Determination of Hydroxyls in Mono- and Diglycerides

R. G. JENSEN and J. SAMPUGNA, Department of Animal Industries, Agricultural Experiment Station, University of Connecticut, Storrs

Abstract

A method for the analysis of hydroxyls employing esterification with 3,5-dinitrobenzoyl chloride, followed by titration with tetrabutylammonium hydroxide, has been applied to mono- and diglycerides. Pure mono- and dipalmitins were recovered quantitatively. A commercial glyceryl dioleate was analyzed by this method and the A.O.C.S. pyridine-acetic anhydride procedure with similar results.

Introduction

ROBINSON *et al.* (7) have recently published a procedure for the rapid determination of organic hydroxyl groups, which involves esterification of hydroxyl groups with 3,5-dinitrobenzoyl chloride (DNBC) to a visual end point. The hydroxyls in a large number of compounds, including cholesterol, glycerol, etc., were determined with speed and precision. Both primary and secondary alcohols were completely benzoylated within 15 min at room temperature. It was thought that the method might be useful for the determination of diglycerides in mixtures of glycerides when coupled with an analysis for monoglycerides.

Experimental

Pure materials used to evaluate the method were tripalmitin (Hormel), 1,3-dipalmitin, and 1-monopalmitin. The tripalmitin did not interfere when analyzed. The 1-monopalmitin was prepared from acetone glycerol (2) and was pure when tested by periodic acid oxidation (4). The dipalmitin was synthesized as described by Malkin *et al.* (5) and crystallized from petroleum ether (30–60C) at 35C (3). The purity of the dipalmitin was established by thin-layer chromatography (TLC) (6). In addition, an impure 1-monopalmitin, containing 78.2% monoglyceride (periodic acid oxidation) and 21.8% diglyceride (TLC) and a commercial glyceryl dioleate were analyzed. The latter was washed to remove free fatty acids and glycerol before analysis.

To establish the precision and reproducibility of the method, pure mono- and dipalmitins and glyceryl dioleate were weighed into volumetric flasks and diluted with pyridine. Ten aliquots of each were analyzed (7). Six aliquots of glyceryl dioleate were also tested for hydroxyls by the A.O.C.S. pyridine-acetic anhydride procedure, except that the samples were weighed on an analytical balance (1). See Table I.

TABLE I

Determination of Hydroxyls in 1-Monopalmitin, 1,3-Dipalmitin, and Commercial Glyceryl Dioleate by the 3,5-Dinitrobenzoyl Chloride (DNBC) Method [a]

	1-Monopalmitin meq OH		1,3-Dipalmitin meq OH		Glyceryl Dioleate meq OH/100 g	
	Analyzed	Recovered	Analyzed	Recovered	A.O.C.S.	DNBC
	0.252	0.248	0.222	0.220	153 [b]	152
% Recovery	.....	98.4	.....	99.1	.....	.....
Std. deviation	.....	0.003	.....	0.007	12	5.1

[a] Results are averages of ten analyses on aliquots.
[b] Average of six determinations by the A.O.C.S. Cd 13-60 method for hydroxyls.

Further analyses were made on the dipalmitin, the impure 1-monopalmitin, and mixtures of the latter and dipalmitin to show the utility of the method. These results, in which the figures for the mixtures have been adjusted to allow for the diglyceride in the monoglyceride, are shown in Table II. For these determinations the samples were weighed directly into 125 ml iodine flasks in which all titrations were made, or appropriate dilutions were prepared in pyridine.

Discussion

From the results of the hydroxyl determinations on aliquots (Table I) it is clear that the procedure quantitatively estimates the hydroxyls in mono- and diglycerides. The % recoveries and standard deviations were: 1-monopalmitin, 98.4; 0.003 and 1,3-dipalmitin, 99.1, 0.007. Results obtained when the commercial glyceryl dioleate was analyzed by the DNBC and A.O.C.S. methods were quite similar. In Table II the average recovery for 1,3-dipalmitin was 97.3%, and the impure monopalmitin 99.4%, and for the mixtures 98.7%. The lower limit of dipalmitin that could be estimated in a mixture of mono- and diglycerides was about 20% on a weight basis (Table II). The method calls for a sample of 0.4 meq of hydroxyl and dipalmitin would contribute about 0.05 meq at 20% concentration. Therefore, the diglyceride hydroxyl in the impure monoglyceride (21.8%) was detectable. To calculate the diglyceride content of the commercial glyceryl dioleate (Table I), a figure of twice the monoglyceride content as meq (40.6) was subtracted from the total hydroxyl content. This resulted in 70.8 meq of diglyceride hydroxyl or 44.0% diolein.

Advantages of the method are speed, simplicity, and relative lack of manipulation. Disadvantages are the necessity for using dry reagents and for titrating while protected from water and carbon dioxide and the expense of the titrant. Tetrabutylammonium hydroxide is available only as a methanol solution. We found it less expensive to prepare our own titrant from either tetrabutylammonium iodide or bromide (7). Although it was not tried in mixtures, glycerol could be determined by analyses before and after washing. Free fatty acids must be removed beforehand, because the tetrabutylammonium acts as a base.

TABLE II

Hydroxyl Contents of Mono- and Dipalmitins, and Mixtures Thereof

1,3-Dipalmitin		Monopalmitin [a]		Mixtures		
				Analyzed		Recovered
Analyzed	Recovered	Analyzed	Recovered	Dipalmitin	Monopalmitin [a]	
			meq of OH			
0.395	0.392	0.424	0.423	0.260	0.116	0.383
0.231	0.216	0.419	0.412	0.104	0.290	0.398
0.178	0.174	0.299	0.299	0.200	0.194	0.387
0.315	0.310	0.186	0.182	0.100	0.323	0.407
0.413	0.410	0.468	0.468	0.050	0.387	0.425
0.300	0.278	.....	.....	.....	.....	.....
Ave.						
0.366	0.356	0.359	0.357	0.143	0.262	0.400
% Recovery	97.3	.....	99.4	.....	.....	98.7

[a] Contained 78.2% monopalmitin and 21.8% dipalmitin. Figures are adjusted.

The results above indicate that the method of Robinson *et al.* (7) is useful in estimating both the total and the diglyceride contents of partial glycerides. The determination of diglycerides, of course, requires an independent analysis for monoglyceride.

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Tempering Triglycerides by Mechanical Working[1]

R. O. FEUGE, W. LANDMANN,[2] D. MITCHAM, and N. V. LOVEGREN,
Southern Regional Research Laboratory,[3] New Orleans, Louisiana

Abstract

The tempering of fat products to convert their components to stable polymorphs is an important and a sometimes troublesome problem in the manufacture of these products, particularly chocolate and chocolate-type confections. It has been found that a solid-to-solid transformation to the stable polymorphs can be effected by mechanical working consisting of extrusion under pressure. With a fat of relatively few components, such as cocoa butter, evidence of the transformation can be obtained from X-ray diffraction patterns. For more complex fats, hardness and melting characteristics must be considered. There is evidence that mechanical working is also effective in the transformation of a cocoa butter-like fat made from hydrogenated cottonseed oil and olive oil, and in the transformation of highly hydrogenated cottonseed oil. Mechanical working to effect polymorphic transformation is also effective with products containing the fats mentioned.

Introduction

THE POLYMORPHISM of triglyceride products used in the solid or semi-solid state frequently poses practical problems. Among these is the tempering of chocolate and chocolate-type confections. The major components of cocoa fat in chocolate exhibit four distinct melting points (1). When chocolate is melted and resolidified, special precautions must be taken to ensure rapid transformation of the major components of the fat to their thermodynamically stable form. Failure to achieve this may result in the physical deterioration of the surface structure and may produce fat bloom, which is the appearance of grey spots on chocolate when the fat recrystallizes slowly into larger crystals. Confections containing cocoa butter-like fats also may bloom if proper precautions are not taken. Shortenings also are tempered and are said to perform best when their solid components are in the beta-prime state (2), the thermodynamically stable form of some of the components.

Customarily the transformation of a fat product to a more desirable polymorphic state is accomplished by one or more of three techniques: seeding of the solidifying melt, tempering of the solid or semisolid fat by holding it at a temperature just below its melting point, and aging of the solidified fat. In the manufacture of chocolate the first two techniques are employed. The melted chocolate is cooled to the point of partial solidification. Finely divided chocolate whose fatty components are largely in the most stable form is introduced. The mixture then is kneaded and mixed at a constant temperature until more seed crystals of the stable form appear. When such a mixture is solidified by passage through a cooling tunnel and subsequently warmed to room temperature, the greater portion of the fatty phase is converted into the stable polymorphic form within about 30 minutes (3).

The customary practice in the manufacture of shortenings is to temper the quickly solidified and plasticized products prior to shipment to the ultimate consumer (2). Tempering here consists of aging for a short time at a given temperature.

The present report is concerned with a rapid procedure for converting solid triglycerides to their thermodynamically stable polymorphic form; i.e., it is concerned with effecting a rapid solid-to-solid transformation. The procedure, which is more applicable to certain types of triglycerides than to others, consists simply of mechanical working by extrusion under pressure to extensively deform the triglyceride crystals.

Experimental

General Procedure. To demonstrate the effectiveness of mechanical working as a means of forcing polymorphic transformations, various fat products were heated to well above their melting points to destroy all traces of seed crystals, then the products were quickly solidified, and portions were worked at temperatures well below their melting points.

Mechanical working was accomplished by repeated extrusion through a sodium press, which consisted essentially of a plunger and a cylinder, the latter measuring 0.66 in. in diameter by 1.75 in. in length. The bottom of the cylinder was fitted with an orifice-containing plate through which the solid fat or fat product was extruded. For the cocoa butter and cocoa butter-containing products a plate having a cluster of three orifices, each measuring 0.0135 in. in diameter, was used. For the other fats and fat products a plate having an orifice measuring 0.25 in. by 0.02 in. was used. Both plates had a thickness of 0.074 in. Pressures up to about 1,000 psi were required for the extrusions. The first extrusions for each sample always required less force than did later extrusions.

X-ray diffraction patterns were obtained for the unworked and worked portions of the quickly solidified samples of the fats and for the same fats after thorough tempering by aging and holding them at

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[2] Fellow, National Confectioners Association.
[3] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.