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Sweet pepper extracts using green technology and application of nanocapsule extracts in preparation of ketchup

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Abstract

Extraction yield with great concentration of sweet pepper has improved by using green technology that reduce environmental cost, and short duration. Results suggested that sweet pepper have higher content of radical scavenging activity and reducing power. Phenolic and flavonoids contents were (86.82 and 38.14 respectively) for Supercritical Fluid Extraction (SCF-CO_2) extracts and increased to (90.14 and 45.11 in their nano-capsules, followed by Ethanol extract (EE) and Microwave-Assisted Extraction (MAE). In HPLC analysis, chlorogenic acid was the largest phenolic compounds in MAE 12275.31 µg/g and Gallic acid was 648.45, followed by SCF-CO_2 extract being 342.11µg/g. SCF-CO_2 nano-capsules recorded high total phenolic, total flavonoids contents, small nano-particles through TEM, more mortality for cancer cell lines under the study followed by MAE, and their stability were less than MAE. The result proved that encapsulation is a very important technique to protect the plant extract from any environmental conditions and aid in the release of these bioactive compounds in the target organs when incorporated in some functional foods. Functional foods produced in this work (ketchup) proved that the addition of 1% sweet pepper extracted by SCF-CO_2 followed by microwave were the most acceptable products from the consumers since they retain their release of these bioactive compounds in the target organs when incorporated in some functional foods.

Keywords: Green Emerging technology, conventional methods extraction, physiochemical properties, nano-encapsulation, cytotoxic effect, application

1 Introduction

Sweet pepper is fruit belongs to*Capsicum annuum species*. In recent years, scientists have turned towards antioxidants which play an important role to retard the free radical induced oxidative stress to body damage [1].Polyphenolsand flavonoids have gained growing interest as natural antioxidants since they can retard many chronic deceases and protect human body from free radical [2].The great challenge is to use the most suitable technology for extracting these compounds. In this study the application of new emerging technologies i.e. Ultrasonic-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE)and Supercritical Fluid Extraction (SFE), in the extraction of sweet pepper are used as alternative to the conventional methods in the extraction of sweet pepper (*Capsicum annuum*). These extractions techniques with encapsulation and nano-emulsion as novel technology are promising and can enhance the bioactive contents by increasing their concentrations, the amount, yields, reduced processing times, environmental damage, bioavailability of components, consumption of organic solvents, and their stability during food processing. The use of these novel green technology have great potential in functional food market as safer product [3].

Carotenoids ,phenolic compounds, capsaicinoids, capsinoids, vitamins and minerals are the main bioactive compounds present in Capsicum peppers [4, 5, 6].

Thus, phenolic compounds and their abundant group (Flavonoids and their glycoside derivatives) can be a good indicator of antioxidant activity in peppers [7, 8, 9, 10, 11, 6].

The use of dry sweet pepper(extracted by theses new technology) containing these bioactive compounds in the formulation of functional foods is a new era to improve health disease status of the population, life style and quality.

The main goal of all food industries is to achieve the consumers' expectations in the final products by reducing the amount of organic solvent waste, quantity, cost and energy used in the food processing.

Therefore, the aim of this study is comparing the use of green emerging technology in sweet pepper as alternative to conventional extracting methods, studying the yield and antioxidant activities as well as their nano-capsulation, cytotoxic effect, stability and its application in Ketchup as functional food..

2. Materials and Methods

Materials

Sweet pepper (*Capsicum annuum*) was purchased from Egypt local markets at Dokki, Giza Governorate.

Methods

Conventional methods extraction

Water extraction

Fifty grams of dried sweet pepper were shaken in a water bath at 24 °C for 24 hours with 200 ml of distilled water. The supernatant following three separate extractions was

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collected, centrifuged, dialyzed, lyophilized and kept till usage at 4 °C. The weight of the sweet pepper water extract divided by the dry weight of the raw plants was used to calculate the percentage of water extract yield [12].

Ethanol Extraction

Dried sweet pepper ethanol extract was carried out according to the method of Sun *et al.,* [13]. Plant powder (200 g) was combined with 200 ml of 95% ethanol with careful stirring for 30 minutes in a water bath at 45°C. The supernatants were collected after numerous extractions and evaporated under vacuum at 45 °C before being kept at 4 °C until use. According to the maximum amount of dried plant, the percentage of sweet pepper production was estimated.

Green methods extraction

2.2.2.1. Supercritical fluid carbon dioxide (SCF-CO_2) Extraction

Dried sweet pepper (100 gm) was mixed with ethanol 15% (w/w) as co-solvent in a separate vessel and extracted by using a laboratory CO_2scale unit

(Spe-ed TM SFE-2/2, Applied separations, Built in conjunction with the USDA1- USA) in National Research at 40 °C and 150 bar pressure.CO_2 apparatus was assessed by two vessels(300 ml), kept 30 min for complete contact between the sample and CO_2 , after which the sample was collected[14]. The following formula was used to get the sweet pepper percentage yield:

Yield (%) = 100 (W1/W2) ………… (1)

W1 is the weight in grams of the plant extracts, and W2 is the mass in grams of the dried, unprocessed plant powder.

Ultrasound-assisted Extraction (UAE)

The sample was stored at 4 °C until use after being sonicated in Ultra sonic water bath (Model Ud 150 SH3.8LQmade in U.S.A.) for 30 minutes at 40 °C with 50 g of dried sweet pepper after diethylether (300 ml) had evaporated [15].

Microwave-assisted Extraction (MAE)

Five grams of sweet pepper powder were extracted with 50 milliliters of ethanol in 80-milliliter capped vials using a mechanically adapted microwave oven (ETHOS 1600, Milestone, Sorisole, Italy) for 60 minutes at 40°C under pressure. After filtering, the extract was collected, cooled to ambient temperature, and then stored at 20 °C until analysis.

Determination of total phenol content (TPC)

According to the method described by Mythili *et al.* [16], the total phenolic content (TPC) of sweet pepper extracts and their nano-capsules were assessed using the Folin-Ciocalteau reagent. Total phenolic content are quantified as gallic acid equivalents (mg GAE/g dry extract) and contrasted with a blank using the same 20ul of clean water.

Determination of total flavonoid content (TFC)

Using a UV/visible spectrophotometer, the total flavonoid content (TFC) of sweet pepper extracts and their nano-capsules were assessed in accordance with the methods of Huang et al. [17], Ebrahimzadeh *et al.* [18], and Nabavi *et al.* [19]. At 415 nm, the mixture's absorbance was measured. The standard for the calibration curve was quercetin (µg/g).

Determination of radical scavenging activity

Radical scavenging activity of tested sweet pepper samples was tested using spectrophotometer at 517 nmaccording to the method of Burits and Bucar,[20].

% DPPH radical scavenging activity = (Ac–A s)/ Ac ×100 ……(2)

Where As represents the sample's absorbance and Ac represents the control's absorbance in the absence of the sample.

Using 500 mg/ml, the radical scavenging activity of nano-capsules was assessed as above.

Reducing power (RP)

The Oyaizu method, developed in 1986 [21], was used to assess the reducing power (RP) of sweet pepper extracts. Using a spectrophotometer set at 700 nm, the absorbance of the tested substances was compared to that of BHT and ascorbic acid at room temperature. As mentioned previously, the decreasing power of nanocapsules was calculated using 500 mg/ml.

HPLC analysis

Using liquid chromatography (HPLC), the chemicals found in sweet pepper extracts were analyzed using 4.6 mm x 250 mm i.d., 5 m, Eclipse C18 column for the separation water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were the components of the mobile phase, which had a flow rate of 1 ml/min. The linear gradient was sequentially programmed into the mobile phase as follows: 0 minutes (82% A), 0-5 minutes (80% A), 5-8 minutes (60% A), 8 minutes (12%), 12 minutes (15%), and 15 minutes (82% A). At 280 nm, the multi-wavelength detector was observed. For every one of the sample solutions, 10 l of injection volume was used. The column was kept at a constant temperature of 35 °C [22].

Differential scanning calorimetry (DSC)

Using a differential scanning calorimeter (DSC), the thermal stability of sweet pepper extracts and their nano capsules was investigated using the method of Hazra *et al.* [23]. A temperature range of -150 to 300°C was used to compute the peak evaporation enthalpies, and the findings were compared with the estimated vaporization enthalpies of the main components.

Transmission Electron Microscopy (TEM)

Sweet pepper extracts morphological characteristics and their nano capsules were measured using TEM after air-dried at room temperature for 3 min. using the method of Abd-Elsalam and Alexei, [24].

Preparation of nano-capsules

Ultrasound and high-speed homogeniser (PRO, USA) were used to encapsulate sweet pepper extracts into nano using the methods of Vasiliki and Constantina, [25].

The encapsulation yield (EY) effectiveness of was measured according to Paviani *et al.* [14] method as follows:

$$EY\ (\%) = \frac{\text{Total weight of the sample after encapsulation}}{\text{Total weight of the sample before encapsulation}} \times 100 \quad ....(3)$$

Cytotoxic effect of nano- capsules

Cell feasibility was assessed using three cancer cell lines from the National Cancer Institute of Egypt (MCF-7 for breast cancer, HEPG2 for liver cancer, and HCT116 for colon cancer) suspended in DMEM-F12 media (for HePG2, MCF-7, and RPE-1) with one normal cell line (BJ1) [26].

Then, using a microplate multi-well reader and a reference wavelength of 620 nm, the absorbance was determined. The following formula was used to get the feasibility change percentage:

((Absorbance of extract/Absorbance of negative control) -1) x 100.................. (4)

Applications

Tomato ketchup fortified with nano-encapsule sweet pepper extracts

Tomato ketchup was prepared according to the method described by Sahin and Ozdemir, [27] with some modification. Tomato paste (350 gm) was mixed in an open pan with 0.47 gm spices (cloves, cinnamon, black pepper, and cardamom), 2.25gm onion, 1gm garlic powder, 44.5gm sugar, 5.85 salt, 20.4ml apple vinegar and0.08 gm paprika powder then 0.5% of pectin was added to the mixture. The ketchup formula was divided into five samples, each was fortified with 1% sweet pepper extracted by water, ethanol, super critical fluid-carbon dioxide (SCF-CO_2) and microwave nano-capsules ketchup samples were sealed in glass jar, and stored at (20–22 °C) for 24 h until analyses.

Physical properties of Ketchup samples

The physical properties of ketchup samples were tested against pH, color as described by Schnell *et al.*, [28] using Hunter Lab colorimeter and water soluble dry matter (Brix°) using the method of Ranganna [29].

Sensory evaluation of tomato ketchup samples

All ketchup samples were sensory evaluated by fifteen trained panels using the method of Piggott, [30]. Panelistsin three training sessions (1hr each) were given a randomly coded samples.. Sensory evaluation was given a nine point hedonic scales with (1 = the lowest or extremely dislike and nine= the highest or extremely like).

Statistical analyzes

Data were reported as means standard deviation (n = 3) and Anova variance analysis with average comparison Duncan Multiple Range adjusted to 0.05 [31].

3. Results and Discussion

Samples extraction Yield

The extraction yields of sweet pepper were shown in (Figure 1). The highly percentage yield observed was for the extraction in water i.e. 11.52% followed by microwave, CO_2, ultrasound and solvent i.e., 10.57, 9.58, 9.05and 8.94% respectively. Results obtained showed approximately similar in total yields between supercritical fluid CO_2, microwave-assisted and ultrasound-assisted extraction.

Antioxidant activity of sweet pepper extracts and their nano-capsules

DPPH radical scavenging

Five distinct extraction techniques i.e. water, ethanol, CO_2, microwave, and ultrasound were investigated in concentrations ranging from 100 to 500 mg/ml. to measure the antioxidant activity of sweet pepper extracts and their nano-capsules using DPPH radical scavenging at 517 nm (Table 1).

Results showed that sweet pepper sample had concentration-dependent increases in scavenging potential. As at a concentration of (500 mg/ml) the scavenging action of pepper was 36.92, 31.28, 30.45 and 24.09% in CO_2 followed by, microwave, solvent and ultra sound respectively. Previous studies have reported that using a high concentration (500mg/mL) of pepper fruits showed increases in scavenging activity [32].

Sweet pepper extracts nano-capsules (Table 1) showed that nano encapsulation, breaks the components into smaller particles and distributing them evenly throughout the mixture and that led to an increase in DPPH in the same order before nano-capsules. Our data agreed with that mention by Sahba and Dariush [33] who stated that the antioxidant activity increased by encapsulations which enhance the bioavailability of natural antioxidants aside with the reduction of the biological system's leakage and volatility.

Table (1). DPPH scavenging activities of sweet pepper extracts and their nano-capsules

Types of Extraction	Conc. $mgmL^{-1}$	DPPH radical scavenging %	
		Extract	Nano-capsules extract
Water extract (Conventional method)	100	8.25±0.24^{a}	11.38±0.46^{ab}
	200	11.08±0.68^{ab}	16.27±1.04^{ab}
	300	14.67±1.24^{ab}	18.69±1.28^{b}
	400	16.28±1.33^{b}	20.25±1.39^{b}
	500	20.19±1.58^{bc}	24.67±2.11b^{c}
Solvent extract (Conventional method)	100	12.29±0.35^{ab}	15.93±1.58^{ab}
	200	18.17±1.26^{b}	21.55±1.93^{b}
	300	23.28±1.87^{bc}	27.12±2.28^{bc}
	400	27.31±1.83^{bc}	31.22±2.34^{c}
	500	30.45±2.11^{c}	35.17±2.56^{c}
*SCF-CO_2 extract (Green method)	100	15.08±1.04^{ab}	19.05±1.27^{b}
	200	19.28±1.38^{b}	23.47±1.75^{bc}
	300	25.32±1.76^{bc}	29.81±2.00^{c}
	400	30.25±2.13^{c}	34.68±2.24^{c}
	500	36.92±2.46^{d}	40.17±3.27^{d}
*UAE extract (Green method)	100	9.13±0.48^{a}	11.28±0.83^{ab}
	200	12.36±0.83^{ab}	16.87±0.94^{ab}
	300	17.11±1.23^{ab}	20.69±1.24^{bc}
	400	20.39±1.35^{b}	25.48±1.67^{bc}
	500	24.09±1.64^{bc}	28.97±2.23^{bc}
*MAE (Green method)	100	13.67±1.05^{ab}	16.87±1.42^{ab}
	200	19.18±1.24^{b}	23.68±2.14^{bc}
	300	23.35±1.36^{bc}	28.61±2.46^{bc}
	400	27.28±1.69^{bc}	31.57±2.44^{c}
	500	31.28±2.03^{c}	36.88±2.71^{d}

Where; *SCF-CO_2: Supercritical Fluid Carbon dioxide, *UAE: Ultrasound-assisted extraction, *MAE: microwave –assisted extraction

Reducing Power

Table (2) showed that the highest reducing power results for sweet pepper extract were observed by SCF-CO_2 0.220mgmL^{-1}, followed by microwave 0.208.The use of water and UAE lower the absorbance at 700nm than the CO_2. In general CO_2 give the higher reduction of active radicals. Reducing power of sweet pepper extracts and their nano-capsules, relatively increased with graded concentrations from (100 to 500 mgmL^{-1}), which means that the electron-donating capability of all of the examined samples is dose dependent. The lowering power of water extract at the same dose (500 mgmL^{-1}) was lower, i.e. 0.168 for the extract and 0.16 for the nano-capsule, respectively. The second and third orders go to sweet pepper MAE and solvent extracts.

Total Phenolic Contents (TPC)

Results in Table (3) illustrated that SCF-CO_2 extracts TPC had highest value 86.82 mg GAE/100g followed by ethanol extraction 81.2mg GAE/100g, while the lower was for water extract35.23mg GAE/100g followed by Ultrasound. Also, microwave is one of the greenest promising methods in terms of extraction quantity and containment of phenolic content, it recorded 76.38 exceeded to 85.68 mg GAE/g extract in nano-capsules. Our results are contrary to the findings of Ahmad *et al.*,[34] who reported a higher TPC of water extract (27.68 mg GAE/g DW), as compared to the ethanol extract17.1 mg GAE/g DW. TPC of sweet pepper extracts nano-capsules following the same trend. These data agreed with that mentioned by Kanatt *et al.*, [35] who illustrated that the stability of the supplemented bioactive component needs nano technique with encapsulation technology to protect them during processing and help in their control release under various conditions.

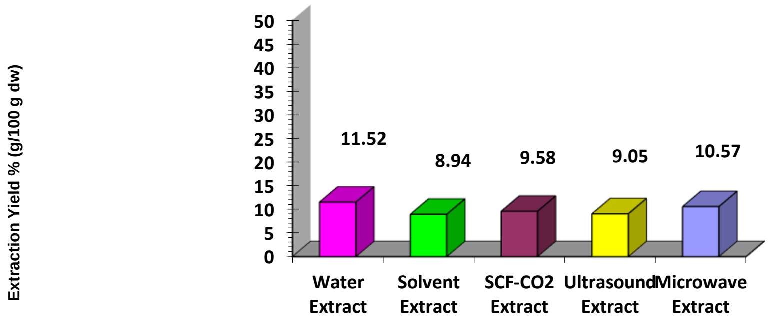


Figure (1). Total yield (%) of sample extracts

Table (2). Reducing power activity of sweet pepper extracts and their nano-capsules

Types of Extraction	Conc. mgmL^{-1}	Reducing power Absorbance at 700 nm	
		Extract	Nano-capsules extract
Water extract (Conventional method)	100	0.123±0.01^{a}	0.129±0.01^{a}
	200	0.137±0.01^{a}	0.145±0.01^{ab}
	300	0.146±0.01^{ab}	0.152±0.01^{ab}
	400	0.154±0.02^{ab}	0.159±0.01^{ab}
	500	0.161±0.02^{b}	0.168±0.01^{b}
Solvent extract (Conventional method)	100	0.162±0.02^{b}	0.169±0.01^{b}
	200	0.173±0.02^{b}	0.181±0.02^{bc}
	300	0.184±0.02^{bc}	0.192±0.02^{bc}
	400	0.193±0.02^{bc}	0.199±0.02^{bc}
	500	0.201±0.02^{c}	0.208±0.02^{c}
*SCF-CO_2 extract (Green method)	100	0.195±0.01^{bc}	0.203±0.02^{c}
	200	0.202±0.01^{c}	0.209±0.02^{c}
	300	0.209±0.02^{c}	0.216±0.02^{c}
	400	0.214±0.02^{c}	0.221±0.02^{c}
	500	0.220±0.02d	0.228±0.02^{d}
*UAE extract (Green method)	100	0.157±0.01^{ab}	0.163±0.01^{b}
	200	0.162±0.01^{b}	0.168±0.01^{b}
	300	0.167±0.01^{b}	0.173±0.01^{b}
	400	0.176±0.01^{b}	0.181±0.02^{bc}
	500	0.182±0.02^{bc}	0.189±0.02^{bc}
*MAE extract (Green method)	100	0.175±0.01^{b}	0.182±0.02^{bc}
	200	0.182±0.02^{bc}	0.188±0.02^{bc}

	300	0.195±0.01bc	0.200±0.02c
	400	0.201±0.01c	0.209±0.02c
	500	0.208±0.01c	0.215±0.02c

Where; *SCF-CO_2 : Supercritical Fluid Carbon dioxide; *MAE: Microwave-Assisted *UAE: Ultrasound-assisted extraction

Table (3): Total phenolic contents (TPC) of extracts and their nano-capsules

Extract types	Total phenolic contents (TPC) (mg GAE /g extract)	
	Extracts	Nano-capsules extract
Water extract (conventional method)	35.23±1.58a	39.82±1.38a
Ethanol extract (conventional method)	81.20±3.45c	82.55±3.54bc
*SCF-CO_2 extract (green method)	86.82±3.68c	90.14±3.67d
*UAE extract (green method)	63.43±2.37b	68.02±2.44b
*MAE extract (green method)	76.38±2.81bc	85.68±2.16c

Where; *SCF-CO_2: Supercritical Fluid Carbon dioxide extraction; *MAE: Microwave-Assisted extraction; *UAE: Ultrasound-assisted extraction, MAE: microwave assist extraction

Total Flavonoids Contents (TFC)

Results in Table (4) revealed that SCF-CO_2 extract of sweet pepper exhibited higher TFC 38.14, followed by ethanol, MAE, UAE and water being 36.88, 36.66, 21.46and 9.37 mg QE/g,respectively.SCF-CO_2 extract nano-capsules showed increase in flavonoid content from38.14 in the extract to45.11 at a percentage of about 18%. Due to the bulk heating irradiation properties of the MAE, an innovative green technology, increased flavonoid content is produced before and after nano-capsules 36.66 and 40.81(11%) at a faster rate. The cell membrane swells and ruptures under the influence of microwave, releasing the bioactive into the aqueous ethanol solvent, according to Jin *et al.* [36]. By merging conventional techniques with those from the UAE and water, the process was made superior to the other two approaches solvent extraction with microwave irradiation.

Table (4): Total flavonoids content (TFC) of extracts and their nano-capsules

Extract types	Total flavonoids content (TFC) mg QE/ g extract	
	Extracts	Nano-capsules extract
Water extract (conventional method)	9.37±	12.64±
Ethanol extract (conventional method)	36.88±	40.24±
*SCF-CO_2 extract (green method)	38.14±	45.11±
*UAE extract (green method)	21.46±	25.52±
*MAE extract (green method)	36.66±	40.81±

Where; *SCF-CO_2: Supercritical Fluid Carbon dioxide; *UAE: Ultrasound-assisted extraction, *MAE: microwave extraction

HPLC analysis of sweet pepper extracts

HPLC for sweet pepper extract Tables (5), identified chlorogenic acid as the largest phenolic compounds in MAE, while Gallic acid was the second largest compound 841.15µg/g followed by UAE extract being 648.45 and CO_2 342.11 µg/g respectively. While the order of richness of other phenolic compounds with the significant amount was in the order of kaempferol > Gallic acid > ellagic acid in UAE and naringenin > chlorogenic acid > ellagic acid in CO_2 and ethanol extract.

Accumulated polyphenol content in sweet pepper is essential and depends on the ripening stage and genotype . [37]. So, obtained results related to the identification of phenolic compounds of sweet pepper extracts were not so easy to compare with literature data. However, our data lightly in agreement with those of Mennat *et al.*,[38], who reported that there is some Egyptian pepper contained higher phenolic compounds than in other depend on their content of pyrogalol, catechin, catechol, ellagic acid, Gallic acid and chlorogenic acid. Ahmad *et al.*, [34] reported that sweet pepper samples showed higher phenolic compounds 178 to 384.9 mg per 100 gram chlorogenic acid as compared to red and green peppers

Table (5): HPLC analysis of sweet pepper extracts

Components	Conc. (µg/ml=µg/g)				
	Conventional method		Green method		
	*SPWE	*SPEE	*SPUAE	*SPSCF-CO_2	*SPMAE
Gallic acid	219.63	239.20	648.45	342.11	841.15
Chlorogenic acid	41.18	126.92	79.53	145.88	12275.31
Catechin	9.34	0.00	4.73	0.00	422.69
Methyl gallate	0.71	5.66	1.45	9.65	18.37
Caffeic acid	7.22	4.96	18.21	7.26	24.09
Syringic acid	1.88	12.31	11.61	19.34	127.72
Pyro catechol	2.14	0.00	0.00	0.00	0.00
Rutin	3.46	28.95	29.23	38.92	80.47
Ellagic acid	23.81	64.58	138.37	76.35	14.42
Coumaric acid	0.00	1.55	0.00	3.68	17.80
Vanillin	0.06	1.57	0.00	2.96	0.00
Ferulic acid	4.95	18.48	7.29	23.68	0.00
Naringenin	19.84	155.90	62.30	169.34	0.00
Taxifolin	5.34	0.00	60.46	0.00	0.00
Cinnamic acid	1.13	13.73	58.43	23.66	10.76
Kaempferol	0.84	0.00	676.60	2.31	0.00

Where; *SPWE: sweet pepper water extract; *SPEE: sweet pepper ethanol extract; *SPUAE: sweet pepper Ultrasound-assisted extraction, *SPSCF-CO_2: sweet pepper Supercritical Fluid Carbon dioxide extraction; *SPMAE: sweet pepper microwave-assisted extraction.

Transmission Electron Microscopy (TEM) of sweet pepper extracts and their nano-capsules

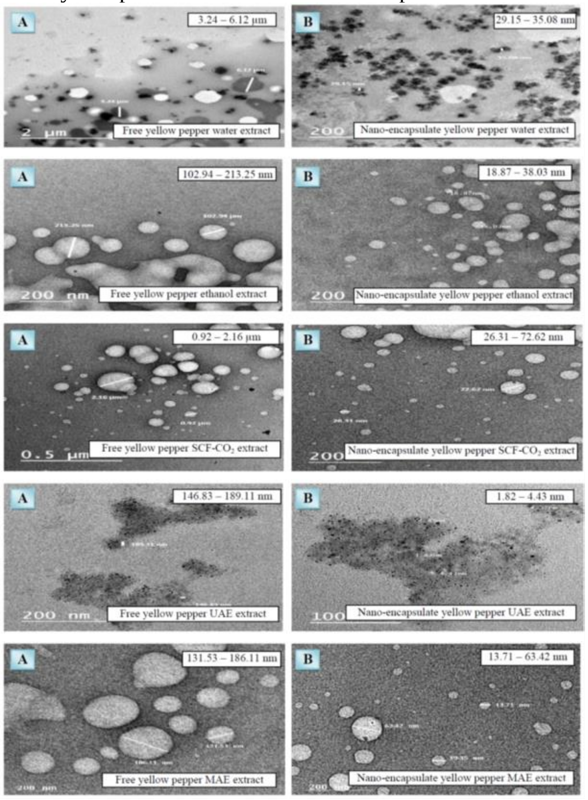
The transmission electron microscopy (TEM) characteristics (Figures 2A and B) was used to investigate the morphology of sweet pepper extracts and their nano-capsules particles sizes. Sweet pepper extracts Figure (2A) showed more spherical shape in water, ethanol, SCF-CO_2 and MAE except that extracted by ultrasound which showed indefinite shape with diameters (3.24-6.12 µm), (102.24- 213.25 nm), (0.92-2.16 µm), (131.53-186.11nm) and (146.83-189.11nm) respectively. The TEM of sweet pepper extracts nano-capsules Fig (2B) followed the same spherical shape as in water, ethanol , SCF-CO_2 and MAE extracts except that extracted by ultrasound which showed indefinite shape with diameters (29.15-35.08 nm), (18.87- 35.40 nm), (26.31- 72.62 nm),(13.71-63.42 nm) and (1.82-4.43nm) respectively.

Differential Scanning Calorimetry (DSC) of sweet pepper extracts) and their nano-capsules

The DSC curves of sweet pepper ethanolic extract Figure (3A) showed sharp endothermic peaks present at 42.3 °C. Also, SCF-CO_2 extract had three small sharp endothermic peaks at melting point 35.5, 37.6 and 42.2 °C respectively. It has been attributed more stable for its decomposition.

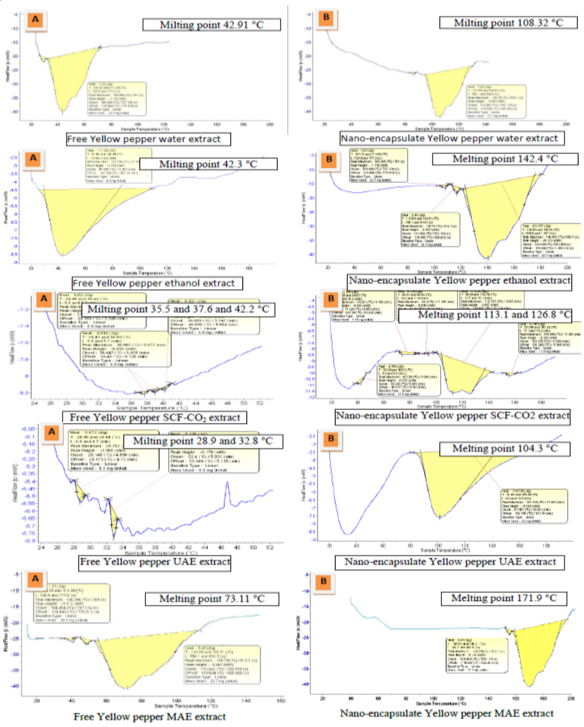
Furthermore, UAE extract had two small peaks at around 28.9 and 32.8 °C and one sharp exothermic peak at 47 °C, probably due to a solid restoration before melting. Also, MAE had wide endothermic peaks at 73.11 melting point. The endothermic peaks pointed to the melting boiling and the latter can be attributed to its degradation. While sweet pepper SCF -CO_2 extract nano-capsules Fig (3B) showed also sharp endothermic peak with two melting point and one for ethanolic , and UAE and MAE i.e. (113.1, 126.8, 142.4, 104.3 °C and 171.9) respectively.

The results of sweet pepper extracts nano-capsules DSC showed higher resistance to temperature observed, and are in agreement with reported by Batra *et al.*, [39] who stated that the encapsulation of the extracts in spontaneous emulsification method increased the antioxidant and temperature of their initiation oxidation activity compared to the same extracts in pure form.



A: TEM images of sweet pepper extracts before encapsulation, B: TEM images of sweet pepper extract capsules

Figures (2A,B): TEM images of sweet pepper extracts and nano-capsules



A: DSC of sweet pepper extracts before encapsulation
B: DSC of sweet pepper extract capsules
Figures (3A,B): DSC analysis of sweet pepper extracts and nano-capsules

Cytotoxic effect of sweet pepper extracts and their nano-capsules

Data in Table (6) indicated that the nano-capsules exhibited potent lethal inhibitory effect against the examined human cell lines (i.e. HePG2, PACA2 and MCF7) with several dilutions (100 to 0.78µg/ ml). The growth inhibition of tested samples after incubation for 48 hrs was tested. Results showed that the maximum inhibition was achieved after 48 hrs. Results also, show that 90 % death of cell was observed in all nano- capsules of SCF-CO_2.

The mortality rate was highest for PACA2 treated, then MCF7 using the same extraction technique, with respective mortality rates of 39.6 and 25.6%.
Human cancer cells treated with nano-capsules experienced morphological collapse, supporting their potent anticancer effect. Our findings supported Kurnijasanti and Fadholly, [40] observation that Capsicum annum L. is an anticancer agent.
From the above results the use of Ethanolic, SCF-CO_2 and MAE extracts nano-capsules will be highlight in the application of ketchup as functional foods due to their physiochemical properties.

Applications

Ketchup fortified with sweet pepper extracts nano-capsules

Food industry usually added functional ingredients which have potential health benefits to enhance the texture, flavor, color, or shelf-life of food products. Nano-encapsulation of sweet pepper extracts stability during processing and storage is important for successful preparation of functional foods.

Table (6): Cytotoxic activity test of sweet pepper extracts nano-capsules on HePG 2, PACA2 and MCF7(normal retina cell line)

Sample Code	IC_{90} (µg/ml)	Remarks (% mortality at 100µl/ml)		
		HePG 2	PACA2	MCF7
*SPWE	46.00	0.8 %	4.6%	2.1%
*SPEE	46.00	3.4%	26.5%	15.9%
*SP SCF-CO_2	46.00	5.9%	39.6%	25.6%
*SPUAE	46.00	2.6%	23.8%	10.8%
*SPMAE	46.00	2.9%	37.9%	16.2%
DMSO	--------	5%	1%	3%
Negative control	--------	0 %	0 %	0 %

Where; *SPWE: sweet pepper water extract; *SPEE: sweet pepper ethanol extract; *SPUAE: sweet pepper Ultrasound-assisted extraction, *SPSCF-CO_2: sweet pepper Supercritical Fluid Carbon dioxide extraction; *SPMAE: sweet pepper microwave-assisted extraction; IC_{50}: Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs; IC_{90}: Lethal concentration of the sample which causes the death of 90% of cells in 48 hrs

Sensory evaluation

Table (7) and Figure (4) shows the sensory evaluation of ketchup with different sweet pepper extracts nano-capsules. Significant differences ($p < 0.05$) were observed in all recipes in taste, appearance, texture, flavor and over all acceptability. The physical properties are one of the factors that will attract customers to buy and accept it.

Table (7). Sensory evaluation of Ketchup samples fortified with 1 % sweet pepper extracts nano-capsules

Samples	Color	Taste	Aroma	Texture	Overall acceptability
Control	9.21 ± 0.1^{a}	8.67 ± 0.2^{a}	8.95 ± 0.1^{a}	8.66 ± 0.1^{a}	8.68 ± 0.1^{a}
1 % SPWEN*	8.51 ± 0.2^{ab}	8.12 ± 0.2^{b}	8.41 ± 0.2^{ab}	8.10 ± 0.2^{b}	8.22 ± 0.2^{b}
1 % SPEEN*	8.62 ± 0.2^{ab}	8.34 ± 0.2^{ab}	8.45 ± 0.2^{ab}	8.23 ± 0.2^{ab}	8.31 ± 0.2^{ab}
1 % SPSCF-SO_2 EN*	9.20 ± 0.2^{a}	8.95 ± 0.2^{a}	8.95 ± 0.2^{a}	8.70 ± 0.2^{a}	9.00 ± 0.2^{ab}
1 % SPUAEN*	8.15 ± 0.2^{b}	8.26 ± 0.1^{ab}	8.16 ± 0.1^{b}	8.12 ± 0.1^{b}	8.14 ± 0.1^{b}
1 % SPMAEN*	8.99 ± 0.2^{a}	8.90 ± 0.2^{a}	8.90 ± 0.2^{a}	8.69 ± 0.2^{a}	8.80 ± 0.2^{ab}

Where; SPWEN*: Ketchupfortified with 1% sweet pepper water extract nano-capsule; SPEEN*: Ketchupfortified with 1% sweet pepper ethanol extract nano-capsule; SPSCF-SO_2EN*: Ketchupfortified with 1% sweet pepper SCF-SO_2 nano-capsule; SPUAEN*: Ketchupfortified with 1% sweet pepper ultrasonic extract nano-capsule; SPMAE*: Ketchupfortified with 1% sweet pepper microwave extract nano-capsule

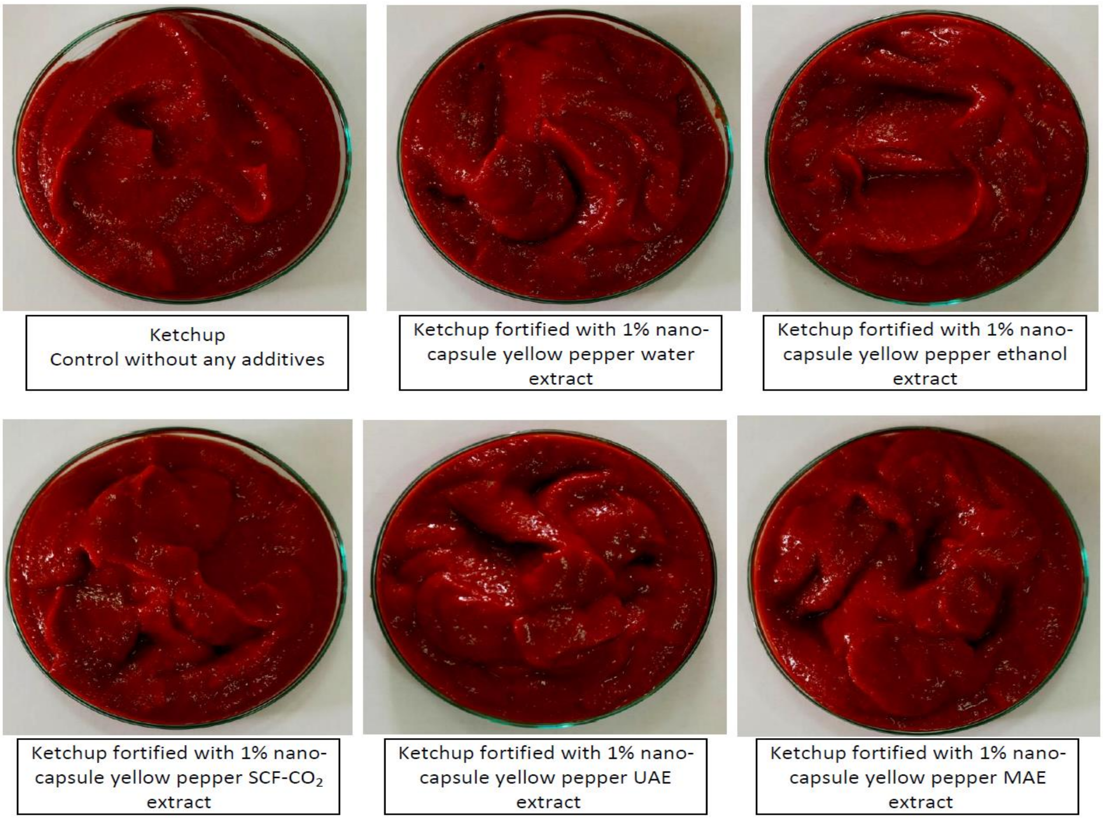


Fig. (4). Ketchup products enriched with sweet pepper extracts nano-capsule

Physical properties of fortified tomato ketchup samples

The results of the physical properties are shown in Table (8). It is clear that the moisture content of ketchup Samples fortified with sweet pepper alcoholic, carbon dioxide, ultrasound and microwave extracts nano-capsules, were varied between 64.35 and 64.12 %.The present results are in agreement with the results showed by Torbica *et al*.,[41] who stated that moisture content ranged from 67.42% to 79.02%.. Thus the lower moisture content in the fortified ketchup samples than the control was accordance with Sharoba, *et al.* [42] for Egyptian tomato sauce. Also minimal shelf stability was achieved with high moisture content [43].

Among the parameters analyzed for the assessment of tomato ketchup quality, pH is a very important factor due to the acidity that affect the thermal processing conditions required for producing safe products. Tomato ketchup samples have a pH value ranged between 4.06 in control sample and 4.04 in all fortified ketchup samples. These results are in accordance with Anthon *et al.*, [44], who stated that pH greatly affect the quality of the final product for both types of ketchup.

Another factor that affect ketchup fortified with sweet pepper extracts nano-capsules is its total solids content (Brix°). The higher the percentage of Brix°, the lower the activity of water (a_w) as found by Porretta and Birzi, [45], that prevents the growth of diseases that cause microorganisms in the innovative ketchup sample compared to a commercial sample.

There was a significant ($p < 0.05$) decreasing of lightness (L*). Lightness in food is related with many factors, including the concentration of pigments present. Addition of 1% sweet pepper extracts nano-capsules led to significantly increase (< 0.05) in redness (a*) in all ketchup fortified samples when compared to control sample. This may be due to nano-encapsulation that protects ketchup from oxidation. In the case of sweetness, results showed that there were an increase in sweetness (b*) by (< 0.05) were the value ranged (16.14,16.15.16.17,16.14 and 16.16) for water, alcohol, super critical , utlrasound and microwave extracts nano capsules respectively when compared to control samples. These results proved that the sweetness of food was increased with using green emerging technologies when compared to the conventional ones [46].

Table (8): Comparison of pH, Brix° and color values (L*, a* and b*) of ketchup samples fortified with sweet pepper extracts nano-capsules

Ketchup samples types	Parameter					
	Moisture %	pH	Brix°	color values		
				L*	a*	b*
Control	64.35 ± 2.1^{a}	4.06 ± 0.3^{a}	29.85 ± 3.1^{a}	39.88 ± 3.5^{a}	29.25 ± 3.0^{a}	16.11 ± 1.3^{a}
Ketchup fortified with SPWEN*	64.14 ± 2.1^{a}	4.04 ± 0.3^{a}	29.43 ± 3.2^{ab}	39.02 ± 3.6^{c}	29.27 ± 3.0^{a}	16.14 ± 1.3^{a}
Ketchup fortified with SPEEN*	64.13 ± 2.1^{a}	4.04 ± 0.3^{a}	29.23 ± 3.2^{b}	39.16 ± 3.4^{ab}	29.28 ± 3.0^{a}	16.15 ± 1.3^{a}
Ketchup fortified with SPSCF-CO_2 EN*	64.13 ± 2.1^{a}	4.04 ± 0.3^{a}	29.63 ± 3.1^{ab}	39.05 ± 3.5^{b}	29.30 ± 3.0^{a}	16.17 ± 1.3^{a}
Ketchup fortified with SPUAEN*	64.12 ± 2.1^{a}	4.04 ± 0.3^{a}	29.43 ± 3.2^{ab}	39.04 ± 3.4^{b}	29.26 ± 3.0^{a}	16.14 ± 1.3^{a}
Ketchup fortified with MAEN*	64.13 ± 2.1^{a}	4.04 ± 0.3^{a}	29.13 ± 3.2^{c}	39.17 ± 3.3^{ab}	29.28 ± 3.0^{a}	16.16 ± 1.3^{a}

Where; Numeric description of color using L*a*b* CIELAB color space; L* (lightness or darkness) ranges from black (0) to white (100); a* color direction in red (a* > 0) or green (a* < 0); b* color direction in sweet (b* > 0) or blue (b* < 0); SPWEN*: Ketchup fortified with 1% sweet pepper water extract nano-capsule; SPEEN*: Ketchup fortified with 1% sweet pepper ethanol extract nano-capsule; SPSCF-SO_2EN*: Ketchup fortified with 1% sweet pepper SCF-SO_2 nano-capsule; SPUAEN*: Ketchup fortified with 1% sweet pepper ultrasonic extract nano-capsule; SPMAN*: Ketchup fortified with 1% sweet pepper microwave extract nano-capsule

4. Conclusions and Prospects

Today, great interest directed towards plants bioactive compounds extracted by novel green technology to build up strong immune system.. Functional food market has expanded over the years, due to consumers demand for healthy food products, reducing the use of synthetic preservatives and improves the products quality [47].

This study proved that SCF-CO_2 extract of all plant samples yielded a high yield, total phenolic content, total flavonoids content and antioxidants activity followed by microwave and ethanolic extraction. Nano-encapsulation of sweet pepper extracts with their highly content of phenolic content enables the minimally invasive delivery of sustained concentrations to a particular site. The future of natural compounds nano-encapsulation such natural compound (i.e. polyphenols) is encouraging for enhancing the effectiveness of pharmaceuticals and functional foods and maximizing the usage of these bioactives' health advantages.

Ketchup (1%) which was tried after number of experiments with different concentrations) incorporated with SCF-CO_2 sweet pepper extract nano-capsules had higher values of color, taste, aroma, appearance, odor, texture and overall acceptability than other extracts nano-capsules.

Supercritical Fluids CO_2 followed by microwave extraction (considering as green emerging technologies) is highly efficient technique that provides good yields and preserves the physico-chemical characteristics of the extracts. They could be with nano-encapsulation good technique to preserve antioxidant activities, total phenolic and flavonoid compounds during food processing. Plant extracts with their bioactive compounds could be used in a variety of industries, including the food, pharmaceutical, and cosmetic ones. In the latter, it might take the role of the synthetic antioxidant now being used to combat the harmful effects of synthetic additives on public health.

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Data Availability

Data sharing is not applicable to the main text. Data in the supplementary figures are available on reasonable request from the corresponding author.

COMPETING INTERESTS

The Authors declare no Competing Financial or Non-Financial Interests

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