

Microencapsulation of Paprika (*Capsicum annum L*) Oleoresin by Spray drying

Krithika V¹, Radhai Sri S², Ravindra Naik³ and Thirupathi V⁴

¹Senior Research Fellow, Central Institute of Agricultural Engineering, IEP Center, Coimbatore-03, TamilNadu, India.

²Associate Professor, Department of Nutrition & Dietetics, PSG College of Arts and Science, Coimbatore-14, TamilNadu, India.

³Senior Scientist, Central Institute of Agricultural Engineering, IEP Center, Coimbatore-03, TamilNadu, India.

⁴Professor, Department of Food and Agricultural Process Engineering, AEC&RI, TNAU, Coimbatore-03, TamilNadu, India.

Abstract

Spice oleoresins exhibit sensitivity to light, heat and oxygen and have lower shelf life if not stored properly. The technique of microencapsulation provides a better stability and in addition controls the release of bioactive compounds. Paprika oleoresin with 1,00,000 CU was encapsulated at three concentrations as 5, 10 and 15 per cent based on carrier. Quality characteristics of the microcapsules revealed that the storage life of Paprika oleoresin was maximized by encapsulating Paprika oleoresin (5-15%) in miniature sealed capsules with a matrix material (Gum Arabic) in order to protect destructive changes and also to convert it into a free flowing powder. Scanning Electron Microscopy study showed that microcapsules obtained from oleoresin encapsulated with 10 and 15 per cent, had superficial indentations and dents similar to honey comb structure, as well as lesser cracks and breakages on the surface, which ensured greater protection to the core material. Of the three concentrations of microencapsulated paprika oleoresin (MPO), oleoresin encapsulated at 10 per cent had better scavenging activity. HPTLC analysis confirmed the presence of major phytochemicals namely alkaloids, flavonoids and carotenoids in the microencapsulated paprika oleoresin.

Keywords: paprika oleoresin, gumarabic, spray drying, antioxidant enzymes, radical scavenging activity, phytochemicals.

1. INTRODUCTION

Microencapsulation is a process by which solids, liquids or even gases may be enclosed in microscopic particles formation of thin coatings of wall material around the substances [1]. The entrapment of sensitive ingredients within a continuous film or coating can protect them from environmental factors such as moisture, air or light [2]. The structure, formed by the microencapsulating agent around the microencapsulated substance (core) is called the wall. The wall protects the core against deterioration and releases under desired conditions. Controlled release of food ingredients at the right place and the right time is a key functionality that can be provided by microencapsulation [3].

Spice oleoresins exhibit sensitivity to light, heat and oxygen and have short storage lives if not stored properly. Destruction of several pigments occurs under exposure to oxygen wherein the hydroxylic groups are converted into unstable ketones, which in turn decompose into colourless compounds with a shorter carbon skeleton. Due to the short shelf life encountered by the active principles in oleoresin, technique of microencapsulation provides a better stability and in addition controls the release of bioactive compounds. Controlled release of the active ingredients can improve the effectiveness of food additives, broaden the application range of food ingredients and ensure optimal dosage. With carefully fine-tuned controlled release properties,

microencapsulation is not just an added value, but is also the source of totally new ingredients with matchless properties. Spray drying technique is quite suited in the encapsulation of oils and oleoresins [4]. Spray drying process have ensured its dominance; these include wide choice of carrier solids, good retention of volatiles, good stability of the finished product and large scale production in continuous mode. It has been used for decades to encapsulate food ingredients such as flavours, lipids, and carotenoids. Spray drying is the most commonly used microencapsulation method in food industry, which is economical, flexible and produces a good quality product [5].

Gum arabic is a hydrocolloid produced by natural exudation of acacia trees and is an effective encapsulation agent. It has high water solubility, low viscosity of concentrated solutions relative to the other hydrocolloid gums and ability to act as an oil-in-water emulsifier. Among the wall materials used, gum arabic stands out due to its excellent emulsification properties and is widely used. Gums have the advantage of being considered natural in all countries [6]. In addition, the wall material is ideally suited to the encapsulation of lipid droplets as it fulfills the role of both surface active agent and drying matrix, thus preventing the loss of volatiles in contact with the atmosphere. Our objective in this study was focused to test the quality attributes and to assess the antioxidant activity of microencapsulated paprika oleoresin.

2. MATERIALS AND METHODS

2.1. Wall and core materials

Paprika oleoresin with 1,00,000 CU (PO 2) (core material) was procured from Chilli Export House, Virudhunagar, Tamil Nadu, India. The sample was stored in air tight aluminium containers and kept in a cool dry place in order to protect the oleoresin from oxidation before conducting trials. Gum arabic- food grade was obtained from M/s. Ponmani and Co, Coimbatore; TamilNadu, India was used as carrier agent for spray drying.

2.2. Preparation of microcapsules by spray drying

A Pilot model vertical co-current spray dryer (M/s Goma Engineering Private Ltd., Mumbai, India) with a water evaporating capacity of 2 kg/h installed at the Post Harvest Technology Centre, Tamil Nadu Agricultural University, Coimbatore, India was used for the spray drying. About 500 g of gum arabic was dispersed in distilled water at 60-70°C and the volume was made up to 1000 ml. To this mixture about 50,100 and 150 g (i.e., 5, 10 and 15 per cent based on carrier) of oleoresin was added. This mixture was emulsified in a shear homogenizer (Homolab 2-FBF Italia) for 5 min at 3000 rpm until the oleoresin dispersed completely [7]. The resultant homogenized mixture was spray dried, using rotary wheel atomizer nozzle at an inlet air temperature of 180°C. A pilot model spray drier (Goma Engineering Pvt Limited) - vertical co-current type with a water evaporation capacity of 1.5 l/h was used for microencapsulation of oleoresin. The essential components of pilot model spray drier are hot air supply system, infeed supply system, rotary wheel atomizer, drying chamber and powder recovery system. The encapsulated oleoresin powder was collected and packed in aluminium foil pouches and sealed air tight. Sealed pouches were stored at room temperature ($28 \pm 2^\circ\text{C}$, 68% RH) to conduct studies on the quality characteristics of encapsulated oleoresin.

2.3. Quality characteristics of microencapsulated paprika oleoresin

2.3.1. Moisture

Moisture content of the encapsulated powder was determined by the toluene distillation method [8]. Ten grams of powder was added to 250 mL toluene in 500 mL flask. The flask was fitted with a Bidwell-Stearling trap and the sample was brought to a boil on a hot plate. The distillation was carried out for 3h, and then the volume of water collected was registered.

2.3.2. Bulk density

Bulk density of the microencapsulated powder was determined by tapping method. Two grams of powder were loosely weighed into 10 ml graduated cylinder. The cylinder containing the powder was tapped on a flat surface to a constant volume. The final volume of the powder was recorded and the bulk density was calculated by dividing the sample weight by volume [9].

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Weight of the microencapsulated powder (g)}}{\text{Volume of the sample (cm}^3\text{)}}$$

2.3.3. Total carotenoids

Carotenoids are active component in the paprika oleoresin. Total carotenoid content of microencapsulated paprika oleoresin powder was determined by AOAC method [10]. One gram of microencapsulated oleoresin powder was dissolved in 25 ml of acetone in 100 ml standard measuring flask and then made up to mark. One ml sample solution from this flask was transferred to another standard flask and again made up to the mark with acetone and shaken well. The absorbance of this solution was taken at 462 nm wavelength using acetone as blank in the spectrophotometer (FT-IR Spectrometer - VERTEX 70). Total carotenoid content of the sample was calculated as follows:

$$\begin{aligned} \text{Colour value (units)} &= \frac{\text{ABS at 462 nm} \times 66,000}{\text{Sample Weight (gm)}} \\ \text{Total carotenoid (g/kg)} &= \frac{\text{Colour value}}{1600} \end{aligned}$$

2.3.4. Capsaicin content

Capsaicin is a protoalkaloid which is responsible for the pungency of chillies. The peculiar pungency in red pepper is due to capsaicinoids (0.01 to 0.09 per cent) present in the fruits. The quality of chilli fruit extracts or oleoresins is determined by the capsaicin content. Capsaicin content of spray dried microencapsulated oleoresin was assessed by following standard procedure (IS 15697: 2006).

2.3.5. Colour value

The colour value of the developed microencapsulated oleoresin powder was assessed using colour meter (M/s. Hunterlab, Reston, VA, USA; model CFLX-45). It works on the principle of focusing the light and measures energy reflected from the sample across the entire visible spectrum. It provides reading in terms of 'L', 'a' and 'b' where, luminance (L) forms the vertical axis, which indicates whiteness to darkness. Chromatic portion of the solids is defined by: a (+) redness, a (-) greenness, b (+)

yellowness, and b (-) blueness. The colour was measured by using CIELAB scale at 10° observer at D₆₅ illuminant and it is supported with universal software v4.10.

2.3.6. Morphology of microcapsules

Morphology of the microencapsulated paprika oleoresin powder samples was analyzed with the help of scanning electron microscope (SEM- JOEL Model JSM 6360). The microcapsules were attached to the SEM stubs of '1' diameter using 2-sided adhesive tape. The specimen was coated with gold-palladium to a thickness of about 100 Å using Hitachi vacuum evaporator, Model HUS 5 GB. Coated samples were viewed in a Hitachi scanning electron microscope operated at 15 kV, and photographed.

2.4. Enzymic and Non-enzymic antioxidants

The Microencapsulated paprika oleoresin powder was analyzed for the presence of enzymic and non-enzymic antioxidants namely catalase [11], SOD [12], glutathione reductase [13], glutathione S transferase [14] and glutathione peroxidase [15], ascorbic acid [16] and vitamin E [17].

2.5. Free radical scavenging activity of MPO

Free radicals are unstable molecules that include the, superoxide (O₂⁻), nitric oxide (NO), hydroxyl radical and molecular oxygen (O₂). In an attempt for free radicals to stabilize, they attack other molecules in the body potentially leading to cell damage. Superoxide radicals are known to be very harmful to cellular components as precursor of more reactive oxygen species [18]. Hydroxy radical has the capacity to cause strand breakage in DNA, which contributes to carcinogenesis, mutagenesis and cytotoxicity. The free radical scavenging activity of MPO was studied as excessive generation of free radicals is implicated in an ever growing number of disease conditions including cancer, atherosclerosis and neurodegenerative diseases. The extent of inhibition of superoxide generation invitro was determined by Beauchamp and Fridovich method [19]. Hydroxyl radical scavenging activity was measured by Elizabeth and Rao, method [20].

2.6. Screening of phytochemicals in microencapsulated oleoresin using HPTLC

Phytochemicals are bioactive, non-nutrient, naturally occurring plant compounds found in vegetables, fruits and spices [21]. The most important of these phytochemicals are alkaloids, tannins, flavonoids, and phenolic compounds. Presence of these compounds in MPO was identified. About 100mg of MPO was taken and mixed with 1ml of warm water, centrifuged at 2000rpm for 5min. About 5µl of the above solution was loaded in the 5 x 10 Silica gel 60 F₂₅₄ TLC plate using Hamilton syringe and LINOMAT 5 instrument.

2.7. Statistical Analysis

Antioxidant activity (enzymic and non-enzymic) of microencapsulated paprika oleoresin was expressed as mean and S.D. One way ANOVA was applied to draw the significant difference between MPO I (5 per cent), II (10 per cent) and III (15 per cent).

3. RESULTS AND DISCUSSIONS

3.1. Quality characteristics of microencapsulated paprika oleoresin

Moisture content of microcapsules decreased with the increase in concentration of oleoresins. The differences in the percentage increase in the moisture content among different powders indicated variations in hygroscopicity and moisture gradient between the sample and the atmosphere. Similarly a decreasing trend in the moisture content of microencapsulated fat powder was reported by Hogan et al. [22]. One of the major characteristics of the bulk density is that it depends not only on the powder's chemical composition, particle size and moisture content, but also on its processing and handling history. Bulk density ranged between 512.6 to 526.3 kg/m³. Higher moisture content at lower concentration of oleoresin would tend to have a higher bulking weight caused by the presence of water which is considerably denser than the dry solid. Similar results were reported by Chegini and Ghobadian [23] for microencapsulated orange juice powder. Beristian et al. [24] reported that bulk density of cardamom oil encapsulated with mesquite gum at oil to gum ratios of 1:5, 1:4 and 1:3 w/w did not vary significantly.

Carotenoid is one of the principle components present in paprika and gives the colour to the fruit body. The total carotenoid content of microcapsules increased with increase in oleoresin concentration. The higher oleoresin concentration might have resulted in greater proportion of total carotenoid content in the encapsulated powder. The principal pungent component of red peppers is a group of acid amides of vanillylamine and C8 to C13 fatty acids, which are known generally as capsaicin. It has many useful properties, such as natural antioxidants and pharmacological.

Table 1 Quality characteristics of MPO

MPO	Moisture (%)	Bulk Density (kg/m³)	Total Carotenoids(%)	Capsaicin (%)
I (5%)	8.2	526.3	48.1	0.26
II (10%)	6.3	518.8	51.6	0.28
III (15%)	5.5	512.6	52.6	0.28

The colour value of the encapsulated powder was assessed using Hunter Lab colour meter. The observation of (Table II) 'L', 'a' and 'b' values of the microencapsulated paprika oleoresin powder revealed that concentration of oleoresin altered the 'a' value (redness) and there was not much change in 'L' and 'b'. The 'a' value increased with increasing paprika oleoresin concentration indicated the bright red colour of the spray dried powder. Govindarajan [25] stated that colour and pungency are the most relevant quality factors in red pepper fruit and their products. In addition, the colour of the end product is an important quality parameter especially in dehydrated products derived from red pepper, like paprika powder (red chilli powder with low pungency).

Table 2 Colour value of MPO

Oleoresin concentration (%)	Colour value		
	L	a	B
5	36.02	30.43	24.63
10	36.11	33.12	24.56
15	36.15	35.52	24.36

(L) whiteness to darkness, a (+) redness, a (-) greenness, b (+) yellowness, and b (-) blueness

Microcapsules prepared by spray drying of paprika oleoresin using gum arabic as wall material was observed for size and shape from the Scanning Electron Microscopy. It was observed that microcapsules produced with minimum concentration of oleoresin (5 per cent) exhibited higher agglomeration, which would affect the free flowing properties of the encapsulated powder. Extensive agglomeration of microencapsulated powder particles obtained from the pressure nozzle could be due to the high levels of surface oleoresin (Plate 1). It was observed that microcapsules had superficial indentations and dents similar to honey comb structure, as well as lesser cracks and breakages on the surface, which ensured greater protection to the core material (Plate 2 and 3).

Surface indentations of the encapsulated powder might be due to the result of shrinkage while cooling after drying, especially at high drying rates which was associated with small particles and usually led to rapid wall solidification [26]. Similar result in morphology of monoterpenes was also observed by Bertolini et al. [27] in microencapsulated monoterpenes using gum arabic as the wall material. Barbosa et al. [28] encapsulated *Bixin* and reported that microcapsule composed of gum arabic was more stable to photodegradation than that composed of maltodextrin, in addition gum arabic presented the highest encapsulation efficiency.

3.2. Levels of Enzymic and Nonenzymic antioxidants in MPO

The data presented in Table III revealed that the activity of enzymatic antioxidant catalase and superoxide dismutase get altered with increase in concentration of oleoresin. Catalase activity for 15 per cent of MPO was observed to be maximum (129.00 ± 0.63 units) whereas the enzyme activity of 5 and 10 per cent concentration was 120.66 ± 1.63 and 121.83 ± 1.60 units respectively. Similar trend was observed in superoxide dismutase (SOD) activity as 15 per cent of MPO provided the maximum activity as 0.673 ± 0.016 units when compared to 5 and 10 per cent with 0.59 ± 0.017 and 0.633 ± 0.023 units respectively. Statistical data showed that there was a significant difference between the different concentrations of MPO both in catalase and SOD activity. Hot peppers are remarkable source of these antioxidant compounds, including vitamins, phenolic compounds, capsaicinoids and carotenoids [29]. Active spice principles namely capsaicin, curcumin, and eugenol, inhibit lipid peroxidation by quenching oxygen free radicals and by enhancing the activity of endogenous antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, and glutathione S transferase.

Table 3 Mean levels of enzymic and non-enzymic antioxidants in MPO

MPO	Catalase (U ^Δ /mg protein)	SOD (U* /mg protein)	Ascorbic acid (mg/g)	α-tocopherol (mg/g)
I (5%)	120.66±1.63 ^c	0.59±0.017 ^b	0.231±0.02 ^b	0.028±0.00 ^b
II (10%)	121.83±1.60 ^b	0.633±0.023 ^a	0.370±0.01 ^a	0.030±0.00 ^b
III (15%)	129.00±0.63 ^a	0.673±0.016 ^a	0.371±0.02 ^a	0.037±0.00 ^a
CD (5%)	1.36	0.048	0.058	0.0021

Values are mean ± SD, A column means followed by common superscripts are not significant at 5% level by DMRT

* Decomposition of μmol of H₂O₂/min/mg protein

Δ 50 % inhibition of NBT reduction/min/mg protein

Ascorbic acid content of encapsulated oleoresin increases with the increase in the ratio of oleoresin with respect to the wall material. Ascorbic acid content of MPO 10 and 15 per cent was equal as 0.37mg/g whereas 0.23 mg/g for 5 per cent MPO. Level of α-tocopherol of MPO ranged between 0.028 and 0.037 mg/g. An increase in the level of ascorbic acid and α-tocopherol was noticed along with the increase in the ratio of the oleoresin, which was proved statistically. Statistical analysis revealed that there was insignificant difference between MPO II and III for ascorbic acid content whereas such difference was observed between MPO I and II for α-tocopherol content.

3.3. Free radical scavenging property of microencapsulated oleoresin

3.3.1. Hydroxyl radical scavenging activity of MPO

Figure 1 revealed that the encapsulated oleoresin at three different ratios of 5, 10 and 15 percent exhibited a concentration dependent scavenging activity. To scavenge the 50 per cent (Inhibitory Concentration-IC₅₀) of hydroxyl radicals generated in the reaction mixture, it was noticed that a level of 360, 350 and 325 μg with 5, 10 and 15 per cent of microencapsulated oleoresin respectively was required. This showed that the activity level was closer in the selected three levels of MPO (5, 10 and 15 per cent). Further, it shows that a better scavenging property was observed in encapsulated oleoresin even at a lower concentration (5 per cent). This could be due to the presence of ascorbic acid in oleoresin as observed by Chatterjee and Nandi [30], who stated that ascorbic acid has better scavenging activities because they are present both intracellular as well as in the extra cellular fluid in addition ascorbic acid spares GSH and prevents its oxidation.

3.3.2. Superoxide radical scavenging activity of MPO

Figure 2 revealed that extracts of microencapsulated oleoresins exhibited a concentration dependent scavenging activity on the superoxide radicals. Activity of

oleoresins encapsulated at 5, 10 and 15 per cent were comparable. IC 50 (50 per cent of the generated free radicals inhibition concentration) of the extracts obtained from the three MPO indicated that 10 per cent encapsulated oleoresin had a better scavenging activity when compared to oleoresin encapsulated with 5 and 15 per cent as only a minimum amount of 255 μg of 10 per cent encapsulated oleoresin was required to scavenge 50 per cent of the super oxide radicals. In case of 5 and 15 per cent the 50 per cent scavenging activity was achieved by 295 and 260 μg respectively. Superoxide anion a reduced form of molecular oxygen is a highly toxic species, which is generated by numerous biological and photochemical reactions. Both anaerobic and aerobic organisms possess SOD enzymes which catalyze the breakdown of superoxide radical [31]. Superoxide plays an important role in the formation of other ROS such as hydroxyl radical and singlet oxygen which induce oxidative damage in lipid, proteins and DNA.

3.4. Screening of phytochemicals in microencapsulated oleoresin by HPTLC

Peppers (*capsicum species*) contain moderate to high levels of neutral phenolics or flavonoids, phytochemicals that are important antioxidant components of a plant-based diet, other than traditional nutrients, that may reduce the risk of degenerative diseases. The interests in phenolic compounds, particularly flavonoids and tannins, have considerably increased in recent years because of their broad spectrum of chemical and diverse biological properties, which include the antioxidant effects and radical scavenging properties [32]. Scanning was done at 500nm for alkaloid, 366 nm for flavonoid and 500nm for carotenoid. An orange-brown colored zone at white light was observed in the chromatogram, confirmed the presence of alkaloid (A), yellow fluorescence zone at UV366nm was observed in the chromatogram, confirmed the presence of flavonoids (B) and blue fluorescence zone was observed in the chromatogram after derivatization, which indicates the presence of carotenoids in MPO II.

UV Spectrum was taken at 366 nm for flavonoid, 500nm for alkaloid and carotenoid and comparison has been done with the known reference standards of HPTLC. Spectral analysis of MPO extract confirmed the presence of three important secondary metabolites namely alkaloids, flavonoids and carotenoids. An orange-brown coloured zone was reported and identified as alkaloid (Figure 3). The presence of flavonoids was confirmed by observing the yellow fluorescence on the TLC plate. For carotenoids similar blue colour spots were observed in the extract of MPO (Figure 3).

4. CONCLUSION

Microencapsulation protects the core against deterioration and releases under desired conditions. The active principles in the encapsulated oleoresin could be retained for longer time, easily stored and handled when compared to oleoresin. The results of the biochemical constituents and radical scavenging activity of MPO indicated that encapsulation process retained the bioactive properties of oleoresin.

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