World Applied Sciences Journal 13 (8): 1821-1826, 2011
ISSN 1818-4952

Morphology, Properties and Safety of Microencapsulated Orange Peel Oil

[1]A.Z.M. Badee, [2]S.A. El-Nawawi, [3]H.M. Aly and [2]A.E. Abd El- Kader

[1]Department of Food Science and Technology,
Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Department of Chemical Engineering and Pilot Plant Laboratory,
National Research Center, Giza,, Egypt
[3]Department of Cytogenetic and Cell Biology, National Research Center, Giza, Egypt

Abstract: Encapsulation is a current, important and broadly used process to retain and protect volatile and flavoring compounds in commercial food products. Unfortunately production of these materials is largely an empirical science. In the present investigation gum arabic was used as carrier and orange peel oil was chosen as a core ingredient because, besides having a great economic importance in food industry, its volatile components are highly sensitive to the oxidation by effect of temperature, light and oxygen presence. Although several encapsulation methods have been reported, the spray drying technology has become one of the most important processes used to produce dry flavors from liquids throughout the food and beverage. Orange oil was encapsulated with gum arabic via spray drying using inlet and exit air temperatures of 200°C and 100 °C, respectively. The resultant powders were analyzed for moisture content (toluene distillation), surface content (Soxhlet extraction) and total oil (Clevenger distillation). The high retention percentage of orange peel oil powder (89%) could be successfully prepared in the gum Arabic materials with low surface content comparing with 20% w/w of conventional materials. As regard to the safety of encapsulated orange oil, it was noticed that glucose serum, cholesterol and aminotransferases enzymes exhibited no significant change as compared to control. Meanwhile, creatinine content (mg/dl) was significantly ($p<0.002$) increased from 0.8 (in control) to 1.06 mg/dl after encapsulated orange oil treatmen

Key words: Encapsulation · Emulsion · Orange peel oil · Spray drying · Gum Arabic and carrier

INTRODUCTION

Flavor has today become an essential ingredient for food developers: it imparts taste and aroma to a variety of foodstuffs, restores the flavoring lost during processing and allows the development of new products with novel tastes. There are three major purposes for encapsulating flavors. The first one is to convert liquid flavors into a dry form and free-flowing powder form. The second is to prevent flavor compounds from easily undergo oxidation leading to a decrease in flavor strength or even the development of off-flavor. Since encapsulation is effective ways to provide barriers against undesirable environmental factors and thereby minimize the changes. Finally, microencapsulated flavors offer the possibility for controlled flavor release during processing or final food preparation [1-3].

Spray drying is the most common technique to produce flavor powders from food flavor emulsion; recipes for spray-dried flavors contain in addition to the liquid flavor, carrier materials such as maltodextrin (MD), gum Arabic and modified starch, etc. Ingredients are mixed, emulsified, homogenized and spray dried; water content is reduced to below 5% and the flavor is encapsulated in an amorphous glassy carbohydrate matrix. Therefore, the aim of the present work was to evaluate the chemical properties and safety of orange peel oil capsules.

MATERIALS AND METHODS

Orange peel oil was obtained from Kato Aromatic Co. Giza (Egypt) .Gum Arabic was obtained from PRS Panreac (Espania).

Experimental animals were obtained from the Animals House of the National Research Center. Swiss Albino mice of five weeks age with an average weight of 25-30 gram were used in the experiment.

Corresponding Author: A.E. Abd El- Kader, Department of Chemical Engineering and Pilot Plant Laboratory, National Research Center, Egypt. E-mail: amalibrahim67@yahoo.com.

Kits for determination of glucose, cholesterol, Aspartate amino transferase (AST), Alanine amino transferase (ALT) and creatinene were purchased from Biodiagnostic Giza Co. Egypt

Emulsion Preparation and Spray Drying: Solutions of Arabic gum containing 30 percent (w/w) solids were dispersed in deionized water and gently heated (60°C) over a steam bath to facilitate solubilization. The solutions were allowed to cool to room temperature before storing under refrigeration overnight (4°C). The orange oil (20% w/w of solids) was added and homogenized vigorously (10, 000 rpm for 5 min) with an Ultra Turrax M-45 homogenizer at ambient temperature (22°C). The obtained emulsion was maintained under slow agitation during spray drying.

The emulsion was spray dried in a BUCHI 190 Spray Dryer with an evaporation rate of 1.5 kg /1 hour and a chamber diameter of 10 cm, equipped with a pressurized nozzle operating at 5 atmospheres. Feed is metered into the dryer by a peristaltic pump. Drying conditions were controlled using inlet and exit air temperatures of 200°C and 100°C, respectively. Powder was collected at the bottom of dryer cyclone and kept in air tight containers at 8°C until analyzed

Separation and Identification of the Chemical Components of Orange Oil: a.Gas chromatography technique was used to separate and identify the volatile chemical components under the following conditions:

A Hewlett Packard 5890 series II Instrument equipped with a flame ionization detector (FID) and a carbowax fused silica column (50 m length, 0.25 mm inside diameter and film thickness of 0.32 μm). The oven temperature was programmed from 60°C to 200°C at the rate of 3°C/min. Helium (1 ml/min) was used as a carrier gas with a split ratio of 100:1. The temperature of injection port and detector were 150°C and 250°C, respectively. Percentages of peak area were calculated with a Hewlett Packard 3396 integrator.

Retention of Individual Aroma Compounds During Spray Drying: The amounts of individual aroma compounds retained by spray drying operation were determined by gas chromatographic (GC) analysis .Fresh powder samples were extracted using an acetone - dissolved in 0.85 g of distilled water in a screw -cap vial and mixed using a vortex mixer. Then 4 g of an acetone solution containing 2- octanone as an internal (0.25mg/g acetone) was added to the vial and mixed again. After settling, the supernatant was transferred to 2 ml screw - cap vial. One microliter of each extract was automatically injected in split less mode into a HP5890 series II GC according to Risch and Reineccius [4]b. Physicochemical properties of orange oil.

Determination of the Properties of Powder

Determination of Total Oil Remainder and Moisture Content: Total oil and moisture content in the spray dried powders were determined by Clevenger distillation [6]

Determination of Surface Oil: Soxhlet extraction apparatus was used for removing the surface oil from the encapsulated samples. Forty grams of powder were placed in an extraction thimble and covered with glass wool .The powder was extracted with hexane for 4 hour, after which the oil retention was determined by Clevenger hydrodistillation. Differences in oil volume between solvent washed samples versus non-washed samples were attributed to surface oil on the powder according to Trubiano and Lacourse [7]

Determination of Bulk Density: Bulk density was determined by the tapping method according to Hall and Hedrick [8] Powder (30 gm) was loosely weighed into a 100 ml graduated cylinder. Cylinder with the powder was tapped on a flat surface to a constant volume. The final volume was recorded. Bulk density was calculated by dividing the sample weigh by the volume

Determination of Scanning Electron Microscopy (SEM): The specimens were mounted on copper stubs with double - side's adhesive tape and coated with gold using Sputter Coater S150A Edwards -England.

The specimens were examined under Scanning Electron Microscope (JXA-840A Electron Probe Micro analyzer- JEOL-, Japan.) imaging mode at an acceleration voltage of 30 kV. Photographs were taken at 500 X magnification [9]

Biological Treatment: Ten of Swiss Albino male mice were given daily gavages doses of encapsulated orange oil. The dose was 1/10 of LD_{50} (LD_{50} = 5600 mg/ kg body weight) which was dissolved in10 ml water. The dose was applied 5 days per week for 30 days. The Standard Balanced Diet used in Animals House of the National Research Center was consisted of (g/100g) casein 15 g/100g, starch 43.7 g/100g, sucrose 21.8 g/100g, fat 15 g/100g, vitamin mixture 3.5 g/100g and salt mixture 1 g/100g according to Vrana *et al.* [10] and water were available ad. libitum.

A-Biological Evaluation: Blood samples were taken from mice after 30 days. The blood samples were collected from the eye plexuses by a fin capillary glass tubes according to the method described by Schemer [11]. The collected blood samples were put into a dry clean glass tubes without anti coagulant to prepare serum sample. Blood was left for 15 minutes at room temperature to coagulate by itself then the tubes were centrifuged at 3000 rpm for 10 minutes. The clean supernatant serum was kept at -20°C to determine blood constituents as serum total cholesterol, glucose, kidney function (creatinine) and liver function enzymes (AST and ALT).

Estimation of Serum Glucose: The glucose content was determined according to the methods of Trinder [12] Barham and Trinder [13].

Estimation of Serum Total Cholesterol: The determination of cholesterol was carried out according to the method of Richmond [14].

Estimation of Alt Activity: (Alanine aminotransferase) The determination of ALT was carried out according to Reitman and Frankel [15].

Estimation of Ast Activity: (Aspartate aminotransferase) The determination of AST was carried out according to Reitman and Frankel [15].

Estimation of Serum Creatinine: Creatinine was kinetically determined according to Houot [16].

RESULTS AND DISCUSSION

Physicochemical Characteristics of Orange Peel Oil: The physicochemical characteristics of an essential oil is closely related to its identity and quality .Thus, the physicochemical properties of orange peel oil used in this study were estimated and the obtained results are shown in table (1).

From the results of physicochemical characteristics of orange peel oil, tabulated in Table (1), it could be noticed that the orange oil used in the present study was matching with the B. P. [17]

Chemical Composition of Cold Pressed Orange Peel Oil: The chemical composition of cold pressed orange peel oil before and after encapsulation with gum Arabic was identified. The identified components are tabulated in Table (2)

The data of Table (2) showed that the major component cold pressed orange peel oil is d-limonene (95.92%). Some data showed that the monoterpene hydrocarbons representing 99.20% of the identified oil components, while the oxygenated components (5 components) representing 0.8% of the identified oil components. Shaw [19] mentioned that d-limonene of orange peel oil ranged between 83-97%.

Properties of Encapsulated Orange Oil Powder after Spray Drying: The properties of encapsulated orange oil powder after spray drying (total oil, surface oil, moisture content and bulk density) we estimated and the obtained results are shown in Table 3.

Table 1: Physicochemical characterization of Orange peel cold pressed oil

Characteristics	Parameters	Results	Reference [17]
Sensory attributes	Appearance	Liquid	Liquid
	Color	pale Yellow	Pale Yellow
	Odor	Characteristic	Characteristic
Physicochemical	Refractive Index (20°C)	1.4740	1.472 -1.476
	Solubility in ethyl alcohol	Soluble in 7 ml.	Soluble at 20°C
		90 % ethyl alcohol	in 7 parts of ethanol (90%)
	Specific gravity (20°C)	0.8451 mg/ml	o.842- o.848 weight per ml
	Optical Rotation	+ 98°	+ 94° : + 99°
	(20°C)		
	Acid value	1.5	1.9 - 2.42
	(mg KOH/100g oil)		[18]

Table 2: Chemical composition of cold pressed orange oil before and after encapsulation over gum Arabic

				After encapsulation	
Peak No	RRT	Major component	Before encapsulation % fresh	GA	Change %
1	0.55	α - pinene	0.59	0.58	- 0.01
2	0.74	B - pinene	0.31	0..27	- 0.04
3	0.81	Camphene	0.21	0.18	- 0.03
4	0.85	Myrcene	1.96	1.74	- 0.22
5	1.00	d-Limonene	95.92	89.8	- 6.12
6	1.01	Cineole	0.23	0.20	- 0.03
7	1.26	γ- Terpiniene	0.03	0.02	- 0.01
8	1.29	P- cymene	0.18	0.16	- 0.02
9	2.47	Linalool	1.37	1.37	0.92
10	3.19	Neral	0.05	2.06	2.01
11	3.22	α - Terpineol	0.05	1.04	0.99
12	3.39	Geranial	0.05	2.44	2.39
		Total oxygenated compound	0.8	7.1	6.3
		Total non oxygenated compound	99.20	92.89	- 6.3

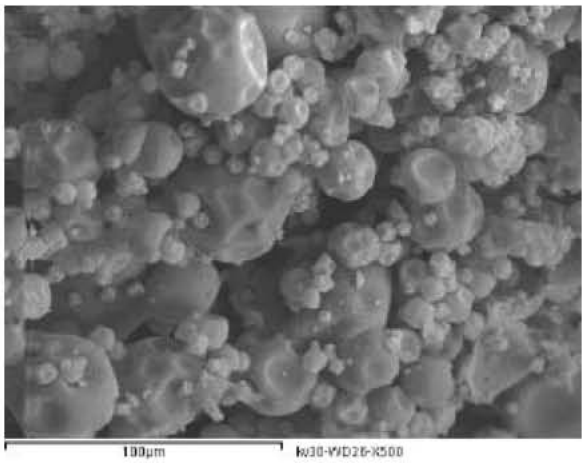
GA: arabic gum

RRT: is the relative retention time of d-limonene (7.916 min) was used as standard retention time equal one

Table 3. Properties of encapsulated orange oil powder after spray drying (Total oil, Surface oil, Moisture content and Bulk density)

Carrier material	Starting (g oil)	Total oil remainder (g oil/100g Powder)	Retention %	Surface oil (g oil/100g Powder)	Moisture Content %	Bulk density (g/ml)
GA	20	17.8	89	0.9	4.6	0.46

GA: gum Arabic



Gum Arabic

Fig. 1: Physical structure of gum Arabic encapsulated orange peel oil powder

Arabic gum (GA) showed 89% retention oil and 4.6% moisture contents. In addition, the surface oil content was 0.9 g oil /100g powder. Moreover, the bulk density results were ranged between 0.42-0.55 mg/ml

Microcapsules External Morphology: The poor retention showed by some encapsulate agents is often attributed to their poor film - forming abilities. One reason for using Scanning electron microscopy (SEM) in the research of microencapsulation is the need to determine the encapsulating ability of the various polymers. Indication of this ability is given by the degree of integrity and porosity of the microcapsules [20]. The morphology of spray dried orange oil microcapsules over gum Arabic as revealed by SEM are given in Figure (1).

Figure (1) shows a typical sample of gum arabic microcapsules. These are spherical bodies with outer surfaces free of cracks and pores. The only defects are dents due to shrinkage of the droplets during the early stages of the drying process. These data are confirmed with those given by Greenwald [21].

The Biological Effect of Encapsulated Orange Peel Oil: The following experiment was conducted to check the safety of encapsulated orange oil for use in human diet. Thus, the changes in some clinical pathological parameters i.e. AST, ALT, glucose, cholesterol and creatinine in blood of five weeks mice, were measured

Table 4: Changes in clinical pathology parameters of mice given daily gavage of encapsulated orange oil

Group	AST U//L	ALT U/L	Glucose mg/dl	Cholesterol mg/dl	Creatinine mg/dl*
Control	25.0±2.0[a]	35±3.0[a]	165.0±1.0[a]	116.0±1.0[a]	0.8±0.02[a]
Orange oil	26.0±1.3[a]	37.75±5.51[a]	155.0±3.50[a]	120.0±3.90[a]	1.06±0.04[b]
Normal range	<30- 40	<30- 40	80-160	90-170	0.3-0.8
Reference	[22]	[22]	[23]	[23]	[23]

Means at the same column with different superscripts are significantly different at $p<0.05$

* means $P<0.002$

SEM (Standard error of mean)

Values are expressed as means ± S.E

after one month (5 days /week) of daily gavage of encapsulated orange oil at a dose of 5600/kg body weight compared with control group. The obtained results are shown in Table (4).

The data in Table (4) (shows that daily gavage of encapsulated orange oil for 30 days did not significantly affect liver function, since no significant changes were detected in AST and ALT before and after feeding on orange oil .In addition, glucose and cholesterol followed the same above mentioned trend, as the changes in both after feeding orange oil were not significant. On the other hand, creatinine content (mg/dl) was significantly ($p<0.002$) increased from 0.8 (in control) to 1.06 mg/dl after feeding on orange oil.

NTP [24] investigated the toxicity of d-limonene (>99% pure) at doses ranged from 413 to 6600 mg/kg, daily administered to rats and mice five days/week for three weeks. No signs of compound-related toxicity were noted at doses <1650 mg/kg daily [24, 25]

D-limonene, the major component of orange peel oil, is considered to have fairly low toxicity. It has been tested for carcinogenicity in mice and rats. Although initial results showed that d-limonene increased the incidence of renal tubular tumors in male rats, female rats and mice in both genders showed no evidence of any tumor. Subsequent studies have determined how these tumors occur and established that d-limonene does not pose a mutagenic, carcinogenic, or nephrotoxic risk to humans. In humans, d-limonene has demonstrated low toxicity after single and repeated dosing for up to one year [26].

Gould [27] mentioned that number of dietary monoterpenes has antitumor activity, exhibiting not only the ability to prevent the formation or progression of cancer, but to regress existing malignant tumors. Limonene has well-established chemopreventive activity against many cancer types.

Moreover, Tisserand [28] classified the toxicity of orange peel oil as belonged to group D. Group D characterized non- toxic materials (LD_{50}>5g /kg body weigh

CONCLUSION

- The orange oil used in the present study was matching with the values recorded in the British Pharmacopoeia, 2007.
- Properties of encapsulated orange oil powder (total oil remainder, surface oil content, moisture content and bulk density) were measured. Arabic gum (GA) showed 89% retention oil and 4.6% moisture contents. In addition, the surface oil content was 0.9 g oil /100g powder. Moreover, the bulk density results were ranged between 0.42-0.55 mg/ml.
- Gum arabic microcapsules are spherical bodies with outer surfaces free of cracks and pores. The only defects are dents due to shrinkage of the droplets during the early stages of the drying process
- The data showed that feeding of mouse in diet containing encapsulated orange oil did not significantly affected liver function, since no significant changes were detected in AST and ALT before and after feeding on orange oil diet. In addition, glucose and cholesterol followed the same above mentioned trend, as the changes in both after feeding orange oil were not significant. On the other hand,creatinine content (mg/dl) was significantly increased from 0.8 (in control) to 1.06 mg/dl after feeding on orange oil.These results prove the complete safety of using encapsulated orange oil in human diet. Further studies are essential to detect the actual effect on renal parameters.

REFFERENCES

1. Beristain, C.I., E. Azuara and E.J. Vernon-Carter, 2002. Effect of water activity on the stability to oxidation of spray dried encapsulated orange peel oil using mesquite gum as wall material. J. Food Sci., 67: 206-211

2. Cadwallader, K.R., D.M. Brehart, S.J. Schnmidt and M.S. Huda, 2002. Volatile release from a sucrose encapsulated flavoring. In Proceedings of 2002 IFT annual meeting and food expo, IFT, Anaheim, CA, USA.
3. Madsen, M.G., R.D. Grypa and J.L. Pratt, 2001. Electronic nose analysis of glass encapsulated diacetyl. In Proceeding of 2001 IFT annual meeting, New Orleans, LA, USA.
4. Risch, S.J. and G.A. Reineccius, 1988. Effect of emulsion size on flavor retention and stability of spray dried orange oil In: Reineccius G.A. and Risch S.J. (eds) Flavor Encapsulation, American Chemical Society Symposium Series 370 Washington DC. ACS. pp: 67-77.
5. Guenther, E.S., 1961. "The essential oils" Vol.I, III, IV,4th.Ed D.Van Nostrand Company, Inc Princeton: New Jersy.Tornto, New York, London
6. A.O.A.C., 1990. Association of Official Analytical Chemist Volatile oil in spices. In Official Methods of Analysis, 15th Edition Helrich, K. Ed.AOAC: Arlington, VA, pp: 1001.
7. Trubiano, P.C. and N.L. Lacourse, 1988. Emulsion-stabilizing starches: use in flavour encapsulation. In: Flavour Encapsulation (edited by S.J. Risch and G.A. Reineccius). ACS Symposium Series 370. Washington, DC: American Chemical Society.Pp. 45-54.
8. Hall, C.W and T.I. Hedrick, 1971. Quality control and sanitation .In Drying of Milk Products; AVI Publishing :Westport, CT, pp: 197-211.
9. Rosenberg, M. and S.L. Young, 1993. Whey protein as microcapsulating agents. Microencapsulation of anhydrous milkfat-structure evaluation. Food Structures, 12: 31-41.
10. Vrana, A., P. Fabry, L. Kazdova and K. Zvolankova, 1978. Effect of the type and proportion of dietary carbohydrate on serum glucose levels and liver and muscle glycogen synthesis in the rat, Nutrition and Metabolism, 22: 313-320.
11. Schemer, S., 1967. The blood morphology of laboratory animals Longmans green and Co. Ltd. pp: 350.
12. Trinder, P., 1969. A method for estimation of plasma glucose . Ann. Clin. Biochem, 6: 24.
13. Barham, D. and P. Trinder, 1972. An improved color reagent for the determination of blood glucose by the oxidase. system.Analyst, 97: 142-145.
14. Richmond, W., 1973. Enzymatic determination of Cholesterol . Clin. Chem., 19: 1350.
15. Reitman, S. and S. Franket, 1957. Colorimetric methods for determing GPT and GOT. Am. J. Clin. Path., 28: 56-63.
16. Houot, C., 1985. In Interpretation of Clinical Laboratory tests. 1985, 220-234, Edited by Siest, G. Henny, J. Schiele, F. and Young, D.S. Biomedical Publications.
17. B.P., 2007. British Pharmacopoeia Published on recommendation of the commission on Human Medicines Pursuant to the Medici cines. Act 1968 and notified in draft to the European Commission in accordance with Directive 98/34/EEC. Effective date: January 2007. London: The stationery office.
18. Emest, G., 1967. The essential oil book . The six printing, U.S.A by Lancaster press, INC. Lancaster, PA.
19. Shaw, P.E., 1979. Review of quantitative analyses of citrus essential oils. J. Agric. Food Chem., 27: 246-257.
20. Rosenberg, M., I.J. Kopelman and Y. Talmon, 1985 . A scanning electron microscopy of micro encapsulation. J. Food Sci., 50: 139-144.
21. Greenwald, C.D., 1980. Particle morphology in the spray drying of foods. PhD Thesis, University of California, Berkeley, CA.,
22. Harris, E.H., 2005. Elevated liver function tests in type 2 diabetes. Clinical Diabetes. 23(3): 115-119.
23. Gad, S.C. and C.P. Chengelis, 1992 .Clinical Pathology Reference range mouse-young Adult,CD-1.In Animal models in toxicology, (Ed by S.C. Gad and C.p. Chengelis.) New York, Basel, Hong Kong. pp: 794 .
24. N.T.P., 1990. National Toxicology Program. Toxicology and Carcinogenesis Studies of d-Limonene (CAS No 5989-27-5) in F344/N Rats and B6C3F1 Mice (gavage studies). Nat. Toxicol. Program Tech. Rep. Ser., 518: 1-165.
25. Whysner, J. and G.M. Williams, 1996. D-limonene mechanistic data and risk assessment: absolute species-specific cytotoxicity, enhanced cell proliferation and tumor promotion. Pharmacology Ther., 71: 127-136
26. Jidong, S., 2007. D-limonene: safety and clinical applications (Clinical report). Alternative Medicine Review.
27. Gould, M.N., 1997. Cancer chemprevention and therapy by monoterpens. Environ. Health Perspect. 104(4): 977-979.
28. Tisserand, R., T. Balacs and J. Caldwell, 1995. Essential oil safety. (Ed) Edinburgh Hong Kong London Madrid Melbourne New York and Tokyo.