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Evaluation of the Turbidity and Thin Layer Chromatographic Tests for Detection of Castor Oil

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ABSTRACT

Detection procedures for castor oil in genuine and treated groundnut oils were screened and false turbidity was noticed when ammonium molybdate/sulfuric acid reagents were added to rancid groundnut oil. Successively bleached and neutralized rancid groundnut oil samples do not respond to the tests. Turbidity did not appear for groundnut oils containing 10 and 20% castor oil and even for pure castor oil sample. Thin layer chromatography (TLC) was found to be quite effective for most of the treated, adulterated, genuine, old and fresh oil and fat samples, but showed a streaking, when applied to rancid groundnut oil. However, streaking could be greatly reduced and TLC could be successfully performed with bleached and neutralized, rancid groundnut oil samples.

INTRODUCTION

Castor (*Ricinus cummunis* L. Euphorbiaceae) is a widely cultivated oilseed crop in India. Castor oil is cheaply priced and is used as an adulterant for many common edible oils. Research on detection of castor oil started as early as 1951 (1) by estimating the hydroxyl value and refractive index of acetylated oils (quite low in the presence of castor oil). Colorimetric testing as reported by Auselmi et al. (2) was found unsuitable. Rajnish (3) has developed a turbidity (ammonium molybdate in sulfuric acid) method which also is said (4,5) to be unsatisfactory as it fails even with pure castor oil. A thin layer chromatographic (TLC) technique was developed by Lakshminarayana and Mani (5) for detection of castor oil which is quite effective in most cases. It is of less use (6), however, when the oil under investigation is highly oxidized.

Work has been done to evaluate molybdic acid, sulfuric acid and TLC methods for detection of castor oil. Effort has been made to verify the reported (6) interference due to high free fatty acid (FFA) content and autoxidation of edible oils using the characteristic triricinolein spot of castor oil on thin layer chromatoplates. Effects of oxidation, neutralization and bleaching have also been studied.

EXPERIMENTAL PROCEDURES

Castor and other oilseeds for the experiments were procured from seed suppliers and local markets. Oils were extracted using hexane in a Soxhlet apparatus. The extract was filtered and the solvent was removed under vacuum. Oils thus free from residual solvent were used for detection and chromatographic determinations. Samples with high FFA contents (sesame, groundnut and safflower oils) were procured from the old stocks of a local oil firm. Palmolein, RBD palm oil and rice bran oil were supplied by CFTRI Employees Consumers' Cooperative Society, pure ghee was from the Mysore dairy and vanaspati (Dalda) was manufactured by Hindustan Lever Ltd. in India.

Ammonium Molybdate Test

One mL of filtered oil sample dissolved in 10 mL of petroleum ether (bp 40-60 C), acidified with (2% v/v) conc. hydrochloric acid in a clean dry test tube. To this, one drop of reagent (1.25 g ammonium molybdate in 100 mL of sulfuric acid, sp. grav. 1.84) was added down the side of the test tube, and turbidity was observed instantaneously.

Thin Layer Chromatography

Thin layer chromatoplates (20 × 20 and 40 × 20 cm) coated with Silica Gel G (BDH grade, 0.25 mm thickness) were prepared following the procedure of Kirchner (7). A 1% chloroform solution of samples (5-10 μL) was spotted onto preactivated plates. Chromatographic grade petroleum ether (bp 40-60 C), ether and benzene were used as developing solvents. Spots were visualized in an iodine chamber and confirmed by charring the components after spraying the plates with 5% aq perchloric acid.

Oxidation

Oil was placed into a round-bottomed flask (500-mL capacity) provided with 2 side tubes. The flask was connected via a suction pump and air was allowed to bubble through the inlet into the oil. The contents were heated at 90 C on a steam water bath and then aerated for 10 hr (6).

Bleaching

Oil that was free from moisture was placed into a round-bottomed flask (500-mL capacity) fitted with an electric stirrer. Activated carbon (J.T. Baker Chem. Co., 10% w/w)

TABLE I

Detection of Castor Oil in Treated and Adulterated Groundnut Oil

Sample oil	Am. molybdate test	Sulfuric acid test	TLC test
Groundnut	Neg.	Neg.	Neg.
Castor	Undissolved	Undissolved	Pos.
Bl. groundnut	Neg.	Neg.	Neg.
Neut. groundnut	Neg.	Neg.	Neg.
Rancid groundnut	Pos.	Pos.	Streaking
Rancid, bl. groundnut	Neg.	Neg.	Neg.
Rancid, neut. groundnut	Neg.	Neg.	Neg.
Rancid, neut. bl. groundnut	Neg.	Neg.	Neg.
Rancid groundnut & 1% castor	Pos.	Pos.	Pos.
Rancid, bl. groundnut & 1% castor	↓	↓	↓
Rancid, neut. groundnut & 1% castor	↓	↓	↓
Rancid, neut. bl. groundnut & 1% castor	↓	↓	↓
Rancid groundnut & 2% castor	↓	↓	↓
Rancid, bl. groundnut & 2% castor	↓	↓	↓
Rancid, neut. groundnut & 2% castor	↓	↓	↓
Rancid, neut. bl. groundnut & 2% castor	↓	↓	↓
Rancid groundnut & 5% castor	↓	↓	↓
Rancid, bl. groundnut & 5% castor	↓	↓	↓
Rancid, neut. groundnut & 5% castor	↓	↓	↓
Rancid, neut. bl. groundnut & 5% castor	↓	↓	↓
Groundnut and 5% castor	Pos.	Pos.	Pos.
Groundnut and 10% castor	Neg.	Neg.	Pos.
Groundnut and 20% castor	Neg.	Neg.	Pos.

was mixed with the oil before operation. The flask was placed on a steam water bath and the temperature was raised to 60-70 C. The contents were stirred slowly and the temperature was further raised to 95 C. The process continued for 40 min and then the bleached oil was separated from the carbon by centrifugation.

Neutralization

A glass column (30 cm long, 3 cm diam.) packed with neutral alumina (BDH grade, 75 g) was used for neutralization (8). The oil to be neutralized was applied onto the top of the column and was slowly adsorbed. Neutral oil was eluted with hexane solvent.

RESULTS AND DISCUSSION

Ammonium Molybdate/Sulfuric Acid Test

Until recently, the molybdic acid (ammonium molybdate in sulfuric acid) test was in practice for detection of castor oil in most of the laboratories, but was found to be unsatisfactory, particularly for sesame, linseed and coconut oils, as well as for highly rancid oils (5,6). A variety of hydroxy and nonhydroxy fatty compounds was used by Lakshminarayana (4) to test applicability of the turbidity test and to investigate its chemical nature. Turbidity was observed in ca. 2% of the hydroxy, hydroperoxy, epoxy fatty acids, their esters, epoxy acetoxy, and hydroxy fatty alcohols. Turbidity was even noticed with sulfuric and chlorosulfonic acids because of the formation of sulfate with oxy-compounds and its precipitation from the medium. The role of the ammonium molybdate was only to sharpen the white turbidity.

During our investigation, ammonium molybdate and sulfuric acid tests were performed with a set of samples (Table I) prepared by mixing groundnut oil with castor oil and by oxidizing, bleaching and neutralizing various mixtures of these oils. Turbidity was observed for samples containing castor oil up to a level of 5% or less by both test reagents, whereas it did not appear at all for the samples having 10 or 20%, or even for pure castor oil. This must be due to the solubility of resultant sulfate in the highly polar triglyceride (triricinolein) medium. Rancid groundnut oil gave fine, pearly turbidity with these reagents due to the presence of peroxides and hydroperoxides in situ and their precipitation as sulfates. But, successively bleached and neutralized, rancid groundnut oil samples did not respond to the tests because of the removal of the oxygenated portion of the oil during these treatments. Therefore, bleaching and neutralization were found to be useful in case a false turbidity was noticed for an oxidized oil during the turbidity test for detection of castor oil.

Thin Layer Chromatography

A TLC technique for detection of castor oil was developed by Lakshminarayana and Mani (5) and is frequently used. Although it is quite effective when applied to a variety of admixtures (Table I), it fails if the oils under investigation is highly oxidized. Streaking was observed during the investigation when the test was applied to a rancid oil having a peroxide value of 124.4, again probably due to the oxy-compounds generated during oxidation. But, subsequently bleached and neutralized samples did not give intense streaking on TLC plate, making the detection process easier, as the range of triricinolein (reference spot R_f 0.44-0.47) was found to be quite clear. A distinguishable co-TLC with castor oil could be performed for these samples. Separation could not be achieved when co-TLC was performed for castor and rancid groundnut oils.

To study the reported interference of FFA (6) with castor triricinolein, sesame, groundnut and safflower oils with 7.5, 9.7 and 13.0% FFA, respectively, were spotted onto a TLC plate along with castor oil as an internal reference standard. Fresh sesame, groundnut and safflower oils were also run alongside. Development of the chromatoplate in petroleum ether/ether/acetic acid, 60:40:2, v/v/v, and its visualization in iodine vapor revealed no difference between fresh and high acidic oils; also, no spot was found in the triricinolein region, ruling out any possibility of interference due to high FFA levels.

To test the applicability of the chromatographic method, various common edible oils and fats, viz., coconut, sesame, rapeseed, mustard, groundnut, cottonseed, soybean, palmolein, RBD palm oil, nigerseed, rice bran, safflower oils, vanaspati and pure ghee, have been screened following the usual procedure (5) along with castor oil as the internal reference standard. Spots were visualized under iodine vapors and were finally confirmed by charring the components on a hot plate after spraying with perchloric acid (5%). No spot in any of the oils was found within the range (Rf 0.44-0.47) of castor triricinolein, indicating that the TLC method is reliable if the oils under investigation are fresh and free from rancidity. The chromatography was repeated using benzene as developing solvent, but showed very slow migration of triricinolein (0.02), diricinolein (0.07), and monoricinolein (0.1) from castor oil compared to the other oil samples.

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Elucidation of the Chemical Structures of Natural Antioxidants Isolated from Rosemary[1,2]

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ABSTRACT

A natural antioxidant extract with activity greater than BHA and equal to BHT was isolated from rosemary leaves. The extract was separated into 7 primary fractions with liquid chromatography, using silicic acid as an adsorbent followed by gradient elution. Each fraction was rechromatographed to yield a total of 16 subfractions. Two compounds, carnosol and ursolic acid, were identified by infrared, mass and nuclear magnetic resonance spectrometry. Carnosol was shown to be one of the active antioxidant components in rosemary. Ursolic acid was not an effective antioxidant. Further fractionation of the most active antioxidant subfractions by high performance liquid chromatography and the elucidation of the chemical structures of these fractions are now in progress.

INTRODUCTION

About 20 million lb of synthetic antioxidants is used annually by food manufacturers in the United States (1). The compounds currently used—primary butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) are added to a variety of foods.

In recent years, there has been increasing concern over the safety of many synthetic food additives. Antioxidants have been one of the major food additive groups which have come under the review of regulatory agencies. In feeding tests, high levels of the antioxidants BHA, BHT and TBHQ cause significant enlargement of the liver (2). BHT, but not BHA and TBHQ, also increases liver microsomal enzyme activity (2). BHT was removed from the FDA Generally Recognized as Safe (GRAS) list (3).

The use of extract from rosemary spice as a natural antioxidant was first reported by Rac and Ostric-Matijasevic (4). Later, Chang et al. (5) reported a patented process for the extraction of rosemary and sage, followed by a vacuum steam distillation of the extract in an edible oil or fat to obtain an odorless and flavorless natural antioxidant. Its antioxidant activity was demonstrated in both animal fats and vegetable oils. Furthermore, it was able to improve the flavor stability of soybean oil, as well as the flavor stability of potato chips. More recently, Bracco et al. (6) also reported the use of double-step, falling film molecular distillation to obtain an active antioxidant from rosemary extract.

This paper reports the fractionation and identification of one of the active antioxidant components in the extract of rosemary.

EXPERIMENTAL

Preparation of Rosemary Antioxidant

The scheme for the preparation of rosemary antioxidant (RA) is shown in Figure 1.

Three kg of rosemary leaves which had been ground to a fine powder was extracted with 18 L of methanol at 60 C for 2 hr. The mixture was filtered and the residue was extracted again with 12 L of fresh methanol. The combined filtrate was bleached with 600 g of active carbon and then filtered to yield a light-brown filtrate. The methanol solution was then concentrated to ca. 2 L by rotary evaporator and then filtered to remove the precipitates (A). The fil-

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