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Microdetection of kusum oil

Recent interest in kusum oil, a fat available from the seeds of the species *Schleichera trijuga*, that is a member of the family *Sapindaceae*, centres upon some prominent features of the oil. The fat is an oleic-rich oil distinguished by the presence of a wide range of fatty acids from acetic to behenic[1–4] and by a remarkably low content of linoleic acid[1]. The oil is further characterised by a high Reichert–Meissl value (R.M.V.) and a high Kirschner value (K.V.)[5]. These figures taken in conjunction with the low Polenske value (P.V.) and the buttery consistency of the oil might render the oil difficult to detect if used as a butter adulterant. Thus, an occasion may arise when the oil in possible admixture with such fats or, maybe, for some other compelling reasons, needs to be identified. Detection or determination of the arachidic acid which is present in a fairly high proportion[1,2] in the oil may, perhaps, indicate the presence of kusum oil, but the mere presence of arachidic acid, in such cases, cannot be considered conclusive for the presence of kusum oil. Proper solution of the problem requires a method that is conclusive and, at the same time, sensitive enough to detect microquantities of the oil with minimum interference in a reasonable time, and it is expected that the method be preferably simple. Now, a notable feature of the oil is the characteristic presence of a cyanogenic compound in a concentration reported to be between 0.03% and 0.5% expressed as HCN[1,6]. The cyano compound has recently been studied by KUNDU *et al.*[3,4] and is shown to be cyanoglyceride (the first and so far the only lipid known to have been characterised in a naturally occurring oil). This communication utilises this unique feature of the oil and devises a procedure that involves the use of chromatography and copper acetate–benzidine acetate as a reagent for the detection of cyanide ion in the vapour phase and that is applicable for detecting microquantities of the oil.

Experimental

Apparatus and reagents

The apparatus used were 10-ml test tubes, 100-ml erlenmeyer flasks, micropipettes, ordinary pipettes, glass plates (20 × 20 cm, 10 × 20 cm), chromatographic chambers, etc. The glassware was thoroughly cleansed and dried prior to use.

The reagents used were 1 *N* aqueous sodium hydroxide, 9 *N* sulphuric acid, chloroform (Merck), Silica Gel G (Merck), petroleum ether (60–66°, C.P.), diethyl ether (C.P., dry and distilled, peroxide-free), acetic acid (A.R.), iodine crystals (Merck). The sample was *ca.* 5% solution in chloroform, and reference substances were *ca.* 1% solution in chloroform. Copper acetate–benzidine acetate reagent was prepared[7] by mixing equal volumes of a 3% solution of copper acetate in water and a 1% solution of benzidine in *ca.* 10% acetic acid solution, prior to use.

Preliminary detection

About 0.1 g of the sample (although lower amounts may be allowed) is taken in a clean 10-ml test tube. The contents are warmed with the sodium hydroxide solution, acidified with sulphuric acid in the cold, and the vapour evolved is allowed to come in contact with a piece of filter paper soaked with the copper acetate–benzidine acetate reagent. The presence of the cyano compound (and hence, generally, of kusum oil) is indicated by the exposed zone of the filter paper being coloured blue.

Confirmation of the presence of kusum oil

A 0.2-ml solution of the crude oil in chloroform (approx. 10 mg) is applied in the form of a band on a silica gel layer (0.8 mm thick) spread uniformly over a glass plate and fractionated into classes, using the solvent system, *n*-hexane–diethyl ether–acetic acid (75:25:1) in a perfectly flat-bottom chromatographic chamber closed at the top with a lid and equilibrated inside with the solvent vapour. Suitable reference substances consisting of a triglyceride, a diglyceride, a fatty acid and a sterol are chromatographed side by side under identical conditions for comparison. The solvent front is allowed to ascend to about 15 cm. The plate is then taken out of the chamber, the solvent removed by evaporation and the bands are located by exposure to iodine vapour. The triglyceride band is marked and the adsorbed iodine removed from the plate by warming. The marked band is then scraped off the plate into a 100-ml erlenmeyer flask, extracted with warm chloroform and filtered into another 100-ml erlenmeyer flask. The solvent is removed and the procedure described under preliminary detection is repeated with the residual mass. The presence of kusum oil is confirmed by the blueing of the exposed zone of the filter paper.

Discussion

The procedure described above is capable of detecting micro quantities of the oil. The sensitivity of the method comes out to about 25 μg with respect to the cyano compound expressed as HCN; the value has been calculated on the basis that 10 mg of the oil have been used and the concentration of the cyano compound in the oil is about 0.25% (mean of the reported values) expressed as HCN. Possible effects of the presence of several selected oils were noted, using model mixtures. Thus, butter fat (fat with comparable R.M.V., P.V. and K.V.), coconut oil (*Cocos nucifera*, oil with high P.V. but low R.M.V., K.V. and I.V.), peanut oil (*Arachis hypogaea*, oil with low R.M.V., P.V. and K.V. but medium I.V.) and safflower oil (*Carthamus tinctorius*, oil with low R.M.V., P.V. and K.V. but high I.V.) were respectively fortified with graded amounts (10, 5, 2 and 1%, by weight) of the kusum oil in chloroform solution, and the procedure given above was repeated in each case. Appropriate control experiments were performed side by side for comparison. Only the oils with added kusum oil gave satisfactory positive response to the test within the limit recorded. The procedure described is applicable in general to any cyano compound capable of liberating HCN under the conditions of the experiment and, in the case of seed oils, is generally indicative of the presence of kusum oil but, on chromatographic resolution as above, becomes specific for kusum oil as so far no other oil is known to contain cyanoglycerides.

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