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Extraction of oleoresin by supercritical fluid extraction, capsulation and its physiochemical properties

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A B S T R A C T

Red pepper oleoresin was extracted from Egyptian food factories waste by using supercritical fluid CO₂. Nano particle was achieved by mechanical process i.e. ultra sonication giving a yield of 24900 µg/mg higher than that extracted by conventional methods and sodium alginate was used as wall material. The physiochemical properties were investigated before and after capsulation. The heat stability of nanocapsules was determined by differential scanning calorimetry (DSC) and was considered as indicative of thermo-oxidative stability and results proved that microcapsulation played an important role in the oxidation prevention process.

Introduction

There is a worldwide growing interest in the application of natural colorants for food products, and red pepper (*Capsicum annum* L.) or paprika has been used since ancient times as a colorant to enhance or change food color. Carotenoids are one of the most important groups of natural colorants, and red pepper oleoresin is an important natural source of these pigments (Jarén-Galán *et al.*, 1999). The main constituents of the carotenoid fraction are capsanthin and capsorubin (Weissenberg *et al.*, 1997), which are almost exclusive to the genus *Capsicum* and are responsible for the final red color. Red pepper varieties differ by its

color, shape, dimension, flavour, degree of hotness, etc. Paprika is often consumed as fresh fruit or dried and used as ground in the food industry, but it is also used in pharmaceutical and cosmetic industries. Spice pepper (*Capsicum annum*) takes the very special among paprika varieties.

Color of ground paprika fruits has been identified: green chlorophylls, yellowish-orange lutein, zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin and β-carotene (Deli *et al.*, 2001). *Capsicum* oleoresin from paprika contains a complex mixture of essential oils, waxes, colored materials, and several capsaicinoids. They

also contain resin acids and their esters, terpenes, and oxidation or polymerization products of these terpenes (www.zarc.com/english/cap-stun/tech_info/oc/). Due to their aroma, flavour, taste and color, they can completely substitute ground paprika in products, in order to improve sensory properties of food products.

Supercritical fluid (SCF) extraction has received increasing attention in a variety of industries, because it can provide high solubility, improved mass transfer rates, and increased selectivity with small changes in process temperature and pressure (Brunner, 1994). Carbon dioxide (CO₂) is probably the most widely used SCF. Advantages include inertness, non-toxicity, non-flammability, non-explosiveness, and availability with high purity at low cost (del Valle *et al.*, 1999; Lang and Wai, 2001; and Luz *et al.*, 2013). Furthermore, relatively low critical properties make CO₂ (TC=304, 1 K, pc=73, 8 bar) an ideal solvent for the extraction of thermally labile components such as carotenoids.

Various investigators have studied the extraction of paprika oleoresin with supercritical CO₂ (SC-CO₂), and concluded that extraction yields increase pronouncedly as the extraction pressure increase. Jarén-Galán *et al.* (1999) established that the fraction of carotenoid pigments in the oleoresin increased 3 times as a result of an increase in pressure from 137,8 to 413,4 bar. Furthermore, pigments isolated at low pressure (137.8 bar) consisted almost exclusively of β-carotene, while pigments isolated at higher pressure (414.4 bar) contained greater proportions of capsorubin and capsanthin. Daood *et al.* (2002) confirmed that the solubility of paprika pigments in SC-CO₂ and their recovery increased pronouncedly when pressure increased from 100-200 to 400 bar (both at

40 °C). Daood *et al.* (2002) for the 35–55°C temperature range, and reported a 5-time increase in recovery of carotenoid pigments as a result of an increase in pressure from 100 to 400 bar. Ambrogi *et al.* (2002) also confirmed an improvement in extraction kinetics of carotenoid pigments from paprika with an increase in process pressure (300–500 bar), and reported a solubility of 7.5 g oleoresin/kg CO₂ at 69 °C and 300 bar.

Oleoresin is susceptible to oxidants, light and heat which can easily deteriorated when exposed to such factors (Lee and Chen, 2002; Pesek and Warthesen, 1987). Therefore oleoresin must be protected by microencapsulation technique which has been widely used in food processing to protect food ingredients.

The objective of this work was to extract oleoresin from food wastes by supercritical fluid CO₂, evaluate the image of the extracts and study the physical properties of both the extracts and its microencapsulated form.

Materials and Methods

Samples preparation

Red pepper samples

Low grade grapes and red pepper were obtained from local market. The material was collected immediately prior to the disposal step, blended and homogenized by using Sonicator (Ultrasonic processor) XL No. 2015–10 in dark place, then placed in petri dish, packed with aluminium foil and stored in a refrigerator at –50°C. All samples were freeze-dried (LABCONCO, Kansas City, USA) at –50 °C & 0.014 mbar for 2 days to reach moisture content 4 %. Grapes and pepper samples powder were ground and stored in a refrigerator at –80 °C in brown glass bottle to prevent oxidative damage until extraction.

Supercritical fluid CO₂ extraction

Oleoresin

Oleoresin was extracted from low grade grapes and red pepper by Supercritical (SCF) as described previously at 40 °C temperature and 350 bar pressure (Uquiche *et al.*, 2004) until no significant amount of extracted oleoresin could be collected.

Physical properties

Transmission electron microscopy

The morphology of the extracted oleoresin was examined by the transmission electron microscopy (TEM) (JED 1230, JEOL Ltd.)

Fourier transforms infrared spectroscopy (FT-IR)

The spectra or finger print of the extracted oleoresin sample were obtained by using FT-IR spectroscopy. Oleoresin extracted sample of FT-IR (FT-IR-6100, Jasco, Japan) were prepared by using potassium bromide disks (Nobuo, 2008).

Thermal properties of oleoresin

The thermal properties of oleoresin extracted from low grade red pepper by supercritical CO₂ under various conditions i.e. pH, temperature and day light were determined according to Van den Berg *et al.* (2000). Extracted oleoresin was protected from light and the stability was calculated from the reduction in the content of oleoresin.

Thermal stability of extracted oleoresin (Differential Scanning Calorimetry) DSC

Thermal stability of oleoresin extracted sample was determined according to the methods described by Pérez-Alonso *et al.*

(2008). The extracted sample between 4 and 5 mg was placed in the furnace of the TA Instruments DSC model 2010 (New Castle, DE. USA), and was subjected to heating rates (β) of 4,6,8 and 10 °C min⁻¹ from 30 to 230°C or 400°C, when required, using an oxygen flow rate of 25 cm³ min⁻¹. A blank was run using N₂ in order to determine if the exothermic peaks of the samples were due to oxidation. Measurements were done in duplicate.

Microencapsulation of extracted oleoresin

Microencapsulation of extracted oleoresin was done with sodium alginate (6 % (w/v), $d_p = 3$ mm) by using standard ionotropic gelation through a syringe as described by Kubik *et al.* (2004).

Transmission electron microscope of encapsulated oleoresin

The morphology of oleoresin microencapsulated sample was examined by transmission electron microscopy (TEM) using freeze-fraction replica method as described above.

Thermal stability of all microencapsulated products (differential scanning calorimetry DSC

The thermal stability of 1 microencapsulated oleoresin was determined as described before.

Results and Discussion

Supercritical Fluid CO₂ extraction of bioactive compound from natural sources

The SCF oleoresin extracts had 24900 µg/mg yields higher than that extracted by conventional methods being 900 µg/gm. This higher yield was achieved at high pressure and high temperature (350 bar, 40

°C) which indicates that the solvent strength of carbon dioxide at higher pressure was sufficient to replace solvent for extraction. One advantage of SCF over solvent is the selective extraction and fractionation of desired compounds made possible by altering the density.

Physical & Chemicals properties

Transmission electron microscope

The morphology of extracted oleoresin after freeze-drying was obtained by using transmission electron microscopy (JED 1230, JEOL Ltd., and Tokyo, Japan).

Figure (1_{a,b}) Shows picture of oleoresin nanoparticles round shapes with diameter range from 11-46 nm and tube shape from 11-46 nm.

FT-IR analysis

Figure 3 shows FTIR spectra of extracted oleoresin by supercritical fluid CO₂. The spectra consist of different groups of absorption bands at wave numbers ranging from 4000-300 cm⁻¹. The band appeared at 3456.74 was assigned to (O-H stretching) which is only identified in the high pressure extracts. A band appearing at 3007.44 cm⁻¹ was assigned to =CH asymmetric stretching. The C-H stretching of methylene (ν_{sym}) and methyl (ν_{sym}) bands were identified at 2925.48 cm⁻¹ and 2856.06 cm⁻¹ respectively.

The region of 1800-300 cm⁻¹ is the fingerprint region of the individual bonds of functional groups. The sharp band at 1743.33 cm⁻¹ is assigned to C=O stretching vibrations and may be characterised by the presence of high amounts of carboxylic acid in the extract. A sharp small peak at 1458.88 cm⁻¹ is assigned to CH₂ cm⁻¹. Small peaks

were observed at 1238.08 cm⁻¹, 1164.79 cm⁻¹ were assigned to stretching C-O and to polysaccharides respectively.

Thermal properties of extracted oleoresin

Effect of PH on the Stability of Oleoresin

The stability of the extracted oleoresin from low grade grapes & red pepper by using Supercritical extraction method was tested at different pH values (i.e. 1-13) as presented in table 2.

Results in (Table 2 and Fig 4) showed that the stability of the extracted oleoresin towards different pH values was affected. The extracted oleoresin showed higher retention values at pH 4 to 6. Beyond these values a great loss in oleoresin content was noticed. The maximum stability was at pH 5 after 30 min, and decreased after 60 and 90 min respectively. Results also revealed that at higher pH values from 10 to 13, the extracted oleoresin completely decreased, while in acid media the percent retention increased from 1 to 5 pH. This showed that the optimal pH values was at 4.5–5.5 and gave a maximum percent retention.

Effect of temperature

The stability of oleoresin at different temperature (i.e. 20, 40, 80 and 120°C) for (30, 60 and 120 min) was evaluated as presented in (Table 3 & Fig 5).

Results in table 3 and figure 5 showed that the stability of crude oleoresin was varied according to the temperature used and time of treatment. The percent retention was decreased by increasing temperature from 40 to 120 °C. The complete loss in oleoresin was noticed at 100°C for 30 min (zero) and at 80 °C, for 60 and 90 min (zero). The maximum retention (99.7 %) of oleoresin

was found at 40°C for 30 min. and reduced to 74.1 and 31.9 at 60 and 90 min. respectively.

This means that oleoresin is stable at 20–40 °C after 30 min. and at 20 °C after 60 and 90 min. The stability was reduced at 40 °C to 74.1 and 31.9 respectively.

Effect of day light

The stability of oleoresin extracted by Supercritical CO₂ from low grade grapes and red pepper as affected by day light was tested as presented in the following (Table 4 and Fig.6).

Results in table 4 and figure 6 showed that the percent retention of the extracted oleoresin was affected by daylight and time of exposure. However storage of oleoresin for 4 and 8 hr. in dark had almost no effect on the percent retention. The less reduction was noticed after 8 hr in sun light being 86.9%.

From table and figure 4 and 6, it could be concluded that oleoresin should be stored in dark conditions to prevent its degradation.

Thermal stability of extracted oleoresin

The thermal stability of oleoresin extracted by supercritical CO₂ was determined by using Differential scanning calorimetry (Figure 7). Oleoresin showed well defined exothermal peaks that corresponded to temperatures of 369.12°C.

Microencapsulation of extracted oleoresin

Microencapsulation technology in food processing has been widely used to protect ingredients against deterioration, volatile losses or premature interaction with other ingredients. The protective mechanism

therein is to form a membrane (wall system) to enclose droplets or particles of the encapsulated material (core).

Transmission electron microscopy

The morphology of Oleoresin microencapsulated was measured by transmission electron using Transmission Electron Microscopy (Fig. 8).

TEM in figure 8 presents the Oleoresin microencapsulated with diameter of 10.0–26 nm.

This microencapsulated Oleoresin was capsulated in hard capsules.

Thermal stability (Differential Scanning Calorimetry) DSC

The thermal stability of the encapsulated oleoresin was determined by using Differential Scanning Calorimetry (Figure 9). Higher exothermal peaks occurred at much higher temperature 304.19°C than that for the pure extracted oleoresin. This result indicated that microencapsulation played a preponderant role in the oxidation prevention process in the biopolymers blend as reported by Rodríguez-Huezo *et al.* (2008).

Conclusion

Low grade grapes and red pepper were broken and ground to the level that allow diffusion of active compounds, intensified mass transfer and extraction.

The extraction of oleoresin from low grade grapes and red pepper by supercritical fluid CO₂ shows that the highest yields of extracted oleoresin was achieved 24900 µg/mg at 350 bar, 40 °C.

Table.1 Standard curve of oleoresin

Absorbance A_{472}	Oleoresin (mg/L)
0	0
0.033	0.5
0.047	1.0
0.071	1.5
0.081	2.0
0.095	2.5
0.116	3.0

Table.2 Effect of PH stability of natural oleoresin extracted by supercritical CO₂

pH	Oleoresin %		
	After 30 min	After 60 min	After 120 min
1	Zero	Zero	Zero
2	2.6	1.4	Zero
3	35.9	30.2	28.4
4	74.6	69.3	64.5
5	94.5	90.6	84.3
6	86.3	79.2	71.4
7	53.1	42.7	31.0
8	36.0	32.5	22.6
9	15.3	11.9	9.4
10	Zero	Zero	Zero
11	Zero	Zero	Zero
12	Zero	Zero	Zero
13	Zero	Zero	Zero

Table.3 Heat stability of oleoresin extracted by supercritical CO₂

Temperature °C	Oleoresin (%)		
	30 min.	60 min.	90 min.
20	99.7	99.1	98.9
40	99.5	74.1	31.9
60	54.0	25.3	1.9
80	12.8	Zero	Zero
100	Zero	Zero	Zero
120	Zero	Zero	Zero

Table.4 Effect of day light on stability of oleoresin extracted by supercritical CO₂

Sample	Oleoresin retention %					
	Darkness			Sun light		
	0	4h	8h	0	4h	8h
Extracted carotenoids	100	99.3	98.9	100	91.8	86.9

Figure.1 TEM of oleoresin nanoparticles extracted by supercritical CO₂

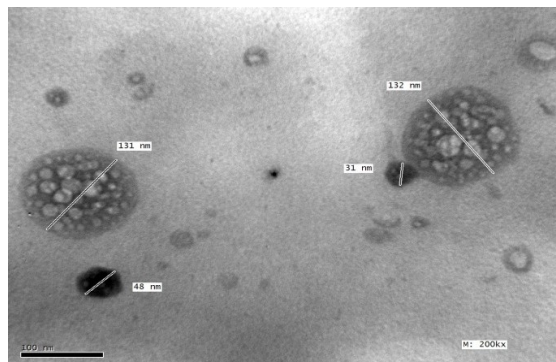


Figure.1a TEM of oleoresin nanoparticles extracted by supercritical CO₂

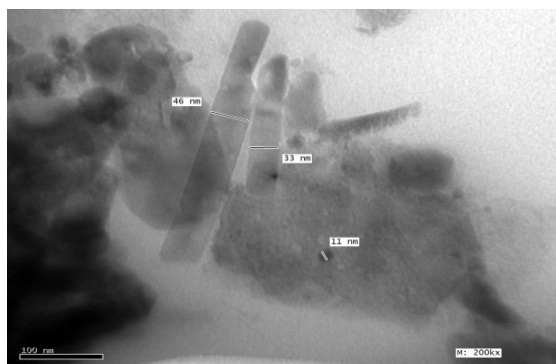


Figure.2 Standard curve of oleoresin

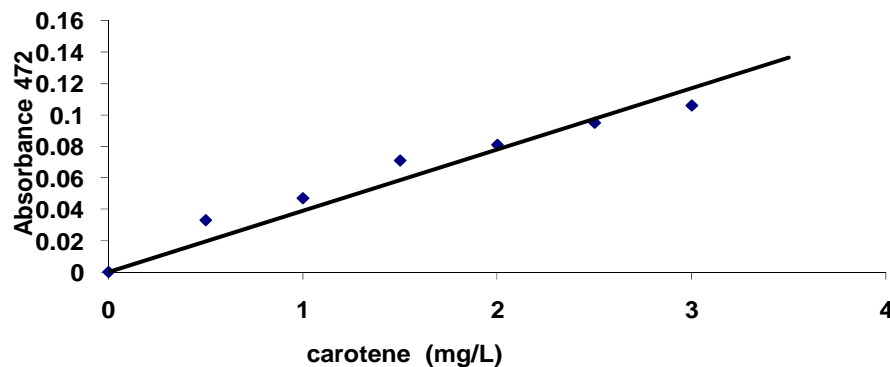


Figure.3 FT-IR analysis of oleoresin

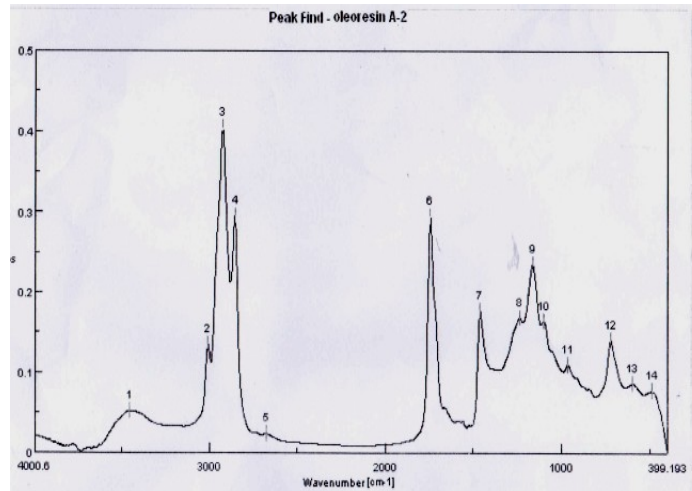


Figure.4 PH stability of natural oleoresin extracted by supercritical CO₂

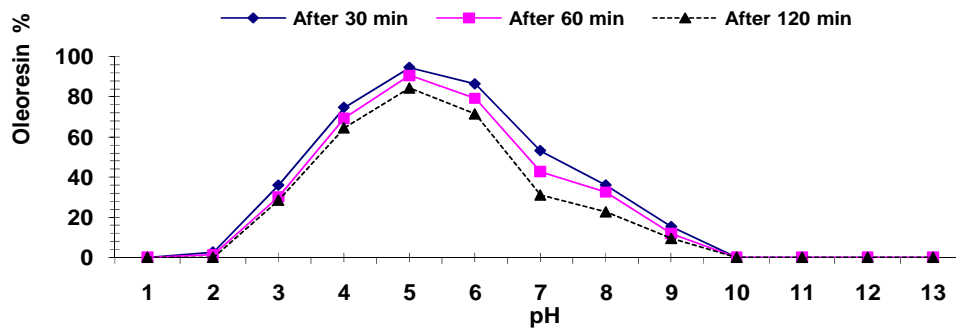


Figure.5 Heat Stability of Oleoresin extracted by Supercritical CO₂

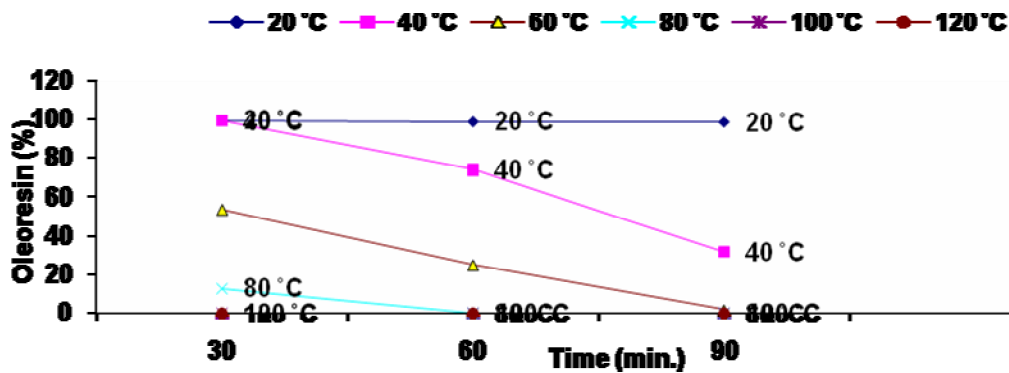


Figure.6 Effect of day light on stability of oleoresin extracted by supercritical CO₂

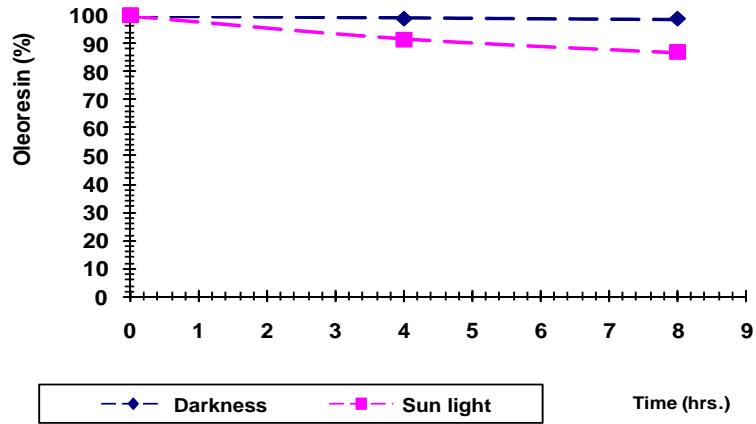


Figure.7 DSC of Nano Oleoresin

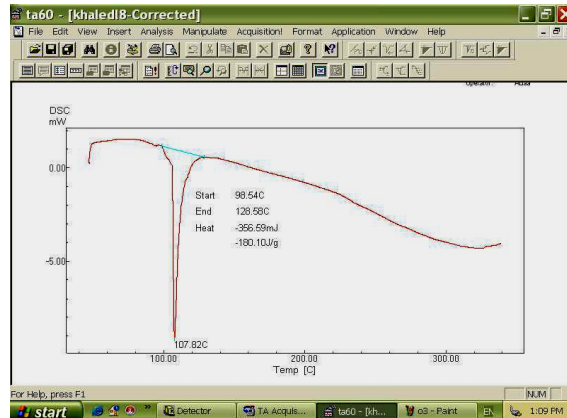


Figure.8 TEM of nano oleoresin microencapsulated

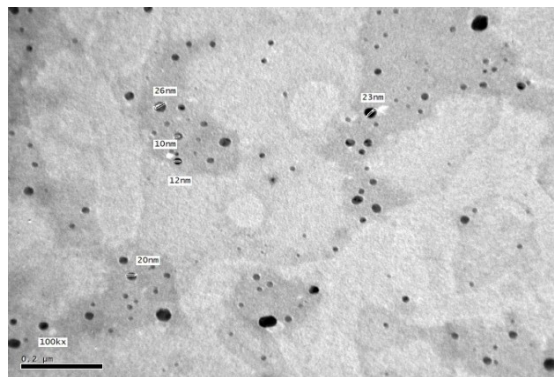
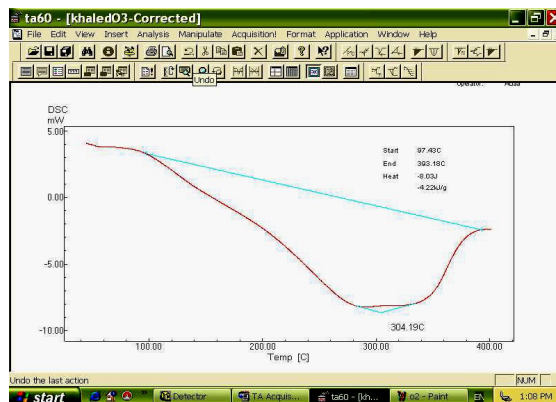


Figure.9 DSC of encapsulated oleoresin



Sodium alginate beads were found to be a better wall material for encapsulation of pepper oleoresin that prolonged its shelf-life and increase its stability. Satisfactory results for prediction of oleoresin functional groups have been shown by using FTIR spectroscopy. The complete FTIR analysis to determine oleoresin can be performed in less than 2.5 minutes per sample. Further advantage of FTIR method is that it is environmentally friendly, as no chemical are needed for the analysis. Oleoresin nanoparticles could be produced by using sonicator that broke its call and increase the surface area.

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