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Effect the Addition of Micro- and Nano-Capsule Cumin and Clove Oils as Antioxidants and Anti-Cancer on Rancidity and Shelf Life in Some Biscuit Products

Ahmed M. S. Hussein; Khaled F. Mahmoud*; Mohie M. Kamil; Nefisa A. Hegazy

Food Technology Department, National Research Centre, Dokki, PO box 12311, Giza, Egypt.

Abstract

The natural properties of clove and cumin oil are well known especially as an antioxidant and anticancer, however, natural variations, thermal instability and low water solubility impose restrictions on harnessing its potential and its use in the food industry. The nano-encapsulates were prepared from clove and cumin oil separately to protect them from exposure high temperatures using natural carboxy-methyl-cellulose (CMC) coating. The results showed that the transmission electron microscopy (TEM) morphology had a particle size between 21 to 20.74 nm for clove oil capsules, while it was from 24.03 to 65.88 nm for cumin oil capsules and the DSC thermal stability of the cumin oil capsules was higher than that of clove oil 212.08 and 175.92 °C, respectively. Two types of biscuits were prepared (sweet and salt biscuits) by adding nano-encapsulates clove and cumin oil, respectively. The results of sensory evaluation showed a general acceptance of salted biscuit samples containing nano-encapsulate cumin oil, followed by sweet biscuit samples containing nano-encapsulate clove oil. The results of the anti-oxidant activity showed the highest activity nano-encapsulate of both oils, and the highest activity was clove oil coated with nano-encapsulate. During the storage stability of the biscuit samples at room temperature, the increase of acid value and peroxide value at room temperature was the least possible in the samples containing nano-encapsulate of clove oil, followed by that containing cumin oil in comparison with other samples. The anti-proliferative effect of clove oil nano-encapsulate in CMC was apparently equally strong with primary clove oil in PC3, HePG2 and MCF-7 cell lines compared to clove oil non-encapsulate.

Keywords: Essential Oils, Antioxidant Activity, Encapsulation, Sweet and Salt biscuits, Storage Stability

1. Introduction

Oxidation is one of the main factors in food degradation and reduces its nutritional value and quality. Moreover, free radicals resulting from the oxidation process are disease-stimulating agents that threaten consumer health [1].

There is great interest in using natural antioxidants instead of synthetic antioxidants due to the health risk of their use [2]. Natural antioxidants have no harmful effect and can also improve human health due to their great role as food additives with nutritional and therapeutic activity [3].

Several previous studies reported the antioxidant bioactivity of natural compounds, which are essential oils in herbs, spices and peel waste [4,5].

Cumin (*Cuminum cyminum* L.) is an annual cultivated herb of the family *Apiaceae*. It is used as a spice in particular due to the special flavor and aromatic effects in foods, cumin seeds are used as a spice commonly in cuisines of Egypt, North Africa, Middle East and the Western China. Cumin seeds have been used in the traditional treatment of toothache, dyspepsia, diarrhea, jaundice and epilepsy [6]. Volatile cumin seed oil imparts a special aroma to the seeds; also contains high amounts of phenolic compounds that show significant radical scavenging, carotenoid/ linoleic acid chelation and reduced power activities.

Clove (*Syzygium aromaticum* L.) is used either in the form of clove flowers or essential oil in Egypt, This is apart from the use of carnations in the

*Corresponding author e-mail: khaledfm69@yahoo.com ; (Khaled F. Mahmoud).
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production of cigarettes, cloves also produce an essential oil with good yield ranging from 10% to 20%. In general, clove oil contains eugenol as the main ingredient, and it has strong antioxidant activity [7] it is able to reduce the effect of free radical [8].

Clove oil is extracted from the spice *Syzigum aromaticaum*, and is characterized by its therapeutic properties as anti-inflammatory, antioxidant and anti-aging [9]. These antioxidant activities are attributed to its content of unsaturated phenolic compounds, especially eugenol, eugenol acetate and beta-caryophylline. Clove oil is also used as a food additive at an approved concentration of about 1500 parts per Million [10, 11]. However, the use of cumin oil or clove oil as a food ingredient is very limited due to the low solubility in water, and poor stability in ambient conditions, especially heat, oxidation, light and acidity [12, 13]. The organoleptic properties also change during conventional storage, as these essential oils are easily subjected to oxidation or dehydrogenation reactions due to chemical or enzymatic reactions [14]. Therefore, to avoid spoilage of cumin or clove oil during storage and processing of packages in carriers, various natural polymers such as lecithin, Gum Arabic, maltodextrin, poly-lactic glycolic acid and serum protein concentrate were used. This has also enhanced its solubility in water [15]. However, carboxy-methyl-cellulose (CMC), which is widely used as a food additive, has not been used to prepare nano-emulsions and nano-encapsulations for cumin and clove oils.

The aim of the present study was preparation of micro- encapsulation and nano-encapsulation form of cumin and clove oils using CMC and evaluation of their antioxidant and anti-cancer activities in-vitro and using cell lines, respectively. Also studying the effect of adding these prepared encapsulation form of both oils in sweet and salted biscuits to extend shelf life and preventing rancidity during the storage period

2. Experimental

Materials and Methods

Study Area:

The study was carried out at Nanotechnology Lab and Food Science & Technology Lab, Food Technology Department, National Research Centre, Egypt from October, 2019 to February, 2021

Materials

Raw materials; the plants used are cumin seeds (*Cuminum* cyminum L.) and clove flowers (*Syzygium aromaticum* L.), which were purchased from local markets spices, Egypt.

Chemicals; Ascorbic acid; ferric trichloride; Folin-Ciocalteu; potassium ferricyanide [$K_3Fe(CN)_6$], 2,2-diphenyl-1-picrylhydrazyl (DPPH); sulphuric acid; sodium phosphate; ammonium molybdate and sodium hydroxide (NaOH); trichloroacetic acid; ammonium molybdate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Methods

Extraction of cumin and clove oils

The essential oil of cumin seeds and clove flowers powder was obtained by Clavenger apparatus (hydrodistillation), using the method of water distillation according to Kiran *et al.,* [16]. Dried cumin seeds or clove flowers (160 g) were placed in a distillation apparatus with 1 liter of distilled water and distilled with water for three hours. The oil was then removed and passed through anhydrous sodium sulfate before being stored at 4 ° C until it was used.

Preparation of nanoencapsulation

Encapsulation of essential oils:

Nano-encapsulation of essential oils (cumin and clove oil) was performed using the emulsion extrusion technique described by Chan [17]. The polymer carboxymethylcellulose (CMC) was dissolved in distilled water to produce polymer solutions at a concentration of 2% w / v%. The solutions were left fixed for 24 hours to disengage before use and form a generative state. Then, the polymer solution (10 ml) and the essential oils (clove and cumin) were homogenized 2 grams of each oils separately, in a 200 ml beaker with stirring at 20000 rpm for 20 minutes with a mixture using High Speed Homogenizer in the presence of emulsifier Span 20 (1%). Oil was gradually added to the polymer solution during mixing until the required oil loading was obtained. Then, the mixture was subjected to ultrasound to form the nano-encapsulate.

While obtaining the micro-encapsulate, it requires the use of calcium chloride solution 2% (w/v in distilled water), and through a syringe, gradually dripping from the previous mixture for each oil separately to produce balls of oil coated with a polymer of CMC at 4 °C until use**.**

Transmission Electron Microscopy (TEM)

Twenty microliters of diluted samples was placed on a film-coated 200-mesh copper specimen grid for 10 min [18].

Thermal stability

The thermal stability of cumin and clove essential oil and their encapsulated profile were determined using a differential scanning calorimeter (DSC). Ten milligram samples were placed in aluminium crucibles. The samples were analyzed under a flow of nitrogen gas (40 mL/min). A dynamic scan was performed at a heating rate of 10 °C/min over a temperature range of 10 to 300°C according to Hazra et al., [19, 20].

Preparing salted cumin and sweet cloves biscuits

Various biscuit samples were prepared according to Serial *et al*., [21]. Sweet biscuits containing non-coated clove oil or microencapsulate and nanoencapsulate were prepared with adding 1.0, 5.0 and 5.0 g to wheat flour, respectively. While the salted biscuits were prepared by adding noncoated cumin oil or microencapsulate and nano-encapsulate to wheat flour 1.0, 5.0, and 5.0 g, respectively, compared to control samples without adding oil or its different formations.

Sensory evaluation

The sensory evaluation was conducted with the assistance of 20 specialists in baking technology in the Department of Food Technology, National Research Centre; Egypt. Sensory evaluation was performed in sweet and salty biscuits after and before addition of clove and cumin oil forms to evaluate color, texture, Oder taste, appearance and overall acceptability of biscuit samples. The objective sensory quality of a biscuit is described by its sensory profile consisting of sensory features according to Lawless and Heymann [22].

Color measurement

The coloration of both sides of the cookies was measured for the sweet and salted biscuit samples fortified with nano-capsule and micro-capsules of clove and cumin oil using Hunter's Lab color analyzer. In the Hunter Lab colorimeter, the color of the sample is indicated by the three dimensions, L*, a*, and b* which give the measurement of brightness, redness / greenness and yellowness / blueness of the product respectively [23].

Extraction of oil from bakery products

The oils from the varieties of bakery product samples sweet biscuits and salty biscuits were extracted through solvent extraction technique as described in the method of AOAC [24]; hexane used as solvent was recovered by Rotary Evaporator.

The different forms of cumin and clove oil were individually extracted by cold percolation method [25, 26] using hexane organic solvent. 3 g of bakery product was taken in 25 ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 2 h. After 2 h, evaporated using a rotary vacuum evaporator to obtain oil forms into capsules and the residues were weighed to obtain the extractive yield of all the oil extract and were stored in air tight bottles at 4 °C.

Phytochemical analysis of oils extracts forms

Determination of total polyphenol content (TPC):

The total polyphenol content of oils and its extracts from bakery product were determined in triplicate in Gallic Acid Equivalents (GAE) by using Folin-Ciocalteu's reagent method [27]. The extract (0.5 ml) and 0.1 ml of Folin-Ciocalteu's reagent (0.5 N) were mixed, and the mixture was incubated at room temperature for 15 min. Then, 2.5 ml of saturated sodium carbonate solution was added and further incubated for 30 min at room temperature, and the absorbance was measured at 760 nm using a UV–VIS Spectrophotometer against a blank sample. The calibration curve was made by preparing gallic acid (10 to 100 μg ml^{-1}) solution in distilled water. Total phenol content is expressed in terms of gallic acid equivalent (mg/g of extracted compounds) [28, 26].

DPPH free radical scavenging assay:

The free radical scavenging activity of essential oil extracted from bakery product was measured by using 2, 2-diphenyl-1- picryl hydrazyl (DPPH) by the modified method of McCune and Johns [27]. (2002). The reaction mixture (3.0 ml) consisted of 1.0 ml DPPH in methanol (0.3 mM), 1.0 ml methanol, and 1.0 ml of different cumin or clove oils extract forms diluted in methanol, was incubated for 10 min, in dark, after which the absorbance was measured at 517 nm using a UV–VIS Spectrophotometer against a blank sample. Ascorbic acid (2 to 16 μg ml^{-1}) was used as positive control [28, 29].

Determination of reducing power:

The reducing power of extract was determined according to the method of Jahanban *et al.* [30]. Different forms of cumin or clove oils extracts in methanol (1.0 mL) were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3 000 g for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm.

Rancidity analysis:

Acid value (FFA%)

Acid value was determined according to PORIM test methods no. p2.5 [31]. 0.5 g of different forms oils extract from bakery product was weighed into an Erlemeyer flask. 50 ml isopropanol was added in a flask and bring the solution to the boil over a hot plate. 0.5 ml of phenolphthalein was added and neutralized by dropwise addition of 0.1 N potassium hydroxide till a faint, but permanent pink colour was obtained. Expression of result in eq (1):

FFA % as oleic acid = (28.2 x N x V)/ W …… (1)

Where: N = normality of NaOH solution; V = volume of NaOH solution used in ml; W = weigh of cumin or clove oil sample.

Peroxide value (PV):

PV determination was performed according to AOAC [24], with some modifications. Cumin and clove oils forms extract from bakery product separitly (2 mg) was dissolved in a blended solution of 30 ml chloroform–glacial acetic acid (3:2, v/v). A saturated solution of KI (1 ml) was added. The mixture was shaken by hand for 0.5 min and kept in the dark for another 5 min. After the addition of 75 ml distilled water, the mixture was titrated against sodium thiosulphate (0.1 M) until the yellow colour almost disappeared. Then, about 0.5 ml of starch indicator (0.05%) solution was added. Titration was sustained until the blue colour just disappeared. A blank was also determined under similar conditions. PV (meq/kg) was calculated as follows in eq (2):

PV (meq/kg) = C × (V-V0) × 12.69 × 78.8 / m ….(2)

where C is the sodium thiosulphate concentration (mol/l), V and V0 represent the volumes of sodium thiosulphate exhausted by the samples and the blank, respectively (ml), and m is the mass of oil extract (mg).

Cytotoxic activity test (Anticancer Activity)

Anticancer activity screening for the cumin and clove essential oils and its nano- and micro-encapsulated were carried out and four different human cancer cell lines including colon HCT116, prostate PC3, liver HepG2 and breast MCF-7 were obtained from the American Type Culture Collection (Rockville, MD, USA).

Cytotoxic activity test (In vitro bioassay on human tumor cell lines) was conducted and determined by the Bioassay-Cell Culture Laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo 12622, Egypt. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [32].

Sample concentration range between (100 to 0.78 µg/ml) using MTT assay.

Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, and Sanford, ME, USA). Cells were suspended in RPMI 1640 medium [(for HePG2- MCF7 and HCT116 – DMEM for A549 and PC3)] , 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine at 37 ºC under 5% CO2.

Cells were batch cultured for 10 days, then seeded at concentration of 10x103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56 ug/ml). After 48 h of incubation, medium was aspirated, 40ul MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO2. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at

37ºC. A positive control which composed of 100µg/ml was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions [33, 34].

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

= ((Reading of extract / Reading of negative control) -1) x 100

A probit analysis was carried for IC50 determination using SPSS 11 program.

Statistical analysis

Experiments and analysis was conducted triplicate. Data were evaluated using the analysis of variance (ANOVA) procedure in SAS, [35] (Version 9.2), and differences between means of parameters were compared using the Duncan's test at the 5% significance level. Statistical analysis was performed using SAS program (Statistical Analytical Systems, Cary, NC).

3. Results and Discussion

Physical properties

TEM

The results of particle shape and size by TEM in Fig. (1a) showed that clove oil nanoparticles encapsulated in **CMC** consist of a core phase trapped in shell material of fairly constant thickness. The nanocapsules appear to consist of spherical particles with a diameter of about 20.74 to 21.00 nm. The outer surface of each particle is almost smooth and regular, which indicates that the CMC polymer film a continuous surrounding the clove oil droplets. Thus, the size and diameter of clove oil drops coated in carboxymethylcellulose capsules are in the nano scale, due to the use of encapsulation technology, which ensures that all clove oil drops are successfully encapsulated and the absence of free oil displacement in the medium indicates a homogeneous distribution of clove oil drops inside the capsule.

On the other hand Fig. (1b) shows that nano-encapsulate of cumin oil in CMC appeared to be irregular shape units and the diameter was ranged from 24.03 to 65.88 nm. The external surface of each unit was regular and in the form of a very thin coating containing cumin oil droplets inside, indicating the presence of a layer of CMC polymer thin film around the cumin oil, indicating the efficiency of the packaging process.

Nagaraju *et al.*, [36] used non-ionic surfactants to stabilize the nano emulsion of clove oil to increase its solubility in water and increase chemical stability. The emulsion particle size was 300 nm after 8 days of storage at room temperature.

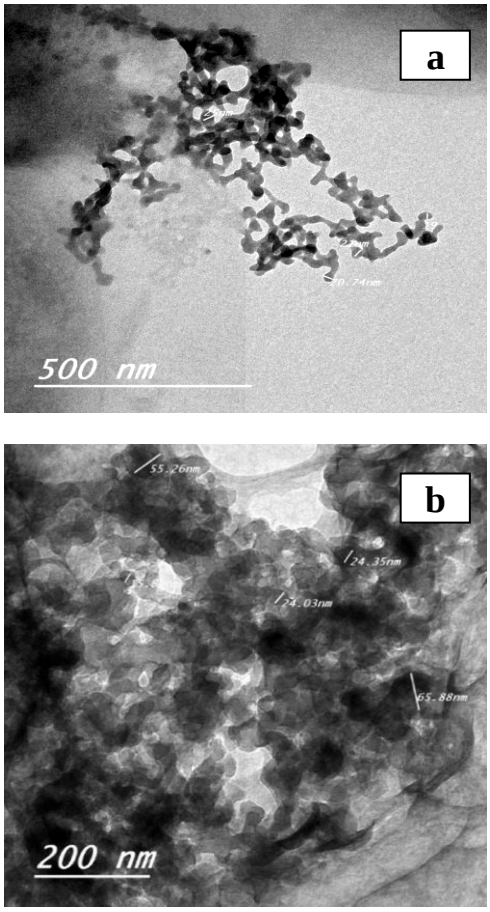

Figure (1): Translasion Electron Microscopy morphology
a: for Clove oil nana-encapsulation in CMC
b: for cumin oil nana-encapsulation in CMC

Differential scanning calorimetry (DSC)

DSC is a technology that reflects the thermal stability was measured by increasing the temperature of oil cloves and cumin samples extracted and it's nanoencapsulates by CMC. In Figure (2) the results showed that the temperature of all samples holder increases linearly as a function of time.

Cumin oil extract exhibited no thermal transition, but gel network structure developed progressively

upon aging. One peak was observed in cumin oil extract Figure (2a) corresponding to melting points for non-encapsulated cumin oil. The melting point enthalpies and solid restructuration for cumin oil extract were 48.91 ºC and 30.74 Jg^{-1}, respectively. This indicates the sensitivity of non-encapsulate cumin oil to temperatures, causes a loss of its natural properties, and cannot be used in food especially when treated more than 24 h heat treatments, which impedes its application as food additives.

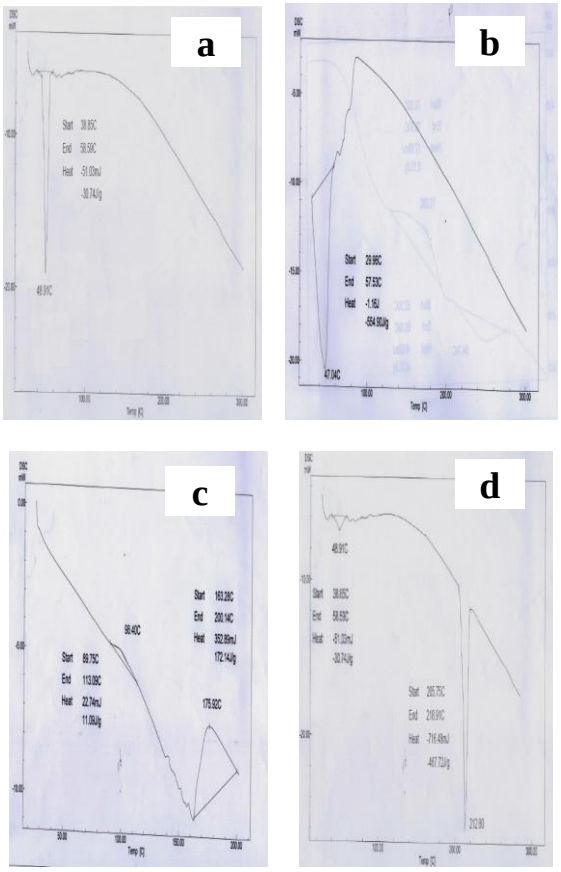

Fig. (2): DSC of clove and cumin oils forms
Where a: DSC of cumin oil extract, b: DSC of clove oil extract, c: DSC of clove oil nano-encapsulated in CMC, d: DSC of cumin oil nano-encapsulated in CMC

Clove and cumin oils extract was measured by DSC and its nano-encapsulated in CMC. Fig. 2(a, b, c and d) showed that the melting point of clove oil (non-encapsulated) was 47.04 ºC which cannot be used as a food additives owing to loss its characteristics especially in bakery products. So, the results showed that encapsulation process increases the thermal stability of oils without change in its antioxidant and anticancer properties. Higher exothermic peaks occurred at a much higher temperature of 175.92 °C in clove oil extracted from nano-capsules in CMC compared to the clove oil extract (Fig. 2b). While it was 212.80 °C in cumin oil coated with nano-encapsulates (Fig. 2d), with another pick at 48.91 °C, an indication of the presence of a part of free cumin oil that is not encapsulated in a small percentage that has been replaced from inside the capsule.

Sensory evaluation of biscuits

Sensory evaluation of biscuit samples fortified with nanometer and micrometer capsules of cumin and cloves oils, and comparison with control samples of salted biscuit and sweet biscuit respectively. The results in Table (1) showed that the salted biscuit samples fortified with micro-encapsulates of cumin oil had less color, texture, Oder, taste and appearance compared to the control sample, while the salted biscuit samples fortified with nano-encapsulates showed a noticeable improvement in the sensory evaluation values and were better than the control sample.

The results showed that sweet biscuit samples fortified with clove oil nano-encapsulate higher values of the sensory evaluation than other samples while salt biscuit fortified with cumin oil micro-encapsulate had the lowest values of sensory evaluation.

Parisa et la., [4] stated that the use of nano-encapsulated *Cumino cyminum* L. essential oil (CCEO) coated nanochitosan coating as strong coating to increase the shelf life of sardines.

Color analysis

The results of the colorimetric evaluation of samples of sweet and salted biscuits fortified with micrometer and nano-encapsulate of clove and cumin oils, respectively (Table 2). The crust of salted biscuit samples is more lightning (L*) value than the control sample, followed by the sample fortified with non-encapsulate cumin oil, then the sample supported with micro-capsules and finally the two varieties of biscuit fortified with nano-encapsulate. Whereas, the crumb was better in the salted biscuit sample fortified with non-encapsulate cumin oil, followed by the sample fortified with nano-encapsulate, then the control, and then the micro-, respectively.

The sample of sweet biscuit fortified with clove oil showed higher crust (L*) values than the salted biscuit, but less than control sample (63.87 and 65.87), respectively.

While the (a*) values showed the highest result in the sample of sweet biscuits in all samples in the crumb and it was highest in the sample supported with nano-encapsulate of clove oil, but the (a*) values were lower in the face of the sample of salted biscuits, especially in the sample containing micrometric capsules of cumin oil.

On the other hand, the (b*) values of crumb for sweet biscuit samples were higher in most samples. Otherwise, the face value of the salted biscuit samples was lower than the rest of the samples.

The values of ΔE* ranged from 73.73 to 79.79 in the face of the salt biscuit samples fortified with cumin oil and thus became less white, especially in the samples supported with micro-encapsulate, while the values in the back ranged from 81.33 to 87.76, which means volatility from light yellow to dark in color, the values of ΔE* ranged from 82.08 to 86.26 in the crust of sweet biscuit samples fortified with clove oil, which means fluctuation from yellow to brown, especially in the control sample, while the values of ΔE* ranged from 95. 75 to 83.39 in the crumb for the same samples, which means change to green especially in the sample containing non-encapsulate clove oil and the overall change to saturation was minimal (P> 0.05).

Chemical properties

All studied of antioxidant activity and reducing power varied according to clove and cumin oils extract and its micro- and nano-encapsulate extracted from bakery product compared to control sample without any addition of oil forms at different during storage periods of 0, 30 and 60 days.

Antioxidant activity

DPPH was used to determine the free radical scavenging capacity in different samples. It was expressed by DPPH with an IC_{50} value which indicates the effective concentration of the extract required to inhibit 50% of free radicals. The results in Table (3) showed that both essential oils whether clove or cumin oil extract from bakery products and their nano-encapsulate extract were displayed good antioxidant activities for different storage periods.

On the other hand, clove oil extract from sweet biscuits sample nano-encapsulate was 54.21 µg/ml higher than that extracted from microencapsulated 52.33 µg/ml at zero time storage, while the oil extract from control sample showed less of DPPH 5.66 µg/ml, while after 60 days storage found that clove oil extracted from nano-encapsulate sample retained for its antioxidant properties result loading and activity was high in comparison all samples at 60 days storage. At the end of storage period (60 days) samples from clove oil extracted retained about 300 % from its antioxidant properties

Table (1): Sensory evaluation of salt and sweet biscuits

Samples	Color	Texture	Oder	Taste	Appearance	Overall acceptability
Salt biscuit (control)	9.0 ^{a}	8.8 ^{a}	8.1 ^{b,c}	8.7 ^{a}	8.6 ^{a}	8.5 ^{a}
Sweet biscuit (control	8.0 ^{c}	8.2 ^{c}	7.9 ^{c}	7.4 ^{c}	7.5 ^{c}	7.6 ^{c}
Salt biscuit with cumin micro—capsule	6.1 ^{d}	7.5 ^{c}	7.1 ^{c}	6.2 ^{d}	7.6 ^{c}	7.7 ^{c}
Salt biscuit with cumin nano—capsule	6.4 ^{d}	7.9 ^{b}	8.3 ^{b}	7.4 ^{c}	8.0 ^{b}	7.9 ^{b}
Sweet biscuit with clove micro—capsule	7.2 ^{c,d}	7.9 ^{b}	8.2 ^{b}	7.8 ^{b}	7.7 ^{b,c}	7.8 ^{c}
Sweet biscuit with clove nano—capsule	8.3 ^{b}	8.9 ^{a}	9.2 ^{a}	7.7 ^{b}	7.9 ^{b}	8.1 ^{b}
LSD	0.533	0.621	0.455	0.524	0.632	0.659

Table 2. Salt and sweet biscuit samples color parameter

Samples	Crust				Crumb			
	L*	a*	b*	**ΔE***	L*	a*	b*	**ΔE***
Control	61.9	9.79	46.63	77.95	57.11	13.65	58.49	82.88
Salt Biscuit with cumin oil forms								
Non-encapsulate	59.90	9.96	47.42	77.04	59.70	12.88	55.96	82.83
Microencapsulated	57.56	8.75	45.23	73.73	54.45	12.47	59.87	81.38
Nanoencapsulated	54.10	14.45	56.84	79.79	57.17	15.27	64.81	87.76

Samples	Crust				Crumb			
	L*	a*	b*	ΔE*	L*	a*	b*	ΔE*
Control	65.87	10.83	54.64	86.26	48.60	24.73	78.38	95.48
Sweet Biscuit with clove oil forms								
Non-encapsulate	63.87	14.14	53.11	84.26	49.04	24.99	78.35	95.75
Microencapsulated	60.88	14.49	64.43	82.08	45.01	28.75	75.64	92.59
Nanoencapsulated	57.53	14.94	59.79	84.31	39.61	29.70	67.10	83.39

Table 3. DPPH radical scavenging activity of clove and cumin oil forms extract from salt and sweet biscuits

Samples	Storage period days	DPPH (IC_{50}) µg/ml			
		Control	Oil extract (non-encapsulate)	Micro encapsulate EO	Nano encapsulate EO
Sweet biscuits (clove)	0	5.66±1.2^{a}	54.67±2.33^{a}	52.33±3.54^{a}	54.21±2.62^{a}
	30	3.21±0.9^{b}	49.28±2.74^{ab}	50.62±2.42^{ab}	53.02±3.10^{a}
	60	2.88±1.0^{c}	40.35±3.18^{b}	48.22±3.06^{b}	51.04±2.93^{ab}
Salty biscuits (cumin)	0	9.55±1.6^{a}	35.24±3.21^{a}	31.57±2.61^{a}	34.88±3.72^{a}
	30	6.31±1.1^{b}	32.31±2.11^{ab}	30.02±2.35^{a}	33.25±2.76^{a}
	60	4.25±1.3^{c}	26.52±2.47^{b}	28.54±2.08^{ab}	31.48±2.62^{ab}

Each value in the table is represented as mean ± SD.

Table 4. Reducing power of essential oils extracted from salt and sweet biscuits

Samples	Storage period (days)	Reducing power (IC_{50}) µg/ml			
		Control	Oil Non-encapsulate	Micro-encapsulated EO	Nano-encapsulated EO
Sweet biscuits (clove)	0	212.15±9.4^{a}	346.25±12.37^{a}	523.41±12.4^{a}	542.66±14.5^{a}
	30	191.52±9.8^{ab}	321.53±11.62^{ab}	476.89±11.3^{b}	509.45±12.5^{b}
	60	180.21±10.1^{b}	296.77±11.51^{c}	422.58±12.5^{c}	468.67±13.1^{c}
Salty biscuits (cumin)	0	113.78±6.5^{a}	161.82±9.4^{a}	243.27±9.5^{a}	262.60±7.5^{a}
	30	98.25±7.8^{b}	142.52±7.1^{b}	226.57±8.6^{ab}	258.22±8.1^{ab}
	60	85.41±8.5^{c}	133.61±5.9^{c}	212.65±7.5^{b}	245.68±9.5^{b}

Each value in the table is represented as mean ± SD

Results showed that the cumin oil extract from salty biscuit bakery product was higher in antioxidant activity in nano-encapsulate form compared with other samples at different storage period. Additionally, the synergy of antioxidants in the mixture creates antioxidant activity not only dependent on concentration, but also on structure and interaction between the antioxidant oils.

Generally, the results in Table (3) observed that the antioxidant activity of clove oil was higher than that of cumin oil, whether in the extracted oil or in the micro or nano extracted from the bakery samples during the different storage periods. This indicates the efficiency of the encapsulating process for clove oil with CMC is better in maintaining the antioxidant activity, especially since the packaging material is able to protect clove oil up to 175 ºC.

The results are consistent with findings by Nagaraju *et al.*, [36] in the antioxidant properties of the original clove oil and nanometer capsule oil in food product development due to the high water dispersion rate but tolerance of 25% oil content on a dry weight basis.

Reducing power

The results in Table (4) showed that the reducing power of the essential oil extracts indicates that it likely contributes significantly to the radical scavenging activities.

The results of the low capacity of oil non-encapsulate as well as that extract from bakery products of both clove and cumin oil showed the same ability to remove free radicals as antioxidants, which confirms their efficiency during different storage periods 0, 30 and 60 days. The results showed that clove oil coated in nano-encapsulate with CMC extracted from sweet biscuits had the highest reducing capacity of 542.66 µg / ml compared to that

extracted from micro-encapsulate 523.41 µg / ml, and both of them are greater than non-encapsulate clove oil after zero storage, while after 60 days of storage, the reducing capacity decreased to varying degrees, and the lowest decrease in the sweet biscuit extract coated in nano-encapsulate was 468.67 µg / ml.

The reducing power of cumin oil extracts correlated well with increasing storage periods. The nano-encapsulate cumin oil extracted showed stronger reducing power than micro-encapsulate extracts after 60 days storage were 245.68±9.5 µg/ml but it was 212.65±7.5 µg/ml for micro-encapsulate cumin oil extract from salty biscuits and 336.24 µg/ml compared to the sample. In both cases, the result was higher compared to the sample containing cumin oil, which was not encapsulated, it was 133.61±5.9 µg/ml, and this is a comprehensive indication of the capsules' ability to protect cumin oil, whether in the nano- or micro- forms.

Rancidity of bakery products

The antioxidant compounds from clove or cumin oils encapsulation forms it not only increases the stability of foods, to prevent lipid peroxidation, but also protects biomolecules from oxidative damage in bakery products. Free fatty acid and peroxide values were determine in clove or cumin oils forms extract from different bakery products with comparison the control samples.

Acid value (Free Fatty Acid %)

The free fatty acid value was estimated for sweet biscuit samples fortified with uncoated clove oil was in the range of 0.58 ± 0.12% to 0.71 ± 0.09% during the storage period from 0 to 60 days, while the value for free fatty acids was detected in sweet biscuit fortified with nano-encapsulate clove oil ranging from 1.42±0.11 to 1.47±0.12 % from 0 to 60 days during storage period. This indicates that the clove oil nano-encapsulation technology was protecting against fatty acid oxidation.

While the results of FFA values for sweet biscuit samples fortified with clove oil micro-encapsulate showed an increase after 60 days of storage, it was 1.52±0.10, which is higher than its counterpart coated with nano-encapsulate. On this basis, the clove oil nano-encapsulate has found desirability and success in preventing oxidation, and it results in prolonging the storage period to preserve these products.

The results also showed that cumin oil nano-encapsulate have a lower level of RI 8.88 % compared to the other salted biscuit samples, either containing or not coated with micrometer capsules of cumin oil, 25.58 and 31.03 %, respectively. In comparison, the control sample had the highest rate increase of RI 55.56 %, which indicates a rapid deterioration of control samples during storage period due to oxidation of fatty materials used in preparation and processing of this product. On the other hand, the biscuits containing cumin oil coated with nano-encapsulate was more stable for oxidative rancidity of the products during the storage period.

Peroxide value (PV)

The peroxide value is commonly used to estimate the volume of oxidation products in oils. The relative increases in PV for sweet biscuits fortification with clove oil non-encapsulate, micro-encapsulate and nano-encapsulate stored under accelerated conditions were presented in Table (6).

The clove oil forms showed characteristic increases in PV after 60 days of storage period, the clove oil non-encapsulate had the highest of RI for PV was 18.54 %, indicating a higher extent of primary oxidation, but the relative increase in PV from clove oil nano-encapsulate was 0.92 %. It is the result of a small increase due to the oil from oxidative stress protection at storage period.

The minimum increase in PV for nano-encapsulate cumin oil sample was stabilized more than the sample containing microencapsulate cumin oil were 2.42 and 5.43 %, respectively. Compared to the sample containing non-encapsulate cumin oil, there was a large difference in RI, which reached 19.42 % after 60 days of storage.

Accordingly, the process of encapsulation with nano-encapsulate by CMC for both clove oil and cumin oil showed significant decrease in RI of FFA and PV values, thus reduces the oil's exposure to oxidation and increases the ability of this products to remain in high quality and a long shelf life compared to other samples that are non-encapsulate or not fortified with clove or cumin oil nano-encapsulate.

In similar results, the composition of the early lipid oxidation products was measured throughout the storage period of the samples using the PV values. Parisa *et al.* [4] found that the effect of nanoencapsulate cumin oil by chitosan lead to changes of PV values in both control samples and nanoencapsulate samples during storage periods after

16 days was significantly higher than that of zero time, and the other treatments did not affect the four days.

Cytotoxic effect on human cell lines

The anti-proliferative activities of the cumin and clove oils and its nano- and micro- encapsulated were evaluated against 4 different human cancer cell lines including liver HepG2, breast MCF-7, prostate PC3, and colon HCT116 using an SRB assay.

The anti-proliferative activities were expressed by median growth inhibitory concentration (IC_{50}) and provided in Table (7). From the results it is evident that although non-encapsulate clove oil displayed potent growth inhibitory activity against HCT116 (89.6 %), PC3 (100 %), HePG2 (56.2 %) and MCF7 (81.2 %) cell line.

While, nano-encapsulate clove oil showed the strong growth inhibitory activity against 88.1, 92.6, 47.3 and 78.6 % for HCT116, PC3, HePG2 and MCF-7 cell line, respectively.

The micro-encapsulate clove oil anti-proliferative activities was 24.5 and 12.1 μgmL^{-1} for HCT116 and HePG2, respectively, it had no activity (NA) against the PC3 and MCF-7 cell line.

It is clear that the anti-proliferative effect of nano-encapsulated clove oil in CMC was similarly potent to the non-encapsulate clove oil in HCT116, PC3, HePG2 and MCF7 cell lines than micro-encapsulated clove oil.

Table (5): Relative increase in acid value (FFA%) (As oleic acid) of clove and cumin oil encapsulated extract from salt and sweet biscuits with its (non-encapsulate) for incubation period days

Samples	Storage period (days)	Acid value (FFA %)			
		Control	Oil Non-encapsulate	Micro-encapsulate oil extract	Nano- encapsulate oil extract
Sweet biscuits (clove)	0	0.32 ± 0.11^{a}	0.58 ± 0.12^{a}	1.41 ± 0.17^{a}	1.42 ± 0.11^{a}
	30	0.49 ± 0.12^{b}	0.62 ± 0.16^{ab}	1.49 ± 0.13^{ab}	1.44 ± 0.12^{a}
	60	0.66 ± 0.15^{c}	0.71 ± 0.09^{b}	1.52 ± 0.10^{b}	1.47 ± 0.12^{ab}
	RI* %	106.25	22.41	7.80	3.52
Salty biscuits (cumin)	0	0.18 ± 0.02^{a}	0.29±0.08a	0.43 ± 0.10^{a}	0.45 ± 0.07^{a}
	30	0.22 ± 0.05^{b}	0.34 ± 0.06^{ab}	0.49 ± 0.09^{ab}	0.46 ± 0.07^{ab}
	60	0.28 ± 0.04^{ab}	0.38 ± 0.08^{c}	0.54 ± 0.08^{b}	0.49 ± 0.06^{b}
	RI* %	55.56	31.03	25.58	8.88

Values are mean ±SD for triplicate determinations

RI*: Relative Increase of FFA

Table (6): Relative increase in peroxide value (PV) of clove and cumin oils extract from salt and sweet biscuits compared with its non-encapsulate at storage period days

Samples	Storage period (days)	Peroxide value (meq O_2/kg)			
		Control	Oil Non-encapsulate	Micro-encapsulate oil extract	Nano- encapsulate oil extract
Sweet biscuits (clove)	0	5.88 ± 0.82^{a}	4.11 ± 0.36^{a}	9.71 ± 1.23^{a}	9.72 ± 1.22^{a}
	30	6.41 ± 0.91^{b}	4.52 ± 0.38^{ab}	9.86 ± 1.30^{ab}	9.75 ± 1.20^{a}
	60	6.97 ± 0.98^{bc}	4.80 ± 0.38^{b}	9.92 ± 1.32^{ab}	9.81 ± 1.21^{ab}
	RI* %	18.54	16.79	2.16	0.92
Salty biscuits (cumin)	0	3.95 ± 0.32^{a}	3.14 ± 0.29^{a}	7.00 ± 1.02^{a}	7.01 ± 1.01^{a}
	30	4.34 ± 0.38^{b}	3.47 ± 0.31^{b}	7.24 ± 1.00^{ab}	7.12 ± 1.03^{ab}
	60	4.82 ± 0.38^{bc}	3.75 ± 0.33^{bc}	7.38 ± 1.08^{ab}	7.18 ± 1.05^{ab}
	RI* %	22.03	19.42	5.43	2.42

Values are mean ±SD for triplicate determinations

RI*: Relative Increase of PV

Table (7): Cytotoxicity of clove and cumin oils and its nano- and micro- encapsulated against HCT116 [Colon], PC3 (Prostate), HePG2 (Hepatocellular) and MCF7 (Caucasian breast adenocarcinoma) of human malignant cell lines**.**

Oil Forms	HCT116 (Colon)		PC3 (Prostate)		HePG2 (Hepatocellular)		MCF7 (Caucasian breast adenocarcinoma)	
	*IC_{50} µg/ml	Remarks % at 100 ppm	IC_{50} µg/ml	*GIA % at 100 ppm	IC_{50} µg/ml	GIA % at 100 ppm	IC_{50} µg/ml	GIA % at 100 ppm
*Non- clove oil	37.6	89.6	23.8	100	37.8	56.2	45.02	81.2
*NE- clove oil	37.6	88.1	23.8	92.6	37.8	47.3	45.02	78.6
*ME -clove oil	21.6	24.5	23.8	NA	37.8	12.1	45.02	NA
Non- cumin oil	24.5	72.8	18.6	99.1	26.7	48.1	36.8	66.4
NE- cumin oil	23.8	65.1	18.6	71.6	26.7	39.8	36.8	61.4
ME- cumin oil	11.2	8.9	18.6	NA	26.7	11.9	36.8	NA
DMSO	---	1.0	---	1.0	---	3.0	---	3.0
Negative control	---	*NA	---	NA	---	NA	---	NA

*IC_{50}: Lethal concentration of the sample which causes the death of 50% of cells in 48 hrs (anti-proliferative activity); *NA: no activity;

*Non: non-encapsulate; *NE: nano-encapsulate; *ME: micro-encapsulate;

*GIA: growth inhibitor activity

Nagaraju *et al.,* [36] showed that the cytotoxicity of nano-capsule clove oil was four times lower than that of the initial oil at a concentration 60 μgml^{-1} when tested on $CaCO_2$ cells.

For cumin oil, the results in Table (7) revealed that non-encapsulate cumin oil shows strong growth inhibitor activity against HCT116, PC3, HepG2 and MCF7 cell lines was 72.8, 99.1, 48.1 and 66.4 %, respectively.

The IC_{50} was 24.5, 18.3, 26.7 and 36.8 μgmL^{-1} for HCT116, PC3, HepG2 and MCF7 cell lines, respectively. It is obvious that the anti-proliferative effect of nano-encapsulate cumin oil was stronger than the micro- encapsulated cumin oil. While, the treatment with micro-encapsulate cumin oil had no activity for PC3 cell lines.

The results revealed that the clove oil under investigation possessed significant anticancer activity more than cumin oil. The results also showed both clove and cumin oils nanoencapsulates have an inhibitory activity for the growth of cancer cells close to non-encapsulate oils, but due to the high sensitivity of oils especially to high temperatures during baking, which exposes them to the loss of activity, whether antioxidant or anticancer, which is the aim of the study to protect those oils with a thin layer of CMC nano-capsules; this basis it can be applied at the level of largest in food industry.

AitM'barek *et al.* [37] reported that clove oil in its thymol content has cytotoxic activity against cancer cells. The oil's activity is often attributed to the specific components of the oil. Tsukamoto *et al.* [38] found that clove oil coated, may be involved in stimulating the active proliferation of fibroblasts compared to non-encapsulated clove oil, in addition to participating with other components of the oil on cytotoxicity against cancer cells. Clove oil has shown strong biological activity. In addition to its cosmetic and food use, clove oil represents a significant potential in anti-cancer therapies and certainly deserves further study [39].

4. Conclusion

Cumin and clove oils are rich in their content of bioactive compounds that have importance as antioxidants and anticancer, but due to their high sensitivity to high temperatures and oxidation and their low solubility in water, one of the important things that lose their activity and limit their addition to food.

The oxidative stability of oil was evaluated with the nano--encapsulation of clove and cumin oils. The nano-encapsulation in CMC would allow for oil to be stabilized for longer periods during storage.

Sweet biscuit sample fortified with nano-encapsulates for clove oil showed more acceptance than the other samples in sensory evaluation of products.

5. Conflicts of interest

"There are no conflicts to declare".

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