvent by these two methods.

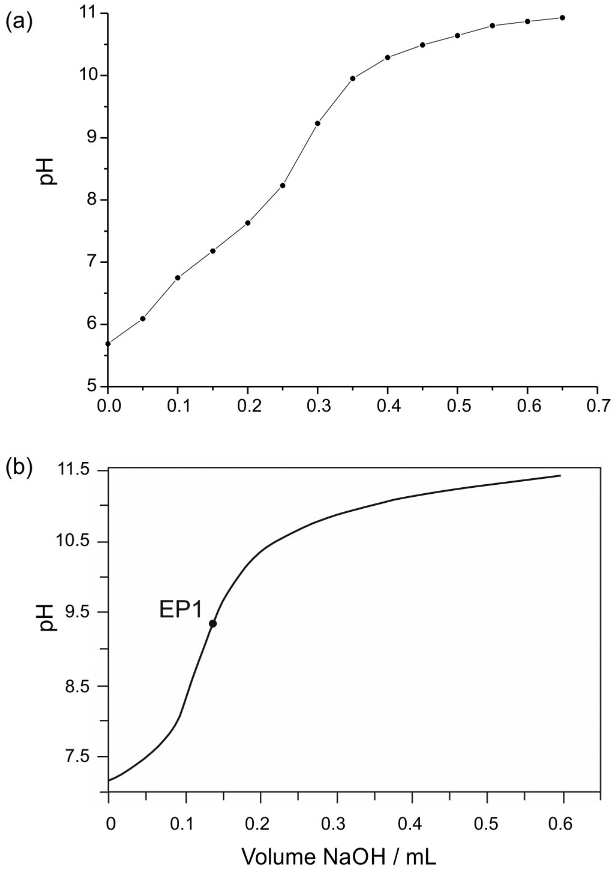

Figure 1. Potentiometric titration curves obtained by the application of the proposed green potentiometric method on the ethanol-water solvent: (a) point-by-point titration and (b) Metrohm Titrando 808 automatic titrator. In both cases, a glass pH electrode (Ecotrode® 3.0 mol L^{-1} KCl) was used.

The potentiometric titration curves obtained for the oils and for swine lard are quite similar to those seen in Figure 1, and therefore, the end point is easily determined. Table 1 shows the acid numbers obtained for swine lard and for the vegetable oils (canola, sunflower, linseed, castor, corn and soy) applying the AOCS Cd 3d-63, the ABNT-NBR 14448, the green visual[25] and the proposed potentiometric methods.

Observing the results in Table 1, it is easy to note that those of the official AOCS method present a tendency to be a little higher than those of the proposed method and also of the others. This can be attributed to the fact that it is more difficult to determine the end point of the titration with the official method than in the other cases. With respect to the precision of the methods, it is clear that the green

Table 1. Values of acid number (± standard deviation) obtained with the ABNT NBR 14448 (ABNT), AOCS Cd 3d-63 (AOCS) and green visual (VIS) methods *vs.* the potentiometric (POT) method proposed herein for oils and fat

	ABNT ± SD / (mg g^{-1})	RSD / %	POT ± SD / (mg g^{-1})	RSD / %	AOCS ± SD[c] / (mg g^{-1})	RSD / %	VIS ± SD[c] / (mg g^{-1})	RSD / %
Canola A[a]	0.137 ± 0.005	3.6	0.140 ± 0.005	3.6	0.155 ± 0.008	5.2	0.146 ± 0.011	7.5
Sunflower A[a]	0.083 ± 0.005	6.0	0.080 ± 0.004	5.0	0.092 ± 0.005	5.4	0.077 ± 0.003	3.9
Linseed A[a]	1.655 ± 0.041	2.5	1.611 ± 0.028	1.7	1.689 ± 0.048	2.8	1.738 ± 0.031	1.8
Corn A[a]	0.084 ± 0.013	15.5	0.085 ± 0.005	5.9	0.119 ± 0.010	8.4	0.089 ± 0.010	11.2
Soy A[a]	0.077 ± 0.008	10.4	0.084 ± 0.002	2.4	0.073 ± 0.005	6.8	0.079 ± 0.002	2.5
Swine lard[b]	0.434 ± 0.008	1.8	0.420 ± 0.007	1.7	0.470 ± 0.034	7.2	0.427 ± 0.015	3.5
Canola B[b]	0.142 ± 0.004	2.8	0.152 ± 0.002	1.3	0.152 ± 0.009	5.9	0.156 ± 0.005	3.2
Sunflower B[b]	0.100 ± 0.008	8.0	0.095 ± 0.002	2.1	0.104 ± 0.009	8.7	0.102 ± 0.007	6.9
Linseed B[b]	5.520 ± 0.281	5.1	5.473 ± 0.053	1.0	5.679 ± 0.215	3.8	5.611 ± 0.192	3.4
Castor[b]	0.758 ± 0.003	0.4	0.739 ± 0.012	1.6	0.815 ± 0.018	2.2	0.743 ± 0.018	2.4
Corn B[b]	0.099 ± 0.005	5.1	0.108 ± 0.003	2.8	0.113 ± 0.004	3.5	0.091 ± 0.008	8.8
Soy B[b]	0.084 ± 0.003	3.6	0.082 ± 0.003	3.7	0.083 ± 0.004	4.8	0.079 ± 0.002	2.5
Mean RSD / %		5.4		2.7		5.4		4.8

[a]Point-by-point potentiometric procedure. [b]Automatic potentiometric procedure. [c]Reference 25. Number of replicates: 5; RSD: relative standard deviation in %.

potentiometric procedure herein proposed presents the best performance. The other methods present similar standard deviation among them with a little lower value for RSD for green visual procedure. In order to verify the accuracy of the potentiometric procedure developed with respect to the AOCS Cd 3d-63 and to the two other applied methods, a statistical weighted linear regression procedure was used. For the comparison of the precisions of the methods, the *F*-test was applied.[30,31]

Table 2 shows the statistical comparison of the results obtained with the ABNT, AOCS[25] and VIS[25] methods *vs.* the POT procedure through the weighted linear regression method at the 95 and 99% confidence levels.[30,31] The results of each method are correlated with those of the POT method through a linear equation: (ABNT, AOCS, VIS) = a + b POT. The limits of confidence of the parameters a and b are calculated as confidence limits: the linear coefficient confidence limit = a ± (SD × *t*) whereas the confidence limit of the slope = b ± (SD × *t*). For the 95% confidence level, the student's *t*-value is 2.23 and for the 99% confidence level, *t*-value is 3.17 (degree of freedom = 10). As can be seen in the results presented in Table 2, the calculated slopes and the intercepts are close to the ideal 1 and 0, respectively, in all cases. However, in the comparison of the proposed method with the AOCS procedure, agreement was observed only at the 99% confidence level. This fact can be attributed to the tendency of the AOCS method to produce higher results because of the difficulty in the accurate determination of the end point of the visual titration of a colored sample. If

Table 2. Statistical comparison through the weighted linear regression procedure of the results obtained with the ABNT NBR 14448 (ABNT), AOCS Cd 3d-63 (AOCS) and the green visual (VIS) methods *vs.* the potentiometric (POT) procedure proposed herein at the 95 and at 99% confidence levels (canola, sunflower, linseeds, castor, corn, soy oils and swine lard). Student's *t*-values are 2.23 and 3.17, respectively (degree of freedom = 10)[30]

	A ± SD	B ± SD	R^2	Confidence limits A ± (SD × t)	Confidence limits B ± (SD × t)	Confidence level / %
POT *vs.* AOCS	0.004 ± 0.005	0.936 ± 0.024	0.993	0.004 ± 0.010	0.936 ± 0.054	95
				0.004 ± 0.014	0.936 ± 0.077	99
POT *vs.* ABNT	0.005 ± 0.003	0.983 ± 0.016	0.997	0.005 ± 0.006	0.983 ± 0.035	95
				0.005 ± 0.009	0.983 ± 0.050	99
POT *vs.* VIS	0.004 ± 0.003	0.964 ± 0.017	0.996	0.004 ± 0.007	0.964 ± 0.039	95
				0.004 ± 0.010	0.964 ± 0.055	99
ABNT *vs.* AOCS	0.000 ± 0.003	0.930 ± 0.007	0.999	0.000 ± 0.006	0.930 ± 0.015	95
				0.000 ± 0.010	0.930 ± 0.021	99

SD: standard deviation.

Table 3. Statistical comparison of the precision of the ABNT NBR 14448 (ABNT), AOCS Cd 3d-63 (AOCS), green potentiometric (POT) and green visual (VIS) methods using the statistical *F*-test. *F*-tabulated = 9.6 ($n_1 = n_2 = 5$; $\nu = 4$; $\alpha = 0.05$); $F = 23.2$ ($n_1 = n_2 = 5$; $\nu = 4$ $\alpha = 0.01$)[30,31]

	F-calculated			
	POT *vs.* AOCS	POT *vs.* ABNT	POT *vs.* VIS	ABNT *vs.* AOCS
Canola A[a]	2.6	1.0	4.9	2.6
Sunflower A[a]	1.6	1.6	1.8	1.0
Linseed A[a]	2.9	2.1	1.2	1.4
Corn A[a]	4.0	6.8	4.0	1.7
Soy A[a]	6.3	16.0	1.0	2.6
Swine lard[b]	23.6	1.3	4.6	18.1
Canola B[b]	20.3	4.0	6.3	5.1
Sunflower B[b]	20.3	16.0	12.3	1.3
Linseed B[b]	16.5	28.1	13.1	1.7
Castor[b]	2.3	16.0	2.3	36.0
Corn B[b]	1.8	2.8	7.1	1.6
Soy B[b]	1.8	1.0	2.3	1.8

[a]Point-to-point potentiometric titration. [b]Automatic potentiometric titration.

the AOCS procedure is compared with the ABNT method, the statistical treatment shows that there is no agreement between the two even at 99% confidence level, because of the tendency of the higher results of the first method as above discussed. The green potentiometric procedure here proposed presents statistical concordance with the ABNT and with the green visual[25] (VIS) methods at 95% confidence level and at 99% confidence level with the AOCS method.[30,31]

Based on the *F*-calculated values in Table 3, the comparison of the precision of the methods two by two can be done and statistical concordance was observed in the majority of the cases at 95% confidence level ($\alpha = 0.05$), with the following exceptions. (*i*) In the POT *vs.* AOCS case, for canola B, sunflower B and linseed B oils, statistical agreement occurs at 99% confidence level ($\alpha = 0.01$). For swine lard, *F*-calculated is higher than the tabulated, even for $\alpha = 0.01$, and therefore, there is no agreement in this case. In conformity with Table 2, this disagreement is caused by the higher imprecision of the AOCS method with respect to the here proposed. (*ii*) In the POT *vs.* ABNT comparison, for the soy A, sunflower B and for castor oils, there is agreement at 99% confidence level. For linseed, no statistical concordance was observed. It is easy to note in Table 2 that the high *F*-values occur due to the larger imprecision of the ABNT method. (*iii*) In the comparison of the POT *vs.* the VIS methods, in two cases, sunflower B and castor oils, the statistical agreement is observed at 99% confidence level. (*iv*) In the comparison between ABNT *vs.* AOCS procedures for swine lard, there is statistical agreement at $\alpha = 0.01$. For the castor oil, however, no concordance was found. Because of the above observations, it is evident that the precision of the proposed green potentiometric method is higher than of the all others here used for comparison.

Despite the fact that the potentiometric methods can be more easily applied, in comparison to the visual method, as they do not depend on the color of the sample, in presence of organic solvents, the glass membrane of the electrode is dehydrated, a fact that results in instability of the measured electric potential. The membrane must be frequently rehydrated or the electrode can be irreversibly damaged. In the green method proposed in this article, the solvent is a 1:1 v/v aqueous solution of ethanol, thus this problem is significantly minimized. Rapid preventive rehydration (1 min) is enough to maintain the membrane of the electrode in good conditions.

The automatic potentiometric titration is usually done in about 5 min, whereas the point-by-point procedure needs about 30 min. The shorter time of the automatic procedure means, as an evident consequence, that the glass membrane remains in contact with the solution for a shorter time interval in comparison with the point-by-point titration. This situation not only reduces the time of the analysis, but also minimizes the possible effect of dehydration of the electrode as it remains for a shorter period in contact with the 1:1 v/v ethanol-water solution.

Conclusion

The green potentiometric method for the determination of the acid number of oils and fats herein proposed furnishes similar but more accurate and precise results to those obtained by the AOCS Cd 3d-63 and by the ABNT-NBR 14448 methods that use organic solvents, such as toluene, and as titrant a KOH solution in isopropyl alcohol. It also furnishes equivalent results to those obtained by the visual green method with the advantage that the end point of the titration is determined instrumentally. Comparison of the AOCS, ABNT and the green visual methods *vs.* the green potentiometric procedure herein presented was done through the weighted linear regression correlation of the results at the 95% confidence level. No evidence for systematic differences between the different sets of results was observed, except in the case of the comparison between the AOCS and the ABNT methods.

The proposed green potentiometric procedure presents less toxic characteristics than the official procedures as it uses ethanol and water as solvents in place of isopropyl alcohol and toluene and therefore is greener.

Considering the above observations, the green method developed in this work can be proposed for the routine determination of the acid number of oils and fats.

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