The decrease in total tocopherol of shea fat with autoxidation

J. K. B. A. ATA & VERONICA SACKEY
Food Research Institute, P.O. Box M.20, Accra, Ghana

SUMMARY

The rate of decrease of the total tocopherol content of shea fat with autoxidation has been studied. The results have shown a close relationship between natural antioxidant depletion and the formation of peroxides. In the initial stages of autoxidation, tocopherols are used up very fast in preferential oxidation until it reaches a level when a second phase of autoxidation sets in. During the latter period, there is not much significant change in the residual tocopherol level.

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RÉSUMÉ

ATA, J. K. B. A. & SACKEY, VERONICA: *La décroissance de la teneur totale en tocophérol du beurre de karité, au cours de l'auto-oxydation.* Les auteurs ont étudié la vitesse de décroissance du tocophérol total contenu dans le beurre de karité, au cours de l'auto-oxydation. Ils ont observé une étroite corrélation entre la disparition de l'anti-oxydant naturel et la formation de peroxydes. Pendant les stades initiaux de l'auto-oxydation, les tocophérols sont oxydés très rapidement, de manière préférentielle, jusqu'à un certain seuil, lorsqu'une seconde phase d'auto-oxydation s'établit. Pendant la durée de cette seconde phase, la teneur résiduelle en tocophérol ne subit pas de changement notable.

Introduction

It is common knowledge in oil technology that when oils are subjected to atmospheric oxygen at high temperatures, the oils develop objectionable flavours and taste. The processes leading to this are divided into two phases:

(a) induction phase,
(b) period of rapid peroxide formation.

In the induction phase, peroxide radicals accumulate slowly and hydroperoxides are built up from the reactions of the peroxides and other molecules having active methylene groups.

In the second phase, the hydroperoxides which have been formed decompose, giving rise to carbonyl compounds (aldehydes and ketones) which are responsible for the objectionable flavour and taste associated with such oils.

Tocopherols (natural antioxidants) as well as other phenolic antioxidants are known to inhibit oxidation by being preferentially oxidised. This preferential oxidation, however, appears to occur only during the induction phase, and, as was shown by Jack & Brunner (1943), antioxidants have no effect after the induction period. This experiment was, therefore, planned to study the behaviour of the natural antioxidants of shea fat with autoxidation.

Materials and methods

Autoxidation

500cm^3 of shea fat was measured into a round-bottom flask. Air was bubbled through continuously whilst it was heated at 140–150°C. 10cm^3 samples of the oil were taken at hourly intervals for 7 h and the peroxide value determined.

Determination of peroxide value

The standard method of analysis adopted by the International Union of Public Analysts Commission (I.U.P.A.C.) was used. The prescribed quantity of each oil was mixed with 25cm^3 of acetic acid/chloroform (2:1) mixture. 4·0g of potassium iodide crystals were added and the mixture was heated to boil in a boiling water bath. Sufficient distilled water

was added to cool the mixture and to avoid any loss of liberated iodine. The mixture was titrated against 0·0002 *N* sodium thiosulphate solution using 1% starch solution as indicator.

Saponification

10cm³ of the oxidised shea butter was refluxed with 40cm³ of 5% pyrogallol solution for 3 min with occasional shaking. 10cm³ of 16% potassium hydroxide solution was then added and refluxed for a further 5 min. The flask and contents were cooled by adding distilled water.

Extraction of unsaponifiable matter

The unsaponifiable matter was extracted with diethyl ether. The ether layer was then washed with distilled water until the aqueous phase was clear or neutral to phenophthalein. The ethereal phase was retained, evaporated on a water bath and the residue dissolved in a mixture of benzene and ethanol (1:1) v/v. This was again evaporated off. The residue was finally dissolved in 5cm³ of benzene. This was retained as the tocopherol mixture.

Thin-layer chromatography

Glass plates (20cm × 20cm) were cleaned with ethanol to remove traces of water. 30g of Kieselgel G in 60cm³ of distilled water containing 1·0cm³ of 0·1% sodium flourescein were prepared. The plates were arranged on the rolling machine. The freshly prepared suspension of Kieselgel G was quickly poured into the spreader, set at 0·5mm and quickly drawn across the plates. The plates were allowed to dry in air and then in a ventilated draft oven set at 105°C for 1 h.

The plates were removed and spotted with the extracted tocopherol mixture using 10^{-6} dm³ micropipettes. The plates were immersed in a glass tank lined with blotting paper and containing a solvent system of the following composition:

Isopropyl alcohol	16·0cm³
Diethyl ether	1·5cm³
Acetic acid	1·5cm³
Acetone	6·0cm³
Petroleum ether (60–80°C) ..	127·0cm³

The plates were run to a solvent front of 14cm and dried in the dark. The spots were identified under the ultraviolet light.

Circles were drawn around the tocopherol spots. The spots were immediately scraped off into a sample tube and extracted with 4cm³ absolute ethanol. 3·5cm³ of 0·6% dipyridyl and 0·5cm³ of freshly prepared 0·2% ferric chloride solution in alcohol were added to the filtrate. The pink colour produced on warming the mixture at 60°C was read exactly 3 min after at $5{\cdot}20 \times 10^{-7}$m in an Optica Milano CF4 spectrophotometer.

The amount of tocopherol in the sample was calculated in micrograms per gram of oil using the formula of the Standard Methods of Oil and Fat Division of the I.U.P.A.C. (1964) given by:

$$\frac{D \times F \times v \times 1000}{V \times G}$$

where D = net extinction, F = spectrophotometric factor for 4cm³, V = volume of benzene used to

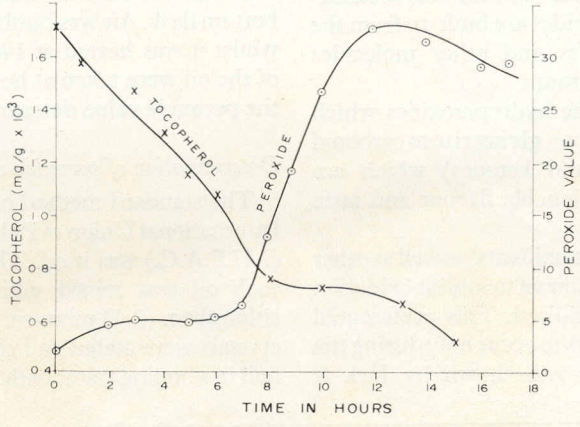

Fig. 1. Relationship between peroxide rise and tocopherol decrease in shea fat at 140°C.

prepare the solution for thin layer chromatography, v = volume in microlitres spotted on plate and G = weight in grams of shea butter used.

The spectrophotometric factor (98) for $\times$-tocopherol was used to calculate the total tocopherol content since experimental difficulties did not permit the resolution of the tocopherols into their separate components.

Results and discussion

The calculated values of the peroxide and tocopherol contents of shea fat on peroxidation are presented graphically in Fig. 1. The graph of autoxidation shows a typical curve for an oil. The tocopherol curve obtained agrees with the views put forward by Jack & Brunner (1943). The initial tocopherol content was very high but this was due to the high unsaponifiable content of shea fat. From the graph, it can be observed that the antioxidant effect seems to have ceased even at a high level of 740μg/g, which is considerably higher than the normal total tocopherol level found in many other oils such as crude groundnut oil (400·0μg/g) and crude palm oil (393μg/g) as shown by Ata & Cobbina (1973).

The results also indicate that the true protective effect of the tocopherols lies within the induction period. It cannot be explained, however, why the high level of anti-oxidant (740μg/g) should not have a longer protective effect. These are areas which need further investigations.

REFERENCES

Ata, J. K. B. A. & **Cobbina, A.** (1973) The level of tocopherol in Ghanaian oils. *Ghana Jnl agric. Sci.* **6,** 45–47.

Jack, E. L. & **Brunner, J. R.** (1943) The relationship between the degree of solidification of fat in cream and its churning time. I. Measurement of the degree of solidification. *J. Dairy Sci.* **26,** 169–177.

I.U.P.A.C. (1964) *Standard methods of the Oil and Fats Division of the I.U.P.A.C.*, 5th ed. London: Butterworths.