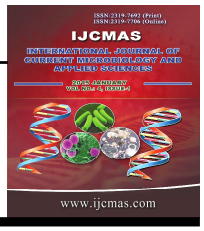
Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 307-320

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 3 (2015) pp. 307-320**
http://www.ijcmas.com



Original Research Article

Physiochemical Characterization and Oxidative Stability of Encapsulated Nano Lycopene Pigments Extracted By CO_2 Fluid Extraction

Manal F. Salama, Effat I. Seliem, Khaled F. Mahmoud* and Azza A. Amin

Department of Food Technology, National Research Centre, Dokki, Egypt
**Corresponding author*

Keywords

Tomato wastes, Ultrasonication, Encapsulation, Niosomes, Heat stability

A B S T R A C T

Supercritical fluid extraction by using CO_2 as a solvent, a relatively new separation technique, can be used as a very efficient in course of analysis or preparation of many useful substances from many of the plant materials. The extracts from these materials are a good basis for the new ingredients in functional foods. This paper deals with supercritical CO_2 extraction of lycopene which is present in natural plants. Lycopene is one of the most important& useful carotenoid found in vegetable or fruits. In this study lycopene in tomato waste skins was extracted by supercritical extraction and the yield and purity were compared with the conventional methods. Nano particle was achieved with Ultrasonication process and were analyzed for their physiochemical properties. Nano lycopene particles were encapsulated by Niosomes entrapment and their heat stability was also studied.

Introduction

Carotenoids represent an important group of natural pigment that are essential for the human health. Their consumption is strongly recommended because, according to epidemiological studies, their intake can be associated with a reduced risk of developing heart diseases or certain types of cancers (Zelkha, 1989; Baysal *et al.*, 2000) Among the several carotenoids, lycopene is one of the utmost importance found in ripe tomato, watermelon and pink grape fruit, giving them a characteristic red pigmentation. Recently, lycopene has been affirmed as generally recognized as safe (GRAS) in US (Crandall, 2003), so that it is now use in food formulations has been made easier, and such foods are now welcome by public and are arriving at more homes recent years. The major sources of lycopene are tomatoes and tomato products and this pigment can represent more than 85% of all the carotenoids present in fruits; its concentration can vary from 30 to 200 ppm in the fresh products or from 430 to 3000 ppm on dry basis (Leoni, 1993). Tomato skins can be a viable source of lycopene, as they contain about five times more licopene than the whole tomato pulp (Sharma and Manguer, 1996), and considering that more than one third of the tomatoes delivered to processing plants ends as processing wastes, mainly constituted by seeds and skins, the recovery of this carotenoid could represent

an alternative for the valorization of the by-products of the tomato industry.

Several extraction and purification methods can be applied to obtain this product, such as solvent extraction (Zelkha *et al..,* 1989), supercritical fluid extraction, distillation membrane separation, chromatography or crystallization. However, from an industrial point of view solvent extraction has been always the first option because of its simplicity and costs. However, techniques that minimize the use of organic solvents to produce food products are always preferred, and as a result techniques like supercritical fluid extraction, which was a laboratory scale technique in 1996 (Shi *et al.,* 2002) is nowadays being used in industrial processes (www.Lycopene.com, 2012; Matteo *et al.,* 2009; Zelkha *et al..,* 1989; Domadia *et al.,* 2013, Silva *et al.,* 2014). Although supercritical fluids are suitable for the extraction of compounds that can easily become degraded by light, oxygen and high pressure like carotenoids.

The solubility of these substances is still relatively low compared to their solubility in organic solvents, and high pressures must be used to obtain suitable extraction yields. Topal *et al.* (2006) studied the effect of operating conditions on supercritical extraction of lycopene from tomato skin in order to find the optimal pressure, temperature and flow rate conditions to obtain all-trans lycopene extracts. They managed to obtain 94 % yield with a pressure of 40 MPa, temperature of 373 K and a flow rate of 2.5 mL/ min.

Regardless of its source, carotenoids need a further treatment to be suitable as industrial colorants. Their non-polar nature limits its application to non-aquas solutions. They are practically insoluble in water, compared with other synthetic colorants. However, different approaches can be taken to minimize this drawback, for example enhance their dissolution rate by lowering the size of lycopene particles (nano particles), and by encapsulating the lycopene with hydrophilic materials in order to obtain controlled release systems that ensure a colorant effect for longer periods.

In food processing field, microencapsulation technique has been widely used to protect food 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 encapsulated material (core). So far, various kinds of microencapsulation techniques such as solvent dispersion/evaporation, phase separation (coacervation), co-crystallization, interfacial polymerization etc., have been developed, among which, spray-drying is the most commonly used one in the food industry due to its continuous production and easiness of industrialization (Jimenez *et al.,* 2004; Rodriguez-Huezo *et al.,* 2004; Schierle *et al.,* 1997).

This work aims to extract lycopene from tomato wastes by supercritical CO_2 fluid extraction, produce its nanoparticle in capsulated form and study their physical and chemical properties.

Sample Preparation

Tomato Samples

Tomato waste (skin & seeds) were obtained from Tomato processing plant (in Cairo). The waste was collected immediately prior to the disposal step, blended then disintegrated by using Sonicator (Ultrasonic processor) XL No. 2015-010 in a dark place. The mixture was placed in Petri dish, packed

with aluminium foil and stored in a refrigerator at -80 °C until freeze.

The tomato waste mixture was then freeze–dried by using (LABCONCO, Kansas City, USA) at -50°C and 0.014 mbar for 2 days to reach moisture content 4 %. Tomato waste powder was ground, packed in brown glass bottle and stored in a refrigerator at -80 °C to prevent oxidative damage until extraction.

Supercritical Fluid CO_2 Extraction

Lycopene

Supercritical CO_2 extraction was used for extraction of lycopene from tomato wastes (skin & seeds) by using Laboratory-scale high pressure extraction plant (Speed SFE Model 7030) and according to the method described by (Baysal *et al.*, 2000).

Lycopene powder (20 gm) was placed in a stainless steel high pressure cell (9.5 cm long with 1.6 cm i.d.) and compressed to the required pressure by a diagram compressor. The extraction temperature (60°C) was monitored by a thermocouple immersed at the centre of the extractor. The extraction pressure (300 bars) was controlled by a back pressure regulator. Flow rate of CO_2 (50 L/min, measured at ambient conditions) passing through the extractor was controlled by manual adjustment of the depressurization valve.

The extraction was continued for 4 hrs. The extract aliquots were periodically collected in pre-weighed glass vials (15 cm^3 capacity), and the obtained extract was assessed gravimetrically by difference with cleaned and dried vials.

The vial was stored in a refrigerator at -80°C until used for physical & chemical properties determination.

Physical and chemicals properties

Transmission Electron Microscopy

The morphology of the extracted Lycopene nanoparticle was examined by the transmission electron microscopy (TEM) (JED 1230, JEOL Ltd., and Tokyo, Japan) using freeze-fraction replica method (Nobuo, 2008).

Fourier Transform Infrared Spectroscopy

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

Thermal properties of lycopene nanoparticle

The thermal properties of lycopene extracted from tomato waste (skin & seeds) by supercritical CO_2 under various conditions i.e. pH, temperature and day light were determined according to Van den Berg *et al.* (2000). The extract was protected from light and the stability was calculated from the reduction in the content of lycopene.

Thermal stability of extracted lycopene DSC (differential scanning colorimetry)

Thermal stability of lycopene extracted sample was determined according to the methods described by Pérez-Alonso *et al.* (2008). All extracted samples between 4 and 5 mg were placed in the furnace of the TA Instruments DSC model 2010 (New Castle, DE.USA), and were 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 cm^3 min^{-1}.

A blank was run using N_2 in order to determine if the exothermic peaks of the samples were due to oxidation. Measurements were done in duplicate.

Microencapsulation of nano lycopene product

Microencapsulation of nano lycopene product was done by using Niosomes entrapment (Shu *et al.,* 2007).

Standard calibration curve of nano lycopene

Preparation of stock reagents

Stock A: of conc. 500 µg/ml, Prepared by dissolving 25 mg of lycopene and completing the volume to 50ml with hexane, "Stock A" was used for scanning the spectrum of lycopene in hexane (Fig. 1). Standard curve was carried out using different concentration of lycopene (Fig. 2) 50 to 250 µg/ml. Spectrum of Nano lycopene in hexane (500µg/ml) and determination of wave length of maximum absorption (λmax).

Preparation of nano lycopene niosomes

Lycopene niosomes using Span 60 (sorbitan monostearates, Merck Schuchardt OHG, Germany) neutral niosomes were prepared using Span 60 and cholesterol, molar ratio (1:1). The niosomes were prepared by the vortex dispersion method). Twenty mg of Lycopene were added to lipid phase (100 mg) which consists of Span 60 then cholesterol and dissolved in 10 ml chloroform in a pear shaped flask of the (Büchi- M, HB- 140, Switzerland) rotary evaporator and rotated at 55°C for 30 minutes. Thin lipid-drug film obtained, under reduced pressure was hydrated using 10 ml distilled water (Baillie and Florence, 1985).

Effect of niosomal surface charge on the percentage of lycopene entrapped

Surface charges were imparted to lycopene niosomal preparations using the charge inducing agent, dicetyl phosphate (DCP). The latter will be used for inducing a negative surface charge. The molar ratio investigated is Span 60/CHOL/DCP (1:0:1). The negatively charged niosomes were prepared using the vortex dispersion method previously mentioned.

Separation of un-entrapped lycopene

The separation of encapsulated lycopene from the free lycopene, using (Hanil, Union 32R, Korea) cooling centrifuge at 7,000 xg, was performed by the use of 10 ml distilled water for either separation or for the washing steps. The amount of lycopene entrapped was measured by using the spectrophotometric assay method mentioned before.

All chemicals used in this assay were obtained from Sigma-Aldrich Chemie GmbH, Germany, Chloroform (HPLC, 99%) from Panreac, Spain and Hexane (HPLC) from Rankem, India.

Assay of nano lycopene niosomes

Nano Lycopene niosomal suspension was diluted to 10 ml with distilled water. One ml of the diluted suspension was centrifuged to discard any excess water. 1 ml of a solution 1% titron-x in methanol was shacked till dissolution of pellet, and then the volume was completed to 10 ml with hexane and sonicated for 30 minutes. Two ml of the solution was diluted to 10 ml with hexane and the absorbance was measured at 470 nm against a blank treated in the same manner.

Transmission electron microscope of encapsulated lycopene product

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

Thermal stability of microencapsulated lycopene product DSC (differential scanning colorimetry)

The thermal stability of microencapsulated lycopene product nanoparticles was determined as described before.

Results and Discussion

Supercritical fluid CO_2 extraction of bioactive compound from natural sources

The food has a need for a new technology to produce valuable additives of natural origin. From the work undertaken in this research, supercritical extraction could be considered with a great valuable and alternative to extraction with conventional solvents. The utilisation of which is being progressively limited in the food industry and other sectors, by the imposition of increasingly demanding regulations for the obtaining the additives needed for many food products.

The possible utilisation of additives that not only serve as colorants but also present antioxidant characteristics, which provide health benefits to consumers, contributes to added value of final product.

The production of these natural additive (as value added) from food plant by using supercritical fluid CO_2 gave a high purity and safety products in spite of high cost compared to conventional methods of extraction.

In this study, one type of additive has been evaluated and already being utilized in food and drink products. This additive was obtained from food processing wastes and showed a greater added value.

In addition, the high degree of purity and safety of the product obtained by means of supercritical extraction is of great importance (Herrero *et al..*, 2010). Results showed that the weight of the extracted lycopene was 14280 µg/mg which is much higher than that extracted by conventional methods being 571.2. These results agreed with the results obtained by (Herrero *et al.*, 2009).

Physical and Chemicals Properties

Transmission Electron Microscope

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

Figure 3 shows pictures of lycopene tube shape nanoparticles with diameter 7nm to 64.8. ± 2.35 nm and round shape 131.6 nm.

Lycopene

FT-IR Analysis

FT-IR spectral peaks of supercritical lycopene sample were shown in figure 5. The spectra consist of different groups of absorption bands at wave numbers ranging from 4000 to 400 cm^{-1}. Figure 7 showed stretching OH at 3441.35 $cm^{-1.}$ The C-H stretching symmetric bands were observed at 2923.56–2856.06 $cm^{-1.}$ The region of 1800–400 cm^{-1} is the finger region of the individual bonds of the functional groups.

The sharp and small bands at 1734.66–1543.74 cm^{-1} are assigned to c=o stetching vibration. Sharp small peaks were observed at 1457.92 and 1382.71 cm^{-1} were assigned to asymmetrical of cH_2 cm^{-1} and deformation cH3 cm^{-1} respectively. A small peak was assigned to stretching C-C at 1165.76 cm^{-1} and OH out of plane at 671.06 $cm^{-1.}$

Thermal properties of extracted

Effect of pH on lycopene stability

The stability of extracted lycopene from tomato wastes by using Supercritical extraction method was tested at different PH values (i.e. 1-13) as presented in table 3.

Results in table 2 and figure 4 showed that the stability of extracted lycopene towards different pH values was affected. The extracted lycopenes showed higher retention values at pH 4. Beyond this value (i.e. 4) a great loss in lycopene content was noticed. The maximum stability for lycopene was noticed at pH 4 after 30 min. and decreased after 60 and 120 minutes respectively. Moreover results also showed that the optimal pH range of lycopene was 3.5–4.5. Results also revealed that beyond 3.5–4.5 pH values the extracted lycopene content was decreased. The lycopene was greatly affected by alkaline media i.e. 10–13 pH.

Effect of temperature

The stability of lycopene (percent retention) after treated at different temperature (i.e. 20, 40, 80 and 120°C) for (30, 60 and 90 min) was evaluated as presented in table 4 & figure 5. Results in table 4 & figure 5 showed that the stability of crude lycopene was varied according to the treatment (temperature values & times). The percent retention of lycopene was decreased by increasing temperature from 40 to 120°C. The high loss in lycopene was noticed when heated at 120°C (i.e. 2.3,0 and 0). The maximum retention of lycopene was 91.9 % at 40°C for 30 min. and reduced to 83.1 and 78.4 % at 60 and 90 min. This indicates that the extracted lycopene by Supercritical had more heat stability at the studied temperature and time (40°C and 30 min). A slight loss in stability was noticed at 20–60°C for 30 min., while it was increased after 60 & 90 min and more than at 40 °C. Such heat stability is greatly important for industrial application.

Effect of day light

The stability of lycopene extracted by Supercritical CO_2 from tomato wastes (skin & seeds) as affected by day light was tested as presented in the following results (Table 5).

Results in table 5 and figure 6 showed that the percent retention of the extracted lycopene was varied according to the periods of daylight exposure. The studied lycopene to day light caused a reduction in the percent retention which also decreased by increasing the time of exposure. However, storage for 4 and 8 hrs in dark had almost no effect on the percent retention for extracted lycopene. The results also showed that the reduction in percent retention was increased by increasing the time of the extracted lycopene to daylight for 4 to 8 h. The retention in lycopene was noticed after 8h in sun light being 78.6%.

From table 5 and figure 6 it could be concluded that storage in dark completely retained all lycopene extract. However this lycopene was lost when subjected to day light. Therefore this lycopene should be stored in dark conditions to prevent its degradation and loss.

The DSC curve of the purest batch of lycopene (96% purity) (Figure 7) shows no other significant artefacts but an endothermic melting curve described by an onset point of 291.24 °C, peak at 293.65 °C and a total enthalpy of fusion of -3.54 J/g (area under peak). Pure substances have their melting point at the onset temperature of the melting curve. Thus, the melting point of lycopene according to DSC measurements is 293.65 °C. The presence of eutectic impurities broadens the main melting peak and lowers the vertex temperature. Furthermore, the impurities yield another smaller peak whose area correlate with the proportion of such impurities in the sample.

From the above results it could be concluded that lycopene was efficiently extracted by supercritical CO_2 (as a recent technology) in a higher yield, higher purity and safety compared to that extracted by the conventional method. The stability of the extracted lycopene towards heat treatment and pH changes was also evaluated. The extracted lycopene was stable at pH 3.5–4.5 and temperature 40–60°C (for 30 min.). Exposure of lycopene to daylight caused a great loss and hence it should be stored in dark conditions for protection against degradation.

Microcapsulation

Microencapsulation/Nanoencapsulation is defined as a technology of packing solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions.

Nanoencapsulation is the technology that can be used to overcome components degradation by oxidation and also permit the dilution of small amounts of the active compound in a uniform dispersion.

Lycopene is sensitive to light, heat and oxygen, and hence has a short storage life if not stored properly (Table 6 & 7).

Microencapsulation in carrier matrices can provide protection against degradative reactions and enhance stability. Various techniques are employed to form microencapsules, including spray dying, extrusion coating, fluidized bed coating, liposomes and niosomes entrapment, centrifugal extrusion and rotational suspension. The most common economical method to carry out microencapsulation is Niosomes entrapment (Shu *et al.,* 2006) (Table 6 & 7).

The data proved that neutral lycopene niosomes exhibited better entrapment (75.12 %) compared to the percentage entrapment of lycopene (61.8 %) performed by the negatively charged niosomes.

Transmission electron microscopy

The morphology of nano lycopene microencapsulated (entrapped in Niosomes) were measured by using Transmission Electron Microscopy (Fig. 8).

TEM in figure 8 present the lycopene microencapsulated with diameter 42.8 ± 3.2 nm.

This microencapsulated lycopene will be capsulated in a hard capsule.

Differential scanning colirimetry

The DSC curve of the encapsulated batch of lycopene (96% purity) in figure 9 shows no other significant artefacts but an endothermic melting curve described by an onset point of 129.65 °C, peak at 306.69 °C and a total enthalpy of fusion of -3.92 k J/g (area under peak).

Table.1 Absorbance of different nano lycopene concentrations in hexane

No.	Kycopene µg/ml	A
1	0	0
2	50	0.096
3	100	0.192
4	150	0.290
5	200	0.388
6	250	0.492

Table.2 Standard curve of lycopene

Absorbance A_{472}	lycopene (mg/L)
0	0
0.15	0.5
0.31	1.5
0.48	2.0
0.65	2.5
0.79	3.0

Table.3 Stability of natural lycopene extracted by Supercritical CO_2 as affected by change in pH values

pH	Lycopene %		
	After 30 min	After 60 min	After 120 min
1	16.3	9.4	2.3
2	23.5	18.7	8.4
3	52.1	40.8	22.0
4	93.6	90.9	87.2
5	75.5	53.1	33.7
6	61.9	34.6	21.5
7	45.7	25.1	15.8
8	38.2	17.4	9.3
9	32.4	10.2	5.6
10	27.1	6.6	Zero
11	14.6	Zero	Zero
12	8.5	Zero	Zero
13	Zero	Zero	zero

Table.4 Heat stability of lycopene extracted by supercritical CO_2

Temperature ˚C	Lycopene (%)		
	30 min.	60 min.	90 min.
20	97.8	92.2	90.6
40	91.9	83.1	78.4
60	89.4	74.6	9.8
80	60.2	37.0	1.2
100	25.1	18.4	Zero
120	2.3	Zero	Zero

Table.5 Effect of day light on stability of lycopene extracted by supercritical

Sample	Lycopene retention %					
	Darkness			Sun light		
	0	4h	8h	0	4h	8h
Extracted Lycopene	100	96.7	95.5	100	85.3	78.6

Table.6 Percentage of nano lycopene entrapped in neutral lycopene niosomes span 60/CHO (1: 1)

Trials No.	Abs.	LYC Entrapped / ml (mg)	LYC Entrapped / 10 ml (mg)	Free LYC	% of Entrapment
1	0.048	1.231	12.309	7.691	61.545
2	0.060	1.539	15.386	4.614	76.931
3	0.062	1.590	15.899	4.101	79.495
4	0.053	1.359	13.591	6.409	67.956
5	0.070	1.795	17.951	2.049	89.753

Mean ± S.D. (S.E.): 75.12 ± 10.865 (4.859)

Table.7 Percentage of nano lycopene entrapped in negatively charged lycopene niosomes span 60 / CHOL / DCP (1:1)

Trials No.	Abs.	LYC Entrapped / ml (mg)	LYC Entrapped / 10 ml (mg)	Free LYC	% of Entrapment
1	0.056	1.436	14.360	5.640	71.802
2	0.056	1.436	14.360	5.640	71.802
3	0.034	0.872	8.719	11.281	43.594
4	0.041	1.051	10.514	9.486	52.569
5	0.054	1.385	13.848	6.152	69.238

Mean ± S.D. (S.E.): 61.8 ± 12.962 (5.797)

Fig.1 Wave length of maximum absorption (λmax)
Where: λmax = 469.9 nm, abs. = 0.8994

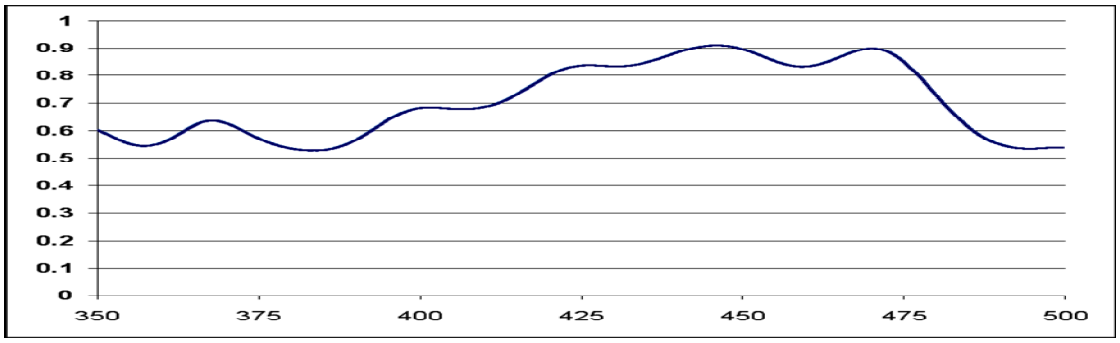


Fig.2 Standard calibration curve of nano lycopene in hexane
Slope = 0.0019498

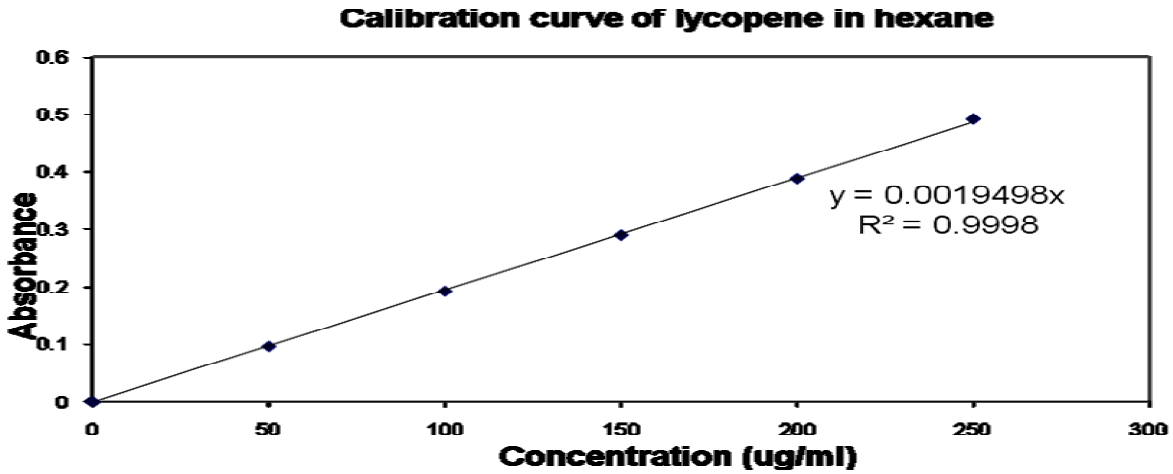


Fig.3 TEM of extracted lycopene nanoparticles by supercritical CO_2 (Tubes and Round shape) extracted by supercritical CO_2

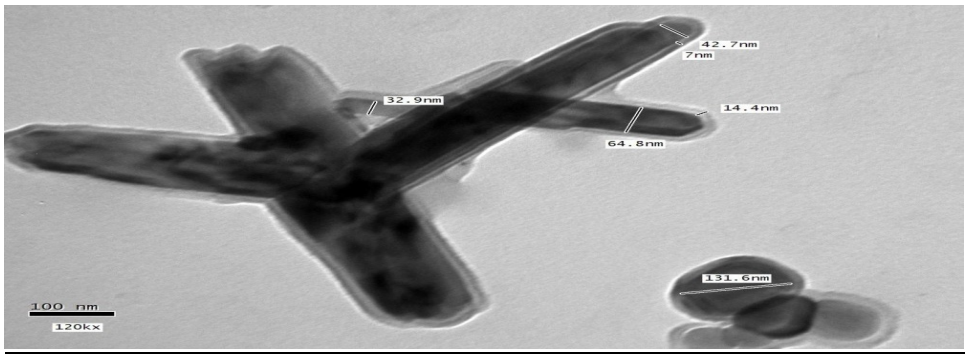


Fig.4 Standard curve of lycopene

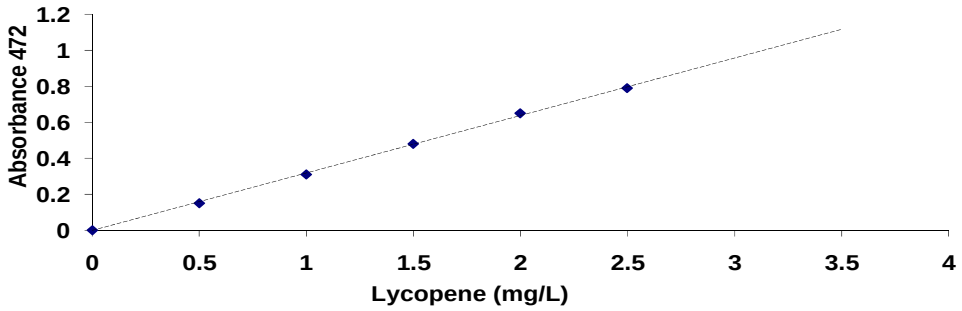


Fig.5 FT-IR spectral peaks of supercritical lycopene sample

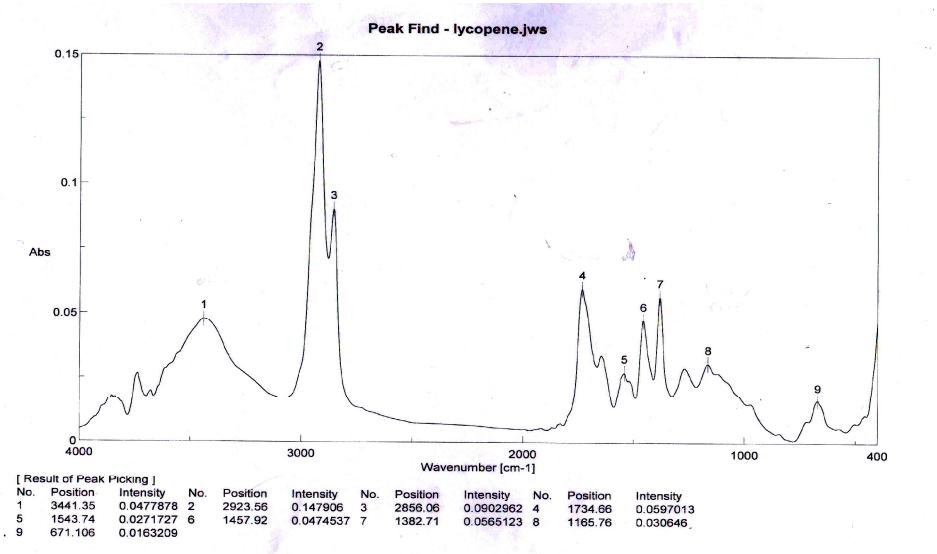


Fig.4 Stability of natural lycopene extracted by supercrirical CO_2 as affected by different pH values

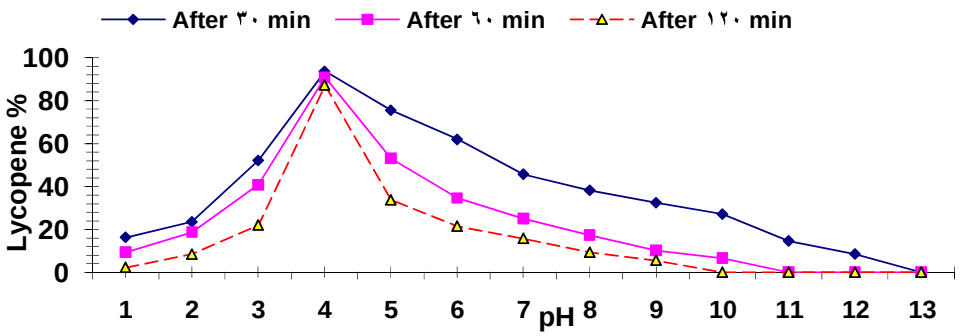


Fig.5 Heat stability of Lycopene extracted by Supercritical CO_2

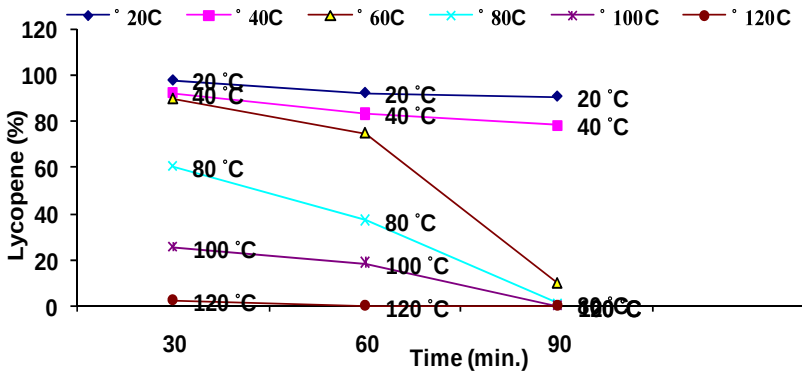


Fig.6 Effect of day light on stability of lycopene extracted by supercritical

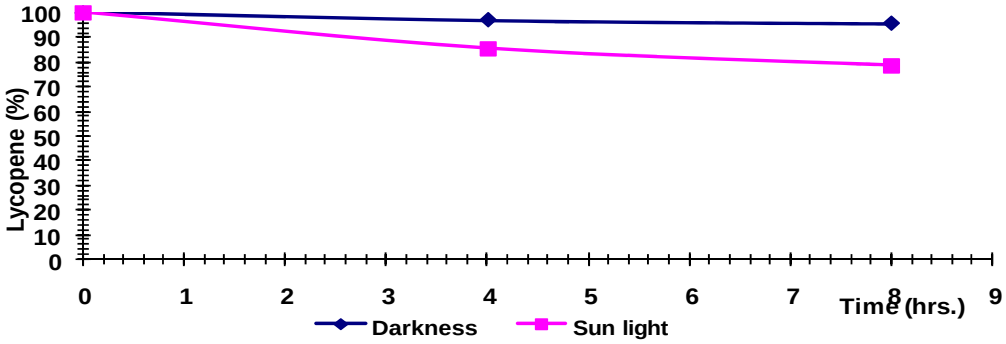


Fig.7 DSC of nano lycopen

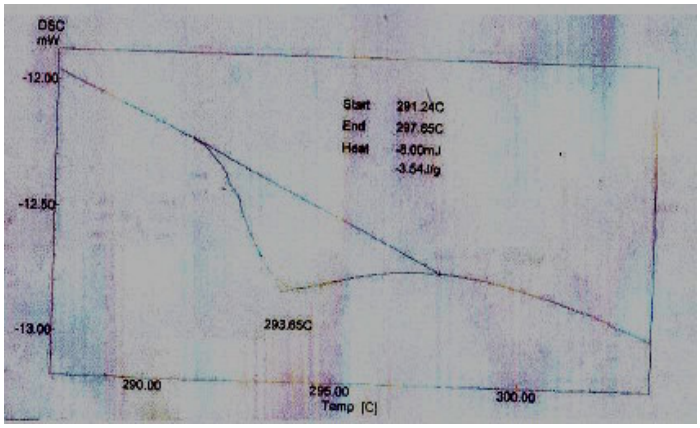


Fig.8 TEM of encapsulated lycopene entrapped in niosome

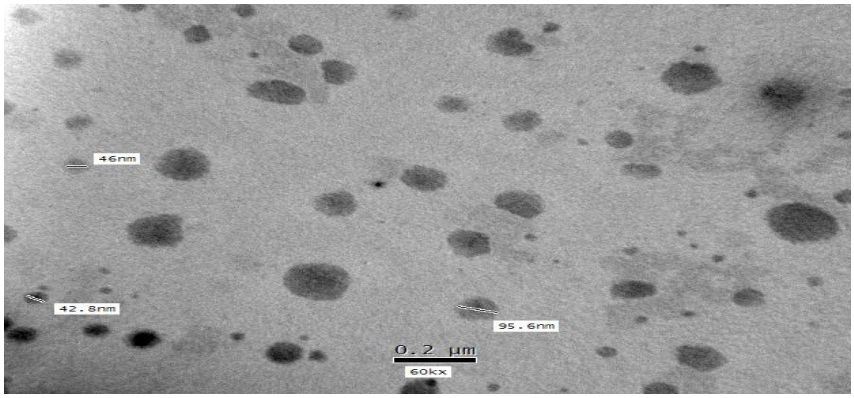
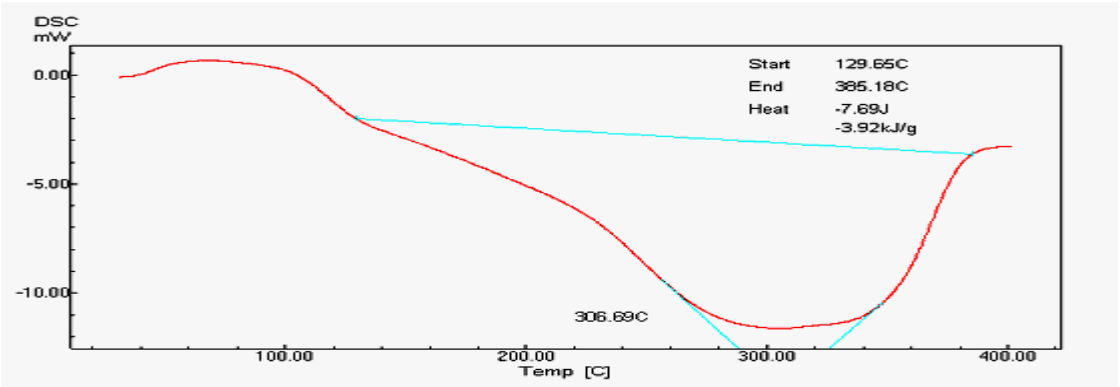


Fig.9 DSC of encapsulated lycopene



Thus the melting point of microencapsulated lycopene increased to 306.69°C compared to the non-encapsulated one being 293.65°C. These results indicate that microencapsulation caused an increase in the thermal stability of lycopene.

Compressed fluids had become interesting methods for extracting lycopene from wastes, ultrasonic processing helped in producing nanoparticles and microcapsulation of lycopene nanoparticles overcome components degradation and enhance its stability.

Acknowledgment

The authors wish to thanks the Science Technology Development Fund (STDF (who support and fund this research throughout a project in title "Food Nano Technology"

References

Baillie, A.J., Florence, A.T. 1985. Preparation and properties of niosomes-non ionic surfactant vesides. *J. Pham. Pharmacol.*, 37: 863–868.

Baysal, T., Ersus, S., Staemans, D.A.J. 2000. Supercritical CO_2 Extraction of β-carotene and lycopene from Tomato Paste Waste. *J. Agric. Food Chem.*, 48(11): 5507–5511.

Crandall, L.L. 2003. Extraction of natural antioxidants. *Inform*, 14: 62.

Domadia, Bhoomika, A., Waghela, N.R. 2013. Supercritical Fluid Extraction of lycopene from tomatoes by using CO_2 as a solvent:

A review. *J. Chem. Pharm. Res.*, 5(4): 188.

Herrero, M., Mendiola, L.A., cifuentes, A., Bamez, I. 2010. Supercritical fluid extraction: recent advances and applications. *J. Chromatogr.,* 1217(16): 2495–2511.

Jimenez, M.H.S., Garc, A., Beristain, C.I. 2004. Spray-drying microencapsulation and oxidative stability of conjugated linoleic acid. *Eur. Food Res. Technol.,* 219: 588–592.

Leoni, C. 1993. Collana di monogra®e tecnologiche. Stazione Sperimentale per l' Industria delle conserve alimentari, Parma, 1993. Pp. 7.

Matteo, F., Martin, A., Cocero, M.J. 2009. Carotenoid processing with supercritical fluids. *J. Food Eng.,* 93: 255–265.

Nobuo, T. 2008. Present status and future prospects of spherical aberration corrected TEM/ STEM for study of nanomaterials (free download review). *Sci. Technol. Adv. Mator.,* 9: 014111. doi:10,1088/1468-6996/8/1/14111.

Pérez-Alonso, C., Cruz-Olivares, J., Barrera-Pichardo, J.F., Rodreguez-Huezo, M.E., Báez-González, Vernon-Carter, 2008. DSC thermo-oxidative stability of red chilli oleoresin microencapsulated in blended biopolymers matrices. *J. Food Eng.*, 85: 613–624.

Rodriguez-Huezo, M.E., Pedroza-Islas, R., Prado-Barragan, LA., Beristain, C.I., Vernon-Carter, E.J. 2004. Microencapsulation by spray drying of multiple emulsions containing carotenoids. *J. Food Sci.*, 69: E351–E359.

Schierle, J., Bretzel, W., Bühler, I., Faccin, N., Hess, D., Steiner, K. 1997. Content and isomeric ratio of lycopene in food and human blood plasma. *Food Chem.,* 59: 459–465.

Sharma, S.K., Le Manguer, M. 1996. Lycopene in tomato and tomato pulp fractions. *Ital. J. Food Sci.,* 49: 1214–1218.

Shi, J., LeMaguer, M., Brya, M. 2002. Lycopene from tomatoes. In: Functional foods: biochemical and processing aspects, Vol. 2. CRC Press, LLC.

Shu, B., Yu W., Zhao Y., Liu X. 2006. Study on microencapsulation of lycopene by spray-drying. *J. Food Eng.*, 76: 664–669.

Silva, E.K., Angela, M., Meireles, A. 2014. Encapsulation of food compounds using supercritical technologies: applications of supercritical carbon dioxide as an antisolvent. *Food Public Health*, 4(5): 247–258. doi:10.5923/j. fph. 20140405.06

Topal, U., Sasaki, M., Goto, M., Hayakawa, K. 2006. Extraction of lycopene from tomato skin with supercritical carbon dioxide: effect of operating conditions and solubility analysis. *J. Agric. Food Chem.,* 54(15): 5604–5610.

Van den Berg, H., Fernando-Granado, H., Hirschberg, J., Olmedilla-Sandmann, G., Southon, S., Stahl, W. 2000. The potential for improvements carotenoids levels in foods and the likely systemic effects. *J. Sci. Food Agrc.*, 80: 880–912.

www.lycopene.com/2012/0423/starbucks-to-stop-using-bug-extract-to-color-rappucci-nos.

Zelkha, M., Ben-Hehuda, M., Hartal, D., Raveh, Y., Garti, N., Ziegler, R.G. 1989. A review of epidemiological evidence that carotenoids reduce the risk of cancer. *J. Nutr.*, 199: 116–122.