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Rapid determination of esterified glycerol and glycerides in triglyceride fats and oils by means of periodate method after transesterification

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Abstract

This paper describes an accurate method to determine esterified glycerol in the glycerides of edible fats and oils and, in general, in all triglyceride fat or oil. Esterified glycerol is released by means of a transesterification reaction with potassium hydroxide in methanol, which simultaneously produces fatty acid methyl esters. Free glycerol is oxidized selectively to formic acid and without any interference in the same environment in which the transesterification reaction occurs by the addition of periodate.

The formic acid produced is then potentiometrically titrated using an acid–base reaction. By knowing the acidic composition and the distribution of the glycerol between triglycerides, diglycerides and monoglycerides, the determination of glycerol also makes it possible to evaluate the content of glycerides present in the fats. The most accurate glyceride determination was obtained by suitably combining the determination of bound glycerol and the distribution of fatty acids obtained by means of gas chromatographic analysis. With little modifications, the proposed method also makes it possible to successfully analyse edible fat in terms of glycerol content in aqueous containing matrixes.

Keywords: Glycerol; Glycerides; Triglycerides; Diglycerides; Fats; Oil; Periodate; Transesterification

1. Introduction

Fatty matrixes found in nature are characterised by well-known parameters. Although glycerides are the largest component in fats, they are not included in the most widely-used quality parameters. The percentage of triglycerides and diglycerides present in glycerides totals is about 97% and 3%, respectively, while only traces of monoglycerides are present (Capella, Fedeli, Bonaga, & Lercker, 1997). Bound glycerol, which is closely correlated to the concentration of glycerides, does not appear among the characteristic quality parameters, most likely due to the lack of relatively simple and accurate methods to determine it.

In well-conserved fatty matrixes, the ratio between the concentration of glycerides and esterified glycerol can be considered constant. In the case of commercially-available butter, it may be stated that this ratio remains constant in so far as it constitutes a typical bulk product (Precht, 1995). A large number of natural fats, such as olive oil, seed oil and fat extracts from pulses and cereals, even when they do not derive from bulk, present a constant glycerides and glycerol ratio (Plank & Lorbeer, 1995). Indeed, in these natural fats the analysis of fatty acids esterified with glycerol indicates that the most commonly found fatty acids were palmitic acid (C16), stearic acid (C18), oleic acid (C18:1)

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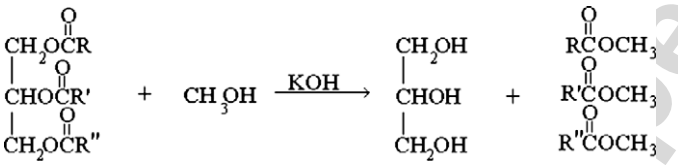
and linoleic acid (C18:2), with the last three representing approximately 90–95% of the total weight of fatty acids, and their molecular weights being almost identical (Capella et al., 1997; Tateo & Bonomi, 2003). As shown below, a method which can determine the glycerol present in the glycerides can also make it possible to determine the glyceride content once the percentage composition of fatty acid and the distribution of glycerol in triglycerides, diglycerides and monoglycerides are known (obtaining them from either experimental data or from the relevant literature).

Free glycerol can be easily analysed by means of chromatographic (Sala & Bondioli, 1998) or volumetric techniques (Weiss Frederick, 1970). Moreover, in the literature a gas chromatographic procedure for the simultaneous determination of glycerol, mono-, di- and triglycerides in vegetable oil methyl esters (Plank & Lorbeer, 1995) and a similar procedure for the determination of monoglycerides, diglycerides, triglycerides and glycerol in fats by means of gel permeation chromatography (Schoenfelder, 2003) have been proposed.

On the opposite, in the literature no simple or accurate procedures to determine esterified glycerol in diary glycerides have been so far reported.

Studies designed to analyse fatty acids deriving from glycerides have shown that the transesterification reaction reported herein is both rapid and quantitative (Nota, Spagna Musso, Naviglio, Romano, & Sabia, 1999):



When the transesterifying reagent, i.e. potassium hydroxide in methanol, is added to the *n*-hexane solution of the glycerides, fatty acid methyl esters are formed immediately and glycerol is released. The methyl esters are dissolved in the hexane phase and the glycerol is quantitatively extracted in the aqueous phase. Therefore, following the transterification reaction, it is possible to quantitatively determine the glycerol by means of the usual procedures. In this paper a volumetric technique is proposed as a simpler, faster and more accurate method with respect to chromatographic techniques reported in the literature (Sala & Bondioli, 1998).

The glycerol formed during the transterification reaction is quantitatively and specifically oxydized into formic acid by adding periodate according to the reaction:

$$C_3H_8O_3 + 2IO_4^- = 2IO_3^- + 2CH_2O + HCOOH + H_2O$$

The formic acid, which is equivalent to the glycerol previously formed, is then determined by an acid–base titration, using a colorimetric indicator or pH-meter or automatic titrator to detect the final equivalent point of titration.

2. Experimental

2.1. Reagents

Glycerol (Baker, Deventer, Holland); anhydrous sodium sulphate (Baker, Deventer, Holland); *n*-hexane (Baker, Deventer, Holland); N/10 titrated sodium hydroxide (Carlo Erba, Milan, Italy); potassium hydroxide (Carlo Erba, Milan, Italy); anhydrous methanol (Lab-Scan, Dublin, Ireland); N/10 titrated hydrochloric acid (Carlo Erba, Milan, Italy); hydrochloric acid 37% (w/v) (Carlo Erba, Milan, Italy); metaperiodated sodium (Baker, Deventer, Holland); ethylene glycol (Lab-Scan, Dublin, Ireland); 1% phenolphtalein solution (Carlo Erba, Milan, Italy), trichloroacetic acid (Carlo Erba, Milan, Italy), all of which were pure for analysis.

2.2. Instrumentation

Glass electrode model DP-100NE (Gibertini, Milan, Italy). Automatic titrator mod. TIM900 equipped with combined Ag–AgCl electrode specifically designed for measuring pH (Radiometer Copenhagen, Cedex, Lyon, France). DANI gas chromatograph, model 86.10 HT, equipped with a PTV (programmed temperature vaporizer) and FID (flame ionization detector) (DANI, Monza, Italy).

2.3. Gas cromatographic conditions for analysing fatty acid methyl esters

RTX 2330 column with stationary phase, 90% bis-cyanopropil, 10% phenylsilicone FAME (fatty acid methyl esters), $l = 50$ m; i.d. = 0.25 mm; f.t. = 0.25 μ (Restek, Bellefonte, CA, USA). Injector (PTV) programme: 50 °C for 15 s, increase of 900 °C/min until 250 °C; hold for 3 min. Oven programme: 50 °C for 2 min; increase of 7 °C/min until 250; hold for 3 min. FID: 260 °C. Carrier gas: helium 2 mL/min. Split: 1:80.

2.4. Analytic procedure to determine esterified glycerol in anhydrous fats by means of potentiometric titration (Procedure A)

One gram of anhydrous fat was accurately weighted and transferred in a 150 mL beaker; 10 mL of *n*-hexane were added and the system was shaked until the fat was completely dissolved; 5 mL of transesterificating reagent (4 N potassium hydroxide in methanol) were added and the system was shaked for 1 min; 25 mL of distilled water were added and glass electrode was inserted into the solution; neutralization was roughly achieved by adding 4.8 mL of HCl 4 N; the pH 7.00 was exactly obtained using either HCl 0.1 N and, if necessary, NaOH 0.1 N. Then 25 mL of sodium meta-periodate solution 10% (w/v) were added and the reaction vessel was kept in the dark for 1 min while shaking vigorously; 10 mL of ethylene glycol aqueous solution 50% (w/v) were added; the system was kept in the dark

for 5 min while shaking vigorously; the resulting solution was potentiometrically titrated by using NaOH 0.1000 N. The titrant volume at the final equivalent point was determined by means of the first derivative technique applied to the obtained titration curve.

2.5. Analytic procedure to determine the esterified glycerine in non-anhydrous fats by means of potentiometric titration (Procedure B)

Two grams of non-anhydrous fat were accurately weighted and transferred in a graduated test tube for centrifuges; 20 mL of *n*-hexane were added and vigorously shaked for 1 min (for fats containing emulsifying substances, 12% w/v trichloroacetic solution in *n*-hexane was added) (Naviglio et al., 2000; Naviglio et al., 2001); the system was then centrifuged at 4000 rpm for 5 min; exactly 10 mL of the hexane phase were withdrawn and the procedure above described for anhydrous fats was applied (Procedure A).

2.6. Determination of glycerides contained in the fats, expressed in percentage (w/w) (Procedure C)

2.6.1. Determination of the aliquota relative to the triglycerides

The procedure for the determination of the triglycerides aliquota is the following: (i) determine the percentage in weight of the glycerol by means of the procedure described above; (ii) determine the distribution percentage of the esterified fatty acids by means of gas chromatography; (iii) transform the percentage of fatty acid methyl esters into the percentage of free fatty acids; (iv) divide the percentage of glycerol determined into two aliquotes (gly1 and gly2) proportional to the percentage of the triglycerides and diglycerides reported in the literature for the type of natural fat being analysed (Capella et al., 1997); (v) determine the FTr factor by applying the following formula:

$$\mathrm{FTr} = \frac{3 \times \mathrm{gly1}}{M \times \sum mpa} \tag{1}$$

where FTr = factor for calculating triglycerides; gly1 = percentage of triglycerides present in the mixture; M = molecular weight of glycerol (g/eq); and $\sum mpa$ = sum of moles deriving from percentage free fatty acid composition.

The determination of the total fatty acids amount in grams (GTr) uses the following formula:

$$\mathrm{GTr} = \mathrm{FTr}(\mathrm{mol\ Cn} \times M(\mathrm{Cn})) \tag{2}$$

Since it is mol Cn = Cn(%)/M(Cn), substituting in the previous equivalence (2) we obtain:

$$\mathrm{GTr} = \mathrm{FTr} \times \sum \mathrm{Cn}(\%) \tag{3}$$

where $\sum$Cn(%) = 100; finally GTr = FTr × 100.

The calculus of triglyceride fraction (Try) can be done utilizing results obtained from (3) and glycerol content related to triglyceride fraction (gly1):

$$\mathrm{Try} = \mathrm{GTr} + \mathrm{gly1} - 18 \times 3 \times \mathrm{gly1}/M \tag{4}$$

where GTr = weight of free fatty acid to esterify to the glycerol in order to reconstitute triglycerides (g); mol Cn = moles of *n*th free fatty acid (mol); M(Cn) = molecular weight of the *n*th acid (g/mol); Cn(%) = percentage weight of the *n*th free fatty acid; and Try = trigliceride aliquote in 100 g of sample.

2.6.2. Determination of the aliquote relative to the diglycerides

The determination of FDi is achieved by applying the following formula:

$$\mathrm{FDi} = \frac{2 \times \mathrm{gly2}}{M \times \sum mpa} \tag{5}$$

where FDi = factor for calculating diglycerides; gly2 = percentage of diglycerides present in the mixture; M = molecular weight of glycerol (g/eq); and $\sum mpa$ = sum of moles deriving from percentage free fatty acid composition.

To determine the number of total fatty acids GDi the following formula is used:

$$\mathrm{GDi} = \mathrm{FDi}(\mathrm{mol\ Cn} \times M(\mathrm{Cn})) \tag{6}$$

Since it is mol Cn = Cn(%)/M(Cn), substituting in the previous equivalence (6) we obtain:

$$\mathrm{GDi} = \mathrm{FDi} \times \sum \mathrm{Cn}(\%) \tag{7}$$

where $\sum$Cn(%) = 100; finally GDi = FDi×100.

To calculate the diglyceride aliquota (Dig) results obtained from (7) are used, and glycerol content related to diglyceride fraction (gly2) is calculated using the relation:

$$\mathrm{Dig} = \mathrm{GDi} + \mathrm{gly2} - 18 \times 2 \times \mathrm{gly2}/M \tag{8}$$

where GDi = weight of free fatty acids to esterify to the glycerol in order to reconstitute diglycerides (g); mol Cn = moles of *n*th free fatty acid (mol); M(Cn) = molecular weight of the *n*th acid (g/mol); Cn(%) = percentage weight of the *n*th free fatty acid; and Dig = diglyceride aliquota in 100 g of sample.

2.7. Calculation of the percentage in weight of the total glycerides in the sample

The relationship used is the following

$$\text{Total glycerides}(\%\ \text{in weight}) = \mathrm{Try} + \mathrm{Dig} \tag{9}$$

3. Results and discussion

3.1. Verify the accuracy of the analytic procedure in order to determine the esterified glycerol (Procedure A)

In order to verify the accuracy of the analytic procedure, three different standard solutions of tributyrin, trimyristin and tristearin representing the short, medium and long-

chain triglycerides were prepared. In addition, a solution was prepared of known concentration of triolein representing the unsaturated triglycerides. These solutions were analysed using the analytic procedure (A) described in Section 2 and results were reported in Table 1. A *t*-test showed no significant differences between theoretical and experimental values at a confidence level of 99%, indicating a good accuracy. Moreover the procedure is precise as the standard deviation does not go beyond 1%, in the case in which the pH-meter is used. It is worth noting that the periodate procedure gives accurate results if the alkalinity deriving from the transesterification reagent is thoroughly neutralised and if the point of equivalence of the formic acid titration formed through the specific oxidation of the glycerine by adding periodate is correctly detected. In the procedure used here, potentiometric titration was used and the point of equivalence was determined by applying the method of the first derivative to the titration curve experimentally obtained. If highly precise results are not required, it is possible to neutralise and titrate using chromatic indicators such as phenolphthalein; in this case the standard deviation does not go beyond 2%. Furthermore, if highly precise results are required using an automatic titrator, it is possible to obtain a gain of an order of magnitude in standard deviation that will not go beyond 0.3%.

3.2. Determination of glycerol in natural fats

In order to verify whether the procedure could be conveniently applied to glyceride mixtures of natural fats, ten samples of commercially-available butter were anhydrified by means of sodium sulphate and filtered through paper filter. As the glycerol content of the ten samples was unknown, each sample was analysed ten times and the

Table 1
Repeatability and accuracy of procedure in analysing standard solutions of tributyrin, trimyristin, tristearin and triolein

Tributyrin	Theoretical (percentage w/w)	Experimental glycerol (%) colorimetric	Experimental glycerol (%) pH-meter	Experimental glycerol (%) automatic titrator
1	30.50	30.8	30.67	30.59
2	30.50	30.5	30.75	30.47
3	30.50	30.2	30.87	30.53
4	30.50	30.8	30.35	30.51
5	30.50	30.1	30.46	30.49
Mean	–	30.5	30.62	30.52
SD	–	0.3	0.2	0.05
Error (%)	–	0.07	0.39	0.06
Trimyristin	Theoretical (percentage w/w)	Experimental glycerol (%) colorimetric	Experimental glycerol (%) pH-meter	Experimental glycerol (%) automatic titrator
1	12.74	12.8	12.85	12.78
2	12.74	12.6	12.72	12.74
3	12.74	12.5	12.57	12.73
4	12.74	12.8	12.66	12.75
5	12.74	12.9	12.73	12.71
Mean	–	12.7	12.71	12.74
SD	–	0.2	0.1	0.03
Error (%)	–	0.16	0.27	0.02
Tristearin	Theoretical (percentage w/w)	Experimental glycerol (%) colorimetric	Experimental glycerol (%) pH-meter	Experimental glycerol (%) automatic titrator
1	10.36	10.5	10.45	10.31
2	10.36	10.2	10.39	10.38
3	10.36	10.6	10.27	10.37
4	10.36	10.6	10.33	10.34
5	10.36	10.3	10.49	10.33
Mean	–	10.4	10.39	10.35
SD	–	0.2	0.09	0.03
Error (%)	–	0.77	0.25	0.14
Triolein	Theoretical (percentage w/w)	Experimental glycerol (%) colorimetric	Experimental glycerol (%) pH-meter	Experimental glycerol (%) automatic titrator
1	10.43	10.5	10.49	10.42
2	10.43	10.2	10.35	10.40
3	10.43	10.5	10.57	10.37
4	10.43	10.1	10.38	10.41
5	10.43	10.6	10.37	10.44
Mean	–	10.4	10.43	10.41
SD	–	0.2	0.09	0.03
Error (%)	–	0.48	0.02	0.21

Table 2
Repeatability of procedure in analysing butter and accuracy evaluated by means of standard additions method[a]

Commercial brand butter samples	Glycerol (%) average	I addition (about 10% of mix)			II addition (about 20% of mix)			I addition (about 30% of mix)		
		Theoretical	Experimental	Error (%)	Theoretical	Experimental	Error (%)	Theoretical	Experimental	Error (%)
Amodio	12.52 ± 0.10	12.86	12.90	0.3	13.20	13.17	0.2	13.55	13.58	0.2
Berna	12.31 ± 0.10	12.95	12.99	0.3	13.15	13.19	0.3	13.59	13.54	0.4
Galbani	12.50 ± 0.10	12.90	12.85	0.4	13.25	13.20	0.4	13.57	13.51	0.4
Granarolo	12.51 ± 0.10	12.88	12.93	0.4	13.23	13.28	0.4	13.51	13.48	0.2
Invernizzi	12.22 ± 0.10	12.99	12.94	0.4	13.18	13.20	0.2	13.54	13.57	0.2
Lupark	12.47 ± 0.10	12.91	12.87	0.3	13.20	13.15	0.4	13.50	13.45	0.4
Matese	12.30 ± 0.10	12.95	12.91	0.3	13.29	13.26	0.2	13.56	13.53	0.2
Optimus	12.36 ± 0.10	12.93	12.93	0.1	13.17	13.14	0.2	13.50	13.45	0.4
Prealpi	12.33 ± 0.10	12.98	12.96	0.2	13.24	13.21	0.2	13.55	13.49	0.4
Yma	12.48 ± 0.10	12.90	12.87	0.2	13.28	13.25	0.2	13.49	13.51	0.1

[a] Results were obtained by means of automatic titrator.

average value was taken as the true value. The results shown in Table 2 indicate that the procedure is easily repeatable, even for natural samples. In order to verify the accuracy of the procedure, a mixture of known concentration of tributyrin, trimyristin, tristearin and triolein in approximately equal parts was prepared. A known amount of the synthetic mixture of triglycerides was added to the ten butter samples previously analysed in measure of about 10%, 20% and 30% w/w, respectively. The results shown in Table 2 indicate that response was linear with the glycerol added to the butter as standard mixture; no significant differences between theoretical and experimental values at confidence level of 99% were found and thus the procedure is also accurate. Similar results were obtained with samples of commercially-available lard and olive oils, indicating that the measurement of the glycerol content obtained by analysing the edible fat matrixes is accurate.

3.3. Determination of glycerol in non-anhydrified butter

Considering that most of commercially-available butter is an edible product, we tested whether or not the procedure proposed could also be applied to fat samples with a relatively high water content. Unfortunately, the results obtained indicate that this kind of butter cannot be easily analysed using the procedure adopted for anhydrous fats, as the water contained in the samples makes the transesterification reaction virtually incomplete. Better results were obtained when the water was eliminated simply and quickly. A suitable quantity of butter was weighed in a graduated test tube and dissolved in *n*-hexane under vigorous shaking. Following centrifugation, the volume of the hexane phase was measured. A known fraction of the hexane phase was accurately removed and analysed according to the procedure described for anhydrous fats (Procedure B). The results obtained, which are shown in Table 3, indicate that the level of accuracy is comparable to that of anhydrous butter and other fatty matrixes (Table 4).

In the cases of lipids containing a large component of polar compounds, such as phospholipids, which act as emulsifying agents, we added a trichloroacetic acid solution in *n*-hexane (Naviglio et al., 2000; Naviglio et al., 2001) or utilized Gerber or Rose-Göttlieb procedures to separate non-polar lipids from polar ones.

Table 3
Determination of glycerides content in non-anhydrous fats and comparison of results with label data

Non-anhydrous fatty matrix	Fat (label) (%)	Experimental glycerol (%)	Glycerides (%)
Butter	Min. 82	10.40	84
Margarine	Min. 70	6.73	73
Cream	Min. 20	2.83	23
Egg yolk	About 26	2.15	28

Table 4
Glycerol content in different anhydrous edible oils and fats

Anhydrous fats and oils	Glycerol (%)
Butter	12.40 ± 0.05
Lard	10.32 ± 0.04
Tallow	10.60 ± 0.04
Olive oil	10.12 ± 0.05
Palm oil	10.50 ± 0.04
Soybean oil	9.78 ± 0.04
Sesame oil	9.80 ± 0.04
Olive husks oil	9.64 ± 0.05
Peanut oil	9.90 ± 0.04
Margarine fat	6.70 ± 0.04
Cream fat	11.55 ± 0.05
Egg fat	8.40 ± 0.04
Flour fat	7.34 ± 0.05
Semolina fat	8.05 ± 0.04

3.4. Determination of glycerides in fatty products by means of glycerol analysis

In many well-conserved natural fats – like butter, olive oil, lard – triglycerides represent approximately 95–98% of the glycerides present; approximately 2–5% is represented by diglycerides. For the reasons outlined in the Introduction, the natural fats present an almost constant ratio between glycerine and glycerides (Capella et al.,

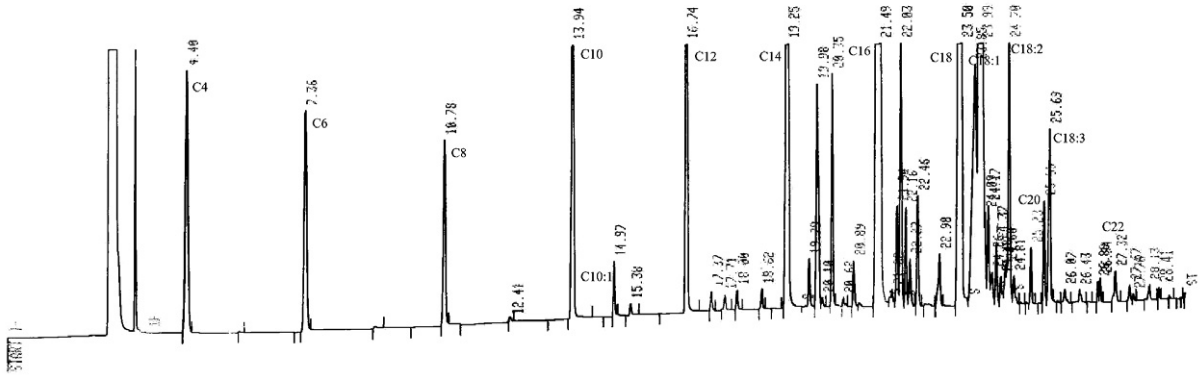


Fig. 1. Gas chromatogram of fatty acid methyl esters of butter.

1997). Consequently, determining the glycerol makes it possible to identify the percentage content of glycerides present in fatty matrixes as the ratio between glycerides and glycerol can be taken from the relevant literature (Capella et al., 1997; Tateo & Bonomi, 2003). Table 4 reports the experimentally found percentage of glycerol in different typical commonly found fats. More accurate analytic results can be obtained by experimentally determining the percentage of glycerol content in fats by means of periodate and the distribution of esterified fatty acids by means of gas chromatography, as indicated in Procedure (C). Fig. 1 reports a gas chromatogram of fatty acid methyl esters obtained after the transesterification of anhydrous milk fat in which appear fatty acid methyl esters ranging from butyric acid (C4) to behenic acid (C22); materials and methods for analysis of fatty acid methyl esters are reported in a previous paper (Nota, Naviglio, Romano, Sabia, & Spagna Musso, 1998).

3.5. Distribution of glycerol between triglycerides and diglycerides

Table 5 shows the analytic results of the same fat sample analysed in terms of glycerides, assuming that in one case, the glycerol is derived exclusively from triglycerides, and in another, from 95% of the triglycerides and 5% of the diglycerides, as in fact reported in the literature. It can clearly be seen that there is a 2% difference between the two analytic results. The same Table shows the results obtained by varying the composition of the triglycerides and diglycerides ranging in the interval 100–95% of triglycerides. As can be seen, there is a difference of less than 2%. This fact guarantees that the distribution of glycerol between triglycerides and diglycerides according to their distribution is a good approximation because the error is negligible. In conclusion, the procedures proposed make it possible to quickly and accurately determine esterified glycerol in glycerides, as well as the content of glycerides present in fats, and thus allow better levels of characterisation of edible lipids.

Table 5
Effect of distribution of glycerol on triglycerides and diglycerides in reconstitution glycerides starting from 100% of triglyceride in fat to 95% in olive oil and in butter

Distribution of glycerol		Calculation		
Triglycerides (%)	Diglycerides (%)	Total glycerides (%)	Triglycerides (%)	Diglycerides (%)
Olive oil (glycerol content: 10.12%)				
100	0	96.13	96.13	0
99	1	95.85	95. 17	0.67
98	2	95.56	94.21	1.35
97	3	95.27	93.25	2.02
96	4	94.99	92.29	2.69
95	5	94.70	91.33	3.37
Butter (glycerol content: 12.40%)				
100	0	99.83	99.83	0
99	1	99.54	98.84	0.71
98	2	99.25	97.85	1.41
97	3	98.96	96.84	2.12
96	4	98.67	95.83	2.83
95	5	98.38	94.85	3.53

4. Conclusions

The method proposed makes it possible to specifically and accurately determine esterified glycerol in the triglycerides of edible fats. Using the procedures outlined in this paper, fatty matrices containing water, such as butter, can be easily analysed. Knowing the acidic distribution of fatty acid and the distribution of glycerol in triglycerides and diglycerides, the experimental determination of esterified glycerol also makes it possible to quantify the glycerides in fats. Better glyceride determination results can be obtained by suitably combining the results obtained from the determination of esterified glycerol with those obtained from the gas chromatographic determination of fatty acids. When the glyceride and bound glycerol content is known, it is possible to obtain a better quality characterisation of edible fats. Good results are obtained utilizing a pH-meter to detect the final point of titration,

while the best results are obtained by means of an automatic titrator.

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