sidered more accurate because no interferences are known to be present at the *trans* absorption. It is for this reason that a 2-component standard mixture using methyl esters was chosen as the preferred method of measurement by IR spectrophotometry. Further, the data in Table IV on intact triglycerides are the same samples analyzed in Table II and the use of prescribed calibration standards makes these *trans* values more accurate than in the AOCS procedure.

REFERENCES

1. Official and Tentative Methods of the American Oil Chemists' Society, AOCS, Chicago, IL, 1946, Tentative Method Cd 14-61.
2. Firestone, D., and P. LaBouliere, J. Assoc. Off. Anal. Chem. 48:437 (1965).
3. O'Connor, R.T., JAOCS 33:1 (1956).
4. Kaufmann, H.P., F. Volbert and G. Mankel, Fette Seifen Anstrichm. 61:643 (1959).
5. Firestone, D., and M. DeLaLuz Villadelmar, J. Assoc. Off. Anal. Chem. 44:459 (1961).
6. Huang, A., and D. Firestone, Ibid. 54:47 (1971).
7. Allen, R.R., JAOCS 46:552 (1969).
8. Huang, A., and D. Firestone, J. Assoc. Off. Anal. Chem. 54:1288 (1971).
9. Official and Tenative Methods of the American Oil Chemists' Society, AOCS, Chicago, IL, 1981, Official Method Ce 1-62.
10. AOAC Official Methods of Analysis, 12th Edn., Assoc. Off. Anal. Chem., Washington, DC, 1975, Sec. 28.052.

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Crystal Structure Modifications of Tristearin by Food Emulsifiers

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ABSTRACT

The effect of several emulsifiers as crystal structure modifiers of tristearin has been investigated. The less thermodynamically stable modification, named α, is preserved when 1-10% of sorbitan monostearate was added before allowing the molten tristearin to cool and crystallize. Several other emulsifiers have been tested and it has been found that the combination of bulkiness of the hydrophilic groups with the right lengths of the hydrophobic chains of a given emulsifier is necessary to preserve the α-modification. Liquid emulsifiers and those having a pronounced hydrophilic character are not efficient as modifiers. The emulsifier has been shown to be incorporated into the tristearin during crystallization from solvent without an immediate effect, but it affects subsequent behavior upon melting and resolidification.

INTRODUCTION

The addition of surface-active agents to chocolate to retard fat bloom has been done for many years. Emulsifiers such as lecithin and monoglycerides have been considered as both viscosity controllers and anti-bloom agents (1-3).

The development of tempering methods allowed an evaluation of the efficiency of a fat-bloom retardant and, as a result, a procedure has been proposed to test the effect of adding a single compound on the chocolate bloom. Sorbitan monostearate (Span 60) was found to be the only effective modifier in preventing blooming in an early study (4). Emulsifiers such as ethoxylated sorbitan esters (Tween 60, Tween 61, Tween 20) were reported to have fair protection of the fat. Several blends of emulsifiers such as Span 60-Tween 60 (40:60) were reported as excellent antibloomers. It has been concluded that the extent of protection provided by the emulsifiers is not fully known and should be further investigated (4).

The polymorphism of triglycerides composed of saturated fatty acids with an even number of carbons has been studied extensively by a number of investigators (5,6). Bailey was the first to compile comprehensive polymorphic data on fats (7) and Lovegren and Gray added significant data on those systems using differential scanning calorimeter to determine the changes that occur (8). Hoerr and Paulicka (9) have studied the various polymorphs using X-ray diffraction and explained the crystal structures of these compounds in terms of molecular orientation in the crystal lattices.

To the best of our knowledge, only a little information has, so far, been published concerning the role of the added surfactants on the crystal structure modification of the saturated fat. Recently, Kawamura (10) has shown the effect of sorbitan monostearate and sorbitan tristearate on the thermal behavior of palm oil using DSC techniques and has demonstrated typical phase transformations of the palm oil in the presence of these emulsifiers. Some other scientific work is connected with the study of the bloom phenomena in chocolate. On the other hand, increasing use is being made of these modifiers in the food industry where their effect is very much related to the modification of the crystal structure of the fat.

In a symposium paper presented by Krog, an attempt was made to find a correlation between the fat and the emulsifier crystal structures. However, no conclusions have been drawn (11).

In the course of our previous studies, we examined the effect of various food emulsifiers on the crystal structure and habit of stearic acid, and have shown the preservation of preferable modification of stearic acid in the presence of modifiers (12,13).

The purpose of this paper, therefore, is to study more carefully the effect of several food emulsifiers on the crystal structure modification of tristearin, to examine X-ray diffractions and thermal behavior (DSC) and to correlate between molecular structure as well as physical properties of emulsifiers and the fat modification formed when crystallization from melt and solvents was induced.

EXPERIMENTAL

Materials

Tristearin was purchased from Sigma and was of 99% purity

(by GLC). The emulsifiers were commercially available from Atlas Europal A.p.S., Italy, Grindsted Products of Denmark, Croda Chemicals, England, and Hamorad Chemicals, Israel. The following types of emulsifiers were tested: sorbitan esters of fatty acid (Spans), ethoxylated sorbitan esters of fatty acids (Tweens), ethoxylated fatty alcohols (Brijs), citric acid ester of monoglyceride (Acidan), diacetyl tarteric acid ester of monoglycerides (DATA), sucrose monostearate (SMS), and polyglycerol esters of fatty acids (PGE).

The emulsifier concentrations were between 1 and 15 wt % of the tristearin.

X-ray measurements were obtained with a Philips diffractometer using Cu radiation and Ni filter.

Thermal measurements were done on a Perkin Elmer differential scanning calorimeter (DSC 2) and a Du Pont 900 differential thermal analyzer. Heating rate was 5 C/min. Sample weights were from 0.5 mg up to 2 mg. ΔH measurements were with an accuracy of ±15%.

RESULTS

Diffractograms of pure tristearin obtained from melt are presented in Figure 1. The first measurement has been done immediately after crystallization (Fig. 1a). It can be seen that the α-form, having only one short spacing at 4. 1 Å has been obtained. After 24 hr of storage at room temperature, another X-ray diffraction analysis has been done and the appearance of β-form has been detected (Fig. 1b). More prolonged storage (96 hr) changed a significant amount of the crystals into the β-modification, which is thermodynamically more stable (Fig. 1d).

The thermal behavior of the tristearin crystals obtained from melt and analyzed immediately after crystallization showed a typical DSC α-form thermogram (Fig. 2a). The thermogram is composed of 2 endothermic and one exothermic peaks.

The first endothermic peak at 55 C is the characteristic α-modification melting point (mp). The caloric data for this polymorph (ΔH_α cal/g) are calculated and presented in Table I. After the melting of α-form, an exothermic peak (ΔH_t) appears, followed by additional melting endothermic peak of the β-form at 73 C ($\Delta H\beta$). The exothermic peak ΔH_t is due to transformation into solid β-form. The energy of this exothermic peak consists of enthalpy of crystallization and enthalpy of transition. In a reverse procedure, the melted tristearin was cooled at a constant rate. It was crystallized at 37 C in the α-form and the ΔH_c of such a process was equal to the ΔH_α in the heating process, indicating that the first peak in the thermogram was due to the complete melting of the α-form.

The results obtained in our laboratory confirm the results of Lovegren and Gray, stating that no additional polymorphs melting at 61 or 64 C could be detected by this method (8). The tristearin in the α-form was stored for 96 hr at room temperature and then a DSC determination was performed. The ΔH_α peak was reduced in comparison to the $\Delta H\beta$ peak (Fig. 2b), indicating that a significant part of the crystals were transformed into the β-form. This result is in excellent agreement with the X-ray determination (Fig. 1d).

The addition of 5 wt % of sorbitan monostearate (Span 60) to the molten tristearin, prior to the crystallization process, caused a significant change in the stability of the

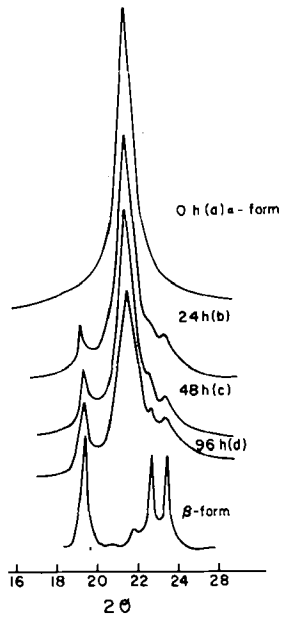

FIG. 1. X-ray diffraction powder pattern of tristearin crystals obtained from melt (a) immediately after crystallization (α-form); (b) after 24 hr of storage at room temperature; (c) after 48 hr of storage; (d) after 96 hr of storage; and (e) pure β-form.

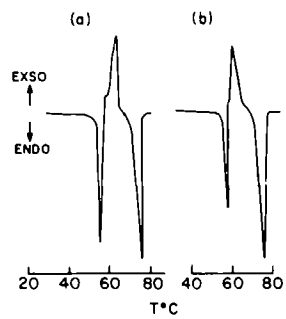

FIG. 2. DSC heating curves of pure tristearin; sample obtained from melt and heated at 5 C/min (a) immediately after crystallization (α-form); and (b) after 96 hr of storage (mixture of α- and β-form).

TABLE I

Calculated Values of the ΔH_α, ΔH_t and ΔH_β of Tristearin Crystallized from Melt in the Presence of Various Amounts of Sorbitan Monostearate (Span 60)[a]

	wt % of Span 60							
	0	1	2	3.3	5	7.2	10	15
	ΔH (cal/g)							
ΔH_α	34.39	29.11	33.87	27.22	26.46	26.17	28.40	25.66
ΔH_t	21.63	17.92	17.30	16.86	8.84	5.56	1.03	0
$\Delta H\beta$	44.36	34.48	34.78	29.26	12.67	8.80	2.26	0

[a]Du Pont 900 differential thermo analyzer. Scale: T = 20 C/in; ΔT = 0.2 C/in.

obtained α-form. Figure 3 presents the X-ray diffraction powder patterns of such crystals, immediately after crystallization and after several hours of storage at room temperature. It can be seen that, even after 96 hr of storage, the α-modification still exists and only very slight traces of β-form can be detected. The sorbitan monostearate served as a crystal structure modifier or as a retarding agent, conserving the α-form modification. Figure 4 shows the thermograms of tristearin obtained from melt in the presence of 1-15% Span 60. The proportion of the ΔH_α to ΔH_β changes from ca. equal lengths (2% Span 60, Fig. 4b) through progressive disappearance of ΔH_t and ΔH_β with the increase of added Span 60, up to their total disappearance (15% Span 60, Fig. 4g).

The disappearance of the second melting peak (4G) indicates that no β-form was obtained or that the less stable α-modification was preserved and melted directly without being first transferred into the β-form. Table I summarizes the results of these experiments, and the ΔH_α, ΔH_t and ΔH_β were calculated from the thermograms.

The ΔH_α, ΔH_t and ΔH_β values, as measured from the DSC curves, were plotted vs the emulsifier concentration (Fig. 5). The ΔH_α values measured for all crystals obtained from the melt process in the absence or presence of various amounts of emulsifiers decrease slowly with increasing amounts of the emulsifier. The ΔH_t values for the crystallization process of the molten α-form decrease sharply with the increasing amount of the emulsifier and reached an almost zero value when 10 wt % of emulsifier was present in the system. In comparison, the ΔH_α line is almost parallel to the x-axis. The crystallization process is exothermic and requires 22 cal/g in the absence of an emulsifier. In the presence of 10 wt % of Span 60, most crystals remain in the α-form before melting and thus almost no exothermic process occurs before transformation into the liquid phase occurs. The ΔH_β values for the melting process best indicate the amount of the α-form crystals existing in the system. The endothermic peak for crystals melting in the absence of an emulsifier is ca. 45 cal/g and is reduced to almost zero in the presence of 10-15% of emulsifier, indicating that no β-form is present in the system and that most crystals are transformed into the liquid phase straight from their α-modification.

Figure 6 is a comparison of the performance of 5 wt % of (a) sorbitan monolaurate (Span 20); (b) sorbitan monopalmitate (Span 40); (c) sorbitan monostearate (Span 60); (d) sorbitan tristearate (Span 65); and (e) sorbitan monooleate (Span 80). It can be seen that while sorbitan monostearate is the most effective modifier in this class, sorbitan tristearate is a fair retardant and the other sorbitan deriva-

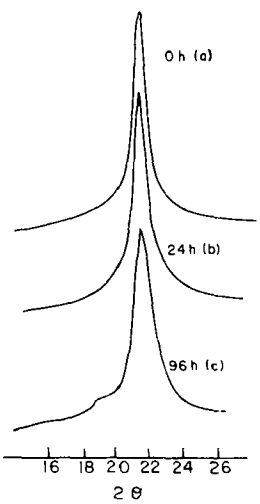

FIG. 3. X-ray diffraction powder pattern of tristearin obtained from melt in the presence of 5 wt % of sorbitan monostearate (Span 60) as crystal modifier (a) immediately after precipitation; (b) after 24 hr of storage; and (c) after 96 hr of storage.

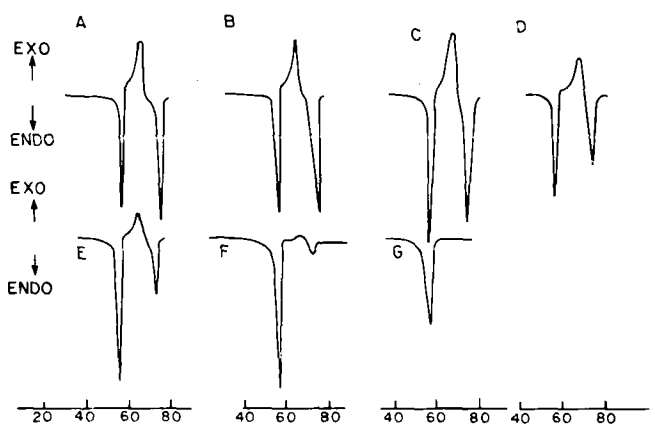

FIG. 4. DSC heating curves of tristearin obtained from melt in the presence of 1-15 wt % of sorbitan monostearate (Span 60): (a) 1 wt %; (b) 2 wt %; (c) 3.3 wt %; (d) 5 wt %; (e) 7 wt %; (f) 10 wt %; and (g) 15 wt %.

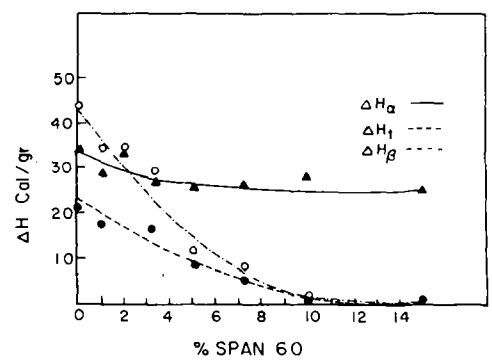

FIG. 5. A plot of the ΔH_α, ΔH_t and ΔH_β values measured by the DSC method for tristearin crystallized from the melt in the presence of increasing amounts of sorbitan monostearate (Span 60) as emulsifier.

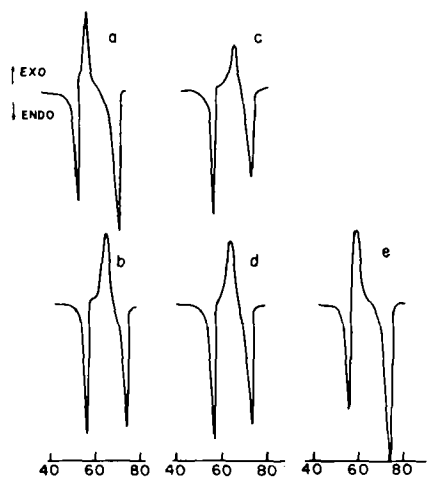

FIG. 6. DSC heating curves of tristearin obtained from melt in the presence of 5 wt % of various emulsifiers: (a) sorbitan monolaurate (Span 20); (b) sorbitan monopalmitate (Span 40); (c) sorbitan monostearate (Span 60); (d) sorbitan tristearate (Span 65); and (e) sorbitan monooleate (Span 80).

tives have almost no effect. It may be inferred that sorbitan monopalmitate (Span 40) will be effective only when relatively high concentrations are provided and that sorbitan monooleate will be ineffective even at high concentrations.

Table II summarizes the screening measurements made on 12 emulsifiers. It is worth noting that our results are in excellent agreement with Krog's statement that only sorbitan monostearate and monoglyceride stearate citrate are effective modifiers.

TABLE II

Calculated Values of the ΔH_α, ΔH_t and ΔH_β of Tristearin Crystallized from Melt in the Presence of 5 wt % of Various Emulsifiers[a]

Emulsifier trade name[b]	ΔH_α (cal/g)	ΔH_t (cal/g)	ΔH_β (cal/g)
Span 40	31.10	17.35	29.04
Span 65	27.54	16.09	26.85
Span 80	31.16	18.15	44.16
Tween 61	31.77	19.77	40.06
Tween 65	36.33	20.93	45.53
Brij 58	31.66	14.64	37.51
Brij 78	34.84	15.08	42.99
SMS	29.63	16.74	38.73
SSL	32.0	21.40	42.39
10G1S (PGE)	32.63	22.18	42.68
Acidan	25.15	9.56	12.85
DATA	32.11	20.09	35.38

[a]Perkin Elmer differential scanning calorimeter. Sensitivity: 5 μcal/sec.

[b]Span 40—sorbitan monopalmitate; Span 65—sorbitan tristearate; Span 80—sorbitan monooleate; Tween 61—polyoxyethylene sorbitan monostearate; Tween 65—polyoxyethylene sorbitan tristearate; Brij 58—polyoxethylene cetyl alcohol; Brij 78—poloxyethylene stearyl alcohol; SMS—sucrose monostearate; SSL—sodium stearoyl-2-lactylate; 10G1S—decaglycerole monostearate; Acidan—citric acid ester of glycerol monostearate; DATA—diacetyl tartaric acid of glycerol monostearate.

In Figure 7, the effect of increasing the amount of Acidan (monoglyceride stearate citrate) is presented. It can be seen that 7.5 wt % of the monoglyceride stearate citrate is sufficient to prevent any formation of the β-form, even in the heating process. No β-form was detected by X-rays even after a few weeks of storage.

As no purification of the commercial emulsifer was done prior to any of the experiments, we have tested the validity of those results and the emulsifiers' performances by comparing the performance of 3 commercial sorbitan monostearates purchased from various sources.

Figure 8 presents those results. It can be seen that emulsifiers from various sources differ in their effectiveness as structure modifiers or retarding agents. Table III summa-

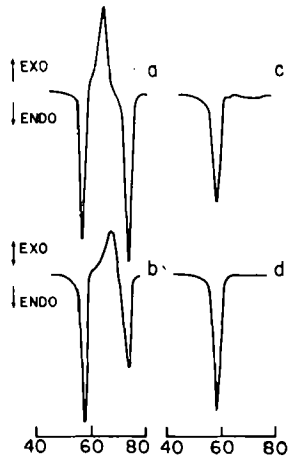

FIG. 7. DSC heating curves of tristearin obtained from melt in the presence of increasing amounts of monoglyceride stearate citrate: (a) 3 wt %; (b) 5 wt %; (c) 7 wt %; and (d) 10 wt %.

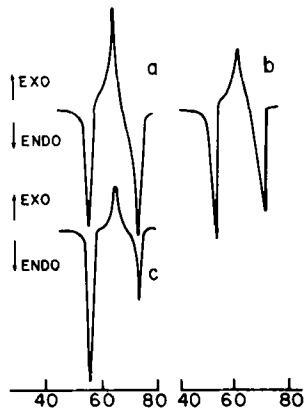

FIG. 8. DSC heating curves of tristearin obtained from melt in the presence of 5 wt % of sorbitan monostearate from various sources: (a) Hamorad, Israel; (b) Atlas Europal A.p.S.; and (c) Croda Chemicals, England.

rizes the analytical data obtained for some emulsifiers. The emulsifiers differ among themselves with respect to some characteristics. It seems that impurities and the distribution of the product components in the emulsifier mixture are affecting the activity of the surfactant. (Further work on this problem is in progress.)

MECHANISTIC CONSIDERATIONS

In order to explain the significance of our results, we have tried to perform several control experiments.

TABLE III

Fatty Acid Composition, mp, X-Rays, Acid and Saponification Values of Several Emulsifiers

Type of emulsifier	Fatty acid composition % C_{14}	% C_{16}	% C_{18}	mp (C)	Short space (Å)	Acid value	Saponification value
Span 60	2	47	50	58.5	4.12	4.48	189
Crill 3[a]	1	45	54	55	4.12	3.36	–
Hamorad[b]	4	25	71	49	4.12	4.49	152
Span 65	–	51	49	46	4.12	–	324
Span 40	9	90	1	57	4.12	4.48	184
Acidan	–	36	64	56	4.12	–	–

[a]Crill 3—sorbitan monostearate from Croda Chemicals, England.

[b]Hamorad—sorbitan monostearate from Hamorad Chemicals, Israel.

The behavior of tristearin obtained from crystallization of solvents in the absence and presence of various emulsifiers at different crystallization conditions has been tested. The results indicate clearly that solvent crystallization under any circumstances will yield only the β-form and that the presence of any of the emulsifiers in any concentration does not affect the crystal structure. The lack of effect in the presence of emulsifiers is quite disappointing, because in our previous study on the effect of modifiers on fatty acids, we detected significant influence of the emulsifier on the fatty acids crystallized from solvents.

In a separate set of experiments, we tried to melt and cool for resolidification the tristearin obtained previously from solvent crystallization in the presence of an emulsifier. Upon resolidification, the α-form was obtained as usual. However, it was found, surprisingly, that when testing subsequent melting by DSC, only part of the α-form converted into the β-form indicating the existence of the emulsifier as an impurity in the tristearin that was previously crystallized from solvent by the analogy in thermal behavior previously described. The fact that the emulsifier was absorbed in the tristearin during the crystallization in sufficient amounts to affect the solidification process in the later stage may serve as a method for evaluation of the amounts of emulsifier capable of incorporation in the tristearin.

This study on the effect of various food emulsifiers on the crystal-structure modifications of tristearin shows that several emulsifiers, such as sorbitan monostearate and other monoglyceride derivatives of citric acid, can serve as α-form crystal preservatives, preventing the transformation of the α-form into the most thermodynamically stable β-form. The enthalpy of α-form melting (ΔH_α) and β-form melting (ΔH_β) and the exothermic transition (ΔH_t) which have been measured and calculated helped to evaluate the amount of β-form obtained upon heating. In our previous study on the crystallization of stearic acid* (13), it was shown that one can predict the activity of a given emulsifier to serve as a modifier if both bulkiness of the hydrophilic groups and the right length of the hydrophobic groups exist in the tested emulsifier. The effective emulsifiers, as found in the present study, have the same characteristics.

REFERENCES

1. Kleinert, J., Int. Choc. Rev. 16:201 (1961).
2. Du Ross, J.W., and W.H. Knightly, Manuf. Confect. 45:50 (1965).
3. Campbell, L.B., and P.G. Keeney, Ibid. 48:77 (1968).
4. Easton, N.R., D.J. Kelly, L.R. Bartren, S.T. Cross and W.C. Griffin, Food Technol. 6:21 (1952).
5. Lutton, E.S., JAOCS 27:276 (1950).
6. Malkin, T., Prog. Chem. Fats Other Lipids, Vol. 2, Academic Press, Inc., New York, NY, 1954.
7. Bailey, A.E., Melting and Solidification of Fats, Interscience Publishers, Inc., New York, NY, 1950.
8. Lovegren, N.V., and N.S. Gray, JAOCS 55:310 (1978).
9. Hoerr, C.W., and F.R. Paulicka, Ibid. 45:795 (1968).
10. Kawamura, K., Ibid. 57:48 (1980).
11. Knog, N., 10th Congress of the International Society for Fat Research, 1968.
12. Garti, N., E. Wellner and S. Sarig, J. Cryst. Growth (in press).
13. Garti, N., E. Wellner and S. Sarig, JAOCS 58:1058 (1981).

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Ethoxyquin in Fish Meal

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ABSTRACT

Four reliable methods are described for the determination of ethoxyquin antioxidant in fresh fish meal. These include an accurate chromatographic laboratory method, a colorimetric method, and 2 rapid factory methods, one a quantitative titration technique and the other suitable for spot checks for ethoxyquin in "go–no go" situations. With the laboratory method, 6 or 8 chromatographic columns can be handled simultaneously. The 2 rapid methods are based on 1,1-diphenyl-2-picrylhydrazyl and may be used routinely in the factory to determine the antioxidant content of several hundred samples.

Fish meal manufactured from the pelagic anchovy, pilchard and mackerel of the Southern Hemisphere contains up to 10% of a highly unsaturated oil which can cause spontaneous heating. Partly for this reason, such meals are treated with 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (ethoxyquin, EQ), a highly effective antioxidant which renders the meal safe for storage and transport, and which maintains a higher calorific and nutritional value than cured meals.

This investigation was motivated by the need for a rapid, reliable method for the determination of EQ in fish meal. Existing methods proved too time-consuming (1) as the number of analyses increased, and all methods suffer from disappearance of the antioxidant in reactive fish meals (2-5). Because the original dosage cannot be determined after a few days, and because the recoverable EQ is changing at a rate which differs for each sample, pin-point accuracy is deemed less important than maximal recovery and simplicity of method. An accuracy of plus or minus 10 mg/kg at the 400 mg/kg level is quite acceptable in meal production.

The official method for EQ of the Association of Official Analytical Chemists (6) involves extraction with petroleum ether (7) chromatography and subsequent measurement by fluorimetry. The method requires extreme care and skill, and is intended for determination at low levels, e.g., on apples. Alternative methods not using chromatography (8) are unsuitable as blank values are large in older meals and not possible to determine, because the "blank" requires an untreated meal, of obviously different history.

This communication describes the methods that are routinely used for determining EQ in fresh fish meal, at both laboratory and factory levels. For laboratory use, the chromatographic method is rapid, relatively simple and reliable. The 2 rapid methods for factory tests, i.e., quantitative titration and spot check for EQ, use 1,1-diphenyl-2-picrylhydrazyl (DPPH). With certain provisos, both can be used by unskilled personnel.

EXPERIMENTAL

Materials and Methods

Chromatography columns, 1.5 × 30 cm, with sintered glass frit (porosity 1) and stopcock (preferably Teflon) were