RAPID PAPER

Identification of Physically Refined Rice Bran Oil and Its Simple Detection in Other Oils

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Abstract: The issue of purity of the edible oils is becoming increasingly important in the food industry. Many branded oil products are being sold at a premium price on the basis of their purity and quality, or on the basis of their health-boosting effects. Undue financial advantage is also taken by deliberately mislabeling or adulterating the oils and presenting them as quality products in the market. In the present paper a new, very simple, rapid, reliable and highly economical qualitative technique is being reported. By means of this test, adulteration of rice bran oil in other oils can be detected within one minute. A small quantity of alkaline solution of suspected oil sample when treated with small quantity of benzenediazonium chloride solution at 0–5°C followed by shaking of the mixture, a brilliant orange-red colour of 5-phenylazo- γ -oryzanol or 5-phenylazoferulic acid (which is indeed a dye material) develops within the next few seconds indicating the presence of rice bran oil adulteration in the test sample. Upto 2.5% rice bran oil adulteration can be detected with this new technique.

Key words: adulteration, rice bran oil, γ -oryzanol, ferulic acid

1 Introduction

Rice bran oil is used as an 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. Rape seed/mustard oil (*Brassica* sp.) is consumed by population groups in countries of Asia, Europe North America and India (1). The higher price gap of rape seed-mustard oil over rice bran oil results in the adulteration of the former. Quite often the mustard

oil shortage in the market creates a stage of black-marketing which is followed by supply of artificially made synthetic mustard oil (2) in the market. The synthetic mustard oil is manufactured by colouring physically refined rice bran oil with an oil soluble yellow dye followed by the addition of a requisite amount of synthetic allyl isothiocyanate ($\text{CH}_2 = \text{CH}-\text{CH}_2-\text{NCS}$). 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.

One of the important minor ingredients of rice bran

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oil identified by T. Tsuchiya of Tokyo is oryzanol (3). Earlier it was considered to be a single component but later oryzanol was found to be fraction containing ferulic acid (4-hydroxy-3-methoxy cinnamic acid) (**I**) esters of triterpenoid alcohol, and plant sterols in crude and physically refined rice bran oil and termed γ -oryzanols (4) (**IIa** = cycloartenyl ferulate, **IIb** = 24, methylene cycloartenyl ferulate, **IIc** = campestryl ferulate, **IId** = cycloartanyl ferulate, **IId** = β -sitosteryl ferulate) by authors (**Structure II, Scheme 1**). The percentage of γ -oryzanol in physically refined rice bran oil samples ranged from 1.2-1.7%.

Methods are required to be established certainly, and to be quick and easy in use. Hitherto no simple technique was available which could easily detect rice bran oil adulteration in mustard oil. Now a simple and reliable qualitative technique has been developed by means of which one can easily detect the presence of rice bran oil in mustard oil. In this new technique when a small quantity of rice bran oil itself or rice bran oil-containing some other oil is allowed to react with benzenediazonium chloride in alkaline medium, a very fine orange-red colour is developed. This colour reaction is specific to rice bran oil. It has been observed by experimentation that the mustard oil and other oils does not give this reaction at all.

First of all aniline undergoes diazotization to form benzene diazonium chloride in presence of sodium nitrite solution and dil. hydrochloric acid at $0 - 5^{\circ}\text{C}$. Subsequently the coupling reaction of benzenediazonium chloride ($\text{C}_6\text{H}_5\text{N}_2\text{Cl}$) with γ -oryzanols or ferulic acid in an alkaline medium that results the formation of an orange-red coloured dye, phenylazo- γ -oryzanol or phenylazoferulic acid.

2 Experimental

γ -oryzanol was obtained from M/s. Vitamic Power, Inc., U.S.A. Physically refined rice bran oil sample was obtained from local industry of Kanpur (India). Aniline, sodium nitrite, sodium hydroxide and hydrochloric acid used for this study were of L.R. grade reagent.

1 mL of oil sample was taken in a dry test tube and was mixed with 2-4 mL of 10% sodium hydroxide solution (w/v) and shake for 5-10 minutes to form an emulsified solution (**I**). Now take 1-2 drops of aniline in an other dry test tube and dissolve it in dilute hydrochloric acid. Thereafter, it was cooled at $0 - 5^{\circ}\text{C}$, and add 2-3

Scheme 1

mL of 5% sodium nitrite solution (w/v) and shaken, this will result in the formation of benzenediazonium chloride solution (**II**). Mix solution (**I**) with solution (**II**) and shaken for few seconds. Development of an orange-red colour within 10-20 seconds indicated the presence of rice bran oil as an adulterant in other oil.

3 Results and Discussion

When well cooled solution of aniline in hydrochloric acid is reacted with sodium nitrite solution at $0 - 5^{\circ}\text{C}$, it results in the formation of an unstable but reactive benzenediazonium chloride.

Diazonium ion is sufficiently electrophilic to couple with reactive phenolic aromatic nuclei in alkline medium to give a coloured azo compound (dye). In the present case the resultant benzenediazonium chloride preferentially couples at ortho position to the phenolic -OH group of the oryzanol to produce an orange-red dye (Scheme 2).

4 Conclusion

This test gives an opportunity of testing adulteration of rice bran oil in mustard oil. The test has also been successfully used in detecting adulteration of rice bran oil in other oils. Down to 2.5% rice bran oil adulteration

can be detected by this highly economical new technique. However, only corn oil gives the test positive and therefore adulteration of rice bran oil in corn oil can not be detected by this method.

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Scheme 2