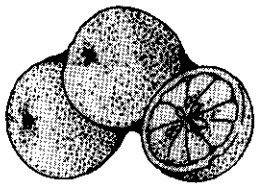

Analysis of Encapsulated Orange Peel Oil

By S. Anandaraman and G. A. Reineccius, Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota

The chemical analysis of encapsulated flavors poses unique problems due to the presence of the encapsulating wall material. The importance of chemical and instrumental analyses of encapsulated flavors in quality control and product evaluation cannot be over-emphasized. Barring a brief mention of surface oil determination by simple solvent extraction and total oil determination by steam distillation of encapsulated lemon oil,[1] there is little scientific literature directed towards methods of analysis of encapsulated flavors. This investigation was undertaken to assess the applicability of existing methods and to develop new methods for analyzing encapsulated orange peel oil. The determinations evaluated were moisture content, total oil, surface oil and peroxide value.

Experimental

Materials

Maltodextrin samples (4, 10, 20, 25 and 36.5 DE, referred to as 040, 100, 200, 250 and 365, respectively) were obtained from the Grain Processing Corporation (Muscatine, IA), and cold pressed orange peel oil was obtained from Universal Flavors (Indianapolis, IN). All organic compounds or solvents and inorganic chemicals used in the analyses were ACS certified grade.

Encapsulation of Orange Peel Oil

Maltodextrin solutions with 30 percent (w/w) solids content were allowed to hydrate overnight. Orange peel oil (20% w/w of solids) was emulsified into the carbohydrate solution using a laboratory homogenizer. The emulsion was immediately fed to a Niro Utility Model spray drier equipped with a centrifugal wheel atomizer. Operating at an inlet and outlet air temperature of 190 ± 3°C and 90 ± 3°C, an evaporative capacity of 6 Kg/hr. was obtained. The powders were stored in airtight glass jars at 4°C prior to analysis.

Moisture Determinations

Moisture determinations were done employing vacuum oven, toluene distillation, Karl Fisher and gas chromatographic methods. The first three methods were AOAC methods,[2] with minor modifications in sample weights, temperatures and/or the durations of determinations. The gas chromatographic method was based on that of Reineccius and Addis for moisture determination in meat.[3] For gas chromatography, a 1.5g sample of spray dried flavoring was extracted with 10 ml

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anhydrous methanol-ethanol mixture (500 ml mixture containing 25g ethanol) for up to 20 hours in order to determine optimum extraction time. Ethanol served as the internal standard. The calibration mixture was composed of 50 mg distilled water in 10 ml of the above solvent mixture.

A Hewlett-Packard Model 5840A gas chromatograph equipped with a thermal conductivity detector was calibrated for the internal standard method and programmed to yield moisture content in milligrams per sample. A 1.25 m x 0.2 cm i.d. glass column packed with Porapak Q was operated at 140°C isothermally for the analysis. A carrier gas flow of 35 ml helium/min. was used. Injector and detector temperatures were 200°C and 225°C, respectively.

Total Oil Determination

Hydrodistillation. The Clevenger hydrodistillation method was used as the conventional procedure for total oil determination.[4] A 12g sample was employed and the distillation done for 2-3 hours. The volumetric estimate was multiplied by the oil density to arrive at the gravimetric estimate.

Gas Chromatography–Acetone Extraction. Encapsulated orange peel oil (1g) was reconstituted with 1 ml distilled water in a test tube. Acetone (4 ml) was added to the reconstituted sample and homogenized using a mini-homogenizer similar to a tissue homgenizer. Upon precipitation of the encapsulating agent, the acetone layer was collected by decantation. A second extraction was done using 4 ml acetone. The acetone fractions were pooled together, dried over anhydrous magnesium sulfate, and 1 ml acetone containing 1 mg or 2-nonanone added as the internal standard prior to gas chromatographic determination.

Calibration standards were prepared by emulsifying 0.85g of each of the encapsulating agents, 150 mg orange peel oil and 1 ml water followed by the above extraction procedure. In order to estimate the recoveries, an additional calibration standard containing 150 mg oil, 1 mg 2-nonanone and 9 ml acetone was analyzed. This permitted an evaluation of the effect of the carrier (maltodextrin) on the analytical result. A Hewlett-Packard Model 5880 gas chromatograph equipped with a single flame ionization detector was used for the quantitative analyses of the extracts. The gas chromatographic conditions were as follows:

Column: 0.2 mm i.d. x 12 m WCOT fused silica (Hewlett-Packard)
Stationary phase: OV-101
Carrier gas: Hydrogen at 35 cm/sec.
Split ratio: 1:60
Column temperature: 60°C to 175°C programmed at 15°C/min.
Injection port temperature: 225°C
Detector temperature: 250°C
Sample size: 2 μl

Gas Chromatography–Adsorption Method. An alternate method to the acetone extraction procedure was examined. The principle of this method involves selective adsorption and/or partitioning of the orange oil components to a hydrophobic phase.

The encapsulated orange peel oil (0.2g) was dissolved in 7.5 ml distilled water and forced through a Sep-Pak® (Waters Associates, Inc., Farmingham, MA) C_{18} reverse phase cartridge by applying a vacuum at the exit to achieve a flow rate of 1 ml/min. This cartridge was subsequently flushed with 5 ml distilled water. Oil was then recovered by elution with 3 ml acetone. The acetone fraction was dried over anhydrous magnesium sulfate followed by the addition of 0.5 ml 2-nonanone solution in acetone (0.5 mg/ml concentration). Calibration mixtures were prepared by recovering the oil from an emulsion of 160 mg encapsulating agent, 40 mg oil and 7.5 ml water. A similar calibration mixture without the encapsulating agent was used to assess recoveries. The gas chromatographic conditons are identical to those described in the acetone extraction method.

Surface Oil Determination

A Soxhlet extraction apparatus was used for extracting the surface oil from the powder. Glass distilled pentane was employed as the extracting solvent. Powder (12-15g) was weighed into an extraction thimble (cellulose, i.d. = 25 mm, length = 80 mm—Whatman Ltd., England), covered with glass wool and placed in the Soxhlet extraction chamber.

Pentane (125 ml) was used in the extraction flask. The flask was heated on a steam bath to get a steady reflux of pentane (1 drop/sec). The extraction was done for 14 hours. Pentane (1 ml) containing 0.5 mg 2-nonanone was added to the extraction flask and then the volume was reduced under a stream of nitrogen to 1 ml. Quantitation of the oil was accomplished by gas chromatographic analysis by the internal standard method.

Peroxide Number Determination

Peroxide content of oxidized orange peel oil was examined by two different methods, the iodometric method[4] and a colorimetric method using titanium sulfate reagent.[5]

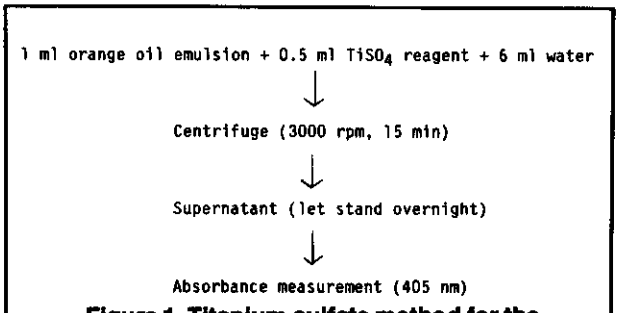

Figure 1. Titanium sulfate method for the determination of peroxide values in encapsulated orange oil

Peroxide analysis was conducted on three different orange oils (representing different levels of oxidation) in the presence of the 10 and 25 DE maltodextrins. Two percent solutions (50 ml) of each of the encapsulating agents were prepared in distilled water. Each (100 mg) of the three different oxidized orange oil samples were dispersed in the solution by vigorous shaking (10 min) in stoppered Erlenmeyer flasks. Aliquots of these oil emulsions were used for peroxide value determinations.

Iodometric Method. To 10 ml of the oil emulsion, 1 ml of 20 percent potassium iodide solution and 1 ml 1 N sulfuric acid were added. The liberated iodine was titrated after 2 min against a 0.005 N sodium thiosulfate solution using a carbohydrate indicator. A blank determination was done using 10 ml of the emulsion containing fresh orange peel oil. Peroxide values were expressed as $\mu g\ H_2O_2$/mg oil.

$$\text{Peroxide value} = \frac{\text{(sample titer volume-blank) (17) (normality of } Na_2S_2O_3)/10^6}{20 \times 1000}$$

Colorimetric Method. Titanium sulfate reagent was prepared by dissolving 2.5g titanium sulfate in 240 ml 12 N sulfuric acid at 90°C with stirring. Upon dissolution (about 6 hours), the reagent was filtered into a 250 ml volumetric flask and made up to volume using 12 N sulfuric acid. The steps involved in the determination are shown below in figure 1.

A standard curve was prepared using 2.5 percent 10 DE maltodextrin solution containing hydrogen peroxide. The supernatant was allowed to stand overnight prior to the absorbance measurement to facilitate further clarification by hydrolysis. All absorbance measurements were made against a reagent blank.

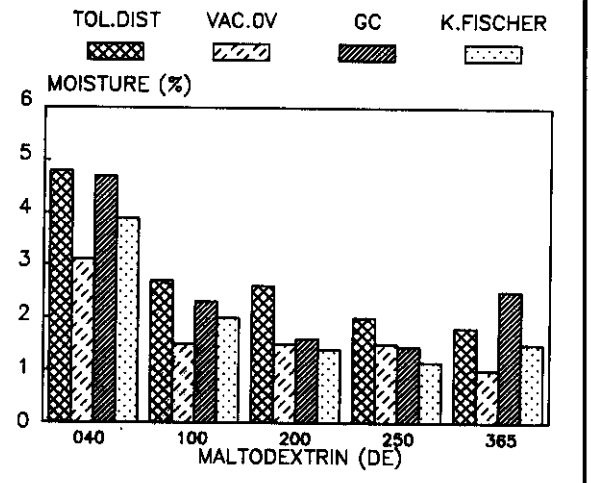

Figure 2. Comparison of moisture determination methods

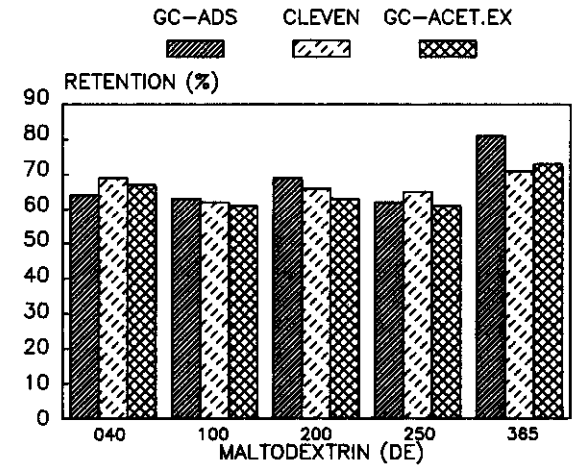

Figure 3. Comparison of oil determination methods

Results and Discussion

Moisture

The high volatile flavor content in encapsulated flavors can interfere in moisture determinations. Loss of volatiles during gravimetric procedures such as the vacuum oven method and water formation via thermal degradation of encapsulating agents by distillation methods are of concern. A comparison of the vacuum oven, toluene distillation, Karl Fisher and GC methods was done using five different samples of encapsulated orange peel oil. The results are presented in figure 2.

Overall, there exists a good agreement among the four methods. The vacuum oven tends to yield lower moisture values. The position of the samples in the oven affected the weight loss and resulted in variations in the determination among replicates. Non-uniform heating can potentially lead to inaccurate results by this method. The toluene distillation method yielded the highest moisture value for most samples, possibly due to the decomposition of low molecular weight sugars during distillation (ca. 110°C).

The gas chromatographic method consistently yielded slightly higher moisture values as compared to the Karl Fischer method.

From a practical viewpoint, for routine moisture determinations, the toluene distillation method is adequate. This method has the advantage of simplicity and reduced analysis time consistent with reasonable accuracy in quality control situations.

Total Oil Determination

Determination of oil content in encapsulated flavors is important in assessing flavor retention. Two gas chromatographic methods were standardized and the results compared to those obtained by conventional hydrodistillation (figure 3). Encapsulation efficiency, in terms of orange oil retention, was chosen as the criterion for comparing the different oil determination methods.

Figure 3 shows that both gas chromatographic methods (ie., adsorption and extraction) yield results similar to the distillation procedure.

The possible loss of these water soluble flavor components in the distillation method, if substantial, would yield convincingly lower retention percentages. Our results indicate that such losses for orange oil are only marginal and are not a significant source of error in total oil determination.

However, a disadvantage of the hydrodistillation method is that it does not yield a profile of individual flavor components without subsequent gas chromatographic analysis. Also, this method is not applicable in the analysis of encapsulated flavors when the flavor contains a large proportion of water soluble components such as pyrazines, low molecular weight alcohols and/or carbonyl compounds.[6]

A comparison of the absolute recoveries by adsorption and acetone extraction procedures are presented in Table I. The acetone extraction procedure showed higher absolute recoveries (91-96%) compared to recoveries achieved by reverse phase adsorption (82-85%) for all the encapsulating agents. So even though both methods yield similar results for total oil with proper calibration in the presence of the encapsulating material, the acetone extraction method is favored due to the higher absolute recoveries.

Table 1. Comparison of Absolute Oil Recoveries from Oil Emulsions by Two Gas Chromatographic Methods

	mg oil		% recovery	
Sample	Absorption[a]	Acetone Extraction[b]	Absorption	Acetone Extraction
040	33.97	136.9	84.9	91.2
100	32.79	140.0	82.0	92.7
200	33.54	144.3	83.9	96.2
250	33.61	142.5	84.0	95.0
365	33.20	144.0	83.0	96.0

[a] 100% recovery = 40 mg oil
[b] 100% recovery = 150 mg oil

The chromatographic profiles of the oils recovered by both methods were identical, qualitatively and quantitatively. Therefore, the lower recovery by the reverse adsorption is not due to the loss of certain flavor components. The loss of fine oil droplets in the effluent stream could explain the lower recoveries by adsorption. A lower flow rate and use of a second adsorption cartridge in series is a possible way to improve the recoveries. Reverse phase adsorbents can adsorb encapsulating agents with hydrophobic groups (gum arabic and chemically modified starches), causing inadequate flow rate and eventual stoppage of fluid flow.[6] Therefore, this method would not be suitable for the analysis of spray dried flavors using these types of carriers.

Acetone extraction and subsequent gas chromatography is a more universal technique. It offers a simple, rapid and precise quantitation method for the anlaysis of encapsulated flavors, irrespective of the nature of the flavor components and/or encapsulating agents.

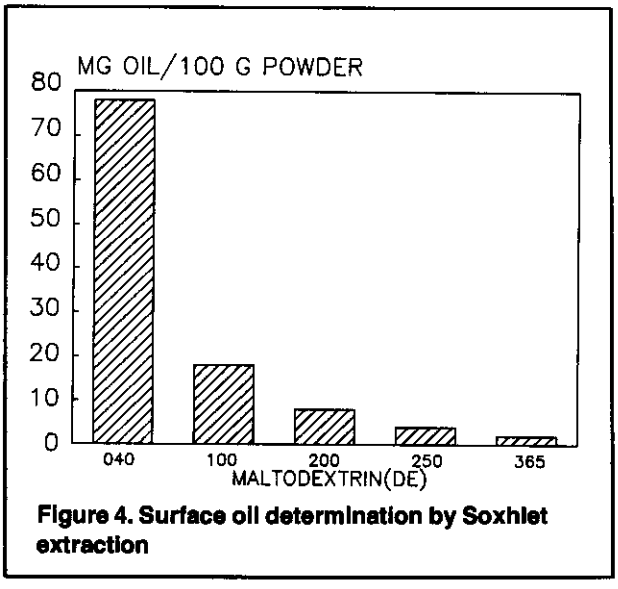

Figure 4. Surface oil determination by Soxhlet extraction

Surface Oil Determination

Surface oil content by Soxhlet extraction method ranged from 3.3 to 75 mg/100 g dry powder (figure 4). It is interesting to note that this surface oil content decreases with increasing dextrose equivalent of the encapsulating agents. This would indicate that the higher DE starches tend to form a tighter and more impermeable matrix during spray drying. Lower surface oil content would enhance storage stability.

Peroxide Number Determination

Comparison of the peroxide value determination by iodometric and colormetric methods on three different oil samples (1, 2 and 3) is presented in Table II. Oil samples 1, 2 and 3 were orange peel oils stored at room temperature for approximately 30, 45 and 70 days, respectively. The colorimetric method consistently yielded higher peroxide values (3 to 4 times) than the iodometric method. This is expected since iodine would be occluded by starch, and potassium iodide may undergo varying reaction rates with peroxides and hydroperoxides.[8] These factors would all potentially result in lower determination values. The three oil samples (1, 2 and 3) showed increasing peroxide contents by iodometry. Results of the colormetric method showed practically no difference in the peroxide content of samples 1 and 2. This is possible because titanium sulfate forms a color complex only with hydroperoxides.[5] During terpene oxidation, hydroperoxides are formed initially in larger amounts compared to the true organic peroxides.[9] Sample 2 could have had a certain amount of terpene peroxides that were not detected by the colorimetric method and, therefore, were not measured.

The colorimetric method yields only hydroperoxide content. However, the hydroperoxides form prior to other organic peroxides

Table II. Comparison of Iodometry vs. Colorimetry for Peroxide Value Determination

	Peroxide value (μg H_2O_2/mg oil)			
	Iodometry		Colorimetry	
Oil Sample No.	Replicates	Mean	Replicates	Mean
	1.49		7.00	
1	1.49		7.00	
	1.70		6.63	
	1.70	1.59	6.50	6.78
	2.13		6.75	
2	2.34		6.63	
	2.55		6.50	
	2.55	2.39	6.75	6.66
	3.19		11.00	
3	3.40		11.00	
	3.40		10.88	
	3.40	3.35	10.88	10.94

during autoxidation and, therefore, the colorimetric procedure would detect early oxidation. Also, it has two other advantages of being more sensitive and free from possible interference from the encapsulating agents when proper precautions are observed.

Conclusions

The toluene distillation method is suggested for the routine determination of moisture in spray dried citrus oils. The technique is rapid and due to large sample size, has minimal sampling error, requires minimal equipment and can be performed by an individual with little technical training.

The determination of total oil is readily accomplished by the hydrodistillation technique. Most of the advantages of the toluene moisture determination also apply to this technique. However, the hydrodistillation technique has the disadvantage of not being applicable to some encapsulated artificial flavors or other flavorings which contain a substantial proportion of water soluble constituents. Also the hydrodistillation technique has a significant disadvantage in providing only "total" oil and does not yield information about the profile of flavor constituents. For encapsulated flavors with significant proportions of water soluble constituents and when information is desired about the flavor profile, acetone extraction is recommended.

We prefer the Soxhlet method for surface oil determination. This technique yields information about total extractable oil. A simple surface wash with organic solvent is sometimes used as an indication of surface oil, but this is not as good an indication of the unprotected oil (i.e., that subject to attack by oxygen) as is the Soxhlet method.

For monitoring peroxide values (or degree of oxidation) we suggest the titanium sulfate method. This technique is more sensitive than the iodometric method and can be done in the presence of the encapsulating polymer. This technique has a further advantage of monitoring hydroperoxides which are early indicators of oxidation.

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References

Address correspondence to G. A. Reineccius, University of Minnesota, Dept. of Food Science & Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108.

1. W. King, P. Trubiano and P. Perry, Food Prod. Dev. **10**:54, 1976
2. Association of Official Analytical Chemists. Official methods of analysis of the Association of Official Analytical Chemists. W. Horwitz, ed., Washington, DC, 1980
3. G. A. Reineccius, and P. B. Addis, J. Food Sci. **38**:355, 1973
4. Essential Oil Association of U.S.A. Inc. Book of Standards and Specifications, New York, NY p. 7 & 9, 1975
5. W. A. M. Ekin and C. J. Frenkel, Amer. Oil Chem. Soc. **53**:746, 1976
6. K. Kernik, G. A. Reineccius and J. P. Scire, Progress in Flavour Research 1984. P. Schreier, ed., Elsevier Publ. Co., Amsterdam. 1985 p. 467-476
7. S. Anandaraman, Encapsulation, Analysis of and Stability of Orange Peel Oil, Ph.D. Thesis, p. 39-40, 1984
8. A. J. Martin, Determination of organic peroxides in organic analysis. J. Mitchell, Jr., I. M. Kollhoff, E. S. Proskauer, and A. Weisberger, eds. Interscience Publishers, Inc. New York, NY Vol. IV:1-63 (1960)
9. G. Windmark and S. Blohm, Acta. Chem. Scand., **11** 392-394, 1957