

Quality Comparison of Rice Bran Oil Extracted with d-Limonene and Hexane

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ABSTRACT

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d-Limonene, a safe agricultural by-product, was used to extract rice bran oil and compared against hexane, a petroleum product widely used as a solvent for extracting edible oil. The yield of crude rice bran oils extracted with both solvents in percentage by weight was obtained. The quality of crude rice bran oil was analyzed. The yield and quality of crude rice bran oil from the limonene-based solvent extraction were almost equivalent to those from the hexane-based operation. The optimum solvent-to-rice bran ratio and extraction time required for d-limonene extraction

of oil, based primarily on crude rice bran oil yield, have been determined to be 5:1 and 0.5 hr, respectively. Despite the absence of antioxidants during the limonene recovery step with vacuum evaporation, the quantity of the oxidation products in the recovered limonene was <1% (wt) of the original limonene solvent. The application of d-limonene solvent as an alternative to hexane in edible oil extraction could potentially eliminate the safety, environmental, and health issues associated with the use of hexane.

Edible oils from low-oil content oilseeds are commonly extracted with solvents. Commercial-grade hexane is the solvent of choice throughout the world for economical reasons. Commercial hexane, a light paraffinic petroleum fraction, has a fairly narrow boiling range of ≈ 63 – 69°C and is an excellent oil solvent in terms of oil solubility and ease of recovery. However, hexane as a solvent is also responsible for serious environmental problems such as fire, explosion, and air pollution, in addition to other health hazards due to its toxicity (Wan et al 1995; Lusas 2000). Over the years, many attempts have made to find alternative solvents, in particular alcohols, halogenated hydrocarbons, hydrocarbons, carbon dioxide (supercritical fluid extraction), and even water (Johnson and Lusas 1983; Lusas 2000). And the search continues.

We have tried to determine whether d-limonene, an agricultural by-product from the citrus industry, was a viable alternative to hexane based on crude edible oil yield and quality. The results of this study would also have other broader implications for the extraction of other lipo-soluble nutraceuticals from agricultural and food by-products including lycopene, where hexane is used as the main solvent. d-Limonene is completely miscible with oils and slightly more polar than hexane as indicated in Table I. It is possible that d-limonene would be able to extract more vitamin E and oryzanols (potential antioxidants) because of its slightly polar nature. It has U.S. Food and Drug Administration's GRAS (Generally Recognized as Safe) status for use as food flavoring. It is relatively fire and explosion safe, nontoxic to humans, and less volatile than hexane and it comes from a renewable source. It was reported that d-limonene administered orally at a dosage level of 150–2,400 mg/kg of body weight/day (equivalent to 10.5–168 g/day for an average human male with a body weight of 70 kg) induced renal alternations in male rats (Kanerva and Alden 1987), however this damage has not been observed in kidneys of male mice, female rats, and female mice. There have been no reports in medical literature to suggest that d-limonene is carcinogenic or mutagenic to humans. As a result, the U.S. National Institute for Occupational Safety and Health (NIOSH) established no recommended exposure limit. d-Limonene is currently used as a degreasing cleaner in places such as restaurant cooking areas and in general custodial applications due to its high oil miscibility (McBride 1990; Braddock 1999).

To use limonene as alternative solvent for extracting edible oils, it is desired that the recovered d-limonene used for extraction be

reusable and its solvent power undiminished as d-limonene is an unsaturated terpenoid compound and therefore vulnerable to oxidation. d-Limonene can be oxidized to its oxidation products carvone and carveol when subjected to higher temperatures in the presence of atmospheric oxygen (Buckholz and Daun 1978). Buckholz and Daun (1978) described the sensory characteristics of oxidized d-limonene as flowery, piney, and minty in their study on the stability of the cold-pressed orange oil. Proctor and Kenyon (1949) studied the terpeny off-notes produced by d-limonene oxidation and concluded that carvone and carveol are the primary products of oxidation which contributed to the terpeny off-notes of the oxidized d-limonene. Ting and Newhall (1965) demonstrated the effect of temperature on the oxidation of d-limonene. Lee and Widmer (1994) studied the evaluation of commercial oleoresins for inhibition of d-limonene oxidation at 50°C in comparison with other food-grade commercial antioxidants and found that the mixture of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) was the most effective in inhibiting oxidation of d-limonene.

In this study, we examined several quality attributes of crude rice bran oil extracted with d-limonene and compared them to those of the oil extracted with pure hexane. Also, the quality of recovered d-limonene from solvent extraction of rice bran oil in the absence of antioxidants after a full cycle was examined and the percentage of oxidative products in the recovered limonene and their composition were determined.

MATERIALS AND METHODS

Materials

Extrusion-stabilized rice bran was obtained from Riceland Foods USA (Stuttgart, AR) in the form of collets (pellets) with an average length and diameter size of 2.5 and 1.5 cm, respectively. Food

TABLE I
Properties of d-Limonene and Hexane^a

Property	Hexane	d-Limonene
Molecular weight (g/mol)	86.17	136.23
Specific gravity (25°C)	0.65	0.84
Viscosity, cP (25°C)	0.32	0.92
Boiling point, °C	68.74	162.78
Latent heat of vaporization, (cal/g)	79.9	84.4
Specific heat (cal/g°C)	0.53	0.44
Solubility in water, wt% (25°C)	0.00123	0.00138
Dielectric constant, 20°C	1.89	2.37
Flash point, °C	-23	48
Surface tension, dyne/cm (25°C)	18.4	27
Renewable	No	Yes
Toxic	Yes	No

^a Riddick et al (1986), Braddock (1999).

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grade d-limonene (96.5% purity) was purchased from Florida Chemical Co. (Winter Haven, FL). HPLC-grade hexane and all the other chemicals and glassware were purchased from Fisher Scientific (Atlanta, GA), Fluka Chemie AG (Buchs, SG, Switzerland), or Aldrich Chemical Co. (Milwaukee, WI).

Moisture Content and Oil Content of Rice Bran

Moisture content of rice bran was determined according to the modified American Oil Chemists' Society (AOCS) official method Ac 2-41 and oil content of rice bran was determined using modified AOCS official method Ac 3-44 (Firestone 1990).

Determining Parameters for Maximum Extraction Yield of Crude Rice Bran Oil

Rice bran was stored in the freezer until used for the extraction to prevent any lipid oxidation. Before extraction, rice bran was ground using a mortar and pestle and the ground bran was sieved using U.S. standard 8- and 70-mesh screens as described previously. Approximately 25 g of the freshly ground and sieved rice bran was weighed into the extraction flask. d-Limonene (50 g) was added to the bran in the flask, which was then attached to the water condenser. The flask was heated on an electrothermal heater (Electrothermal Engineering Ltd, Essex, UK) set at the boiling point of d-limonene. The extraction was conducted at the boiling point.

After extraction, the oil miscella (oil and d-limonene) was separated from the defatted rice bran using a porcelain funnel and Whatman No. 1 filter paper. Vacuum was applied to increase the filtration rate. The extraction flask was washed twice with fresh d-limonene to run down any leftover of d-limonene, oil, and rice bran slurry into the funnel. The cake of defatted meal retained on the filter paper in the funnel was washed with fresh d-limonene to run down any oil that might have retained with the meal. The total volume of fresh d-limonene used in the washings was 55 mL.

The contents of the filtration flask were emptied into the evaporation flask, and the filtration flask was washed twice with hexane to make sure no oil was left behind in the flask and the washings were added to the evaporation flask. The contents of the evaporation flask were subjected to vacuum evaporation using a rotary evaporator at 4 kPa and water bath at 90°C. The evaporation was continued for \approx 1 hr until no further separation was observed. The residual oil still contained traces of d-limonene. The contents of the evaporation flask were then emptied into a tared beaker, and the evaporation flask was washed with a small amount of hexane to run down any leftover oil and d-limonene slurry into the beaker. The miscella in the beaker was subjected to further evaporation in a vacuum oven (Isotemp vacuum oven, model 281 A, Fisher Scientific Co., Springfield, NJ) maintained at 95°C and 4 kPa for 2 hr to remove the remaining traces of d-limonene. The conditions for evaporation in a vacuum oven were obtained using the vapor pressure and temperature data from Doolittle (1994). The beaker with the crude rice bran oil was cooled to room temperature in a desiccator and weighed. The percentage of crude rice bran oil yield was then calculated.

These extraction experiments were conducted at preselected solvent-to-bran ratios (w/w) of 2:1, 3:1, and 5:1 and extraction times of 0.5, 1, 2, and 3 hr. These experiments were repeated for hexane as the solvent at its boiling point for comparison studies. Because the boiling point of hexane is \approx 68°C, no further evaporation in vacuum oven was necessary after evaporation using the rotary vacuum evaporator. All the experiments were duplicated.

GC Measurements

GC 3500 series equipped with a split/splitless injector, flame ionization detector, and peak simple data collection software (SRI Instruments, Torrance, CA) was used for analyzing the crude rice bran oil after evaporation to make sure that there was no d-limonene. The injector and detector temperatures were maintained

at 350 and 400°C, respectively. The column used was a 60 m \times 0.25 mm 5% di-phenyl, 95% di-methyl siloxane column (J&W Scientific, Folsom, CA) with film thickness of 25 μ m. High purity helium gas was used as a carrier with the flow set at 34 cm/sec at 50°C. The temperature of the column was programmed initially to hold at 50°C for 5 min and then ramped at 20°C/min to 250°C, followed by 10°C/min to 380°C, and then held at 380°C for 10 min. The crude rice bran oils were diluted 100 \times in hexane and 1 μ L of this solution was injected with a split ratio of 100:1. The split mode was programmed to be turned on for the first 0.5 min.

The injector and detector temperatures for limonene oxidation study were maintained at 220°C and 275°C respectively. High purity helium gas was used as a carrier with the flow set at 41 cm/sec at 90°C. The temperature of the column was programmed as follows: initially the column was held at 90°C for 3 min and then the temperature was ramped at 4°C/min to 140°C; followed by 15°C/min to 240°C and then held at 240°C for 4 min. An injection volume of 1 μ L was used with a split ratio of 80:1. The split mode was programmed to be turned on for the first 0.5 min. Standards of (+)-*trans*-d-limonene-1, 2-epoxide, (+)-*cis*-d-limonene-1, 2-epoxide, (+)-carvone, and (-)-carveol mixture of isomers were obtained from either Fluka Chemie AG or Aldrich Chemical. HPLC-grade hexane from Fisher Scientific was used as solvent for standards.

Hexadecane was chosen as the internal standard because the retention index showed that it eluted just after the carvone on a polar column. Internal standard solution (0.014263 g/mL) was prepared by dissolving 1.4263 g of hexadecane in 100 mL of hexane solution. A solution containing standards of 0.013128 g/mL of (+)-*cis* and (+)-*trans*-d-limonene-1, 2-epoxides, 0.00678 g/mL of (-)-carveol mixture of isomers, and 0.005406 g/mL of (+)-carvone was prepared in hexane. Different measures (5, 3, 2, and 1 mL) of this standard solution were pipetted into separate 50-mL flasks and 1 mL of internal standard solution was added to each of these flasks and then the volume was made up with hexane. The calibration curve was obtained by plotting the concentration of standard solution against the ratio of area of standard to area of internal standard and the response factor (RF) was determined as (concentration of standard \times area of internal standard)/(concentration of internal standard \times area of standard).

Statistical Analysis

The percentage of crude rice bran oil yield and quality attribute data were analyzed using the general linear models (GLM) procedure (SAS Institute, Cary, NC). Student–Newman–Keuls (SNK) test with $\alpha = 0.05$ was used to test whether there was any significant difference between the data for the respective solvents.

Free Fatty Acid Determination

The total free fatty acid content in the crude rice bran oil was determined according to the modified AOCS method Ca 5a-40 (Firestone 1990). A freshly extracted crude rice bran oil sample (\approx 3.5 g) was weighed into Erlenmeyer flask and \approx 75 mL of hot neutralized alcohol was added to the flask. Phenolphthalein indicator (2 mL) was added to the mixture, titrated with 0.25N standard sodium hydroxide, and shaken vigorously until the appearance of the first permanent pink color. The percentage of free fatty acids was expressed as percent of oleic acid.

Determination of Phospholipids

Phosphorus content of the weighed amount of crude rice bran oil was determined according to the modified AOCS method Ca 12-55 (Firestone 1990). The standard stock solution of phosphate was prepared by dissolving 1.0967 g of dry potassium dihydrogen phosphate in distilled water and diluting to 250 mL in a volumetric flask (0.01 mg of phosphorus/mL). Stock solution (5 mL) was pipetted into a 500-mL volumetric flask and the volume was diluted with distilled water to obtain a final concentration of 0.01

mg of phosphorus/mL. The above standard solutions of 0, 1, 2, 3, 4, 6, 8, and 10 mL were pipetted into separate 50-mL volumetric flasks. All these solutions were diluted to 10 mL with distilled water using a measuring pipette. Previously prepared 0.015% hydrazine sulfate solution (8 mL) and 2 mL of 2.5% sodium molybdate solution of sodium molybdate in distilled water and concentrated sulfuric acid were added to the flasks in the order given. The flasks were sealed with stoppers and inverted three to four times. The stoppers were then loosened and heated in a vigorously boiling water bath for ≈ 10 min. Then the contents were cooled to room temperature in a water bath and diluted to volume with distilled water and mixed thoroughly. The solutions were immediately transferred to a cuvette and the absorbance was measured at 650 nm with the spectrophotometer (model U-3110, Hitachi Instruments, Tokyo, Japan) adjusted to read 0% absorbance for a cuvette containing distilled water. These aliquots correspond to 0.00, 0.01, 0.02, 0.04, 0.06, 0.08, and 0.10 mg of phosphorus, respectively. Then the absorbance of each standard was plotted against its phosphorus content in mg to obtain the standard curve.

Crude rice bran oil (≈ 3 g) was weighed into a porcelain crucible and 0.5 g of zinc oxide was added to it. The crucible with the contents were heated slowly on a hot plate until the sample thickened and then the heat was gradually increased until the mass was completely charred. The crucible was then placed in a muffle furnace and heated to 600°C and held at that temperature for 5 hr. The crucible was removed from the muffle furnace after it cooled to room temperature. Distilled water (5 mL) and concentrated hydrochloric acid (5 mL) were added to the ash. The crucible was covered with a watch glass and heated on a hot plate (type 1900, Thermolyne Sybron Corp., Dubuque, IA) to gentle boiling for ≈ 5 min. This solution was filtered into a 100-mL volumetric flask using Whatman No. 42 filter paper and a glass funnel. The inside of the watch glass and the crucible were washed twice with distilled water. The solution was then neutralized to a faint turbidity by the drop-wise addition of previously prepared 50% aqueous potassium hydroxide solution. Concentrated hydrochloric acid was added to the turbid solution drop-wise until the zinc oxide precipitate just dissolved and then diluted to volume with distilled water and mixed thoroughly. This solution (10 mL) was pipetted into another 100-mL volumetric flask and diluted to volume with distilled water. Next, 10 mL of this solution was pipetted into a 50-mL volumetric flask, and 8 mL of previously prepared 0.015% hydrazine sulfate solution and 2 mL of 2.5% sodium molybdate solution of sodium molybdate in distilled water and concentrated sulfuric acid were added to the flask in the order given. The flask was sealed with a stopper and inverted three to four times. The stopper was loosened and the flask was heated in a vigorously boiling water bath for ≈ 10 min. The contents of the flask were cooled to room temperature in a water bath and diluted to volume with distilled water and mixed thoroughly. The solution was immediately transferred to a cuvette and the absorbance was measured at 650 nm with the spectrophotometer (model U-3110, Hitachi) adjusted to read 0% absorbance for a cuvette containing distilled water. The absorbance of reagent blank with no added oil was determined in a similar fashion by following the same steps. Phosphorus content of the crude rice bran oil sample and the blank were obtained by the use of the standard curve obtained earlier.

Color Measurement

The UV-visible spectrophotometer was calibrated according to the modified procedure laid out in AOCS official method Cc 13c-50 (Firestone 1990). The instrument was previously adjusted to read 100% transmittance using distilled water. The transmittance of nickel sulfate solution (4.4 g of nickel/100 mL) was measured against distilled water at various wavelengths of 400, 460, 510, 550, 620, 670, and 700 nm. Then the recorded transmittance data,

and those specified by AOCS, were plotted against the wavelength for comparison.

The crude rice bran oil extracted was centrifuged (model CL, International Equipment, Needam Heights, MA) for ≈ 1 hr at 2,300 rpm to settle the wax. Then the upper layer was decanted into another vial. Solutions of 1, 5, and 10% crude rice bran oil in hexane were prepared.

The spectrophotometer was adjusted to read 0 absorbance with hexane in both the cuvettes. The 1% crude rice bran oils in hexane were scanned at wavelengths of 400–250 nm to obtain the absorption spectra. The absorbance of 10, 5, and 1% crude rice bran oil in hexane were measured against hexane as a reference at a wavelength of 430 nm in a 1-cm cuvette according to the method adopted by Zhao et al (1987).

Oxidation of Recovered Limonene

The recovered limonene (9 mL) was pipetted into a 10-mL volumetric flask and 1 mL of internal standard solution was added to the flask. Then 1 μ L of this solution was injected into GC and the responses were detected. The oxidation products were quantified as Concentration of oxidation product = $(1.11 \times \text{RF} \times \text{area of oxidation product} \times \text{concentration of internal standard})/\text{area of the internal standard}$.

RESULTS AND DISCUSSION

Initial average moisture content of rice bran used in this study was determined to be $5.96 \pm 0.73\%$ (modified AOCS official method Ac 2-41) and average oil content was $28.42 \pm 1.82\%$ (modified AOCS method Ac 3-44) according to the procedure described earlier. Extraction studies were conducted for preselected solvent-to-rice bran ratios of 2:1, 3:1, and 5:1 at extraction times of 0.5, 1, 2, and 3 hr both for d-limonene and hexane at their respective boiling points. Quality of the crude rice bran oil was analyzed for the oils extracted under the selected conditions of solvent-to-rice bran ratio and extraction time.

The residual crude rice bran oil, obtained after evaporation of d-limonene, was analyzed for the presence of d-limonene using gas chromatography as described previously. The absence of d-limonene peak in the chromatogram was interpreted as complete removal of d-limonene from the crude rice bran oil.

Effect of Solvent-to-Rice Bran Ratio on the Amount of Oil Extracted

The crude rice bran oil yield from solvent extraction was expressed as a percent of rice bran used and tabulated in Table II. The data in Table II show the effect of solvent-to-rice bran ratios on the amount of crude edible oil extracted with d-limonene and hexane at preselected extraction times of 0.5, 1, 2, and 3 hr. For

TABLE II
Oil Yield (% w/w)^a

Ratio of Solvent-to-Bran	Time (hr)	d-Limonene	Hexane
2:1	0.5	15.87 \pm 0.22d	15.17 \pm 0.08d
2:1	1	18.29 \pm 0.12c	16.95 \pm 0.12c
2:1	2	18.97 \pm 0.48c	17.03 \pm 0.45b,c
2:1	3	19.20 \pm 0.30c	17.31 \pm 0.68a-c
3:1	0.5	19.20 \pm 0.19c	15.75 \pm 0.13d
3:1	1	20.30 \pm 0.40b	18.20 \pm 0.59a-c
3:1	2	21.11 \pm 0.11b	18.39 \pm 0.96a-c
3:1	3	21.07 \pm 0.09b	18.89 \pm 0.42a
5:1	0.5	20.73 \pm 0.11b	17.31 \pm 0.56a-c
5:1	1	22.48 \pm 0.15a	18.17 \pm 0.36a-c
5:1	2	22.92 \pm 0.08a	18.97 \pm 0.02a
5:1	3	22.98 \pm 0.73a	18.64 \pm 0.15a,b

^a Values followed by the same letter in the same column are not significantly different ($P < 0.01$). Values are means of independent duplicate determinations \pm standard deviation.

hexane extraction for 1 hr, an increase in solvent-to-ricer bran ratio (w/w) from 2:1 to 3:1 extracted 8.52% more crude rice bran oil, while the increase was almost negligible from 3:1 to 5:1. Hu et al (1996) reported 10.8% increase when the solvent-to-ricer bran ratio was raised from 2:1 to 3:1 with extraction for 0.5 at 60°C. On the other hand, for d-limonene extraction for 1 hr, an increase in solvent-to-ricer bran ratio from 2:1 to 3:1 and 3:1 to 5:1 produced 10.95 and 10.73% more crude rice bran oil, respectively.

Effect of Time on the Amount of Oil Extracted

The data in Table II also demonstrate the effect of extraction time on the amount of crude edible oil extracted with d-limonene and hexane at preselected solvent-to-ricer bran ratios of 2:1, 3:1, and 5:1. For hexane extraction at 3:1 solvent-to-ricer bran, 1 and 2.7% increase in percent of oil yield was observed when the extraction time was increased from 1 to 2 hr and from 2 to 3 hr, respectively. Bhagya and Srinivas (1992) observed a 2.1% increase and a 0.5% decrease when the extraction time was increased from 1 to 2 hr and from 2 to 3 hr, respectively, for soybean oil extraction with hexane as solvent conducted at 60°C and solvent-to-ricer bran ratio of 1.5:1. On the other hand, for d-limonene extraction conducted at 5:1 solvent-to-ricer bran, an increase in extraction time from 0.5 to 1 hr, 1 to 2 hr and 2 to 3 hr produced 8.5, 1.9, and 0.2% more crude rice bran oil, respectively.

Statistical Analysis

As can be observed from Table II, d-limonene extracted significantly more oil than hexane under any given experimental conditions. This might be due to the higher dissolving power of d-limonene for triglycerides when compared with hexane. Higher extraction temperature used during extraction by d-limonene, resulting in the lowering of the viscosities of the d-limonene and oil, thereby increasing the diffusion rates (Krishnamurthy 1982) might also be the reason. Moreover, it can also be observed that with further increase in the time of extraction after 1 hr, the increase in the oil yield both in d-limonene and hexane for all solvent-to-ricer bran ratios was statistically insignificant. The slow

extraction rates observed after 1 hr might be attributed, at least in part, to the decreased solubility of the last portions of the oil, specifically the phosphatides and other nonglyceride materials (Krishnamurthy 1982). Also, 5:1 solvent-to-ricer bran yielded a statistically significant amount of oil with d-limonene as solvent compared with 2:1 and 3:1 ratios. In hexane, no statistically significant increase was observed when solvent-to-ricer bran ratio was increased from 3:1 to 5:1. It can be concluded that solvent-to-ricer bran ratio of 5:1 and extraction time of 1 hr for d-limonene and solvent-to-ricer bran ratio of 3:1 and extraction time of 1 hr for hexane yielded maximum crude rice bran oil for the respective solvents under the conditions of this experiment.

Quality Characterization

The quality of crude rice bran oil extracted was analyzed for the oils extracted at solvent-to-ricer bran ratio of 2:1 and extraction time of 2 hr; solvent-to-ricer bran ratio of 3:1 and extraction time of 1 hr with hexane as a solvent; solvent-to-ricer bran ratio of 3:1 and extraction times of 1 and 2 hr; solvent-to-ricer bran ratio of 5:1 and extraction times of 0.5, 1, and 2 hr with d-limonene as a solvent; and also for the crude rice bran oil obtained from Riceland Foods. The crude rice bran oil extracted under these conditions were chosen either because they represented the next best conditions other than that obtained for the maximum yield or the crude rice bran oil obtained under these conditions was not statistically different from that of the maximum yield. Crude rice bran oil from Riceland Foods was analyzed for comparison studies.

The total free fatty acids (FFA) content extracted under different conditions was expressed as a percentage of oleic acid and tabulated in Table III. The values were slightly higher in d-limonene-extracted oils compared with those of hexane. This might be due either to the slightly polar nature of d-limonene when compared with hexane, with the result that fatty acids are more soluble in d-limonene than in hexane, or the high temperature of extraction used with d-limonene might have degraded the triglycerides to FFA. It may also be noted from Table III that the increase in the time of extraction had no significant effect on the percentage of total FFA extracted while the increase in the solvent-to-ricer bran ratio from 3:1 to 5:1 for 1 hr of extraction with d-limonene-extracted oil resulted in a 9.2% increase in % total FFA extracted. This might also be due to the increase in the extraction of FFA with an increase in the amount of d-limonene available with the corresponding increase in the solvent-to-ricer bran ratio. The % total FFA for the crude rice bran oil obtained from Riceland Foods was higher when compared with hexane-extracted oil under laboratory conditions. Enzymatic hydrolysis of triglycerides might be the reason, as the crude rice bran oil might have been exposed to room temperature after production during storage and transportation.

The phosphorus content of the oil sample has been used to estimate the phospholipid content because the phosphorus content of the oils has been attributed to the presence of phosphatides (Sonntag 1979a,b). The standard curve was obtained by plotting absorbance against the phosphorus content of the standard solu-

TABLE III
Effect of Solvent-to-Rice Bran Ratio and Extraction Time of d-Limonene-Extracted and Hexane-Extracted Oil on Yield (%) of Total Free Fatty Acids (FFA)^a

Solvent	Ratio of Solvent-to-Bran	Time (hr)	% FFA
Hexane	2:1	2	1.81 ± 0.12
Hexane	3:1	1	1.83 ± 0.15
d-Limonene	3:1	1	2.45 ± 0.07
d-Limonene	3:1	2	2.51 ± 0.17
d-Limonene	5:1	0.5	2.74 ± 0.03
d-Limonene	5:1	1	2.68 ± 0.06
d-Limonene	5:1	2	2.79 ± 0.12
Crude rice bran oil	–	–	2.63 ± 0.03

^a Values are means of independent duplicate determinations ± standard deviation.

TABLE IV
Effect of Solvent-to-Rice Bran Ratio and Extraction Time of d-Limonene-Extracted and Hexane-Extracted Oil on Yield (%) of Phospholipids Extracted^a

Solvent	Ratio of Solvent-to-Bran	Time (hr)	Phosphorus (%)	Phosphorus (ppm)	Phospholipids (%)
Hexane	2:1	2	0.05 ± 0.00	477.26 ± 25.04	1.57 ± 0.08
Hexane	3:1	1	0.04 ± 0.00	415.76 ± 21.81	1.37 ± 0.07
d-Limonene	3:1	1	0.07 ± 0.00	696.95 ± 52.36	2.33 ± 0.17
d-Limonene	3:1	2	0.05 ± 0.00	483.13 ± 15.05	1.56 ± 0.05
d-Limonene	5:1	0.5	0.04 ± 0.00	433.64 ± 4.44	1.38 ± 0.01
d-Limonene	5:1	1	0.05 ± 0.00	529.69 ± 38.06	1.59 ± 0.12
d-Limonene	5:1	2	0.04 ± 0.00	399.20 ± 18.14	1.31 ± 0.06
Crude rice bran oil	–	–	0.03 ± 0.00	309.81 ± 9.74	0.96 ± 0.03

^a Values are means of independent duplicate determinations ± standard deviation.

tions. The percentage phosphorus values obtained under experimental conditions of extraction time and solvent-to-rice bran ratio were tabulated in Table IV. The acceptable conversion factor of 31.7 (Friedrich et al 1982) was used to convert the percentage phosphorus values to percentage phospholipids. The increase in the solvent-to-rice bran ratio from 3:1 to 5:1 for both 1 hr and 2 hr d-limonene-extracted oils showed a decrease in the percentage phospholipids extracted. It may also be noted from Table IV that for d-limonene-extracted oils with the solvent-to-rice bran ratio of 5:1, increase in the time of extraction from 0.5 to 1 hr resulted in an increase in the percentage of phospholipids extracted while increase in time of extraction from 1 to 2 hr resulted in a decrease in percentage phospholipids extracted. Krishnamurthy (1982) noted that the phospholipids are extracted in later stages of extraction, as they are less soluble than the triglycerides. As the phospholipids are amphiphilic in nature, the back-binding of phospholipids with the meal due to the van der Waal interactions might have resulted in a decreased yield of phospholipids extracted with an increase in extraction time from 1 to 2 hr. The percentage phospholipids extracted for crude rice bran oil obtained from Riceland Foods was lower when compared with those obtained under the laboratory conditions. This might be due to the difference in the extraction method used for production of crude rice bran oil obtained from Riceland Foods to that extracted under laboratory conditions.

The absorption spectrum of 1% crude rice bran oil, obtained under the conditions of solvent-to-rice bran ratio of 5:1 and extraction time of 0.5 hr in hexane, is presented in Fig. 1. The large absorbance observed between 250 and 400 nm may be due to the presence of unsaturated fatty materials (Formo 1979). The absorbance values, as measured by the spectrophotometer, of d-limonene- and hexane-extracted oils under experimental conditions were tabulated in Table V. The color of the d-limonene-extracted oils was slightly darker compared with those of hexane-extracted oils, as indicated by higher absorbance values. Crude rice bran oil extracted with d-limonene under the conditions of solvent-to-rice bran ratio of 5:1 and extraction time of 0.5 hr recorded the lowest absorbance of the d-limonene-extracted oils. The reason behind d-limonene-extracted oil being slightly darker compared with hexane-extracted oil might be due to the higher temperature used during extraction when d-limonene is used as the solvent, leading to the formation of oxidative materials including polymers and other oil-soluble products as a result of Maillard reactions (Formo 1982; Guhe and Bhowmick 1998). This might be overcome by using vacuum to lower the temperature of extraction. Bleaching

might also aid in reducing the darker color of the crude rice bran oil. As can be noted from Table V, the increase in the time of extraction produced darker oil with both the hexane and d-limonene. But increase in the solvent-to-rice bran ratio for d-limonene yielded lighter oil, as expected. The combination of higher temperature and longer extracting time seems to have an effect on the coloring of defatted rice bran meal. The quality of the defatted rice bran meal as well as overall composition of the rice bran oil treated with limonene is also very important and we plan to study it in the next phase of the research.

The chromatograms of fresh d-limonene and recovered d-limonene with oxidation products labeled are shown in Figs. 2 and 3, respectively. The 5:1 solvent-to-meal ratio and 1 hr extrac-

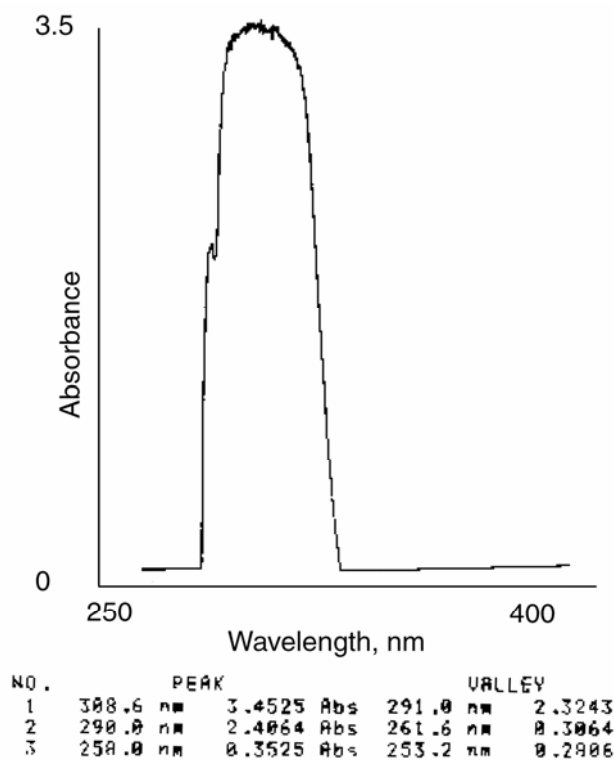


Fig. 1. Absorption spectrum of 1% crude rice bran oil (5:1 solvent-to-rice bran ratio and 0.5 hr extraction time) in hexane.

TABLE V
Color Analysis of d-Limonene-Extracted and Hexane-Extracted Oils Under Given Experimental Conditions^a

Solvent	Ratio of Solvent-to-Bran	Time (hr)	Absorbance Units		
			10% Oil	5% Oil	1% Oil
Hexane	2:1	2	0.19 ± 0.00	0.10 ± 0.00	0.02 ± 0.00
Hexane	3:1	1	0.18 ± 0.02	0.09 ± 0.01	0.02 ± 0.00
d-Limonene	3:1	1	0.75 ± 0.03	0.37 ± 0.02	0.07 ± 0.01
d-Limonene	3:1	2	0.86 ± 0.00	0.53 ± 0.01	0.13 ± 0.03
d-Limonene	5:1	0.5	0.30 ± 0.08	0.13 ± 0.00	0.04 ± 0.02
d-Limonene	5:1	1	0.58 ± 0.01	0.25 ± 0.09	0.05 ± 0.00
d-Limonene	5:1	2	0.71 ± 0.00	0.38 ± 0.01	0.07 ± 0.03
Crude rice bran oil	-	-	0.07 ± 0.00	0.04 ± 0.00	0.01 ± 0.00

^a Values are means of independent duplicate determinations ± standard deviation.

TABLE VI
Oxidation Products of d-Limonene (5:1 solvent-to-rice bran ratio and 1 hr extraction time)

Component	Concentration (ppm) in Fresh d-Limonene	Concentration (ppm) in Recovered d-Limonene
Cis and trans limonene-1, 2 epoxide	1835.2 ± 140.2	2443.4 ± 14.4
Carveol	657.5 ± 55.5	1336.1 ± 0.8
Carvone	429.3 ± 26.5	539.3 ± 3.9

^a Values are means of independent duplicate determinations ± standard deviation.

tion time was chosen for studying the extent of oxidation, as this would represent a true picture at the extreme conditions. The concentration of *cis* and *trans* limonene-1, 2 epoxide, carveol mixture of isomers, and carvone in fresh and recovered d-limonene as measured by gas chromatography are summarized in Table VI. The percentage increase of *cis* and *trans* limonene-1, 2 epoxide, carveol, and carvone from that in fresh to recovered d-limonene were 33, 103, and 26%, respectively. This might be the reason for a slight terpeny off-note observed in the recovered d-limonene. However, even with this increase, the total quantity of oxidation products in recovered d-limonene represents <1% of d-limonene. And with either the use of vacuum or by addition of antioxidants during extraction, the extent of oxidation could be reduced. Thus, it might be concluded that recovered d-limonene could be suitable for reuse as a solvent in the subsequent cycle. Obviously, the long-term impact of limonene stability with repeated cycles is needed for future studies.

CONCLUSIONS

The solvent extraction data collected under experimental conditions of solvent-to-rice bran ratio and extraction time proved that d-limonene was capable of extracting oil from rice bran. Also, the comparison studies proved that d-limonene-extracted significantly more crude rice bran oil than hexane under any given experimental conditions. There was no significant increase in the percentage oil yield when the extraction time was increased >1 hr both with hexane and d-limonene. For d-limonene, solvent-to-rice bran ratio of 5:1 and extraction time of 1 hr represented the conditions for maximum yield of crude rice bran oil under the given experimental conditions.

The percentage of total FFA, phospholipids, and color of the crude rice bran oil extracted under given experimental conditions were analyzed to assess its quality. There was a slight increase in the percentage of total FFA with increase in the solvent-to-rice bran ratio and the extraction time. On the other hand, percentage of phospholipids decreased with increase in solvent-to-rice bran ratio and when the extraction time was increased from 1 to 2 hr for solvent-to-rice bran ratio of 5:1. The color was significantly lighter at extraction time of 0.5 hr and 5:1 solvent-to-rice bran

when compared with other conditions of extraction. Moreover, bleaching might reduce the darker color of the crude rice bran oil. Hence, it may be concluded that 5:1 solvent-to-rice bran ratio and extraction time of 0.5 hr were the optimum conditions for extraction with optimal quality under the given experimental conditions and that limonene was a viable alternative to hexane based on oil yield and quality.

Even though d-limonene extracted more crude rice bran oil than hexane under any given experimental condition, the total composition of crude rice bran oil has to be analyzed to determine the refining loss. The defatted rice bran was darker in color when compared with hexane-extracted meal. This may be due to the Maillard reaction occurring at high extraction temperature (Guhe and Bhowmick 1998). This might become an issue when defatted rice bran is used as an animal feed. Hence further studies need to be made to assess the quality of defatted bran. The extent of oxidation of d-limonene used for extraction was studied by analyzing its oxidation products using gas chromatography. The slight terpeny off-note observed in the recovered d-limonene may be explained by the increase in the amount of oxidation products when compared with fresh d-limonene. However, it should be emphasized that this slight increase should not affect the solvent characteristics of d-limonene as it represents <1% of d-limonene. Hence, it may be concluded that d-limonene is suitable for reuse as a solvent. The other obvious issue with potential use of limonene as a solvent for edible oil extraction is the problem of high-energy cost that is mainly due to high boiling point and slightly high latent heat of limonene. Energy-saving solvent recovery technology such as membrane separations might eventually overcome this obstacle and render limonene-based oil extraction affordable.

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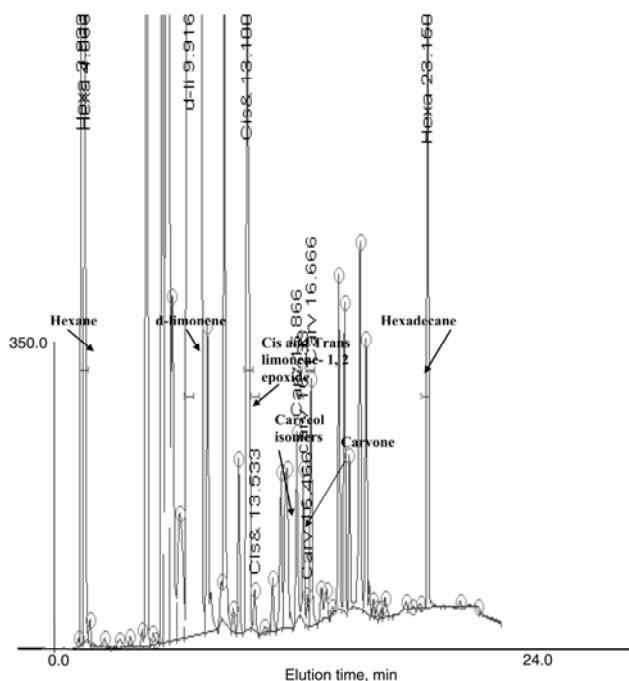


Fig. 2. Chromatogram for fresh d-limonene.

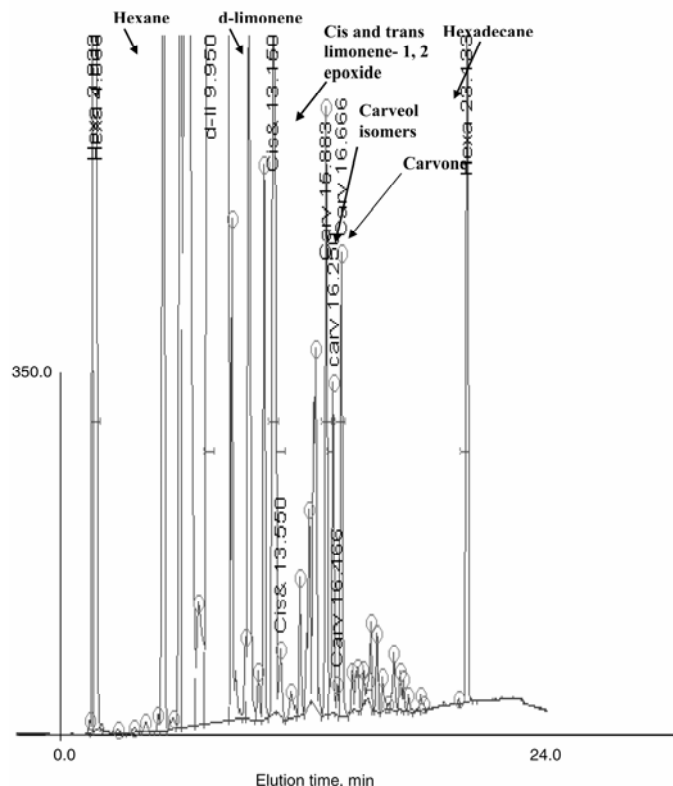


Fig. 3. Chromatogram for recovered d-limonene (5:1 solvent-to-rice bran ratio and 1 hr extraction time).

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