VALIDATION OF A MICROWAVE-ASSISTED PROTOCOL FOR THE DETERMINATION OF SAPONIFICATION NUMBER: A COMPARISON TO ISO 3657:2020

Saponification number in oils measures the amount of alkali required to convert fats into soap and it is a crucial parameter for formulation of industrial or artisanal soap. Microwave assisted solvent extraction is a well-established sample preparation technique and offers a reliable and efficient approach for its quantification. Milestone ETHOS X equipped with fastEX-24 eT rotor was used in this study to demonstrate its effectiveness in comparison with official ISO 3657:2020 method and European Pharmacopoeia.

INTRODUCTION

One of the most commonly used analytical techniques to evaluate the quality of oils and fats is the saponification number (or value). It is a measure of the free and esterified fatty acids present in a lipid-containing matrix.

The analysis, based on the ISO 3657:2020 method[1], consists in titrating the amount of KOH in excess for the complete saponification of one gram of oil or fat. The saponification number is an indirect measure of the length of the fatty acids in the lipid. A high saponification number, such as that of coconut or palm oil, is desirable for soap production. Another important use of the parameter is quality control for possible adulteration. [2]

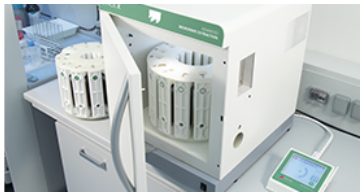
Although the ISO 3657:2020 reference method is simple and accurate, it is time consuming and requires adequate laboratory space if this analysis is to be performed routinely in the laboratory.

Thus, with the aim of optimizing analysis, Microwave Assisted Extraction is a good candidate as a new time saving and reliable method for determining the saponification value. [3]

In the present work we have developed a simple, accurate and faster method for the determination of the saponification value using ETHOS X together with the fastEX-24 eT rotor, reducing the preparation time and the space required to carry out the analyses.

The results obtained were compared with the ISO 3657:2020 method, firstly on some reference vegetable oils and then on the most widely used oils on the market.

Finally, the microwave method is also applied to some widely used cosmetic raw materials, obtaining a comparison also with the official method described by the European Pharmacopoeia. [4]



EXPERIMENTAL

EQUIPMENT

- Milestone's ETHOS X [5] with easyTEMP
- FastEX-24 eT rotor.
- 100-mL disposable glass vials.

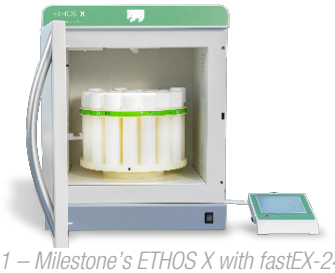


Figure 1 – Milestone's ETHOS X with fastEX-24 eT.

STANDARD AND REAGENTS

Potassium hydroxide (KOH) 0.5 M solution in ethanol and hydrochloric acid (HCl) 0.5 M standard volumetric solutions were used for saponification and titration respectively with phenolphthalein solution (0.1 g / 100 ml of 96 % ethanol) as indicator. All chemicals and solvent used are analytical grade and were purchased from Sigma-Aldrich (Milan, Italy).

SAMPLES

The reference vegetable oils used in this study were purchased from BIPEA (Proficiency testing programs Paris – FRANCE) while commercial vegetable oils come from the local market or supplied directly by Associazione Granaria-Milan (Italy); for cosmetics raw materials laboratory samples with certified value were employed.

SAMPLE PREPARATION

Sample amount depends on the expected saponification value and it is suggested respectively by the ISO 3657:2020 method for vegetable oils reported on Table 1 and by Pharmacopeia 01/2008:20506 for cosmetic raw materials reported on Table 2:

Expected saponification value	Sample amount (g)
150 to 200	2.2 to 1.8
200 to 250	1.7 to 1.4
250 to 300	1.3 to 1.2
> 300	1.1 to 1.0

Table 1 Oil sample quantities as reported by ISO 3657:2020 method based on expected saponification value.

Expected saponification value	Sample amount (g)
< 3	20
3 to 10	15 to 12
10 to 40	12 to 8
40 to 60	8 to 5
60 to 100	5 to 3
100 to 200	3 to 2.5
200 to 300	2 to 1
300 to 400	1 to 0.5

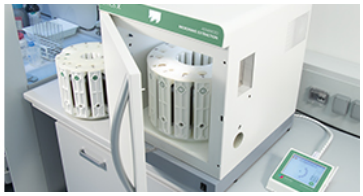
Table 2 Cosmetic sample quantities as reported by Pharmacopeia 01/2008:20506 based on expected saponification value.

MICROWAVE-ASSISTED SAPONIFICATION

Sample to be saponified was added to the glass vials of the fastEX-24 rotor and 25 mL of ethanolic KOH 0.5 M solution was added followed by stirring bar. The following temperature method was employed under continuous stirring:

Step	Time (min)	Power (W)	Temperature (°C)
1	5	800	120
2	15	800	120

Table -3 Microwave saponification program



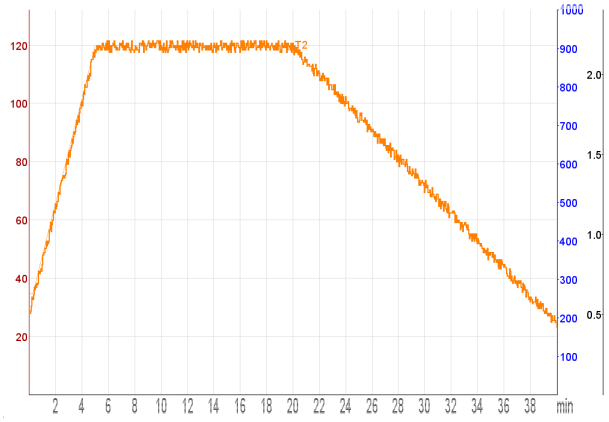


Figure 2 – Microwave run profile

After cooling, the exceeding amount of KOH is directly titrated with HCl 0.5 M into the glass vial, adding also 0.5-1 mL of Phenolphthalein color indicator solution. The equivalence point is reached when the color changes from pink/purple to colorless.
While most of the vegetable oils are titrated at room temperature; coconut oil, palm oil and cosmetic raw material should be titrated still warm to avoid sample solidification and erroneous quantification.

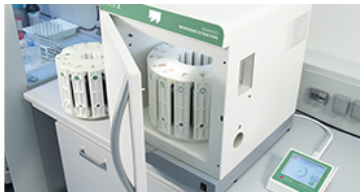
RESULTS AND DISCUSSION

The first tests conducted were made on eight different reference materials and a comparison was made between saponification results obtained with ETHOS X and following ISO 3657:2020 method:

Reference sample	Reference Value (mg KOH / g)	ETHOS X method			ISO Method		
		Obtained Value (mg KOH / g)	SD (mg KOH / g)	RSD (%)	Obtained Value (mg KOH / g)	SD (mg KOH / g)	RSD (%)
Conventional Sunflower Oil	190.5	190.5	0.4	0.2	190.0	0.3	0.1
Sesame Oil	189.2	189.2	0.6	0.3	188.9	0.2	0.1
Mix Refined Oils	189.2	189.6	0.3	0.2	189.7	0.4	0.2
Fish Oil	188.6	189.2	0.6	0.3	188.8	0.6	0.3
Grapeseed Oil	191.3	192.2	0.7	0.4	191.2	1.0	0.5
Crude Rapeseed Oil	190.7	191.1	0.5	0.2	190.4	0.3	0.2
Palm Oil	197.6	201.9	0.7	0.3	200.5	0.4	0.2
Coconut Oil	255.3	259.4	0.7	0.3	257.1	0.3	0.1

Table 4- Summary table of comparison of the results obtained for both methods on reference oils; for ISO method n =3, for ETHOS method n=6

The precision of the method was determined by carrying out six analyses under repeatability conditions on three reference materials, where the tests were carried out on the same day and by the same operator.
The results of the precision study are presented in Table 5:

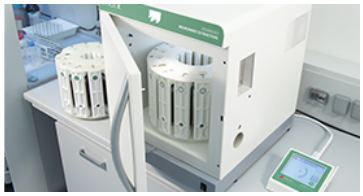


Parameter	Rapeseed Oil	Palm Oil	Coconut Oil
Mean Value (of six different days)	191.2	200.4	259.6
SD	0.4	0.6	0.2
RSD	0.2	0.3	0.1
RSD % Horwitz	0.49	0.51	0.64
HORRAT Value	0.38	0.55	0.10

Table 5 – Parameter considered for precision assessment of the method.

The ratio between the relative standard deviation % (RSD%) under intermediate precision and the RSD% calculated by the Horwitz equation is an indicator of the precision of the analysis and is known as the HORRAT value.
The HORRAT is usually used to indicate the presence of analytical problems that affect the precision of the analysis: values below 1 indicate good analytical precision, values between 1 and 1.5 are acceptable results, while values above 2 indicate analytical problems.
After this validation of the effectiveness of the method, it was applied again, together with ISO 3657:2020, on several commercial oil matrices. The results are shown in Table 6:

Sample	ETHOS X method			ISO Method		
	Obtained Value mg KOH / g	SD mg KOH / g	RSD %	Obtained Value mg KOH / g	SD mg KOH / g	RSD %
Cocoa Butter	192.6	0.3	0.2	192.3	2.6	1.4
Extra Virgin Olive Oil	193.6	0.1	0.1	191.5	1.7	0.9
Conventional Soybean Oil	190.8	0.3	0.2	189.6	4.8	2.5
Mais Oil	191.2	0.5	0.3	190.7	0.5	0.3
Conventional Sunflower Oil	189.9	0.7	0.4	188.0	2.7	1.5
Olive Oil	191.3	0.4	0.2	191.8	1.3	0.7
Peanut Oil	189.3	0.7	0.4	186.2	2.2	1.2
Coconut Oil	259.7	0.3	0.1	258.2	2.3	0.9
Palm Oil	197.4	0.1	0.0	195.9	3.8	1.9
HO Sunflower Oil	193.8	0.2	0.1	187.4	3.2	1.7
Avocado Oil	190.8	0.2	0.1	190.5	2.8	1.5
HO Soybean Oil	190.8	0.2	0.1	189.6	1.4	0.8
HO Rapeseed Oil	188.2	0.5	0.3	187.0	2.3	1.2
Safflower Oil	192.0	0.1	0.1	189.1	2.3	1.2



Conventional Rapeseed Oil	190.9	0.2	0.1	188.4	0.6	0.3
Sesame Oil	188.1	0.4	0.2	187.6	0.4	0.2
Linseed Oil	189.3	0.8	0.4	190.8	0.3	0.2

Table 6- Summary table of comparison of the results obtained for both methods on commercial oils; n=3 for both methods.

Finally, the ETHOS X approach was also tested for saponification on cosmetic ingredients and the results are shown in Table 7:

Sample	Reference Value mg KOH / g	ETHOS X method		
		Obtained Value mg KOH / g	SD mg KOH / g	RSD %
Trioctyldodecyl citrate	145.5	142.5	0.4	0.3
Hydrogenated castor oil dimer dilinoleate	188.2	186.7	1.7	0.9
Vegetal Stearine	207.3	206.0	2.3	1.1
Blend of Mono-, Di- and Triglycerides	284.0	277.4	0.3	0.1
Isostearyl isostearate	103.0	103.1	0.6	0.6
Dipentaerythrityl tetrabehenate/polyhydroxy stearate	184.0	183.5	0.4	0.2
Glyceryl Undecilenate	207.3	209.5	0.5	0.2
Polyglyceryl-10 Pentahydroxystearate	130.0	126.9	0.5	0.4

Table 7- Summary table of the results obtained on cosmetic formulations ingredients; n=3.

CONCLUSION

The results show that the ETHOS X with fastEX-24 eT approach for saponification gives comparable results to both reference material values and the ISO 3657:2020 standard method.
Microwave saponification showed clear advantages over the official ISO methods. In fact, the use of microwave as a heating source allows to significantly reduce the time of saponification (20 min compared to 1 h of the official ISO method) and to obtain generally better results in terms of reproducibility (RSD). This observation can be attributed to the better homogenisation provided by the constant stirring of the magnetic stirring rod during both the saponification and the titration processes.
In addition, the rotor, which can hold up to 24 samples, reduces the laboratory space and time required for the analysis of several samples.
For all these reasons, the ETHOS X has proved to be a successful and innovative approach to oil and fats saponification.

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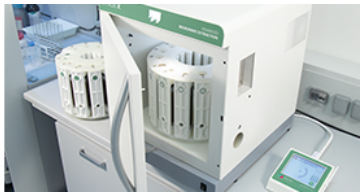

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