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REVIEW

Detection of Adulteration in Edible Oils

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Abstract: In the present paper, simple, rapid and reliable colour tests such as Sodium azide test, Modified nitric acid test, Azo dye test, Boudouin test, Hexabromide test, Halphen's test, Molybdate method and Solvent partition test have been reported for the detection of adulterants in edible oils. These are performed for synthetic mustard oil, argemone oil, physically refined rice bran oil, sesame oil, linseed oil, cottonseed oil, castor oil and palmolein respectively (respective sensitivity level as 0.1%, 0.1%, 2.5%, 0.2%, 1.0%, 0.5% and 2.0%).

Key words: adulteration, synthetic mustard oil, physically refined rice bran oil, sesame oil, linseed oil, cottonseed oil, castor oil and palmolein

1 Introduction

Adulteration has been a problem in the oil and fat trade for a long time (1). It is some times deliberate and occasionally it is accidental. Indeed, accidental contamination is hard to avoid in modern bulk handling installations, where oils of different qualities must be pumped through common valves and pipelines. However, generally expensive oil is intensively adulterated with the cheaper one! For checking purity chemical tests were developed long ago for their characterization. Some tests are so useful that even today they are widely used, and are part of the common language of our non-technical colleagues who buy, sell, and trade the oils. We also have more sophisticated methods of analysis, however, the instruments required are too expensive and beyond reach of a mediocre industrialists, but we can not overlook importance and utility of some of the more traditional tests in detecting the presence of specific oils in suspected blends (2). The Halphen's test (3), for instance, can detect as little as 0.1% of crude cottonseed oil, or stearine in oil mixtures. Oils containing as little as 1% sesame oil will give a crimson colour in the Boudouin (4) or modified Villavechia (5) test, while the Fitelson test gives a positive indication in the presence of tea seed oil (6). The Evers (7) and modified Renard tests (8) claims to be able to detect as little as 5-10% groundnut oil in mixture, and are used as a criterion of purity for groundnut oil itself (9). Picric acids, ferric chloride, benzidine Cu-acetate are the more common reagents for detection of hydrocyanic acid and cyanolipids in naturally occurring oils like kusum (10). Adhikari *et al*. (11) developed three methods for detection/estimation of admixed vegetable oils. The percentage of their fatty acids can serve to detect semi-quantitatively the proportion of Indian rape-mustard oil when present in rice bran oil (12). Ghee is a popular indigenous product in India prepared from cream or butter by a heat clarification process. Adulteration of goat body fat in ghee (13) can be detected using differential thermal analysis (DTA). Ramachandraiah *et al*. (14) have reported a test for detection of neem oil (0.5%) in other oils by observing the change in colour of a thin copper strip on heating at 200℃. Number of instrumental tech-

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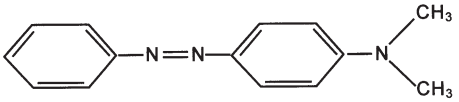
niques such as thin layer chromatography (TLC), near-infrared spectropscopy (NIR), differential scanning colorimetry (15-20) etc. are available for the detection of adulteration of olive oil and butter. Chakraborty *et al*. (21) reported thin layer chromatography of glycerides as a tool for the detection of adulteration in fats and oils. The direct separation of natural glycerides is occasionally not satisfactory due to inadequate resolution on a single chromatoplate. In such cases preliminary fractionation of pure and adulterated oils into groups of glycerides become very useful in confirming the presence of adulterants. It has been possible to detect hydrogenated groundnut, tallow and mahua oils in ghee (butterfat). Raut *et al*. (22) also reported that thin layer chromatography method has been evolved to detect the presence of water-melon seed oil in groundnut and sesame oils. The method is suitable for detection of water-melon seed oil upto 5% in groundnut and sesame oils. Provedi *et al*. (23) have observed color reactions of vegetable oils with Carr and Price's reagent. Number of methods are available in literature for the detection of coconut oil and palm kernel oil in butter (24-29).

2 Methods of Tests and Their Mechanism

The standard qualitative methods for detection of adulterants have been covered under IS: 548 (Part-II)-1976. Most of these methods are based on the development of a characteristic colour or appearance turbidity/precipitate (30).

2·1 Detection of Synthetically Made Artificial Mustard Oil (31) (Sodium Azide Test)

The mustard oil shortage in the market creates quite oftenly a state of black-marketing which is followed by supply of adulterated mustard oil in the market. The synthetic mustard oil is manufactured by colouring certain low-priced vegetable oils with an oil soluble yellow dye (**I**) followed by the addition of requisite amount of synthetic allyl isothiocyanate (CH_2=CH-CH_2-NCS).

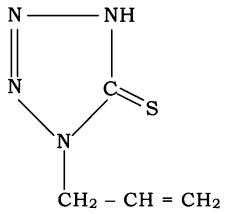


(p-Dimethylaminoazobenzene)
(Butter Yellow)
(I)

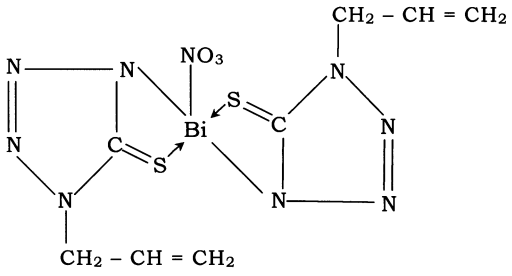
The finished oil looks and smells exactly like the natural mustard oil, but price-wise its manufacturing is quite cheaper and profitable to the unscrupulous manufacturers and retailers.

For performing this test, take 100 mL of the commercial oil sample suspected to be artificial/synthetic mustard oil, add 100 mL of sodium azide solution (2.0 gram per 100 mL), and reflux the mixture on the hot plate or by direct heating for about 3 hrs. Cool the flask and transfer the contents of the flask to a 250 mL capacity separating funnel, and allow the aqueous and oily layer to separate. Collect the lower aqueous layer in a beaker, and remove the oily layer. Wash the aqueous layer twice with 50 mL of diethylether each time in order to remove any residual oily content. Now filtrate this aqueous solution and boil it to concentrate upto around half its volume. Now take 1 mL of bismuth nitrate solution in a test tube and mix with it 1 mL or more of the above concentrated solution. Immediate formation of a deep yellow precipitate will indicate that the tested oil sample contains artificial or synthetic mustard oil. Natural mustard oil when tested in the similar manner will give a negative reaction.

This new technique is based on the reaction (32) of allyl isothiocyanate (CH_2=CH-CH_2-NCS) with sodium azide (NaN_3) that results in the formation of a hetrocyclic compound called 1-allyl-2-tetrazoline-5-thione



1- Allyl-2-tetrazoline-5-thione
(II)



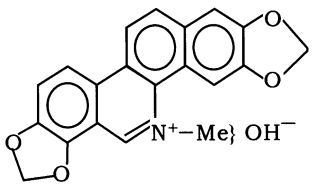
Bis (1-allyl-2-tetrazoline-5-thionato)
(nitrato) bismuth (III)
(yellow)
(III)

(**II**). The compound (**II**) upon treatment with a neutral or very slightly acidic bismuth (III) nitrate solution produces a characteristic deep yellow coloured co-ordination complex bis [(1-allyl-2-tetrazoline-5-thionato) (nitrato) bismuth (III)] (**III**).

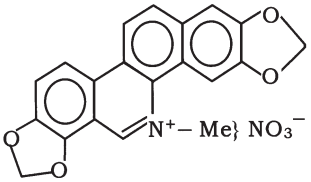
This test gives positive reaction with artificial/ adulterated oil containing down to 0.1% of added allyl isothiocyanate.

2·2 Detection of Argemone Oil

Argemone oil (*Argemone mexicana Linn*) oil is occasionally found as a contaminant in edible oils, particularly in mustard oil (33). However, argemone oil causes dreadful maladies like dropsy, necrosis, high tension glaucoma, diarrhea, vomiting and anemia (34). The oil is reported to cause toxic symptoms and toxicity is ascribed to the presence of alkaloid sanguinarine (**IV**) and dihydro-sanguinarine (35-38). No simple and reliable colour test is available in literature (39-43) for the detection/identification of argemone oil or its chief constituent, sanguinarine (**IV**). In the literature (39), there is a description of colour test for argemone oil present in other oils by means of its reaction with conc. nitric acid in which a yellow to orange colour is developed. This reaction, however, is not very sensitive, and intensity of colour formed by nitric acid test is also not very high. Yellow to orange colour is likely to be due to the formation of sanguinarine nitrate salt (**V**).



(IV)

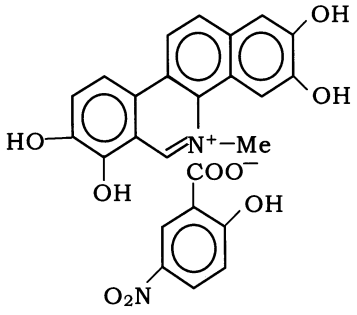


(V)

2·2·1 Modified nitric acid test (44, 45)

In this test, take 5 drops of the oil in a dry test tube and mix successively 0.5 mL of 2% salicylic acid in methanol, 2 mL of conc. nitric acid, followed by 2-4 drops of conc. sulfuric acid, and shake. A crimson red or deep orange-red colour develops within 20-30 seconds if argemone oil adulteration is present. The crimson red or orange-red colour is likely to be due to the formation of nitrosalicylate salt of hydrolysed sanguinarine (**VI**).

First of all salicylic acid undergoes nitration to form nitro salicylic acid in presence of conc. HNO_3 and H_2SO_4. The quantity of H_2SO_4 added is also sufficient to cause hydrolysis of sanguinarine (**IV**) to convert it to tetra hydroxyl compound. The later subsequently reacts with nitro salicylic acid to produce the coloured product (**VI**).



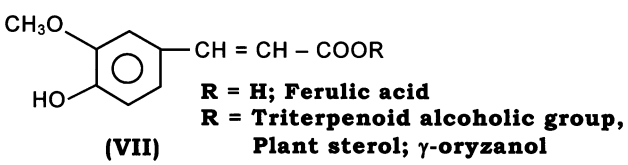
(VI)

Down to 0.1% argemone oil adulteration can be detected with the help of above technique.

2·3 Detection of Rice Bran Oil (46) (Azo Dye Test)

Rice bran oil is used as edible oil in Japan, China, India and other rice producing countries. Physically refined rice bran oil is similar to mustard oil in colour and density. Rice bran oil is price wise cheaper to mustard oil so it is frequently used as an adulterant in mustard oil.

A simple and rapid colorimetric method for the detection of rice bran oil in vegetable oil is described in literature (47). One of the important minor ingredients of rice bran oil identified by Tsuchiya is oryzanol (48). Earlier it was considered to be a single component but later oryzanol was found to be fraction containing ferulic acid (4-hydroxy-3-methoxy cinnaimic acid) esters of triterpenoid alcohol and plant sterols in crude and physically refined rice bran oil and termed (γ-oryzanols (49) (**VII**).

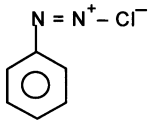


(VII)

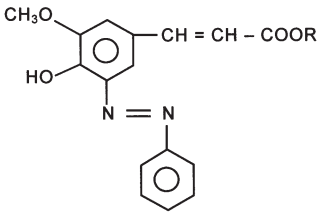
A simple and reliable qualitative technique has been developed by means of which one can easily detect the

presence of rice bran oil in other oils. In this new technique 1 mL of rice bran oil it self or rice bran oil - containing some other oil is taken in a dry test tube and is mixed with 2-4 mL of 10%(w/v) sodium hydroxide solution and shake for 5-10 min to form an emulsified solution (a). Now take 1-2 drops of aniline in another dry test tube and dissolve it in dilute hydrochloric acid. Thereafter, it is cooled at 0-5℃, followed by addition of 2-3 mL of 5%(w/v) sodium nitrite solution and shake, this will result in the formation of benzene diazonium chloride solution (b). Mix solution (a) with solution (b) and shake for few seconds. Development of an orange-red colour within 10-20 seconds indicates the presence of rice bran oil as an adulterant in other oil.

In this technique, first of all aniline undergoes diazotization to form benzene diazonium chloride in presence of sodium nitrite solution and dil. hydrochloric acid at 0-5℃. Subsequently the coupling reaction of compound (**VII**) with compound (**VIII**) in an alkaline medium that results in formation of an orange-red coloured dye (**IX**), phenylazo-γ-oryzanol or phenylazoferulic acid.



(VIII) Bezenediazoniumchloride



R = Triterpenoid alcoholic group, plant sterol; 5-phenylazo-γ-oryzanol

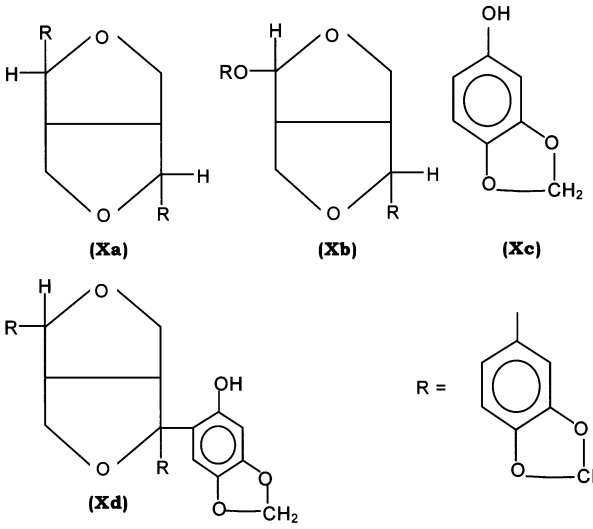
R = H; 5-phenylazoferulic acid

(IX)

Down to 2.5% rice bran oil adulteration can be detected by this highly economical new technique. The adulteration of rice bran oil in corn oil can not be detected by this method due to the presence of ferulic acid ester in this oil also.

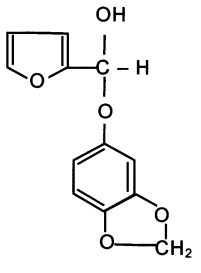
2·4 Detection of Sesame Oil (30)

Sesame oil is extracted from the seeds of *Sesamum indicum*, an herbaceous plant of the Pedaliaceale family. The unsaponifiable fraction contains a class of products specific to sesame; the sesamine (**Xa**) (mean value of 360 mg/100 g oil) and the sesamoline (**Xb**) (average of 270 mg/100 g oil) which leads to the formation of sesamol (**Xc**) (10 mg/100 g oil) and sesaminol (**Xd**) during refining or hydrogenation, sesamol is considerably depleted during deodorization, whereas sesaminol remains less affected at this refining step. Sesamol and sesaminol are two powerful antioxidants (50).



2·4·1 Modified baudouin test (30)

In this test, take 5 mL of the sesame oil or melted fat in a 25 mL measuring cylinder (or test tube) provided with a glass stopper, and add 5 mL of hydrochloric acid and 0.4 mL of furfural solution. Insert the glass stopper and shake vigorously for two minutes. Allow the mixture to separate. The development of a pink colour in the acid layer indicates the presence of sesame oil. Con-



(XI)

firm by adding 5 mL of water and shake again. If the colour in acid layer persists, sesame oil is present. If the colour disappears it is absent.

In above test the pink colour is formed likely due to be formation of addition compound (**XI**) by the reaction of compound (**Xc**) with furfural in presence of hydrochloric acid.

This test is sensitive to the extent of 0.2% of sesame oil in other oils.

2·5 Detection of Linseed Oil (30, 51, 52) (Hexabromide Test)

When highly unsaturated fatty acid such as linolenic acid (**XII**) containing oil, e.g. linseed oil, is treated with bromine in chloroform and then with alcohol and ether, the formation of a precipitate of hexabromide indicates the presence of linseed oil.

In this test 1 mL of the oil is poured in a dry test tube with the help of a pipette add 5 mL of chloroform. Thereafter add about 1 mL of bromine dropwise with the help of a Lunge-Ray pipette, the solution should become deep red in colour indicating the presence of excess bromine and cool the test tube in an ice water-bath add about 1.5 mL of rectified spirit dropwise. The mixture is shaken simultaneously, and the alcohol is added till the precipitate first formed just dissolves. Ten milliliter of ether is added and shake test tube gently to mix the solution. The tube is again kept in the ice-water bath for 30 minutes. Appearance of a precipitate indicates the presence of linseed oil.

The red colour precipitate is likely to be due to the formation of 9, 10, 12, 13, 15, 16 - hexabromo octadecanoic acid (**XIII**).

$$CH_3–CH_2–CH=CH–CH_2–CH=CH–CH_2–CH=CH–(CH_2)_7–COOH$$

(XII)

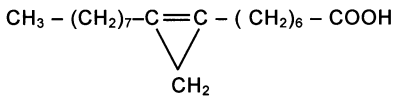
$$CH_3–CH_2–\underset{\underset{Br}{|}}{\overset{\overset{Br}{|}}{CH}}–\underset{\underset{Br}{|}}{\overset{\overset{Br}{|}}{CH}}–CH_2–\underset{\underset{Br}{|}}{\overset{\overset{Br}{|}}{CH}}–\underset{\underset{Br}{|}}{\overset{\overset{Br}{|}}{CH}}–CH_2–\underset{\underset{Br}{|}}{\overset{\overset{Br}{|}}{CH}}–\underset{\underset{Br}{|}}{\overset{\overset{Br}{|}}{CH}}–(CH_2)_7–COOH$$

(XIII)

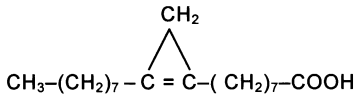
The test is also given by fish oils, and oils and fats containing highly unsaturated fatty acids. Some times soyabean oil and low-erucic rapeseed mustard oil depending upon linolenic acid content may also give positive response to this test. This test is not applicable when linseed oil is present in 'mahua oil'. This test is sensitive to the extent of 1.0% of linseed oil in other oils.

2·6 Detection of Cottonseed Oil (3, 30) (Halphen's Test)

Crude cottonseed oil contains two unusual fatty acids, i.e. malvalic acid (**XIVa**) and sterculic acid (**XIVb**). These belong to the category of cyclopropenic acids (53) responsible for the traditional Halphen reaction. High levels of cyclopropenic acids (specially sterculic acid) could show toxicity.

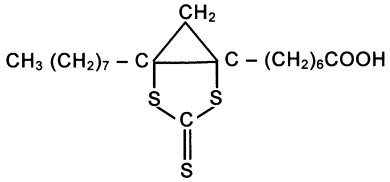


(XIVa)

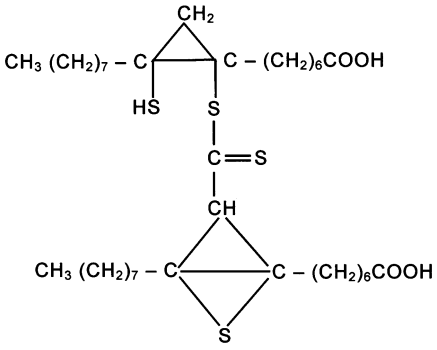


(XIVb)

Take 5 mL of filtered and dried oil in a dry test tube and add to it an equal solution of the sulphur {1.0%(w/v) solution of sulphur in corbon disulphide and then add equal volume of amyl alcohol}. Mix thor-



(XVa)



(XVb)

oughly by shaking and heating gently in a water-bath (70-80℃) for a few min with occasional shaking till carbon disulphide is boiled off and foaming ceases. Place the tube in an oil-bath or a saturated brine-bath maintained at 110-115℃, and hold for 1 to 2 hrs. A red colour at the end of this period indicates the presence of cottonseed oil.

The formation of red colour is due to the formation of addition red coloured compound (**XVa**, **XVb**), a reaction adduct of cyclopropenic acids (**XIVa**, **XIVb**) with sulphur solution.

This test is sensitive upto 0.5% level adulteration of cotton seed oil in other oils. This test is also given by hempseed oil, kapok oil, and oils and fats containing fatty acids of cyclopropenoid structure.

2·7 Detection of Castor Oil (30) (Molybdate Method)

Instrumental techniques are available in literature (54-56) for detection of castor oil in other oils. No simple and relaible method is present in literature which can easily detect the presence of castor oil.

Molybdate method is a rapid test for the detection of castor oil to an extents of 1.0% or more in other oils. In this test take 1 mL of the oil in a dry test tube and dissolve it in 10 mL petroleum ether. Shake vigorously for 2 minutes and add 1-2 drops of molybdate reagent (dissolve 1.25 g of ammonium molybdate in 100 mL of conc. sulphuric acid). Instantaneous development of white turbidity indicates presence of castor oil as an adulterant in test sample.

In the chemistry of test, ricinoleic acid (**XVI**), which is present in castor oil, reacts with ammonium molybdate in presence of conc. sulphuric acid to form white turbid compound (**XVII**).

$$CH_3-(CH_2)_5-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-CH=CH-(CH_2)_7-COOH$$

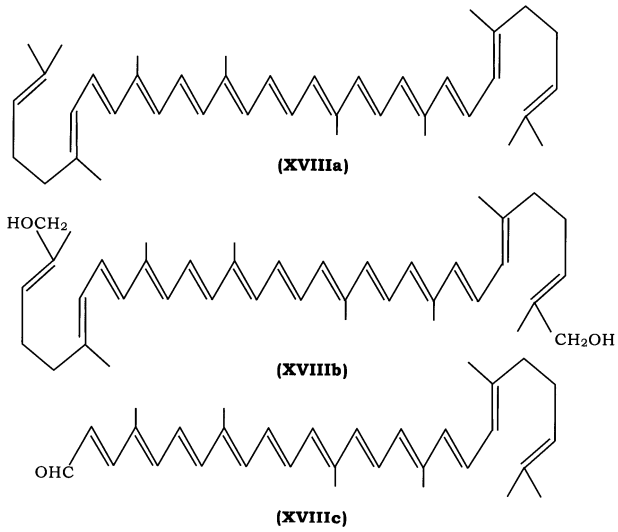
(XVI)

$$CH_3-(CH_2)_5-\underset{\displaystyle OM_0}{\underset{|}{CH}}-CH_2-CH=CH-(CH_2)_7-COOH$$

(XVII)

2·8 Detection of Palmolein (57) (Solvent Partition Method)

Palmolein is the liquid fraction of palm oil, obtained from the fleshy mesocarp of fruits of palm trees (*Ealeis guineenisis*) by the method of expression or solvent extraction. During expression or solvent extraction, it contains pigments like carotenoid (57) lycopene (**XVIIIa**) lycophyll (**XVIIIb**) and licopinal (**XVIIIc**). Lycopene is destroyed to a large extent during process of refining, bleaching and deodorization (58) but it seams that a detectable amount of the pigments still remains in the palmolein fraction. Palmolein can be detected by the phytosterol acetate test, gas liquid chromatography and thin layer chromatography (59).



In the present technique, a simple method based on solvent partition technique for detection of polmolein oil used as adulterant has been reported. In the test, 5 mL of the sample is taken in a beaker and dissolved in an equal volume of hexane. The solution is transfered to a separating funnel after passing through anhydrous sodium sulphate. 3 mL of dimethylformamide (DMF) is added to the solution and shake the mixture gently for about one min and allow to settle till the two layers are clearly separated. The lower DMF layer is drawn off and rejected. A second washing is done if the DMF layer is found deeply coloured. The hexane solution is collected in a porcelain dish and the solvent evaporated on a water-bath. The treated oil sample is then transferred to a test tube. The above experiment is repeated with pure groundnut oil and the treated sample is collected in a second test tube as a reference sample. The two test tubes containing the treated oil samples are then observed under Ultraviolet (UV) light. In case of pure groundnut oil sample, there is no greenish yellow

fluorescence, however, groundnut admixed with palmolein gives greenish yellow fluorescence under UV light.

Palmolein even at 2.0% concentration in groundnut oil can be detected by this method.

3 Conclusion

The above rapid colour reactions give an opportunity of testing adulteration of synthetic mustard oil, argemone oil, physically refined rice bran oil, sesame oil, linseed oil, cottonseed oil, castor oil and palmolein. Moreover, these tests are utmost useful as the cost per test is extremely low whereas GLC, TLC, HPLC and other sophisticated equipments require a high infrastructure and running cost. A further study on other oils must be carried out to develop suitable qualitative tests in the present day context.

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