• *Letter to the Editor*

Improvements in the Azeleoglyceride Analysis Technique

Sir: It has been found possible to introduce the following improvements in the different stages of the azeleoglyceride technique for determination of the glyceride structure of natural fats (Kartha, JAOCS *30,* 280, 1953; Kartha and Narayanan, Ibid. *44,* 350, 1967): 1. Improved procedure for the oxidation of fats: When fats are oxidized at 0.5% concentration or less in acetic acid-acetone (Kartha, JAOCS *30,* 280, 1953), the entire quantity of permanganate (10 g/g fat) and acetic acid (12 ml/31 g of permanganate) required can be added in a single lot before oxidation is started, and the oxidation is complete when the mixture is refluxed with occasional shaking for 4 hr. Under these conditions chain scission is practically complete and analysis of oxidation products for unoxidized triglycerides (Kartha, JAOCS *30,* 28, 1953) can be omitted. Proportions of fats now required need not be more than 200–250 mg when saturated acid (S) content is above 40% and not more than 1.0–1.5 g when S content is below 15%.

2. Single stage determination of total bertram acids from non-glyceride substances: Many fats contain non-glyceridic substances, namely unsaponifiable matter, unesterifiable resin acids and esterifiable resin acids, which give rise, on oxidation, to long chain acids giving water insoluble magnesium salts (bertram acids) just like higher saturated acids. For example, *Myristica attenuata* seed fat produced 14.1% Bertram acids (all percentages given are on refined fat basis) from unsaponifiable matter and a further 1.2% from esterifiable and unesterifiable resin acids while *Myristica fragrans* seed and mace fats produced 0.4 and 2.9% of these acids from the non-glyceridic substances present therein (Kartha and Narayanan, J. Sci. Ind. Res. *21B,* 442, 494, 1962). These acids separate quantitatively with the GS_2A in the insoluble azeleoglycerides (IAG) (Kartha, JAOCS *30,* 280, 1953), and have to be estimated and corrected for to get the true GS_2A contents. The proportions of these acids derived from each source was separately determined earlier (Kartha and Narayanan, J. Sci. Ind. Res. *21B,* 442, 494, 1962). However the total bertram acids from all non-glyceridic substances is now determined in a single step: The mixed acids are esterified as such (without separation of unsaponifiables) for 8 hr with methanolic sulfuric acid and the crude esters isolated by usual procedures. They will contain the esters of all the esterifiable acids along with the unsaponifiables and unesterifiable acids. This mixture is directly oxidized as described in section 1 and the products of oxidation separated into acidic and neutral fractions by washing in di-ethyl ether solution with dilute ammonia. The acidic fraction is submitted to the separation a second time. The purified acidic fraction is submitted to hydrolysis with alcoholic potash and the Bertram acids present are precipitated twice as usual (Kartha, JAOCS *30,* 280, 1953). The Bertram acids thus obtained are derived exclusively from the non-glyceridic substances in the fat.

If the percentage proportions of IAG, Bertram acids from IAG, and Bertram acids from non-glyceride substances are A, B and C respectively; in fats wherein GS_3 is absent, the proportions of $GS_2A + GSA_2$ in IAG = $A - C$ and the proportions of saturated acids in $GS_2A + GSA_2 = B - C \times 100/A - C$.

3. Improved procedure for azeleoglyceride separation: In fats consisting predominantly of GU_3 and GSU_2 (say 80% and above), the precipitation of GS_2A magnesium salts in the IAG may not be complete in 30 min at 25 C (1) due to intersolubility effects exerted by the magnesium soaps of GA_3 and lower saturated acids produced during oxidation on those of GS_2A. Error due to this is minimized in the following procedure. The total oxidation products of fat isolated as usual (Kartha, JAOCS *30,* 280, 1953) is heated in a current of air on a water bath till all the acetic acid and a major portion of the lower mono-basic acids produced during oxidation are volatilized (odour). The residue is then dissolved in very dilute ammonia, if necessary with the aid of minimum heat, to give a clear solution cooled to room temperature and precipitated with 15 ml of 15% ammonimum chloride and 35 ml of 15% magnesium sulfate solutions, keeping a minimum solution volume of 100–150 ml/g fat oxidized. The precipitated mixture is cooled at 10–15 C for 2 hr, filtered off on a funnel cooled to same temperature in the usual filter paper cone, and washed well with cold water (10–15 C) to separate the IAG fraction. The soluble azeleoglyceride (SAG) and IAG fractions are recovered as usual (Kartha, JAOCS *30,* 280, 1953).

The SAG is then washed in ethereal solution with excess of freshly prepared (neutral to phenolphthalein) 3% solution of sodium bicarbonate three times to remove all GA_3 present and the bicarbonate insoluble fraction is submitted to a second azeleoglyceride separation under the same conditions as the first. Material recovered from precipitated magnesium soaps, if any, is added to the IAG from the first separation, since in some cases it may still contain some GS_2A. The bicarbonate washings and filtrates from the second azeleoglyceride separation are combined for recovering the final SAG fraction. Analysis of the final SAG and IAG fractions is conducted as before (Kartha, JAOCS *30,* 280, 1953). The second azeleoglyceride separation may be omitted when the Bertram acids isolated from the SAG in the first separation does not amount to more than 1% on fat basis.

Fats with S content above 30% have not so far shown any significantly higher GS_2U contents by the improved procedure. However oils with S content below 30% as a rule show 1% to 3% higher GS_2U contents by the improved procedure. This increase does not depend on the S content alone, and is apparently influenced by other aspects of component acid composition, notably the mean chain length of the saturated acids. The uniform use of the improved procedure in all cases will avoid all possibility of error due to this source.

There is no alteration in the overall scheme of analysis and methods of calculation which remain exactly the same as before (Kartha, JAOCS *30,* 280, 1953).

A. R. S. Kartha
Division of Chemistry,
Indian Agricultural Research Institute,
New Delhi, India

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