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SPECTROPHOTOMETRIC DETERMINATION OF 1-MONOGLYCERIDES IN FATS

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Abstract. A method for the determination of a small amount of 1-monoglycerides in fats has been described. The method is based on a quantitative periodic acid oxidation—the resultant formaldehyde giving magenta colour with Schiff's reagent is determined spectrophotometrically. The method is quite accurate for samples containing both small as well as large amounts of 1-monoglycerides.

Commercial monoglycerides are invariably prepared by the interesterification of fats or fatty acids with glycerol in the presence of catalysts.[1,2] The reaction product always contains, in addition to tri and diglycerides, a substantial amount of 1-monoglycerides. The periodic acid oxidation procedure has been widely used for the quantitative determination of 1-monoglycerides.[3] Several modifications in the method have also been suggested.[4–6] Recently, monoglycerides of long chain fatty acids have been analysed as their trimethylsilyl ethers by gas liquid chromatography.[7] However, monoglycerides in amounts less than 1–5% cannot be determined accurately by these methods except by GLC. In 1964, Szonyi and Sparrow reported the colorimetric determination of small amounts of monoglycerides in fats.[8] According to their method glycolaldehyde fatty esters produced by periodic acid oxidation are converted to their 2,4-dinitrophenylhydrazine derivatives and determined colorimetrically. The method is cumbersome and the results are corrected after using an assumption of standard deviation obtained in the absorbance.

In the present procedure the formaldehyde produced by periodic acid oxidation is determined spectrophotometrically by Blaedel and Blacet method.[9] The magenta colour given by Schiff's reagent with formaldehyde in the presence of sulphuric acid does not fade appreciably during 6 hr, whereas the colour given by the glycolaldehyde fatty acid ester also produced during oxidation, fades completely within 2 hr. Since the oxidation is quantitative, the increase in absorption in colour is proportional to the 1-monoglyceride content of the sample. The method is accurate with a standard deviation of $\pm$ 1–2%.

Experimental

Reagents and Materials

Chloroform. Chloroform is of A.R. quality or comparable purity.

Periodic Acid. Periodic acid solution is prepared by dissolving 5.4 g acid in 100 ml distilled water and then adding 1900 ml glacial acetic acid. After mixing thoroughly, the solution is stored in a dark glass-stoppered bottle.

Schiff's Reagent. The reagent is prepared by dissolving 0.5 g Fuchsine (rosaniline hydrochloride) in 500 ml distilled water and then adding 5.15 g sodium bisulphite. About 15 min later, 17 ml 6N HCl is added and the mixture is allowed to stand for 3 hr before use. During this time the solution fades to a permanent pale yellow colour. The reagent is stable at least for a month.

Glycerol monostearate (B.D.H.) is further purified by column chromatography and then the purity is checked by thin layer chromatography.

Purification of 1-Monostearate. Hydrated silica gel, 30 g (100–200 mesh, adjusted to contain 5% water) is covered with petroleum ether (b.p. 60–80) in a beaker. The mixture is stirred and brought to the column (19 nm I.D. $\times$ 290 nm) with additional increments of solvent. When the solvent level falls to 2 cm above the top of the absorbent, add 1 g mono glyceride dissolved in 15 ml chloroform slowly so that channels are not formed. The flow is adjusted so that 1.5–2 ml eluent are collected per minute. This rate is maintained throughout the experiment.

Elution of Tri, Di, and 1-Monostearate. Traces of tri and diglycerides, present as impurities in the monostearate, are eluted with 200 ml benzene and 200 ml 10% ethyl ether in benzene (v/v) respectively. The last fraction of pure monoglyceride is eluted with 250 ml ethyl ether. The eluates are evaporated on a steam-bath under a stream of nitrogen or clean dry air. The purity of the monoglyceride is also checked by TLC.

Thin Layer Chromatography. Thin layer plates are prepared according to Stahl.[11] Silica gel G (30 g Merck Darmastadt) is thoroughly mixed with water (60 ml). The resulting slurry is applied to glass plates (20 $\times$ 20 cm) in a thickness of 250μ. The plates are dried at 110° for $\frac{1}{2}$ hr before use. Better separation is obtained on fresh plates. Solution of monoglyceride in chloroform (1%) is applied to the plate at places 1 cm apart on a line 1–2 cm from the bottom. The plate is developed (40 min) by ascending solution in air-tight tanks containing benzene–ether (80:20). The tank is also lined with solvent-soaked filter papers.

After development, the plate is dried in a current of nitrogen and sprayed with 0.2% ethanolic solution of dichlorofluorescein. The separated monostearate and traces of di and tri if present, then appear as bright yellow fluorescent spots when viewed under UV light. Batches which indicated traces of impurities are rechromatographed by the column method.

Procedure

Three sample solutions are needed for the monoglyceride determination: (1) a solution containing known amount of pure 1-monostearate which has been oxidized to the corresponding glycollic ester and formaline to obtain the typical working curve, (2) a solution containing an aliquot of pure fat and 1-monostearate and (3) a solution of pure fat as used in No. 2.

Periodic Acid Oxidation. Pure glycerol monostearate (238.67 mg) dissolved in chloroform and transferred to a 250-ml volumetric flask and volume made up to 250 ml with chloroform. Sample solution (25 ml), containing 23.86 mg monostearate, gives 2 mg formaline after periodic acid oxidation. Periodic acid solutions (31.2 ml) pipetted out into a 250-ml glass-stoppered separating funnel and mixed well. After 30 min the aqueous layer of the separated phases is transferred to 100 ml volumetric flask. The chloroform layer is exhaustively extracted (5×10 ml) with distilled water, collected and diluted to volume with water. Five ml of this oxidised solution contain 0.1 mg Formaline. Similarly 5 ml stock solution containing 0.02, 0.04, 0.06, 0.14, 0.16 mg Formaline are prepared on the basis that 1 mole monoglyceride liberates 1 mole formaldehyde (Table 1).

In a single determination, 5 ml solution of each concentration to be analysed, and 6 ml water are added to a mixture of 10 ml Schiff's reagent and 1.2 ml 75% H_2SO_4 in stoppered test tubes. After addition of reagent the tubes are allowed to stand for 2 hr at 30°C and the transmission is determined at 580 nm alongwith a reference in which all the reagents are added except monoglyceride (Fig. 1).

For verification of the procedure, known amount of 1-monoglycerides are added to fat samples and percentages are determined after obtaining the transmission, from the typical working curve. The results are given in Table 2.

TABLE 1. DATA OF THE GRAPH BETWEEN TRANSMISSION AND FORMALINE OBTAINED AFTER PERIODIC ACID OXIDATION OF 1-MONOSTEARATE AT ROOM TEMPERATURE.

Concn of 1-monostearate (mg)	Concn of liberated formaline (mg)	Transmission 580-nm
2.39	.01	96
4.78	.02	95
7.16	.03	92.5
9.55	.04	90
12	.05	85
14.32	.06	81
16.70	.07	74
19.09	.08	69.5
21.48	.09	63
24.00	.10	54
28.80	.12	44
33.60	.14	33
38.40	.16	18
43.20	.18	10
48.00	.20	4

TABLE 2

Monostearate (known) (%)	Transmission at 580nm	Determined from typical working curve (%)	Error (%)
1.0	98	.98	2.0
2.0	97.5	1.98	1.0
3.0	97	3.0	—
4.0	95	3.95	1.25
10.0	89	9.8	2.0
20.0	68	19.6	2.0
30.0	42	25.5	1.6
40.0	18	39.4	1.5

Discussion

When colour is developed by adding Schiff's reagent to a known amount of Formaline, obtained after periodic acid oxidation, and the transmission or the logarithm of the transmission is plotted against the concentration, a definite and reproducible curve rather than a straight line is obtained. Other colour reagents for Formaline, such as phloroglucinol in basic solution and phenylhydrazine hydrochloride in either acidic or basic solution, fail to give straight lines and the curves are not as nearly reproducible as those obtained with Schiff's reagent.

Best results are achieved when the amount of 1-monoglycerides are selected to yield, after periodic acid oxidation, a concentration of formaldehyde approximately 0.04–0.16 mg in 5 ml solution. It is also desirable to set up a series of standards containing known amount of formaldehyde ranging from 0.01 to 0.2 mg in 5 ml solution. The colours are developed in them at the same time as in the unknown oxidised mixtures of 1-monoglycerostearate and after comparison, the approximate amount needed in the procedure is determined.

Quantitative aspects of the method are also studied. Standard mixtures in fat (free from monoglycerides etc). are prepared by accurately weighing pure monostearate which has previously been purified by combination of column and thin layer chromatography. The percentage compositions as determined from the typical working curve are in close agreement with the actual contents of standard mixtures 2. The small deviation of ± 1–2% between the actual and observed values could have been caused by lack of absolute purity of monostearate used in the standard mixtures.

The method is very useful for determining small amount of monoglycerides where it is used to impart staling to bread, cakes etc.

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