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Nano-Capsulation of Ginger, Red Cabbage and Broccoli Ball Mill Extracts As Sources of Anti-Oxidant and Anti-Cancer and Application in Lentil Soup Powder

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Abstract

The objective of this study was carried to evaluate the possibility of replacing synthetic antioxidants with natural ones. The conventional method and ball mill process were used for grinding dried red cabbage, ginger, and broccoli with respect to their antioxidant activity, total phenolic, and flavonoid contents. Ball mill processes improve efficiency yield and therefore antioxidant capacity. Encapsulation efficiencies of extracts were found to be higher significantly for phenols and flavonoid content as in HPLC analysis showed. DPPH radical scavenging activity of broccoli nano-capsule gave the highest results followed by the red cabbage nano-capsule extract. Chelating capacity Fe^{2+} and iron reducing ability showed that ginger nano-capsule extract increased significantly after ball mill than its extract. Samples extracts and their nano-capsules were evaluated as an effective anticancer for HepG-2 and colon caco-2 cells. Thermogravimetric analysis (TGA) was used to study the thermal stability of all extracts and its nano-capsule which showed an enhancement in the thermal stability of broccoli, red cabbage, and ginger nano-capsules at about 320.33, 402.91, and 319.60°C, respectively. Accordingly, it was possible to use the broccoli nano-capsule extract with the highest thermal stability in preparing lentil soup powder as a functional food to deliver its purpose as an antioxidant and a high anti-cancer.

Keywords: red cabbage, broccoli, ginger, ball mill process, nano-encapsulation, antioxidant activity and anti-cancer, application in lentils soups powder

1. Introduction

Food is a main source of many important compounds for humans to obtain nutrients provide the body's needs for growth and energy production, in addition to that, food is a source of many active biological compounds that have many protective effects. Vegetables and spices are among the most important foods rich in bioactive compounds such as red cabbage, broccoli and ginger [1].

Broccoli (*Brassica oleracea*) is characterized by containing many important nutritional compounds such as minerals, dietary fibers, phenolic compounds and glucosinolates, which makes broccoli and its derivatives an important role as an antioxidant, anti-microbial, anti-cancer and has a role in regulating many vital processes in the body [2].

Red cabbage (*Brassica oleracea*) is considered one of the most widely consumed vegetables due to its high nutritional value and many active vital compounds such as vitamins and dietary fiber. In addition, it contains many flavonoids and phenols, which give the extract, prepared from red cabbage the antioxidant, anti-inflammatory, anti-carcinogenic, bactericidal, antifungal, and antiviral activities, which contribute to the prevention of many chronic diseases, heart disease and diabetes [3].

Ginger (*Zingiber officinale*) is a herbaceous plant and its roots are used as one of the most famous spices due to its pungent taste and aromatic smell due to the

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fact that it contains volatile organic compounds, carbohydrates, protein, fats, dietary fiber, vitamins and many phenolic compounds, the most important of which are gingerols, shogaols, zingiberene, terpenes and parado such as bisabolene, sesquiphellandrene and farnesene. This composition gives ginger many biological properties, as gingerol is antimicrobial, anticancer, antioxidant, anti-inflammatory and anti-allergic analgesic. Also, shegole is anti-coughing effects, and recent studies have proven many health benefits of ginger [4].

Many of the causes of oxidative stress, such as free radicals and smoking etc, lead to the disintegration of collagen, the spread of protein and the decomposition of fats in living cells, which causes apoptosis, as well as the emergence of aging and many diseases such as cardiovascular, diabetes, cancer and many neurological diseases. Many recent studies have mentioned the role of active biological compounds found in food sources reducing the effect of oxidative stress [5].

The different grinding processes that were done to powder the plant parts to a small particle size were obtained by vigorous grinding, with an increase in the extraction yield [6].

Reducing particle size by grinding not only increases the diffusion of bioactive compounds, but also leads to the rupture of cell walls. Because particle size is one of the critical factors affecting extraction [7], this may raise the question whether the extraction of bioactive compounds (phenolic compounds) as reported by others was achieved through the extraction process or simply during sample pretreatment (grinding).

Nanotechnology represents the modern scientific trend to increase the use of elements and compounds by reducing particle size to the level of nano-particles. Recent research has used nanotechnology to convert natural active compounds in food sources like natural plant extracts into nano-particles to increase their potency as an antioxidant, anti-inflammatory, anti-cancer and to reduce the occurrence of oxidative stress in living cells [8].

The aim of this study was to extract and characterize new natural ingredients (bioactive ingredients) from natural sources such as red cabbage, broccoli, and ginger that can be used in functional foods after being encapsulated in nano-capsules with a natural wall material (Arabic gum) to protection and enhance its anti-oxidant and anti-cancer efficiency, and trying to apply it as a supplement in lentil soup powder.

2. Materials and Methods

2.1. Materials

Raw materials: Red cabbage (*Brassica oleracea* var. capitata), Broccoli **(***Brassica oleracea* var. italic) and Ginger (*Zingiber officinale*) were obtained from local market in Cairo, Egypt.

2.2. Chemicals

Ethanlo 70%, Arabic gum, Tween 20, aluminum chloride, sodium nitritr, quercetin, 2,4,6-tripyridyl-5-triazin (TPTZ), acetate buffer, ferric chloride hexahydrate, 2,2-diphenyl-2-picrylhydrazyl radical (DPPH), Folin-Ciocalteus phenol reagent and Gallic acid were purchased from Sigma, Chemicals Company.

2.3. Preparation of plant powders (broccoli, red cabbage, ginger).

2.3.1. Drying process

To facilitate the extraction of bioactive compounds from the plants under study, the drying process must be carried out as follows: broccoli inflorescences (edible buds and florets), red cabbage leaves and ginger were cleaned, washed and cut into small pieces, and dried in a hot air oven at 40°C for 24hr over night, then grinding used a conventional lab mill to get a powder of broccoli, red cabbage and ginger. The powders were obtained and packed into polyethylene bags and kept in refrigerators at 4°C until analysis and use.

2.3.2. Ball milling processing

Techniques were used to reduce the particle size of the powders which differ from the conventional mixing method by ball mill (model: PQ-N2 planetary ball mill, gear drive 4 station - planetary ball mill, 220V) to prepare ultrafine particles from Red cabbage, ginger and broccoli powders according to Joni *et al.,* [9] and Inkyo *et al.,* [10]. 25g of powder for each sample was individually ball milled into a 200ml agate beaker containing 130 numbers of zirconia beads in the range from 0.5 to 1.5 mm (75 beads 0.5mm, 30 beads 1.0mm and 25 beads 1.5mm diameter) and grinding at 40,000 rpm in a high power planetary ball mill. The ginger, broccoli and red cabbage powders were crushed separately for 90 minutes to get a accurate powder. The ball mill process was used as a dispersing agent to prevent the particles from clumping and their contents could easily escape during ethanol extraction step.

2.3.3. Extraction of bioactive compounds

The bioactive compounds of the plant powder for both conventional or ball milling process were obtained with ethanol 70% (500gm for each powder in 1500ml ethanol), then the extracts exposure to

ultrasonicator for 30min and centrifugate at 3000 rpm for 20min , then evaporated by using rotary evaporation at 40°C to obtained bioactive compounds in supernatant phase and preservation in brown bottle at 4°C [11].

2.3.4. Preparation of Nano-encapsulation

The extract of the bioactive compounds of red cabbage, ginger and broccoli was encapsulated in Arabic gum using nanotechnology techniqe. 3% solution of Arabic gum was prepared by dissolving 3g of Arabic gum powder in deionized water (100ml) according to the method of Vasiliki and Constantina [12] with continuous stirring by magnetic stirrer at 2000rpm for 60min and storage in refrigerator at 4°C for 12hr to forms the jelly state and swelling.

Nano-emulsions were prepared using an oil in water system, 10g of sample extract was added separately to 10 ml of deionized water (1:1 w/v ratio) and 1% emulsifier (Tween 20) was added. The mixture was done by adding water gradually to avoid formation of air bubbles during homogenization by magnetic stirrer for 2000 rpm at room temperature until complete dissolution and then exposing the mixture to high-speed homogenizer (Model: 400ELPC, PRO Scientific Inc., 01- 02411ELPC HOMOGENIZER, USA) at 20,000rpm for 20min in the presence of an ice water bath to reduce the temperature of the mixture, all samples stored for 24h prior to encapsulation at 4°C.

To prepare the nanocapsules, 50ml of 3% Arabic gum solution was gradually added to the previously prepared nanoemulsion (10ml) (5:1 v/v ratio) and stirred with an ultrafast homogenizer at 20,000 rpm for 10 min. The mixture was applied with an ultrasonic probe (MTI Corporation, Model UD150SH3.8LQ, USA) for 20min. at 30°C. Then store in tightly closed brown containers at a temperature of 4°C until use and study the physical and chemical properties of the nanometer capsules.

2.3.5. Solvent extraction

Five grams from each plant samples ground either conventional or ball mill were extracted of bioactive compounds with 100 ml of 70% ethanol according to Junka *et al.,* [13]. The mixture was stirred with a magnetic stirrer at 2000 rpm for 30 min., then filtered with Whatman Paper No. 1 to obtain the collected aqueous fractions and then evaporated using a rotary evaporator at 40 °C for 5 min. and determine the bioactive compounds contents as anti-oxidants and anti-carcinogens. The extracted samples were kept in a refrigerator at 4 °C until use.

2.4. Chmical properties

2.4.1. Total phenolic content (TPC)

The estimation of TPC in previous extracts was done using Folin-ciocalteu procedure of Radosevic *et al.,* [14]. Folin-Ciocalteu reagent 2.5ml (10%) and 2ml of sodium carbonate solution (7.5%) were added to 0.5 ml of each extract. The mixture was incubated for 5min at 50°C, cooled at room temperature and the absorbance was measured at 765nm using distilled water as a blank. The amounts of TPC were calculated using a calibration curve for Gallic acid within the range of 50-400 mg/100ml. The results were expressed as mg of Gallic acid per 100g of dry matter.

2.4.2. Total flavonoid content (TFC)

TFC was determined by aluminum chloride method described by Bhandari and Kwak, [15]. One ml of the extracts was kept in a 15ml Falcon tube containing 5ml of distilled water, and then 0.3ml of 5% sodium nitrite was added. After 5min, 0.6ml of AlCl3 (10%) was added to the solution, then 2ml of 1M NaOH was also added, and the volume was completed to 10ml using distilled water. The solution was mixed, and the absorbance was measured at 510nm. TFC amounts were calculated using quercetin calibration within the range of 20-200mg/100ml. The results were expressed as mg of quercetin equivalent per 100g of dry matter.

2.4.3. Antioxidant activity

2.4.3.1. Ferric-reducing antioxidant power (FRAP).

The FRAP assay was conducted according to Junka *et al.,* [13]. This method is based on the reduction of 2,4,6-tripyridyl-s-triazin (TPTZ) with ferric chloride hexahydrate which is almost colorless. This reaction forms a blue ferrous complex after its reduction.

The reagent used was prepared from; solution (1): 10 mmol/L- solution of TPTZ in 40mM of hydrochloric acid, solution (2): 20 mM ferric chloride hexahydrate, solution (3): 20mM acetate buffer pH (3.6). The three solutions were mixed at 1:1:10 ratios. 1500µL of fresh FRAP reagent was added to 10µL of each of the above extracts, then, the mixture was kept in the dark for 30 min, and the absorbance was measured at 593 nm.

2.4.3.2. DPPH radical scavenging activity

The free radical scavenging activity of the bioactive compounds extracts was evaluated by 2,2-diphenyl-2-picryl-hydrazyl (DPPH), according to Alachaher *et al.,* [16]. Ten µL of extract were mixed with 1ml of 0.1mol/L DPPH in methanol. After incubating at room temperature for 30 min in the dark, the absorbance of the mixture was measured at 517nm

using a spectrophotometer (Shimadzu model 1601, Japan). The percent of antiradical activity (ARA) against DPPH was calculated according to the following equation:

$$ARA\% = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.4.4. HPLC analysis

The phenolic compounds content of broccoli, red cabbage, and ginger either ball mill extracts or its nano-capsulate were analyzed using HPLC in Agricultural, Cairo University, the environmental conditions were 25°C and humidity: 38% hr. Agilent 1260 Infinity HPLC Series (Agilent, USA), equipped with Quaternary pump, the column used akinetex-R 5µm EVO C18 100mm×4.6mm. (Phenomenex, USA), operated at 30°C. The separation is achieved using a ternary linear elution gradient with (A) HPLC grade water 0.2% H3PO4 (v/v), (B) methanol, and (C) acetonitrile. The injected volume was 20µL. Detection: VWD detector set at 284nm.

2.5. Physical properties

2.5.1. Encapsulation efficiency (EE)

Encapsulation efficiency (EE) was estimated spectrophotometrically following the procedure of Benzie and Strain [17] by FRAP assay. Fifteen milliliters of ethanol absolut were added to 1.0g of broccoli extract nano-capsule not-ground (B-NCE), broccoli extract nano-capsule ground by conventional method (B-NCCE) and broccoli extract nano-capsule ground by ball mill (B-NCBME) in a glass jar with a lid and was shaken by hand for 2min, at room temperature. The solvent mixture was filtered through a Whatman filter paper no.1 and the solvent was left to evaporate at room temperature, until constant weight. Encapsulation efficiency (EE) was calculated as follows:

EE (%) = (Wt/Wi) ×100

Where: Wt is the total amount of bioactive compounds extract in the capsule suspension and Wi is the total quantity of the extract added initially during preparation.

2.5.2. Transmission Electron Microscopy (TEM)

The morphological characteristics for the ball milled plant extracts capsules [broccoli nano-capsule ball mill extract (B-NCBME), red cabbage nano-capsule ball mill extract (RC-NCBME) and ginger nano-capsule ball mill extract (G-NCBME)] were measured by using transmission electron microscopy (TEM) according to the methods described by Saloko *et al.,* [18]. Twenty microliters of diluted extract-capsule were placed on a film-coated 200-mesh copper specimen grid for 10 min and the fluid excess was eliminated using filter paper. The grid was then stained with one drop of 3% phosphotungstic acid and allowed to dry for 3 min. The coated grid was dried and examined under the TEM microscope.

2.5.3. Thermogravimetric analysis (TGA)

The thermal stability of plant ball milled extracts and its nano-capsules as follows: broccoli ball milled extract (B-BME); broccoli nano-capsule ball milled extract (B-NCBME); red cabbage ball milled extract (RC-BME); red cabbage nano-capsule ball milled extract (RC-NCBME); ginger ball milled extract (G-BME) and ginger nano-capsule ball milled extract (G-NCBME) were estimated by Thermogravimetric analysis (TGA). The TGA analysis was performed using the device model STDQ600 (TA company, USA). The test method was followed; the fine powder was heated in Pt-holder together with another Pt– holder containing AL_2O_2 as a reference material under inert gas. The analysis was carried out under controlled conditions included, including employing a rate of heating rate of 10°C/min under a nitrogen atmosphere and from 25 to 600°C [19].

2.6. Cytotoxicity effect (Cell line)

The effect of red cabbage, ginger and broccoli ball milled extract (RC-BME, G-BME and B-BME) and its nano-capsules (RC-NCBME, G-NCBME and B-NCBME), respectively on colorectal (caco-2) cancer cells and human liver cancer cells (Hepg2) was carried out at the Faculty of Agriculture, Cairo University; and analyzed, as previously described by Repetto *et al.,* [20], and Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nature Protocols, (2008) 1×106 cells (caco-2) cells and (Hepg2) were treated with the IC_{50} of investigated extract values for 24hr, washed twice with PBS, fixed in 70% ethanol, and stored at 4°C, then add 200ml staining solution and incubated for (30min) in dark at room temperature.

2.7. Application

2.7.1. Preparation of different lentils soups powder

The lentils soups powder were prepared from (lentils, water, onion powder, garlic powder, salt, and spices), then boiled together for 10-15 minutes at 110 °C. According to Safaa, [21], the prepared soup samples were then poured. The prepared lentils soup were supplemented with different concentrations (0.1, 0.2, and 0.3%) from broccoli nano-capsulate ball milled extract (B-NCBME). The supplemented prepared

lentil soups were dried in an air oven at 40°C overnight, and then 10g of different supplemented lentil powders were rehydration with 100 ml water and ready for sensory evaluation.

2.7.2. Sensory evaluation of soups

The sensory characteristics of the prepared soups were evaluated according to Farzana, *et al.* [22]. Twelve panelists from the staff members of Food Quality Assurance, National Organization for Drug Control and Research were chosen to evaluate the products. The panelists were asked to score color, odor, taste, Homogeneity, Precipitation, and overall acceptability each on a scale of 1(extremely poor) to 10 (excellent).

2.8. Statistical analyzes

The results were statistically analyzed according to the statistical analysis system SAS, [23] (1999). Duncan's 5% level of significance was used to compare between means according to Sendecor and Cochran, [24].

3. Results and Discussion

The plant extracts were obtained from red cabbage, ginger and broccoli samples in two different grinding. The first method depends on a conventional grinding procedure for dry samples, and the second method depends on a grinding procedure using ball milling to increase the extraction yield with raising the efficiency yield of bioactivity compounds.

3.1. Chemical Analysis

3.1.1. Total phenolic Content (TPC)

Total phenolic content (TPC) for extracts of three plant samples and its nano-capsules in Table (1) showed that the ball milling process for samples have a higher antioxidant capacity compared to the milled samples by conventional methods. While, the nano-capsulation of samples ball milling extract achieved the highest values of total phenolic content.

Table 1

Total phenolic content of plant conventional mill extracts, plant ball mill extracts and plant nano-capsule ball mill extracts

Plants	Total phenols contents (mg GAE /100 g DM) of extracts		
	Conventional grind extract	Ball mill extract	Nano-capsule ball mill extract
Red cabbage	4300^{cC} ±4.1	5730^{cB} ±3.2	6890^{bA} ±3.4
Broccoli	6100^{aC} ±3.6	6620^{aB} ±4.8	7800^{aA}±2.4
Ginger	5850^{bC} ±6.4	6130^{bB} ±3.6	7730^{aA}±2.3

(n=3) Means ± SD; Means in the same column across sample with different small letters are significantly different ($p<0.05$).
Means in the same raw across treatments with different capital letters are significantly different ($p<0.05$).

The results showed that the effect of ball milling treatment and the nano-encapsulation of ball milling extracts was found the highest values of TPC for broccoli extract, followed by ginger and red cabbage extracts being 6620, 6130 and 5730 mg GAE/100 g DM, respectively, compared to the TPC values from extracts that grinded by conventional method.

The lowest of TPC values were found for extracts of samples grinded by conventional methods being 4300 mg GAE /100 g for red cabbage extract, followed by ginger extract 5850 mg GAE /100 g, and the highest TPC value was found for broccoli extract 6100 mg GAE /100g DW.

Significant differences were observed between the TPC values of samples extract that nano-capsulated after ball mill process which recorded higher values was reached a maximum increase in the TPC values for broccoli extract being 7800 mg GAE /100 gm followed by ginger extract 7730 mg GAE /100 g and finally red cabbage extract 6890 mg GAE /100 g. Accordingly, there were no significant differences between the extracts nano-capsulated in the last column under the same treatment. While, there were significant differences in the extracts grinded by conventional methods. Therefore, it can be pointed out that the used of nano-capsulation technique and ball milling process for plant extracts able to transform the particles into nano-scale and increased of yield extraction and its effectiveness as antioxidants, due to its high content of TPC values compared to its other counterparts. The results were in agreement with Soo *et al.,* [25] they found that the encapsulation process lead up to increase of bioactivity compounds and extraction yield.

3.1.2. Total Flavonoid Content (TFC)

The TFC values were determined in Table (2) for three samples extracts for red cabbage, broccoli and ginger grinded by either conventional method or ball mill process and its nano-capsulated, the ginger extract grinded by conventional method had the highest values of TFC being 2410 mg QE (quercetin equivalents)/100 g DW, and the lowest values of TFC was found for broccoli extract 2220 mg QE/100g,

while the TFC values was increased after ball mill process and higher than broccoli extract 2850 mg QE/100 g DW followed by ginger extract 2600 mg QE/100 g DW compared with that obtained after nano-capsulate ball mill extracts. The highest of TFC values in extract of broccoli nano-capsule was 2600 mg QE/100 g DW, while the values were almost equal for extracts of red cabbage and ginger nano-capsulated being 3130 and 3100 mg QE/100 g DW, respectively.

Table 2

Total flavonoid content of plant conventional grind extracts, plant ball mill extracts and plant nano-capsule ball mill extracts

Plants	Total Flavonoid (mg QE /100 g DM)		
	Conventional grind extract	Ball mill extract	Nano-capsule ball mill extract
Red cabbage	2300^{bC} ±4.1	2435^{cB} ±3.2	3130^{bA} ±3.4
Broccoli	2220^{cC} ±3.6	2850^{aB} ±4.8	3600^{aA}±2.4
Ginger	2410^{aC} ±7.4	2600^{bB} ±3.6	3100^{bA}±2.3

(n=3) Means ± SD; Means in the same column across sample with different small letters are significantly different ($p<0.05$).
Means in the same raw across treatments with different capital letters are significantly different ($p<0.05$).

In this context, Yan *et al.,* [26] reported that arabic gum micro capsulated were more efficient in capsulation of citrus flavonoid, stable structure, and highest values yield (72 – 74%) and encapsulation efficiency (97.60 - 99%). Also, Toni *et al.,* [27] suggested that the Arabic gum was suitable for the encapsulation of red cabbage anthocyanin.

3.1.3. Antioxidant activities

3.1.3.1. Ferric reducing antioxidant power

The Ferric reducing antioxidant power of different extracts for ability decrease convert (Fe^3 + Fe) to (Fe^2 + Fe) was showed in Table (3).

The results showed that the nano-encapsulation for red cabbage, broccoli and ginger extracts was conferred bioactive compounds high reductive capacity compare to taht non-capsulation ones. The ginger extract grinded by conventional method had reducing ability in the range of 0.380 g AAE/100g followed by red cabbage extract being 0.310 g AAE/100g while the reducing ability of these extracts were increased after ball mill process being 0.432 and 0.420 g AAE/100g, respectively.

However, the nano-capsules of these extracts showed a high reducing power compared to the others extracts, the ginger nano-capsule ball mill extract being 0.440 g AAE/100 gm and the reducing power was slightly lower in both broccoli and red cabbage extracts that nano-capsule ball mill being 0.340 and 0.410 g AAE/100g, respectively compared to the same extracts without encapsulation.

Table 3

Ferric reducing antioxidant power of different extracts of plants and its nano-capsule

Plants	Ferric reducing antioxidant power (g AAE/100 g)		
	Conventional grind extract	Ball mill extract	Nano-capsule ball mill extract
Red cabbage	0.310^{bB}±0.02	0.420^{bA}±0.02	0.410^{bAB}±0.03
Broccoli	0.291^{bC}±0.02	0.320^{cB}±0.01	0.340^{cA}±0.03
Ginger	0.380^{aB}±0.03	0.432^{aA}±0.05	0.440^{aA}±0.06

(n=3) Means ± SD; Means in the same column with different small letters are significantly different ($p<0.05$).
Means in the same row with different capital letters are significantly different ($p<0.05$).

The correlation between phenolic content of bioactive compounds and antioxidant activity being positive relationship was observed by Busra *et al.* [28]. The results revealed that Arabic gum and capsulation of phenolic compounds can be added to food industries to enhance the phenolic compounds. Also, the percentage of Arabic gum used in wall materials for encapsulation process had no effect on the effectiveness of antioxidants.

3.1.3.2. DPPH radical Scavenging Activity %

The results in Table (4) show that the effect conventional method, ball mill process and its nano-capsulation on the antioxidant activity of red

cabbage, broccoli and ginger extracts. The Red cabbage conventional grinded extract was 69.4%; but the inhibition of the scavenging activity after ball mill process for extract was increased to 74%, the highest increase for the extract after the nano-encapsulation process being 79.2%.

The DPPH radical scavenging activity of ginger extract ground is 72.1%, but the increase of inhibition for the ginger nano-capsule ball mill extract was relatively low at 74% and it was 75.2% for the ginger ball mill extract, this is due to the efficiency of nano-encapsulation and also to the extent of the extract particles in small sizes.

The broccoli extracts showed a lower inhibition of free radical scavenging activity at 65 and 66 % of broccoli ground by conventional method extract (B-CE) and broccoli ground by ball mill process (B-BME), respectively. While the broccoli nano-capsule ball mill extract (B-NCBME) showed the highest inhibition of free radicals, it was 80% compared to the other extracts.

The Increasing ability of scavenging activity for free radicals is due to the distribution of the molecules of a substance in a trend that allows its diffusion within the extract, which allows the emergence of a higher activity of antioxidant compounds.

The results were corroborated by Negi *et al.* [29] who studied the antioxidant and antimutagenic activities of extracts; they suggested that it can be used after the encapsulation process in food applications and nutraceuticals.

Table 4

Radical scavenging activity (%) of different extracts of plants and it's nano-capsulate

Plants	DPPH radical scavenging activity %		
	Conventional grind extract	Ball mill extract	Nano-capsule ball milling extract
Red cabbage	$69.4^{bB} \pm 0.58$	$74^{aC} \pm 0.30$	$79.2^{aA} \pm 0.60$
Broccoli	$65^{cC} \pm 0.85$	$66^{bB} \pm 0.61$	$80^{aA} \pm 0.68$
Ginger	$72.1^{aA} \pm 0.47$	$75.2^{aA} \pm 0.40$	$74^{bA} \pm 0.40$

(n=3) Means ± SD Means in the same column with different small letters are significantly different ($p<0.05$). Means in the same row with different capital letters are significantly different ($p<0.05$).

3.1.4. HPLC Analysis

HPLC analysis of the phenolic compounds for three plant extracts either plant ball mill extracts or plant nano-capsules ball mill extracts were shown in Figure 1 (a, b, c, d, e, and f) and Table (5). The results of broccoli ball mill extract (B-BME) and its nano-capsules showed a significant increase in the concentration of phenolic compounds, especially after nano-encapsulation with Arabic gum. For example, the quercitin concentration of broccoli extract (B-BME) before the ball mill process was 58.192 mg/L, but it reached 1146.729 mg/L for nano-capsule ball mill broccoli extract (B-NCBME). Also, 3-Hydroxytyrosol was 256.604 mg/L before grinding and reached 1005.732 mg/L after grinding and encapsulation, and this increase reached three times the result before milling at RT 4.792 min., and also p-Hydroxybenzoic acid was missed in broccoli extract. While, it appeared at a concentration of 238.855 mg/L after grinding by ball milling and its nano-capsules, and this includes all ingredients with the dimension of mixing and encapsulation changed significantly. This explains that the process of nanoscale encapsulation after ball milling led to an increase in the excretion of biologically active compounds from inside plant cells of the broccoli sample, and brings about an increase in antioxidant activity content as a result of increasing the surface area exposed to interactions with increased solubility and ease of absorption. This is due to the small size of the particles (nano-scale) and the increase of charges on the molecules' surface. HPLC analysis results showed a significant increase in the phenolic compounds of the red cabbage extract. The concentration of benzoic acid before grinding with ball milling was 583.797 mg/L, while it reached 1862.543 mg/L for B-NCBME after the nano-encapsulation process. Also, the Ellagic concentration was 295.091 mg/L, and it reached 1374.175 mg/L, respectively.

In addition, the phenolic compounds of ginger ball mill extract (G-BME) showed a significant increase compared to ginger nano-capsule ball mill extract (G-NCBME). For example, the concentration of Neringein in the G-BME was 124.343 mg/L, while the concentration of Neringein in the B-NCBME was increased after the nano-encapsulation to 204.416 mg/L, which is a twin increase. The Myricetin concentration also increased from 36.126 to 185,432 mg/L, after nano-encapsulation.

This high increase in the phenol content concentration is due to nano-technology in conducting the encapsulation process, which used

ultrasonication and high-speed homogenization to reduce the size of particles and occurrence of bioactive compounds [30].

3.1.5. Total bioactive ingredients extracted and nano-capsulated

The bioactive compounds concentration of extracts and its nano-capsules of all samples for red cabbage, ginger and broccoli after ball mill process showed a significant increase especially after nano-capsule with Arabic gum compared to its extracts in Figure (2). The highest concentration were found in broccoli ball mill extract (B-BME), and broccoli nano-capsule ball mill extract (B-NCBME), which reached to 5480.88 mg/l, and then followed by red cabbage nano-capsule ball mill extract (RC-NCBME) was 4366.05 mg/l, and followed by ginger nano-capsules ball mill extract was 926.2 mg/l of biologically active ingredients.

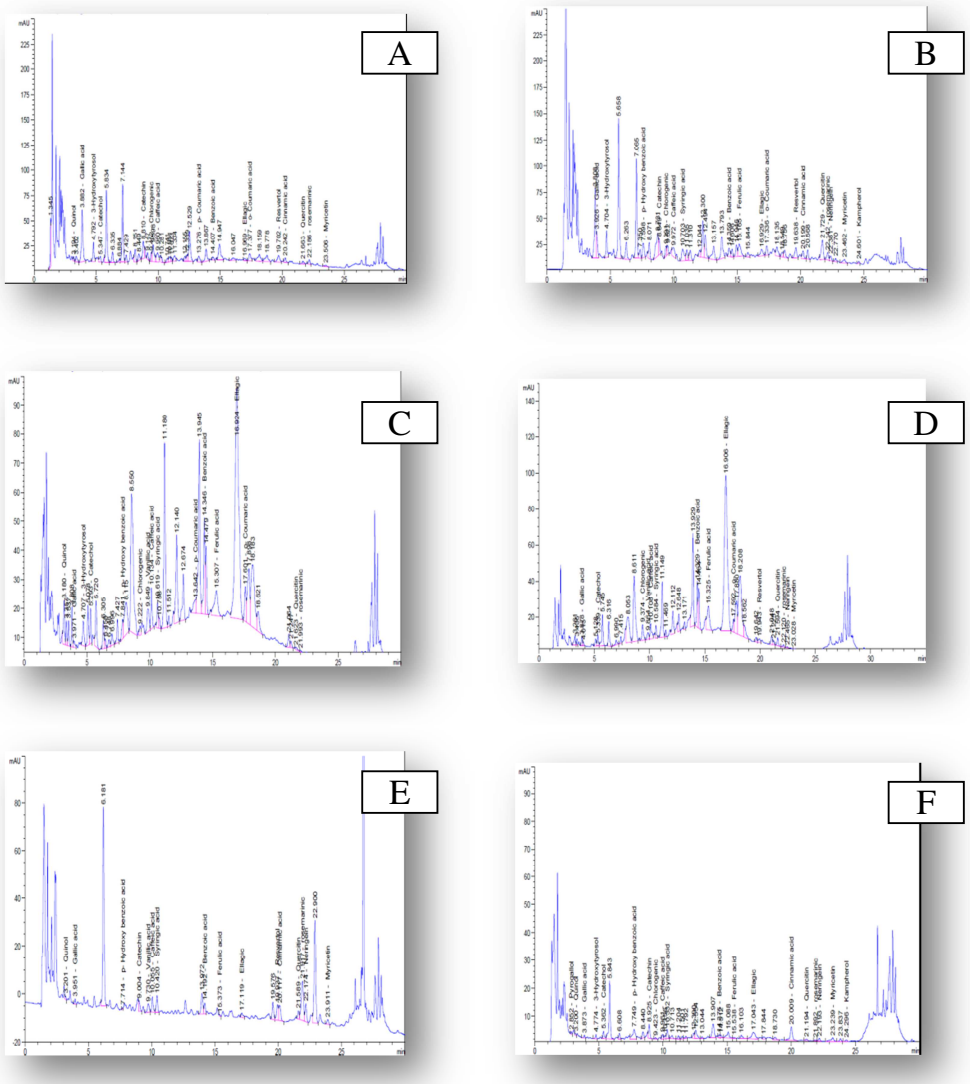

Fig. 1: HPLC Chromatogram diagrams for Broccoli, Res Cabbage and Ginger extracts after and before nano-capsulation

Where: A: HPLC diagrams for Broccoli; B: HPLC diagram for Broccoli extract nano-capsulate; C: HPLC diagram for Red Cabbage extract; D: HPLC diagram for Red Cabbage extract; E: HPLC diagram for Ginger extract; F: HPLC diagram for Ginger extract nano-capsulate

Explanation of the increased concentration of bioactive compounds due to the ball milling process, which causes the dispersal of plant cells and breaks the bonds between the molecules of the material and facilitates the exit of all the contents of plant cells to convert them into nanoparticles, allowing the release of higher amounts of their contents compared to the conventional grinding method that uses lower speeds and mechanical force. In addition, the nano-encapsulation process of these extracts leads to an increase in the surface area of particles with an increase in water solubility as a result of the formation of nano-emulsions before encapsulation using high speed homogenizer with ultrasonic to cause nano- particles dispersion with increased diffusion.

Table 5

Typical high-performance liquid chromatography chromatograms of red cabbage, broccoli and ginger extracts and its Nano-capsules after ball mill process

Bioactive component	Red cabbage				Broccoli				Ginger			
	Ball Mill Extract (RC-BME)		Nano-capsule Ball mill Extract (RC-NCBME)		Ball Mill Extract (B-BME)		Nano-capsule Ball mill Extract (B-NCBME)		Ball Mill Extract (G-BME)		Nano-capsule Ball mill Extract (G-NCBME)	
	*RT	*Con.	*RT	*Con.	*RT	*Con.	*RT	*Con.	*RT	*Con.	*RT	*Con.
3-Hydroxytyrosol	4.707	103.386	4.760	-	4.792	256.604	4.704	1005.73	4.760	-	4.774	7.134
Catechol	5.293	32.065	5.339	91.183	5.347	31.007	5.400	-	5.400	-	5.362	41.447
p-Hydroxybenzoic acid	7.841	24.188	7.700	-	7.700	-	7.598	238.855	7.714	1.116	7.749	109.291
Catechin	8.900	-	8.900	-	8.810	12.024	8.849	29.051	9.004	1.929	8.925	10.919
Chlorogenic	9.222	3.675	9.374	59.130	9.452	11.763	9.391	47.594	9.300	-	9.423	2.570
Vanillic acid	9.849	23.403	9.864	42.737	9.800	-	9.800	-	9.730	7.626	9.800	-
Caffeic acid	10.134	8.452	10.108	32.176	9.960	13.142	9.972	44.554	10.055	4.120	9.961	5.387
Syringic acid	10.619	8.164	10.548	26.897	10.500	-	10.703	77.668	10.420	6.631	10.332	15.537
Coumaric acid	13.642	4.434	13.450	-	13.276	5.34	13.450	-	13.450	-	13.450	-
Benzoic acid	14.346	583.797	14.329	1862.543	14.407	80.995	14.359	682.445	14.192	71.156	14.375	69.037
Ferulic acid	15.307	19.870	15.325	109.327	15.400	-	15.165	76.043	15.373	3.392	15.538	1.591
Rutin	16.700	-	16.700	-	16.700	-	16.700	-	16.700	-	16.700	-
Ellagic	16.924	295.091	16.906	1374.175	16.969	4.621	16.929	66.790	17.119	3.179	17.043	38.252
Coumaric acid	17.601	-	17.592	20.787	17.377	7.775	17.336	23.491	17.400	-	17.400	-
Resvertol	19.800	295.091	19.943	120.059	19.702	137.054	19.638	537.310	19.937	67.790	19.800	-
Cinnamic acid	20.200	7.250	20.200	-	20.242	2.688	20.199	14.960	20.117	3.774	20.009	14.830
Quercitin	21.623	-	21.594	221.586	21.663	58.192	21.729	1146.72	21.589	35.826	21.194	46.915
rosemarinic	21.993	-	22.120	148.940	22.186	122.298	22.142	626.132	21.917	412.16	21.892	65.734
Neringein	22.400	17.563	22.465	176.158	22.400	-	22.381	392.725	22.174	124.34	22.193	204.416
Myricetin	23.481	18.894	23.028	62.864	23.506	44.611	23.462	309.861	23.911	36.126	23.239	185.432
Kampherol	24.700	-	24.7001	-	24.700	-	24.601	65.245	24.700	-	24.296	36.858

*RT: Retention time (min), *Con.: Concentration (mg/L)

Alessandra *et al.,* [31] reported that the use of acerola by products are encapsulated products rich in bioactive compounds with antioxidant activity as functional components and are polyphenols while showing particle size, distribution and dispersion consistently.

3-2-Physical Analysis

3-2-1- Encapsulation efficiency (%)

The efficiency of the Arabic gum nano-capsules for three formula of broccoli extract (B-NCE) not-ground, milled by conventional method (B-NCCE) and ground by ball mill (B-NCBME), was estimated by FRAP test by calculating the residual activity and deducting it from the original added amount of extracts as a percentage with a more conservative observation of the biologically active components.

The efficiency of the Arabic gum nano-capsules of three formulas of non-milled broccoli extract, milled by normal methods and milled in ball mill, was estimated by FRAP test by calculating the residual activity and deducting it from the original added amount of extracts as a percentage, with a more conservative observation of the biologically active components.

The results in Figure (3) showed that the non-ground broccoli extract (B-NCE) had the lowest efficiency in nano-encapsulation due to the large particles of the

extract was 68.52%, while the broccoli extract (B-NCCE) ground by conventional milling increased the encapsulation efficiency reached 92.11%, while the highest efficiency in encapsulation was 97.81% after grinding with a ball mill (B-NCBME), which is due to the small particles that reached the nano-size, which allows to preserve the capsules of Arabic gum with high efficiency. Based on these results, the study is completed on the samples extracts previously grinding by ball mill and its nano-capsules.

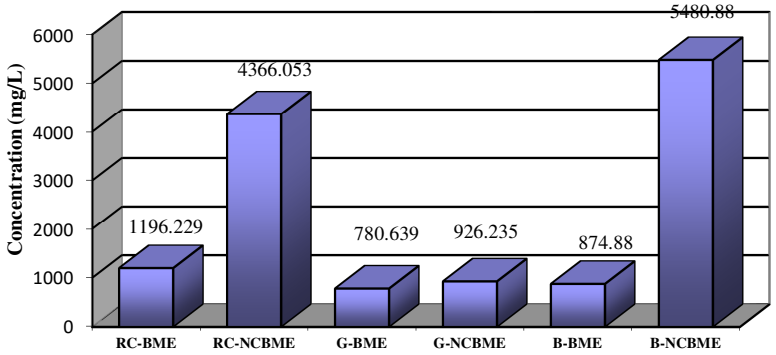

Fig. 2: Concentration of bioactive ingredients extracted and encapsulated from red cabbage, ginger and broccoli samples
Where: RC-BME is red cabbage ball milled extract; RC-NCBME is red cabbage nano-capsule ball milled extract; G-BME is ginger ball milled extract; G-NCBME is ginger nano-capsule ball milled extract; B-BME is broccoli ball milled extract; B-NCBME is broccoli nano-capsule ball milled extract.

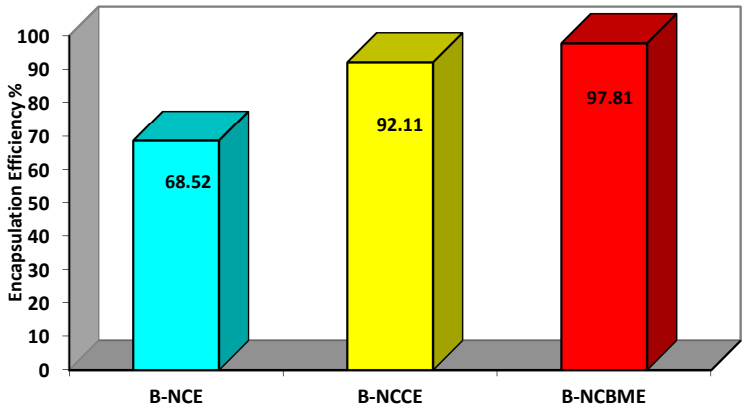

Fig. 3: Encapsulation efficiency of encapsulate broccoli extract formula
Where: B-NCE is broccoli nano-capsule without grinded extract; B-NCCE is broccoli nano-capsule grinded by conventional method extract; B-NCBME is broccoli nano-capsule ball milled extract

3-2-2- Morphology evaluation of plant extracts and encapsulated by TEM

The results in figure 4 (a, b and c) showed that the TEM of sample ball mill nano-capsules extracts (B-NCBME, RC-NEBME and G-NCBME) extents of the particles distribution for particles within into wall material.

Broccoli sample (B-NCBME) was showed in figure (4a) has a small spherical shape with a thin wall around it and most of the capsules are in the nano-scale range and have particle size diameters from 20.72 to 22.36 nm.

While, the results of TEM in figure (4c) showed that the ginger extract (G-NCBME) had the largest diameter arrived to 96.85 nm, the shape of particles was spherical, oval, and completely rotated in a homogeneous distribution within the wall material, but it was in the nano-scale range due to the inefficiency of the grinding process, which leads to poor breakage, and the grinding time had to be

increased [32]. This is due to the lower antioxidant activity of ginger extract (G-NCBME) compared to the other samples.

The TEM results in figure (4b) showed that the red cabbage extract (RC-NCBME) has particles sizes between 79.11 to 89.42 nm, which are spherical, oval and imperfectly round in random distribution within the wall material. Also, the particles in the nano-scale range.

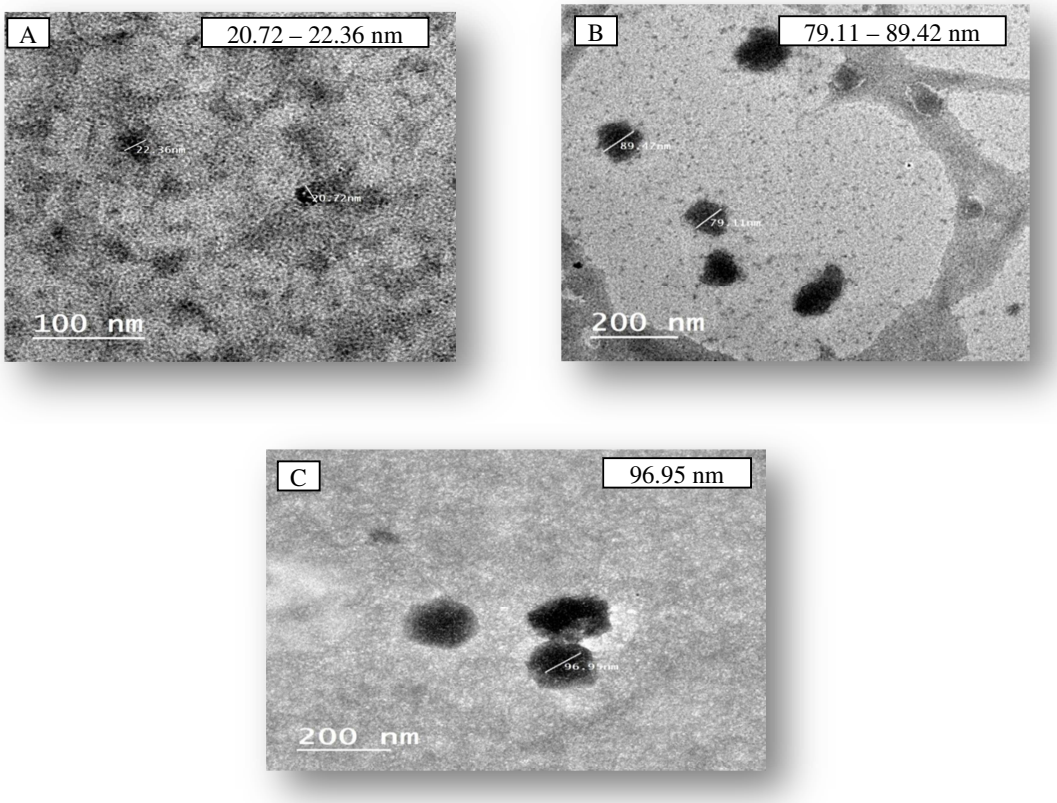

Fig.4: Transmission electron microscopy (TEM) images of bioactive compounds extract
Where: A: Brocooli extract (B-NCBME); B: Red Cabbage extract (RC-NCBME); C: Ginger extract (G-NCBME)

3.3. Thermo Graphic Analysis (TGA)

Thermal gravimetric analysis (TGA) provides useful information about the behavior of a material or bioactive ingredients during heating conditions. TGA can estimate the impact of temperature on the material's weight change that helps in understanding the thermal properties of a material.

The temperature at which the material undergoes the highest rate of weight loss is called the temperature of decomposition (Td), which is determined by estimating the first derivative of the TGA curve at a large change in weight, which is called the derived thermogravimetric (DTG). The thermal properties of the plant ball mill extract and nano-capsules ball mill extract were evaluated by TGA analysis, the results of TGA and DTG curves were showed in Figure (5a, b and c) for broccoli, red cabbage and ginger extracts, respectively. Broccoli extract (B-BME) showed a one-step weight loss with an initial temperature (Ti) of 105.23 °C and peak decomposition temperature (Td1) of 121.11 °C while the wall material (Arabic gum) was lost the weight at 163.57 °C in Figure (5; A1). A similar TGA curve was found for broccoli extract (B-NCBME) in Figure (5; A2)**.** The increase of thermal stability of B-NCBME treatment by nano-encapsulation process was retained its activity and properties from exposure to high temperature and reached to 320 °C and then occurred in weight, which makes it bear high temperatures, whether when applied as a food additives or food supplemented that has a functional to reaching its purpose, especially foods that are exposed to baking, pasteurization or sterilization temperatures.

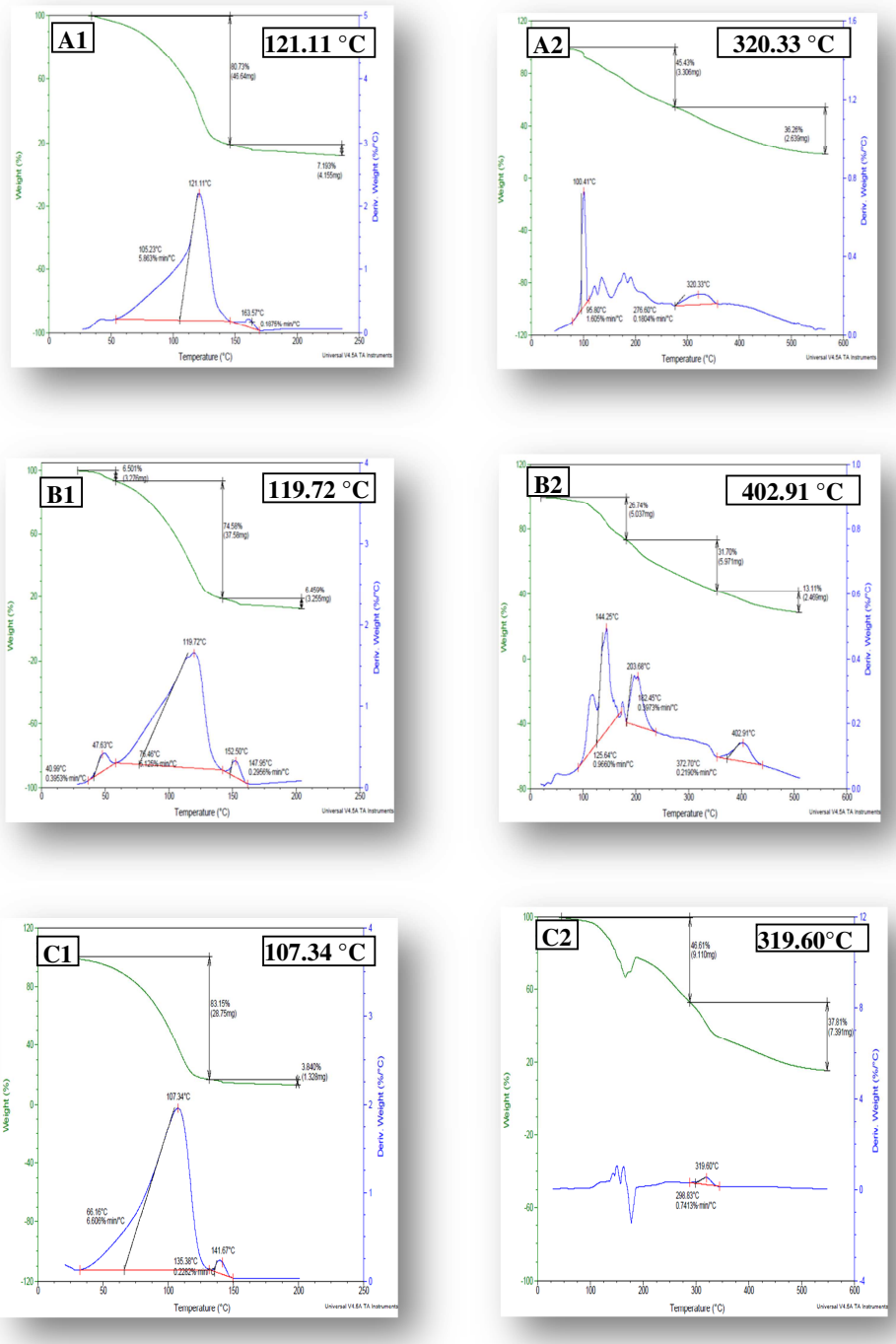

Fig. 5: Thermograms TGA for plant ball mill extracts and its nano-capsules

Where: A1 is broccoli ball milled extract (B-BME); A2 is broccoli nano-capsule ball milled extract (B-NCBME); B1 is red cabbage ball milled extract (RC-BME); B2 is red cabbage nano-capsule ball milled extract (RC-NCBME); C1 is ginger ball milled extract (G-BME); C2 is ginger nano-capsule ball milled extract (G-NCBME)

The result was agreement with that reported by Rezvan *et al.,* [33] who suggested that DSC results evidenced the thermo stability of bioactive ingredients' micro-capsules. TGA analysis was performed as represented by Figure (5 B1 and 2) to study the thermal stability of both bioactive compounds extract from red cabbage and its nano-capsule.

The TGA heat graph of RC-BME showed a one-step loss of mass at 119.72 °C (Fig. 5b1), the decomposition temperatures (Td) was 47.63°C and wall material (Arabic gum) was between 119.72°C and 152.50°C. The previous results indicate the low thermal stability of RC-BME.

On the other hand, the RC-NCBME exhibited a three-step loss of mass (Fig. 5b2) at 144.25 °C due to moisture evaporation, at 203.56 °C and at 402.91 °C, which is dedicated to the decomposition of wall martial and its cor. The wall material of RC-BME can be ability to protect the RC-NCBME to 402.91°C compared to the RC-BME, this results leads to the expansion of its use in food additives or food supplements as antioxidants.

Surprisingly, both the G-BME and the nano-capsule G-NCBME showed a single-step loss of mass was between107.34 and 319.60 °C (Fig. 5c1 and 5c2), respectively, with a degradation temperature (Td) of 83 .15% for G-BME, as it reached to 46.61o% for the G-NCBME. It may reflect the one-step loss of mass G-NCBME and wall material which decomposes at a higher temperature 319.60°C.

3.4. Cytotoxicity of bioactive compounds towards human cell lines

The bioactive compounds ball mill extracts and its nano-capsules were cytotoxic against liver (HepG-2) cell line and caco-2 cell line (Table 6).

Extracts, both nano-capsules and extracts, were individually evaluated for their effect on the viability of HepG2 cells. All tested bioactive compounds showed a cytotoxic effect on HepG2 cells of liver cancer in a concentration-dependent manner, the result also showed that the bioactive compounds ball mill nano-capsules were more active than ball mill extracts against HepG2 cells (2-fold). The most cytotoxic effect against HepG2 cells was also observed with red cabbage extract (RC-BME) at IC50 value was1.90 mg/ml, ginger extract (G-BME) was 1.40 mg/ml and broccoli extract (B-BME) was 1.60 mg/ml; while, the IC50 values after encapsulation process of RC-NCBME, G-NCBME and B-NCBME were 0.22 and 0 .18 and 0.19 mg/ml, respectively. The results was agreement with that mentioned by Li *et al.,* [34] who reported that there is combination between the antioxidant of herbs and anticancer.

Table 6

Cytotoxicity of bioactive compounds extracts and its nano-capsules against Liver and Caco-2 of human malignant cell lines

Sample	*IC_{50} (mg/ml)	
	HepG2 Liver cancer cell	Caco-2 Colon cancer cells
Red cabbage extract (RC-BME)	1.90^{a} ±0.20	2.30^{a} ±0.20
Ginger extract (G-BME)	1.40^{c} ±0.11	1.90^{b} ±0.20
Broccoli extract (B-BME)	1.60^{b} ±0.20	1.60^{c} ±0.20
Red Cabbage extract nano-capsulate (RC-NCBME)	0.22^{d} ±0.05	0.31^{d} ±0.20
Ginger extract nano-capsulate (G-NCBME)	0.18^{e} ±0.04	0.28^{e} ±0.20
Broccoli extract nano-capsulate (B-NCBME)	0.19^{e} ±0.05	0.28^{e} ±0.20

(n=3) Means ± SD Means in the same column with different small letters are significantly different ($p<0.05$). *IC_{50}: Half maximal inhibitory concentration

The human epithelial cell line Caco-2 has been widely used as a model of the intestinal epithelial barrier. The Caco-2 cell line is originally derived from a colon carcinoma. To assess the specificity of the extracts and nano-capsules for Coca-2 cancer cells, the bioactive compounds extracts showed a cytotoxic effect on Coca-2 colon cancer cells, while the bioactive compounds extracts nano-capsules were showed more active than the extracts against Coca-2 cells (6-fold) Compared to its effect on HepG2 cells. Also, the most cytotoxic effect against Coca-2 cells was observed with red cabbage extract (RC-BME) an IC_{50} value was 2.30 mg/ml, ginger extract (G-BME) was 1.90 mg/ml and broccoli extract (B-BME) was 1.60 mg/ml, while the IC50 values after nano-encapsulation process were 0.31, 0.28 and 0.28 mg/ml for RC-NCBME, G-NCBME and B-NCBME, respectively.

The decrease values of IC_{50} can be allow the use a little of concentrations of RC-NCBME, B-NCBME

and G-NCBME which allow higher inhibition of cancer cells. In this context Faezeh *et al.,* [35] suggested that the phytochemicals are associated with a prophylactic effects against cancer after protecting it with nano-capsules.

3.4.1. Liver cancer-cell

Various concentrations of red cabbage, broccoli and ginger extracts (50, 100, 200 and 400 mg/ml) were used against viability of HepG2 cancer cells. All forms of extracts for all three types of plants used in the study were decreased the viability of HepG2 cancer cells and that was more noticeable after the nano-capsules process (Table 7).

Table 7

Effect of concentration of non-encapsulated and nano-capsules extracts on the viability of liver cancer cells (HepG2)

Conc. mg/ml	Viability of HepG2 cancer cells					
	Plant Ball mill Extract			Plant Nano-capsule Ball mill extract		
	RC-BME	G-BME	B-BME	RC-NCBME	G-NCBME	B-NCBME
50	100^{aB} ±0.60	95^{aB} ±0.80	110^{aA} ±0.77	81^{aC} ±0.91	70^{aD} ±0.77	77^{aCD} ±0.49
100	92^{bAB} ±0.54	88^{bB} ±0.79	95^{bA} ±0.90	77^{bC}±0.66	62^{bE} ±0.44	70^{bD} ±0.81
200	90^{bA} ±0.49	80^{cB} ±0.68	87^{cAB} ±0.71	60^{cC} ±0.50	48^{cD} ±0.30	52^{cCD} ±0.63
400	75^{cA} ±0.43	61^{dB} ±0.58	70^{dAB} ±0.87	34^{dC}±0.60	12^{dE} ±0.20	22^{dD} ±0.22

Where: RC-BME is red cabbage ball milled extract; G-BME is ginger ball milled extract; B-BME is broccoli ball milled extract; RC-NCBME is red cabbage nano-capsule ball milled extract; G-NCBME is ginger nano-capsule ball milled extract; B-NCBME is broccoli nano-capsule ball milled extract. (n=3) Means ± SD; Means in the same column with different small letters are significantly different ($p<0.05$). Means in the same row with different capital letters are significantly different ($p<0.05$).

The results showed an inverse relationship between the concentration and viability of HepG2 cancer cells at higher concentration of extracts and lower of viability for HepG2 cancer cells (inhibiting cancer cells). For example, the G-BME at a of 400 mg/ml concentration was decreased the viability of HepG2 cancer cells from 95 to 61% compared the viability of HepG2 cancer cells for G-NCBME at same concentration, and the viability of the cancer cells was decreased from 95 % for G-BME and reached to 12% for G-NCBME after nano-encapsulation process**.**

The effect of plant extracts and its nano-capsules on HepG2 cancer cells were more effective on inhibiting the vitality of cells, and the better effect of them was G-NCBME, followed by B-NCBME and finally RC-NCBME. The results were identical with the conclusion of Khan *et al.,* [36] about the anticancer therapy of herbal plants**.**

3.4.2. Caco-2 cancer cell

The results in Table (8) showed that the relationship between concentrations of different extracts for the three types of plants (50, 100, 200 and 400 mg/ml) were an inverse relationship against Coca-2 colon cancer cells and their viability.

The decrease in viability of Coca-2 colon cancer cell was more effective for the plant nano-capsules extracts compared to the plant extracts, especially in the ginger nano-capsule extract, but the viability of Coca-2 cancer cells was decreased from 90 to 30%, a decrease of up to 60% after the nano-encapsulation process at 400 mg/ml concentration. While, 50 mg/ml concentrate of G-BME for viability of cells was 145 and decreased to 92 when used G-NCBME at the same concentrate.

The results agreed with those obtained by Alok *et al.,* [37] and suggested that the natural bioactive antioxidants may mitigate combined toxicity.

3.5. Application

Lentil soup contains low calories and is a good choice for dieters in the winter because it is one of the foods that warm the body, give it a feeling of satiety, and help it lose weight. It is also one of the popular diets for most countries of the world, especially North African countries. For this importance, it was fortified with B-NCBME in different concentrations in the form of a powder after drying and adding to the previously prepared and dried lentil soup to prolong the storage period for a long time outside the refrigerators in a dried form as a healthy food that contains anti-oxidants and anti-cancers with its full activity.

Lentil soup powder was selected as a food product and enhanced by adding B-NCBME as an example of application in food industries, the lentil soup powder was subjected to heat treatments during processing in order to study the extent ability of the nano-capsules to protect the B-BME in order to reach its functional properties as an antioxidant & anticancer in a safe form and retaining its activity.

Lentil soup was prepared in vitro fortified with different concentrations of 0.1, 0.2 and 0.3% of B-

NCBME, and then sensory properties and acceptability of the prepared products were evaluated.

Table 8

Effect of concentration of plant extract and its nano-capsules on viability of colon cancer cells (Coca-2)

Conc. mg/ml	Viability of Coca-2 colon cancer cells					
	Plant Ball mill extract			Plant Nano-capsule Ball mill extract		
	RC-BME	G-BME	B-BME	RC-NCBME	G-NCBME	B-NCBME
50	210^{aA}±1.20	145^{aC}±0.89	180^{aB}±1.20	110^{aD}±1.10	92^{aE} ±0.70	90^{aE}±0.69
100	180^{bA} ±0.90	120^{bB}±0.79	180^{aA}±1.30	95^{bC}±0.90	80^{bCD}±0.89	75^{bD}±0.70
200	160^{cA}±1.10	110^{cC}±0.10	150^{bB}±1.50	82^{cD} ±0.90	45^{cF} ±0.71	55^{cE}±0.48
400	100^{dA} ±1.11	90^{dB} ±0.97	100^{cA}±0.90	40^{dC} ±0.60	30^{dD} ±0.42	40^{dC}±0.49

Where: RC-BME is red cabbage ball milled extract; G-BME is ginger ball milled extract; B-BME is broccoli ball milled extract; RC-NCBME is red cabbage nano-capsule ball milled extract; G-NCBME is ginger nano-capsule ball milled extract; B-NCBME is broccoli nano-capsule ball milled extract; (n=3) Means ± SD; Means in the same column with different small letters are significantly different ($p<0.05$). Means in the same row with different capital letters are significantly different ($p<0.05$).

3.5.1. Sensory evaluation

Sensory evaluation of dry lentil soup samples after rehydration with hot water and supplemented with different concentrations of broccoli extract B-NCBME in Table (9). The results showed significant differences between the lentil soup samples compared to the control sample. The taste evaluation of fortified samples with concentrations of 0.1 and 0.3% was lower than the sample fortified with 0.2%, but the best of them was the control sample. The sensory evaluation of the color test of lentil soup products at 0.1% was low compared to other samples. While the concentration increase of more than 0.1 brings to decrease in sensory evaluation degrees for color products compared to the control sample. Homogeneity evaluation does not show any significant differences between samples such as Precipitation. The overall acceptance of lentil soup samples was relatively less than the control sample and the lower acceptable at 0.3%.

Broccoli extract B-NCBME shows a strong flavor and aroma in the product, and the increase in its concentration decreased sensory evaluation scores. The concentration of 0.1% fortified was better accepted by consumers compared to the control sample.

The sensory evaluation of the formulated phytochemical used is suitable for encapsulation and can be fortified hot soup [25].

Table 9

Sensory evaluation of lentils soups samples fortified with different concentrations of B-NCBME

Lentils soup samples	Color	Taste	Odor	Homogeneity	Precipitation	Overall Acceptability
B1	9.8^{a}±0.3	6.2^{c}±0.2	9.1^{b}±0.3	9.8^{a}±0.2	9.9^{a}±0.3	9.0^{b}±0.3
B2	9.8^{a}±0.3	7.0^{b}±0.2	8.6^{bc}±0.2	9.8^{a}±0.3	9.8^{a}±0.3	9.0^{b}±0.3
B3	9.7^{a}±0.2	6.1^{c}±0.2	8.0^{c}±0.2	9.3^{b}±0.3	9.4^{b}±0.2	8.5^{c}±0.2
Control	10^{a}±0.4	8.6^{a}±0.3	10^{a}±0.4	10^{a}±0.3	10^{a}±0.4	9.7^{a}±0.3

(n=10). Means in the same column with different small letters are significantly different ($p<0.05$).

B1: B-NCBME (0.1 %); B2: B-NCBME (0.2 %); B3: B-NCBME (0.3 %); Control: without any addition

Figure (6) showed that the lentil soup powders were fortified with B-NCBME at concentrations of 0.1, 0.2 and 0.3% previously prepared and the final product statue to be presented to shops was shown in Figure (6a). While Figure (6b) shows the products of lentil soup after rehydration with hot water and the sensory evaluation was conducted.

DPPH radical scavenging activity %

The chemical properties of lentil soup samples including antioxidant capacity of prepared and supplemented with different concentrations with B-NCBME were estimated compared to a control sample. The results showed in the Figure (7) that increasing the concentration of B-NCBME added to the samples increases the antioxidant capacity compared to the control sample. Broccoli extract B-NCBME exhibited dose-dependent free radical scavenging ability at concentrations 50, 100, 200 and 500 ppm was 49.88, 62.75, 70.38 and 71.22 %, respectively.

While, the control sample not supplemented with B-NCBME had its ability to scavenge free radicals 47.3%.

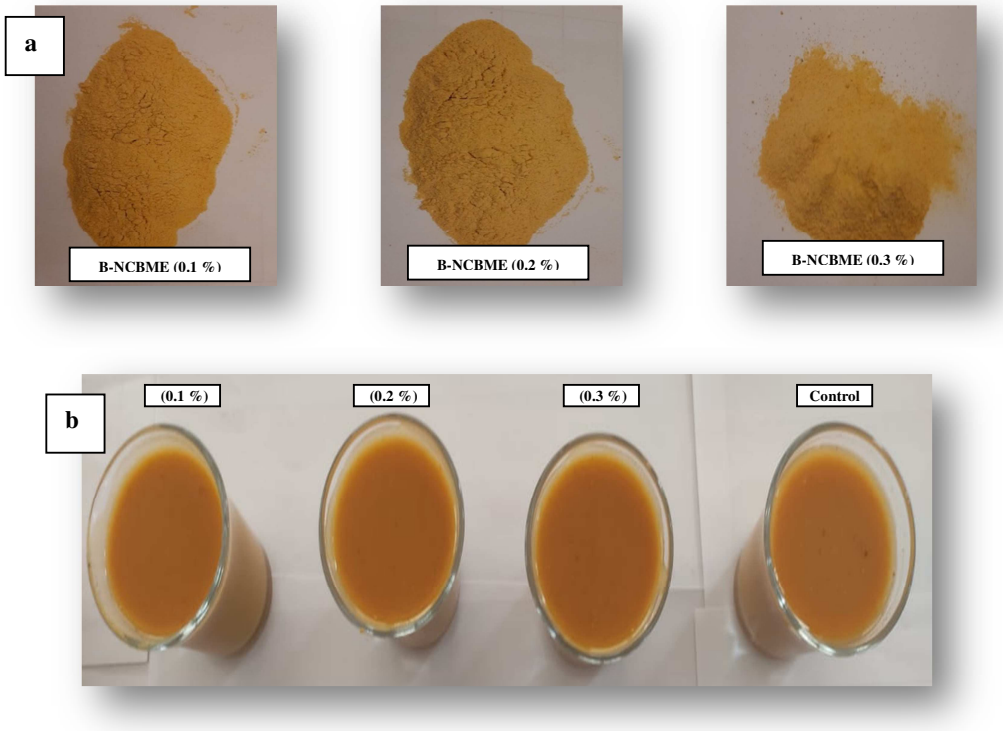

Fig. 6: Lentils soup samples supplemented with different concentrations of B-NCBME (0.1, 0.2 and 0.3 %)

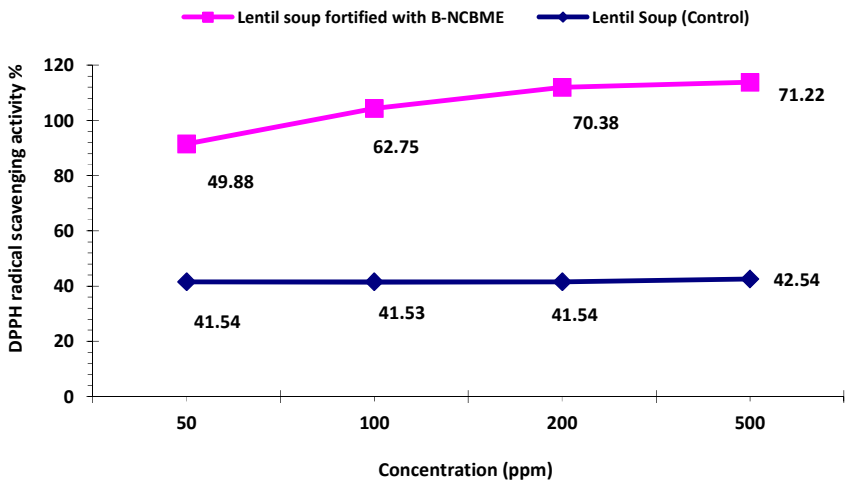

Fig. 7: DPPH Radical scavenging activity (%) of fortified B-NCBME concentrations in lentil soup samples

The radical scavenging activity (%) for different concentrations of B-NCBME in lentil soup samples showed the highest free radical scavenging activity of broccoli by 71.22% when the concentration was added 500 ppm with a scavenging increase of 28.68% compared to the control sample, while at the added concentration of 200 ppm The increase in scavenging was 28.84 % compared to the control sample, which is the same result. Therefore, the addition of B-NCBME at a concentration of 200 ppm is better economically and to prevent affecting the physical properties of product, especially odor.

4. Conclusion

Plant sources rich in their content of bioactive compounds that have antioxidant and anti-cancer

activity due to their high content of phenolic compounds and flavonoids have been utilized using nano-capsules technology as one of the most important nanotechnologies to protect biologically active ingredients. In addition, nano-emulsions were implemented before nano-capsulation.

This study confirmed that traditional methods of broccoli, red cabbage and ginger extracts and their nano-capsules formula are rich in antioxidant content and can replace synthetic antioxidants. While, the ball mill method of the samples before extraction increased their content of phenolic compounds and antioxidants for more than double the amount.

The nano-capsules process of biologically active ingredients extracts from red cabbage, ginger and broccoli were succeeded in protecting their activity due to wall material (Arabic gum) for nano-capsulation process in the ability to protect from high temperatures and also during pasteurization and sterilization on an industrial scale, especially in bakery products. This indicates that the use of Arabic gum packaging materials was better able to protect these bioactive compounds, rather than each one individually.

The nano-capsule method showed high protection for those bioactive compounds extracted from plants, with increased thermal stability, and a high protective effect against the viability of HepG2 and Coca-2 cancer cells.

The broccoli extract ball mill nano-capsule (B-NCBME) was applied in the food industry (lentil soups) as food additives and reaching the target and enhancing immunity.

5. Conflicts of interest

"There are no conflicts to declare".

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