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Effect of Spray-Drying on the Physical, Sensory and In-vivo Parameters of Orange Peel Oil and Limonene

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Abstract

The valorization of agro-industrial waste like orange peel presents an economic and environmental due to the enormous amounts generated during orange juice production. This study focuses on the encapsulation of orange peel oil and its main constituent, limonene, using different wall combinations using spray drying. The increase of gum Arabic (GA) provided higher viscosity (136.33 cP) and stability (0.9%) in the orange oil emulsion before spray-drying, whereas the predominance of maltodextrin (MD) led to the reverse, 58.67cP, and 13%, respectively. In the spray-dried powder, MD as a prominent in-wall mixture resulted in higher bulk density for orange oil powder (0.33 g/cm3), better results in the wet ability test (31.67 sec.), and the highest oil retention (92.22%) for limonene powder. The encapsulation efficiency was positively influenced by the 10% GA with 85.35 and 85.77% for limonene and orange oil powders, respectively. Significant color changes were undergone according to different CIE-LAB characteristics, especially for limonene powder (b* 7.91), where MD is predominant in the wall combinations. Increasing the GA concentration affects the morphology of the particles with more agglomeration, while the predominant MD leads to more spherical and smooth particles. In the sensory analysis, supplemented sponge cake with encapsulated limonene showed the highest overall preference score (7.87/9), while orange oil flavoring in jelly candies had the superiority (7.9/9) compared to the control. The nutritional data and biochemical parameters showed non-significant results in all groups supplemented with the spray-dried flavorings compared to normal control. The spray-drying of orange peel oil and limonene did not show any adverse effects on the nutritional or the biochemical parameters with enhancing the sensory attributes of the final food products.

Keywords: Encapsulation, orange peel oil, limonene, non-enzymatic browning, SEM, Biological parameters, Sensory Evaluation.

1. Introduction

Spray-drying is one of the oldest encapsulation techniques widely used in the food industry. It is a unit operation used to dry various food products, such as milk powder, and special microencapsulation of different food ingredients, such as flavorings agent, coloring agent, and essential oil [(1)]. Preservation of the chemical, physical, biological, and sensory propertiesoftheactiveingredientsisthemaintargetofthee ncapsulation process, in addition, to controlling their release or delivery [(2)] and protecting them from the external environment [(1,3)], such as light, temperature, oxygen, humidity, and from interaction with other substances[(4)].

Flavors containing a broad spectrum of aroma compound scan effectively increase the acceptability of foods and beverages by masking or reducing unpleasant tastes and smells. Generally, aroma compounds are unstable when exposed to environmental factors [(5)]. Additionally, the hydrophobic properties hinder the aroma utilization in foods[(6)]. Therefore, finding suitable and non-toxic carrier material for flavors has become urgent for further applications [(7)]. **Gharsallaoui *et al.*** [(8)] reported that maltodextrin, gum arabic, and proteins like sodium caseinate and whey protein concentrate were the most suitable for microencapsulation using a rapid drying process formation of dense skin and good protection of core materials against oxygen transfer.

The valorization of agro-industrial waste like fruit peel constitutes an essential source of essential oils used as a flavoring presents an economic and environmental necessity [(9)]. An enormous amount of *Citrus sinensis* L. peel waste is generated during orange juice production, and its extracted essential oil is widely applied in food, pharmaceutical, and

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perfumery industries [10]. The orange peel oil has been reported to have many biological activities like antioxidant, anti-cancer, anti-inflammatory, cardio-protective, neuroprotective, anti-bacterial, and anti-mycotic activities [11].

Most of the previously published studies deal with encapsulation of flavorings and oils focused on the physicochemical properties of the micro- or nano capsules formed and the quality of the product [12-13]. However, to our knowledge, nothing was reported concerning the effect of the encapsulation process on the sensory properties of food products used spray-dried flavorings for fortification, in addition to the presence of any drawbacks in the nutritional and biochemical parameters associated with these formulated flavorings. The above information provides the rationale behind this study that aimed to develop spray-dried flavorings containing orange peel oil compared to its main constituent, limonene, and find out the effect of the encapsulation technique on the sensory properties of food products like sponge cake and jelly candies containing the spray-dried orange peel oil. Additionally, the influence of the encapsulation technique on the nutritional and biochemical parameters was studied extensively to assess the effect of spray-dried oil on health.

Materials and Methods

Materials

Limonene and natural orange peel oil were supplied from Sigma-Aldrich (St.Louis, MO) and Ernesto Ventós S.A. (Barcelona, Spain). The coating agents applied were Maltodextrin (MD) DE12-15(National Co. for corn products 10th of Ramadan, Egypt), sodium caseinate (SC) (Fonterra, New Zealand), and the Gum Arabic GA (Avonchem, Cheshire, UK).

Methods

Emulsion preparation:

Hydration of GA indeionized water was conducted with 5-15% (w/v) and left overnight at 4°C. Different concentrations of MD (15.0-30.0% w/v) were added to the gum solution, followed by 5% of SC dispersed in warm water and stirred overnight with a total solid content (35.0% w/v) (Table1). Under intensive mixing, Tween 80 (1.0% w/v, based on water) and aroma compounds (15% w/v) were added. Homogenization was performed at 500.0 W, and 50 kHz for 20 minutes in a cold-water bath [14-15].

Table1: Coating blends used to encapsulate flavorings

Aroma Compound*	Wall materials (%)		
	Gum Arabic (GA)	Sodium Caseinate (SC)	Maltodextrin (MD)
C1-F1	5	5	25
C2-F2	10	5	20
C3-F3	15	5	15
C4-F4	-	5	30

*C: Limonene, and F: Natural orange oil

Emulsion characteristics:

Emulsion viscosity: Bohlin Visco 88 BV (Bohlin Rheology UK Ltd., UK) was employed. Three measurements were conducted three times at 29.5 ± 0.1 °C.

Emulsion stability: According to **Klinkesorn *et al.*** [16], the creaming index was used to calculate the emulsion stability based on the equation [15-17]:

$$CI = (SH/TH).100\%$$

CI is the creaming index of the oleoresin emulsion, SH: is the height of the serum layer formed at the bottom of the glass tubes (mm), and TH is the total height of the emulsions in the tubes (mm). The closer the value of CI to zero, the more stable the emulsion against creaming. Values reported are arithmetic mean of three tests ± SD.

Emulsion Total solids: Moisture content was determined according to the method described in AOAC. [18], then calculated the total solid in emulsion by

$$\%Total\ solid = (100 - moisture\ content)$$

Spray drying process:

The co-current Mini Spray Dryer B-290 (BÜCHI, Flawil, Switzerland) was used. The inlet and outlet temperatures were 160.0 °C and 80.0 °C (±1.0 °C), respectively. The drying process was conducted twice for two different batches of each aroma compound emulsion. Then the collected, dried samples were packaged in polypropylene bags and kept directly in the desiccators until analysis.

Powder characteristics:

Moisture content: 3-5g of powder was heated at 105.0 °C until a constant weight was achieved. The percentage of powder moisture was calculated on a wet basis [18], and the arithmetic mean of three determinations was calculated as ±SD.

Bulk density: The tapping method of **Kausadikar *et al.*** [14] was applied where the sample weight was divided by the volume. The arithmetic mean of three determinations was calculated as ±SD.

$$\text{Bulk density} = \frac{\text{Wight of spray powder}}{\text{Volume of spray powder}}$$

Powder wett ability: It was evaluated according to **Quek *et al.*** [19] method. The necessary time in seconds for the completed isolution was recorded. The arithmetic mean of three determinations was calculated as ±SD.

Non-enzymatic browning: The extent of non-enzymatic browning NEB was expressed as the b *value. The color was measured with spectra measurement– JASCO high performance UV/VIS/NIR/D070061801 (JASCOV-770 Spectrophotometer, NO. D070061801, Japan). The total parameters measured during this analysis were: L* — expressed the lightness (in %), a* value—redness (positive (+ve)) to greenness (negative (-ve)), and b*value —yellowness (+ve) to blueness (-ve).

Encapsulation efficiency:
The total volatile oil content was extracted by Clevenger type apparatus for 3 hrs. [20]. The oil encapsulation retention (%) was calculated using Equation 1:

$$\text{Oil retention (\%)} = \frac{\text{Total oil in the powder}}{\text{Initial oil load}} \times 100$$

Varavinit *et al.* [21] described a modified method to determine the surface oil, where 30 mL of hexane were added to 5 g of powder, followed by stirring at 300 rpm for 10 min. After filteration and wash with hexane, the solvent was vaporized under vaccum to obtain a constant weight. Finally, the encapsulation efficiency of volatile oils (EEVOs) was calculated using Equation 2 [22]:

$$\text{Encapsulation efficiency (EEVOs)} = \frac{\text{grams of oil inside the microcapsules}}{\text{total grams of oil in the system}} \times 100$$

Powder morphology using scanning electron microscopy (SEM): Aroma powder was analyzed using the field emission scanning electron microscope (Quanta FEG 250, FEI, Czech Republic) at an accelerating voltage of 10 kV. The powder was previously gold-sputtered by mounting on aluminums tubs with double-sided adhesive tape and coated with gold using an Edwards sputter coater S150 A (Crawley, England). FE-SEM images were taken with magnification ranges of 1000-15000x and an accelerating voltage of10 kV.

Biological analysis:
Experimental animals: Sixty-six Sprague-Dawley male rats, 1 - 2 months age, weighing (150-180 gm) were obtained from the Animal House Colony of the National Research Centre and used following the guidelines for Animal Experiments approved by the Ethical Committee of Medical Research, National Research Centre, Cairo, Egypt. For acclimatization and to ensure normal behavior, the animals were kept on a standard laboratory diet and water for one week before the experiment at 23 ± 1 ^{0}C), 40-60% relative humidity, and 12 h dark/light cycle.

Diet Composition: The basal synthetic diet for the control (negative) group was designed according to the AIN-93M diet [25] and composed of casein (150g/1 kg diet), unsaturated fat (100 g/1 kg diet), sucrose (220 g/1kg diet), maize starch (440 g/1 kg diet), cellulose (40 g/1 kg diet)[23-24], salt mixture (40 g/1 kg diet) and vitamin mixture (10 g/1 kg diet). The other groups fed on the same above basal synthetic diet but supplemented with natural and microencapsulated flavorings as follows: limonene is 600 mg/kg BW/day [26-27], and orange peel oil is 600 mg/kg BW/day [26-27].

Experimental design: 11 groups (6 rats per group) were designed as follows: normal Control group (negative), group C where basal synthetic diet supplemented with natural limonene (600 mg/Kg BW /day), groups C1-C4 where basal synthetic diet supplemented with microencapsulated limonene (600 mg/Kg BW /day), group F where basal synthetic diet supplemented with natural orange peel oil (600 mg/Kg BW/day), and groups F1-F4 where basal synthetic diet supplemented with microencapsulated natural orange oil (600 mg/Kg BW/day).

Samples collection: After one month, the animals fasted for12 hours followed by anesthesia and were euthanized by cervical dislocation. Blood samples to evaluate biochemical parameters (5ml) were collected from the tail where serum and plasma were separated by centrifugation (Sigma labor centrifuge GMBH, West Germany) at 4000 rpm for 15 min and preserved at -20 ^{0}C.

Biochemical Parameters: Glucose was evaluated by the enzymatic colorimetric method [28]. Hb was assessed by the enzymatic colorimetric method [29]. Total cholesterol [30], HDL [31], LDL [32], and Triglycerides [33] were evaluated as lipid profiles by the enzymatic colorimetric method. Plasma alanine aminotransferase (ALT) [34], aspartate aminotransferase (AST) [35], and alkaline phosphatase ALP [35] activities were assessed as liver function indicators by colorimetric techniques. Plasma total protein [36] and plasma albumin (A) [37] were determined by colorimetric methods as different indicators of liver function. Colorimetric methods assessed creatinine [38], urea [39], and uric acid [40] as kidney function indicators.

Sponge cake and jelly candies preparation
The cake samples were prepared according to **Rizk *et al.*** [41]. The flavor of Limonene 0.11 mg/kg/day and orange oil 0.05 mg/kg/day [42] was added to the creaming stage. The dough was scaled into two aluminum olds (20X10cm) and baked in an electric oven (Universal, Cairo, Egypt) at 175 °C for 25 min. The cakes were immediately removed from the molds and left to cool for 30 min at ambient

temperature, then stored in air tight polyethylene pouches and stored until analysis at 4 °C. According to **Cano Lamadrid *et al.*** [43], the jelly candies samples were prepared with some modifications**.** 100 gm sugar added to 200 ml water, then boiling after adding 20 gm of gelatin. After boiling for 2 min, remove and add Limonene 0.11 mg/kg/day and orange oil 0.05 mg/kg/day [42].

Sensory evaluation

Trained panelists assessed flavoring powders, spongecake, and jelly candies samples using anine-poin the donic scale (9= like immensely, 5= neither like nor a dislike, and 1= dislike extremely). Color, flavor, taste, softness, and overall acceptability were evaluated as the primary sensory attributes[44].Twenty-six panelists carried out the different sensory qualities from Food Technology & Nutrition Division, National Research Centre, Cairo, Egypt.

Statistical analysis:

Statistical Package for the Social Sciences (SPSS 22) was applied for statistical analysis using the analysis of variance (ANOVA) and the Duncan test. The data were expressed as Mean ± SD. Differences were considered significant if $p < 0.05$.

Result and Discussion

Emulsion characterization

Table 2 shows the viscosity, the emulsion stability index (CI %), and the total solids content of homogenized emulsions before spray drying. The viscosity of the emulsion is an important factor because this parameter affects the size of microcapsules and the thickness of their walls. A constant trend could be observed in all samples, where the increase of GA from 5– 15% provided higher viscosity in the examined emulsions C1-C3 (74.67–118.00 cp) and F1-F4 (98.33–136.33cp). Whereas the presence of MD in the maximum concentrations, in the absence of GA, led to the lowest viscosity as shown in emulsions C4 (56.33 cp) and F4 (58.67 cp). Using a constant lower concentration of SC (5%) was based on our preliminary experiments, as SC negatively affects the system's viscosity, resists the feed stock's flow rate, and may clog the spray nozzles.

The above results agree with **Bednarska** and **Janiszewska-Turak** [45], who reported the impact of carrier materials on the apparent viscosity of the solutions. They showed the highest viscosity for GA solution as a carrier, while the one with MD (15 DE) had the lowest viscosity. Again, the replacement of MD with GA caused an increase in the viscosity values, whereas the increase was proportional to the concentration of GA. The rise in dextrose equivalent caused a decrease in viscosity values.

The emulsion stability (CI %) data revealed that most emulsions were kinetically stable, especially for the GA and SC wall systems, due to their excellent emulsifying capacity [12]. According to **Table 2**, the increasing GA concentration causes a decrease in creaming index (CI %), which means a more stable emulsion against separation or creaming. Conversely, the poor emulsifying properties of MD were proven by forming a small separation layer with low emulsion stability and higher CI %, as shown in samples C4 (2.00%) and F4 (13%). The above results agree with **Kausadikar *et al.*** [14], who indicated that GA and modified starch were excellent emulsifiers for lemon oil, in contrast to MD, which lowers the emulsion stability. A thin separation layer and a foam phase were observed by **Carneiro *et al.*** [46] upon microencapsulated flaxseed oil with whey protein concentrate and MD.

Table (2): Effect of the wall combinations on emulsion characteristics

Sample code*	Viscosity(cP)	Emulsion stability
C1	$74.67^{f} \pm 0.58$	$2.70^{b} \pm 0.10$
C2	$95.00^{e} \pm 1.00$	$1.00^{d} \pm 0.10$
C3	$118.00^{b} \pm 1.00$	$1.00^{d} \pm 0.01$
C4	$56.33^{h} \pm 0.58$	$2.00^{c} \pm 0.10$
F1	$98.33^{d} \pm 0.58$	$2.80^{b} \pm 0.10$
F2	$110.00^{c} \pm 1.00$	$1.70^{c} \pm 0.10$
F3	$136.33^{a} \pm 0.58$	$0.90^{d} \pm 0.10$
F4	$58.67^{g} \pm 0.58$	$13^{a} \pm 1.00$

* C: Limonene and F: Natural orange oil; 1, 2, 3 and 4 referred to wall mix number in Table (1)

Powder Characteristics

Bulk density: Bulk densities were determined by the tapping method for all spray-dried samples. They ranged from 0.27 to 0.33g/cm^3 (**Table 3**). A different trend was observed for samples Limonene (C) and orange oil (F), where the presence of maltodextrin (MD) as a prominent in-wall mixture resulted in higher bulk density were C1, C4 (0.29, 0.30 g/cm^3), and F1, F4 (0.33,0.31 g/cm^3) which could be related to the higher moisture detected in the same samples compared to the others. The higher density can store large amounts in small volumes compared to products with lower densities. Moreover, higher Bulk density may indicate lower air cavities, which can help prevent oxidation and deterioration of food products.

Values close to the above findings were obtained in spray-dried vegetable oil (0.32-0.34 g/mL), soy milk powders production (0.21-0.22 g/mL), and oregano essential oil microcapsules (0.34-0.45 g/mL) [47]. The high molecular weight of the biopolymer used as a carrier is responsible for differences in bulk density observed upon increased carrier concentrations [48].

Wet ability: In the current study, the spray-dried flavorings showed an excellent dissolve in water, allowing for a different application in various products due to both hydrophobic and hydrophilic sites. The samples: C1 (56.67s), C4 (31.67s), F1 (64.67s), and F4 (36.00s), which have the lowest or no GA, dissolved faster than those which have higher or maximum concentrations of GA like C3 (67.67s) and F3 (84.00s). The samples with MD as a predominant in the wall mixture F4 (36.00s) took up more than two times faster to dissolve in water than those with the maximum GA concentrations F3 (84.00s). Therefore, the use of GA as a carrier increased the dissolution time (**Table 3**). Conversely, the presence and abundance of the hydrophilic hydroxyl groups in MD are responsible for the complete and fast reconstitution of the powder in water [15]**.** In agreement with the above findings, **Aragüez-Fortes *et al.*** [49] reported that guava powder was more soluble with a higher MD concentration where the rehydration times were 90-145s.

Table (3): Bulk density, wett ability, Moisture content, Encapsulation Efficiency (EE), and Oil Retention of spray-dried flavorings

No. Sample *	Bulk density (g/cm3)	Wett ability (s)	Moisture%	EEVOs%	Oil retention%
C1	$0.29^{c} \pm 0.01$	$56.67^{b} \pm 5.77$	$3.40^{bc} \pm 0.03$	$80.66^{de} \pm 0.95$	$88.85^{b} \pm 1.83$
C2	$0.27^{d} \pm 0.00$	$65.67^{b} \pm 9.81$	$3.34^{c} \pm 0.03$	$85.35^{ab} \pm 2.59$	$89.40^{ab} \pm 1.34$
C3	$0.28^{d} \pm 0.00$	$67.67^{b} \pm 9.29$	$3.04^{e} \pm 0.05$	$84.62^{abc} \pm 1.52$	$86.33^{bc} \pm 1.74$
C4	$0.30^{bc} \pm 0.01$	$31.67^{c} \pm 2.89$	$3.39^{bc} \pm 0.02$	$81.81^{cd} \pm 1.07$	$92.22^{a} \pm 1.76$
F1	$0.33^{a} \pm 0.01$	$64.67^{b} \pm 8.08$	$2.97^{e} \pm 0.13$	$78.53^{e} \pm 0.69$	$81.51^{d} \pm 1.29$
F2	$0.29^{c} \pm 0.00$	$66.00^{b} \pm 10.39$	$3.19^{d} \pm 0.02$	$85.77^{a} \pm 1.73$	$86.02^{bc} \pm 1.63$
F3	$0.29^{c} \pm 0.00$	$84.00^{a} \pm 7.94$	$3.48^{b} \pm 0.03$	$82.47^{bcd} \pm 1.77$	$83.97^{cd} \pm 2.02$
F4	$0.31^{b} \pm 0.01$	$36.00^{c} \pm 3.00$	$3.62^{a} \pm 0.04$	$81.84^{cd} \pm 2.02$	$86.97^{bc} \pm 2.55$

*C: Limonene and F: Natural orange oil; 1, 2, 3and 4 referred to wall mix number in Table (1)

Moisture content: The moisture contents of the formulated spray-dried capsules were 2.97-3.62 % (**Table 3**). According to **Chew *et al.*** [50], the moisture content in food powders suitable for long-term storage should be lower than 6% to extend the powder's usefulness for technological purposes and increase its stability and quality. The represented results of the current study indicated that the obtained flavor powders had an appropriate moisture content which is expected to minimize the chance of microbial contamination and lipid oxidation. The decrease in moisture content was observed in C1 (3.40%) - C3 (3.04%) samples, with a higher increase in C4 (3.39%) and F4 (3.62%) (**Table 3**). In contrast to MD, GA does not seem to affect the moisture, forming the particle shell very quickly, avoiding water diffusion during the drying process.

The above results are in agreement with those obtained in studies on spray drying of essential oils (1.70-4.16%) [17-51] and d-limonene (1.20-2.70%) [52]. Despite the drying temperature, **Bednarska** and **Janiszewska-Turak** [45] reported that moisture content in chokeberry juice encapsulated with GA: MD 10 (3:1) found as 2.9 % compared to 0.9 % for chokeberry juice with AG: MD 15 (1:1), which is agreed with the trend of the current studies for samples orange oil (F) (**Table 3**). On the other hand, the rise in moisture content showed by C4 (3.39 %) and F4 (3.62%) samples which contain limonene and orange oil, are associated with the higher content of MD, as reported by **Rodríguez *et al.*** [53].

Encapsulation Efficiency: **Table 3** gives encapsulation efficiency and total oil retention of flavorings under investigation using GA, MD, SC, binary, and ternary blends. The data showed that oil retention % was maximum for C4 (92.22%) and F4 (86.97%) compared to other samples in the corresponding classes with a significant difference, where C4 was significantly different from C1 and C3 samples, but the non-significant difference with C2 sample. However, F4 was a significant difference from the F1 sample, but the non-significant difference between F2 and F3 samples. Sample C4 and F4 are associated with predominant MD as a wall material. The variation in wall blend used in the encapsulation process significantly affected the oil retention % among the samples after spray drying.

This study investigated the effects of carrier materials and their mixtures in different concentrations on the encapsulation efficiency (EE %) (**Table 3**). EE % after spray drying was positively influenced by 10% GA concentration, as shown in samples C2 (85.35%) and F2 (85.77%). The previous values constitute nearly the maximum peak in EE % line-trend in all samples, where the concentration of GA in the wall mixture of these samples seems to be the optimum in feed solids for flavor retention based on solubility and viscosity in solution according to the hypothesis of **Reineccius** [54]. The non-significant differences between C2-C3 lead simply because the optimum GA concentration in the Limonene (C) samples could be between 10-15% GA in the wall blend. In agreeing with **Charve** and **Reineccius** [55] and **Pratiwi *et al.*** [56], the GA: MD ratio is an essential factor in the encapsulation of flavoring

Where, the ratio of 3:1 is not sufficient here for samples C1 and F1 showed a lower EE% due to the ratio of GA: MD which was 5:1. The general trend of decrease or non-significant difference in EE% from C2-C3 might have been due to the thinner layers of wall material between encapsulated oleoresin droplets in addition to the high in feed viscosity (**Table 2**), which delays particle formation during atomization, consequently favoring volatile losses during drying [57]. In the same context, the encapsulation efficiency was found to increase significantly until a GA/ MD blend ratio reached 40/60. After that, the efficiency was found to decrease significantly, which is consistent with the current study's findings [58].

According to **Charve** and **Reineccius** [55], the loss of aldehydes like citral is severe, while retention of limonene is described as moderate, which indicates the effect of core chemical structure. The addition of MD to the wall mixture increases the glass transition temperature of the blend and exhibits stronger resistance to a humid environment during storage [59]; in addition, GA in optimum concentration leads to less void volume or surface cracks [55].

Non-enzymatic Browning: Results are reported in **Table 4**. According to the different CIE-LAB characteristics, significant differences were observed in all samples for all color parameters, except for L*, which showed a non-significant difference between F1 (87.68) and F3 (87.65), F4 (87.72). The orange oil (F) series had the highest lightness (L*), while the limonene(C) series has been described by the lowest lightness values (**Table 4**). A reverse relation is shown between L* and a* and b* values. Regarding the color coordinate a*, it offers slightly negative values, which indicate green color. The increase of a*and b* corresponds to the rise in redness (a*) and yellowness (b*) occurring at the beginning of NEB. NEB influences food systems in terms of nutritional and sensory attributes. The stability of the food systems should be considered during processing; especially when using proteinous biomaterials in volatile encapsulation like aldehydes, it is essential to determine if the system will be stable during processing [60].

The caramelization of MD could be responsible for the higher values in limonene samples. Orange oil (F-samples) represents many aldehydes, ketones, and alcohols that prevent NEB reactions. Products with more intensive green and blue color notes are formed only later due to secondary reactions. They cannot occur in a short encapsulation time and at relatively low temperatures (120–160°C) [12].

According to the results of different CIE-LAB characteristics reported in **Table 4**, all spray-dried flavorings under went significant changes in color, especially in b*. However, such changes in color parameters are not dramatic and are believed to change the final products' sensory properties or quality. The changes in b* were negative in many samples, i.e., absence of browning or oxidation. In contrast, a* values change to remain in the negative value (**Table 4**).

Table (4): Non-enzymatic Browning of spray-dried flavorings

Sample*	L*	a*	b*
White blank	20.37	0.02	-0.04
Black blank	19.77	-0.09	0.46
C1	$85.56^{e} \pm 0.01$	$-1.19^{f} \pm 0.00$	$7.13^{b} \pm 0.01$
C2	$85.50^{e} \pm 0.00$	$-1.49^{h} \pm 0.01$	$6.78^{c} \pm 0.01$
C3	$85.24^{f} \pm 0.00$	$-1.35^{g} \pm 0.01$	$6.09^{d} \pm 0.01$
C4	$85.68^{d} \pm 0.01$	$-1.04^{e} \pm 0.00$	$7.91^{a} \pm 0.01$
F1	$87.68^{ab} \pm 0.01$	$-0.95^{d} \pm 0.01$	$3.98^{e} \pm 0.00$
F2	$86.45^{c} \pm 0.02$	$-0.48^{a} \pm 0.00$	$2.57^{g} \pm 0.01$
F3	$87.65^{b} \pm 0.00$	$-0.54^{b} \pm 0.02$	$2.03^{h} \pm 0.01$
F4	$87.72^{a} \pm 0.10$	$-0.94^{c} \pm 0.01$	$3.36^{f} \pm 0.01$

* C: Limonene and F: Natural orange oil;1 , 2, 3 and 4 referred to wall mix number in Table(1)

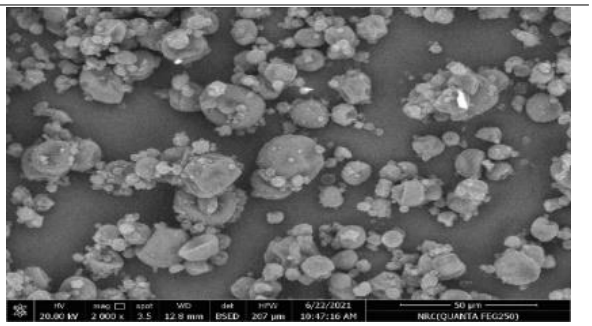
Powder morphology by Scanning electron microscopy (SEM): The analysis of the surface of flavorings obtained with different wall blends was performed using SE Mall owing three-dimensional characteristics to be visualized at different extensions; 5, 10, and 50µm (**Figures 1, 2**). In both C and orange oil (F) series samples, increasing the GA concentration affects the morphology of the particles with more agglomeration, while the predominant MD leads to more spherical and smooth particles. However, small bulbs with semi spherical particles were shown in F1 samples on a wide scale. In general, neither cracks nor porous were found in the micro structure of the samples.

Silva *et al.* [2] showed that MD exhibited excellent encapsulating properties, which enabled the formation of homogeneous capsules, while microencapsulation of jaboticaba peel extract using GA/MD had similar structures to those obtained with MD with few wrinkles and smooth surfaces. In the same context, MD used to encapsulate cheese aroma formed a more spherical shape and smoother surface

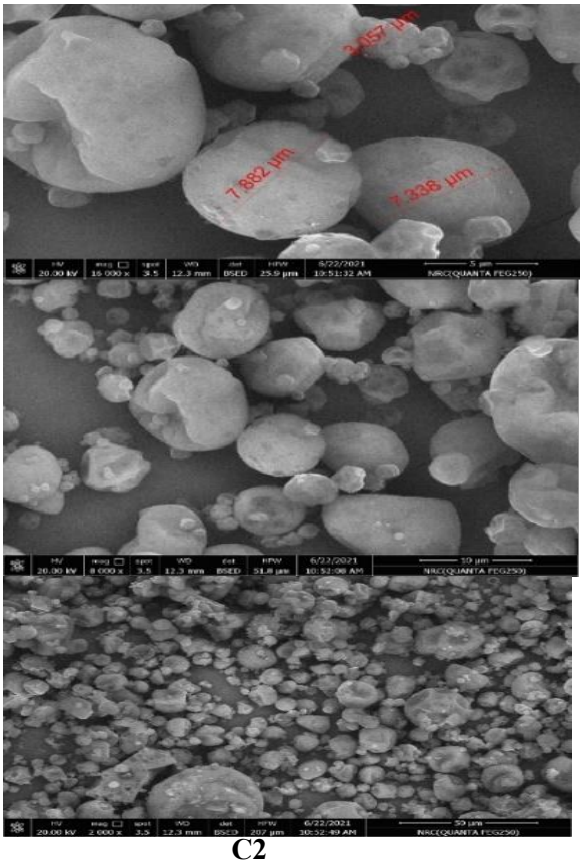
than modified starch which exhibited highly dented surfaces [61]. The previous results agree with the present study's findings, where MD represents an efficient and economical but with less emulsifying properties than GA or SC in the wall blend. The adherence of small particles to the surface of larger particles which was observed in most of the microstructures (**Figure 1**), was also reported by **Silva *et al.*** [2] and **Cano-Chauca *et al.*** [62] on using MD and GA/MD as a wall material during the preparation of jaboticaba peel extract and mango powders, respectively.

Focusing on the nature of the particle's surfaces, rough surfaces with a larger contact area, which is noted in the C4-sample at 5µm (**Figure 1**), are an obstacle to the flow of the powder and enables degraded reactions like oxidation and therefore affect negatively on the quality of the final product [63]. On the other hand, the wrinkled surface that appeared in many samples like F4 (**Figure 2**) could be due to the rapid shrinkage of the emulsion droplets during the early stages of spray drying [15]. The wrinkled surface may resist the free-flowing properties of the powder but to a small extent.

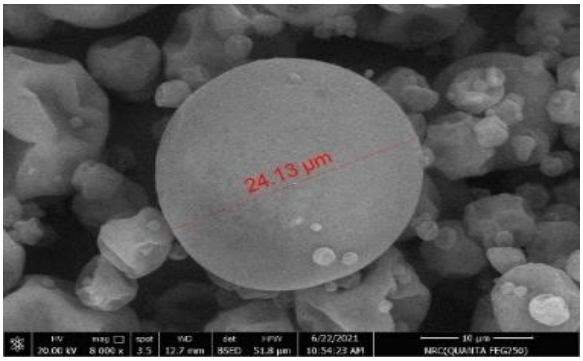
Tiny bubbles or craters on the skin of the particles appeared on some SEM microstructures like that of C1-C4 and F1 (**Figure 1, 2**). The formation of the secretors was explained based on the rapid solidification of minute particles compared to the large droplets; consequently, craters formed due to collisions in the drying tower among solid and semi-solid particles, which are different in sizes [64]**.** According to **Jones *et al.*** [65], craters may form on the surface of particles as an artifact due to the application of high vacuum during the preparation of the sample for SEM imaging and the evaporation of surface oil droplets. The concavities are shown by **Subtil *et al.*** [66] during spray drying of hydrolyzed
case in using MD and GA as a wall material; however, it can negatively affect the flow conditions.

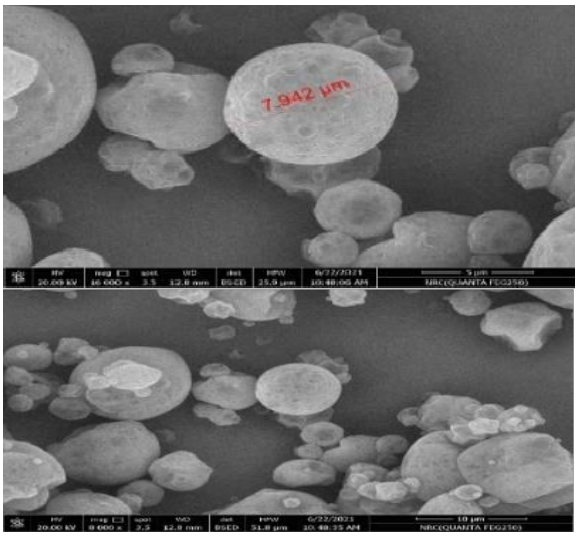


C1



C2





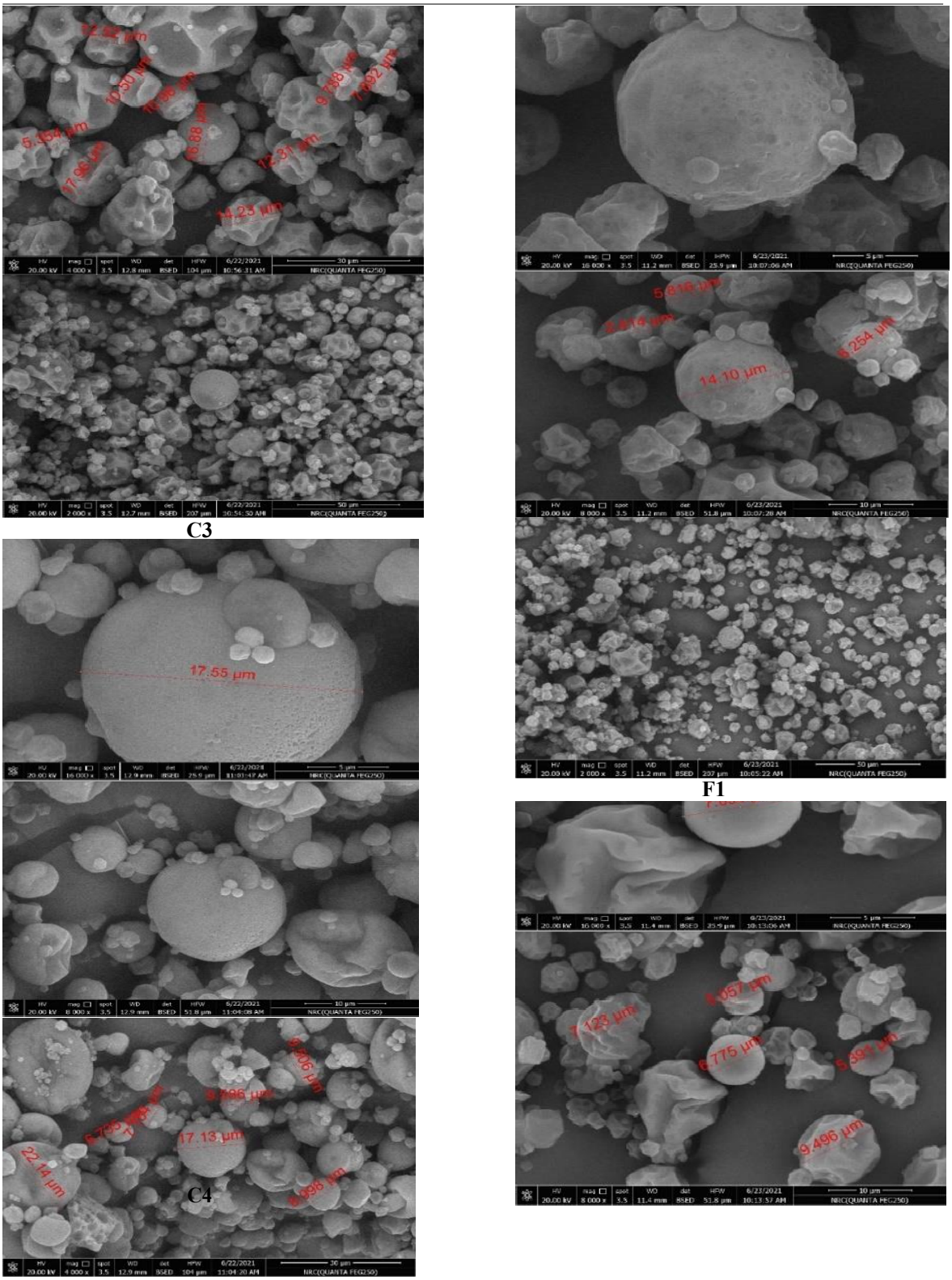
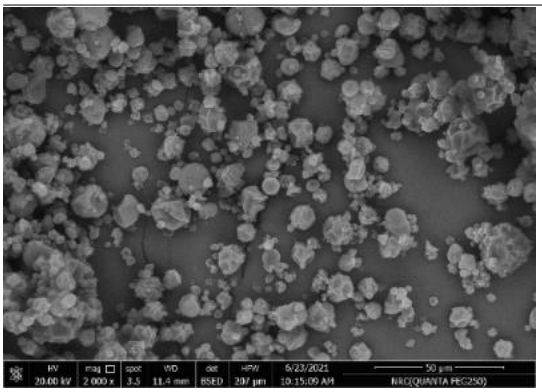
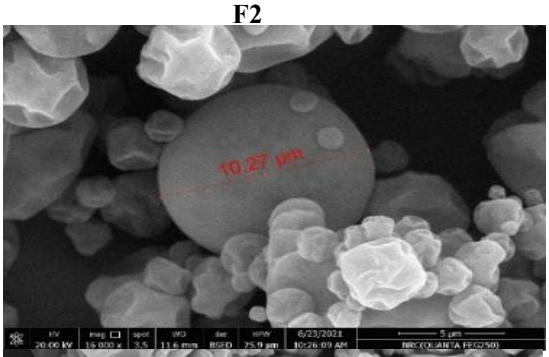
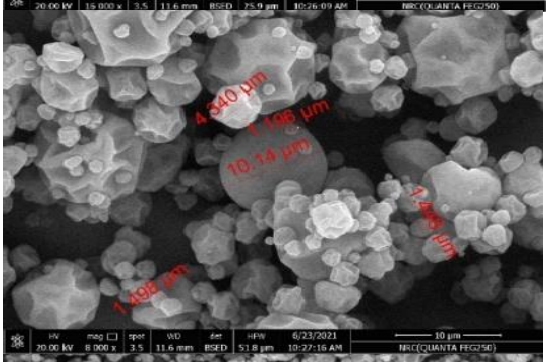


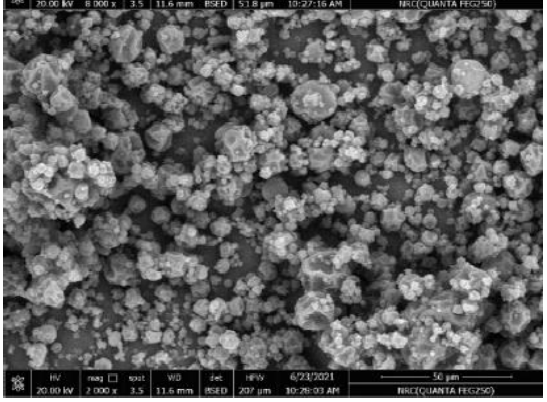
Figure 1. SEM images of spray-drying limonene (C) particles at different magnifications at 5, 10, and 50 µm using different wall material combinations



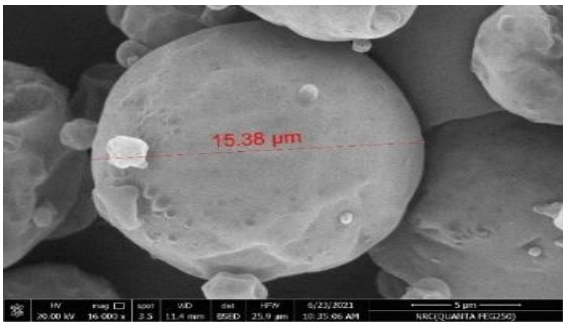
F2

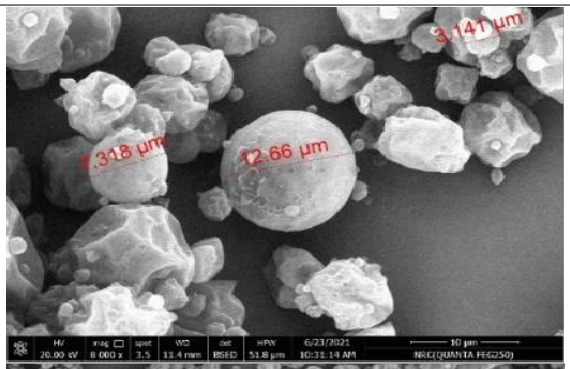


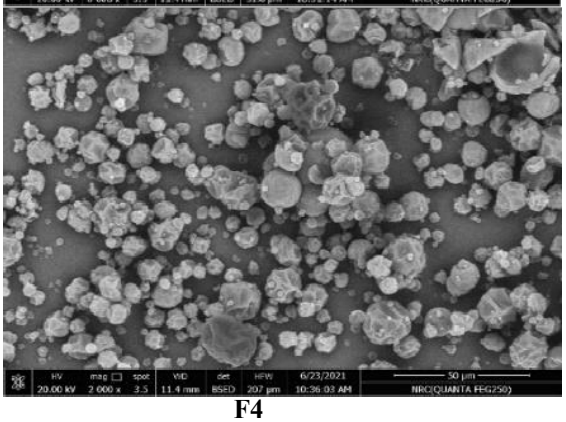




F3







F4

Figure 2. SEM images of spray-drying orange oil (F) particles stored for 6-months at different magnifications (5, 10, and 50 µm) using different wall material combinations

Sensory evaluation:

The flavor attributes for the spray-dried flavorings were evaluated, as shown in Table 5. C4 and F4 recorded the highest scores with significant differences for flavor attributes but not for color. Therefore, they were chosen to fortify and supplement sponge cake and jelly candies as food product examples. Color, flavor, taste, softness, and overall preference of control sponge cake and the cakes supplemented with spray-dried flavorings were evaluated, and the results are presented in Tables 6-7. Higher scores were shown for the sponge cake samples supplemented with spray-dried flavorings than the control but without significant differences (Table 6). Samples supplemented with limonene were the highest preferred in all attributes, but not the softness. Upon evaluation by panelists, higher scores for the fortified jelly candies samples fortified with spray-dried flavorings were detected compared to the control with statistically significant differences ($P < 0.05$) **(Table 7)**. In contrast to the sponge cake samples, jelly candies samples fortified with spray-dried orange peel oil were appreciated the most, except for the color, but no significant difference was observed ($P > 0.05$).

Table (5): Sensory evaluation of spray-dried samples

No. sample*	Color (9)	Flavor (9)	Overall preference (9)
C1	$7.06^{a} \pm 1.11$	$6.17^{abc} \pm 1.89$	$5.83^{a} \pm 2.09$
C2	$7.06^{a} \pm 1.30$	$6.11^{abc} \pm 1.81$	$5.56^{a} \pm 1.46$
C3	$6.89^{a} \pm 1.32$	$6.39^{abc} \pm 1.91$	$5.61^{a} \pm 1.91$
C4	$7.06^{a} \pm 1.16$	$7.22^{a} \pm 1.44$	$6.00^{a} \pm 1.88$
F1	$6.78^{a} \pm 1.00$	$5.44^{c} \pm 1.25$	$5.89^{a} \pm 1.64$
F2	$6.22^{a} \pm 1.31$	$5.94^{bc} \pm 1.47$	$5.94^{a} \pm 1.55$
F3	$6.33^{a} \pm 1.14$	$5.89^{bc} \pm 1.28$	$6.00^{a} \pm 1.61$
F4	$7.11^{a} \pm 1.49$	$6.67^{ab} \pm 0.97$	$6.39^{a} \pm 1.54$

* C: Limonene and F: Natural orange oil;1, 2, 3 and 4 referred to wall mix number in Table (1)

Table (6): Sensory evaluation of Sponge Cake

No. sample	Color (9)	Flavor (9)	Taste (9)	Softness (9)	Overall preference (9)
Control	$7.52^{a} \pm 1.25$	$6.88^{a} \pm 1.56$	$7.08^{a} \pm 1.70$	$7.46^{a} \pm 1.53$	$7.38^{a} \pm 1.44$
Limonene	$7.67^{a} \pm 1.27$	$7.42^{a} \pm 1.21$	$7.67^{a} \pm 1.19$	$7.83^{a} \pm 0.81$	$7.87^{a} \pm 0.78$
Orange oil	$7.63^{a} \pm 1.58$	$7.12^{a} \pm 1.08$	$7.25^{a} \pm 1.09$	$7.90^{a} \pm 0.72$	$7.50^{a} \pm 0.86$

Table (7): Sensory evaluation of Jelly Candies

No. sample	Color (9)	Flavor (9)	Taste (9)	Texture (9)	Overall preference (9)
Control	$6.68^{b} \pm 1.49$	$5.96^{b} \pm 1.62$	$6.48^{b} \pm 1.53$	$7.80^{a} \pm 1.44$	$6.38^{b} \pm 1.29$
Limonene	$7.84^{a} \pm 1.17$	$7.58^{a} \pm 0.98$	$7.48^{a} \pm 0.91$	$8.12^{a} \pm 1.05$	$7.72^{a} \pm 0.95$
Orange oil	$7.80^{a} \pm 1.04$	$7.84^{a} \pm 0.75$	$7.56^{a} \pm 0.96$	$8.16^{a} \pm 0.85$	$7.90^{a} \pm 0.89$

Biological parameters

Nutritional parameters: The results showed a non-significant initial body weight, final body weight, total food intake, and food efficiency compared with normal control **(Table 8)**. A significant reduction observed in body gain in all groups (C: 39.4±0.38, C1: 34.4±2.7, C2: 33.8±1.23, C3: 31.5±0.83, C4: 28.7±3.23) when compared with normal control (49.5±3.06).

The results in table **(9)** showed a non-significant initial body weight, final body weight, total food intake, and food efficiency in all groups compared with normal control. A significant reduction was observed in body gain in all groups (F: 41.4 ± 0.35, F1: 39.7 ± 1.28, F2: 35.7 ± 1.3, F3: 34.2 ± 2.75, F4: 32 ± 2.73) when compared with normal control (49.5 ± 3.06).

Biochemical parameters: The results showed a non-significant in all biochemical parameters in all groups when compared with normal control **(Table10)**.

The results didn't record any significant changes in all biochemical parameters in all groups when compared with normal control **(Table11)**.

Table (8): The different effects of Limonene (C) flavor pure and microencapsulated on body weight, Body gain, total food intake, and food efficiency

Group	Initial body weight (g)	Final body weight (g)	Body gain (g)	Total food intake (g)	Food Efficiency
Normal Control (negative)	$156.2^{a} \pm 10.25$	$205.7^{a} \pm 13.21$	$49.5^{a} \pm 3.06$	$3535.5^{a} \pm 5.16$	$0.014^{a} \pm 0.59$
Limonene(C)	$163.2^{a} \pm 11.31$	$202.6^{a} \pm 11.68$	$39.4^{a} \pm 0.38$	$3529.5^{a} \pm 7.75$	$0.011^{a} \pm 0.05$
C_1	$165.4^{a} \pm 10.62$	$200.3^{a} \pm 13.32$	$34.4^{b} \pm 2.7$	$3525.5^{a} \pm 4.93$	$0.009^{a} \pm 0.55$
C_2	$166.1^{a} \pm 10.43$	$199.9^{a} \pm 11.66$	$33.8^{c} \pm 1.23$	$3520.4^{a} \pm 5.68$	$0.096^{a} \pm 0.005$
C_3	$167.2^{a} \pm 11.13$	$198.7^{a} \pm 11.96$	$31.5^{d} \pm 0.83$	$3518.6^{a} \pm 5.98$	$0.009^{a} \pm 0.14$
C_4	$169.1^{a} \pm 10.22$	$197.8^{a} \pm 13.45$	$28.7^{e} \pm 3.23$	$3515.5^{a} \pm 8.45$	$0.008^{a} \pm 0.38$

Values are represented as Mean ± SD (n=6) in which the same letters in each column reflect a non-significant difference across varieties, whereas different letters reflect a significant difference at $P \leq 0.05$. Food efficiency=Body Gain / Total Food Intake.

Table (9): The different effects of orange oil (F) flavor pure and microencapsulated on body weight, Body gain, total food intake, and food efficiency

Group	Initial body weight (g)	Final body weight (g)	Body gain (g)	Total food intake (g)	Food Efficiency
Normal Control (negative)	156.2^{a}±10.15	205.7^{a}±13.21	49.5^{a}±3.06	3535.5^{a}±5.16	0.014^{a}±0.59
Orange oil (F)	162.7^{a}±11.23	204.1^{a}±11.58	41.4^{a}±0.35	3533.4^{a}±5.78	0.012^{a}±0.06
F_1	163.9^{a}±10.17	203.6^{a}±11.45	39.7^{b}±1.28	3530.3^{a}±6.14	0.011^{a}±0.21
F_2	164.8^{a}±11.37	200.5^{a}±12.67	35.7^{c}±1.3	3528.4^{a}±7.25	0.01^{a}±0.18
F_3	165.5^{a}±10.54	199.7^{a}±13.29	34.2^{d}±2.75	3525.7^{a}±6.36	0.01^{a}±0.43
F_4	166.8^{a}±10.43	198.8^{a}±13.16	32^{e}±2.73	3524.6^{a}±5.32	0.009^{a}±0.51

Values are represented as Mean ± SD (n=6) in which the same letters in each column reflect a non-significant difference across varieties, whereas different letters reflect a significant difference at $P \leq 0.05$. Food efficiency=Body Gain / Total Food Intake

Table (10): The effects of Limonene (C) flavor pure and microencapsulated on different biochemical parameters

Groups / Parameters	Normal Control (negative)	Limonene (C)	C_1	C_2	C_3	C_4
Glucose (mg/dL)	98.3^{a}±2.93	102.2^{a}±2.49	99.2^{a}±1.86	104.3^{a}±2.35	105.4^{a}±2.41	111.3^{a}±2.14
GHb %	5.5^{a} ±1.25	6.1^{a}±1.62	6.4^{a}±1.35	6.5^{a}±1.42	5.8^{a}±1.63	6.9^{a}±1.22
Cholesterol (mg/dL)	89.8^{a} ±4.23	88.3^{a}±4.12	88.5^{a}±5.03	87.3^{a}±4.12	89.4^{a}±5.11	90.1^{a}±4.67
HDL (mg/dL)	26.8^{a}±1.91	24.6^{a}±2.92	25.6^{a}±2.12	26.1^{a}±1.83	25.5^{a}±1.78	25.7^{a}±1.95
LDL (mg/dL)	41.8^{a}±2.03	41.3^{a}±1.27	41.1^{a}±1.39	41.5^{a}±1.55	42.2^{a}±1.77	42.5^{a}±1.65
Triglycerides (mg/dL)	49.5^{a}±5.16	48.5^{a}±5.66	43.7^{a}±5.23	46.6^{a}±5.14	47.2^{a}±5.16	51.2^{a}±5.45
Creatinine (mg/dL)	0.89^{a}±0.21	0.88^{a}±0.17	0.86^{a}±0.23	0.87^{a}±0.35	0.87^{a}±0.16	0.90^{a}±0.18
Urea (mg/dL)	36.5^{a}±3.03	35.7^{a}±1.77	35.6^{a}±2.32	37.3^{a}±1.63	36.5^{a}±2.11	36.8^{a}±1.61
Uric Acid (mg/dL)	2.97^{a}±0.28	2.87^{a}±0.31	2.76^{a}±0.21	2.84^{a}±0.19	2.63^{a}±0.25	2.99^{a}±0.28
AST (IU/L)	135.2^{a}±2.79	134.5^{a}±2.45	131.5^{a}±1.99	131.8^{a}±2.11	129.6^{a}±1.98	137.5^{a}±1.79
ALT (IU/L)	96.9^{a}±2.71	96.6^{a}±2.59	95.4^{a}±2.34	94.4^{a}±2.18	93.2^{a}±2.78	100.9^{a}±2.89
ALP (IU/L)	69.8^{a}±12.46	68.6^{a}±11.71	69.2^{a}±10.02	67.4^{a}±11.14	65.5^{a}±11.23	70.9^{a}±12.24
Albumin (g/dL)	2.75^{a}±0.32	2.35^{a}±0.51	2.12^{a}±0.44	2.14^{a}±0.26	2.16^{a}±0.23	2.38^{a}±0.43
Total Protein (µmol/L)	12.17^{a}± 0.83	12.24^{a}±0.58	11.77^{a}± 0.54	11.85^{a}± 0.062	12.03^{a}±0.49	13.47^{a}±0.076

Values are represented as Mean ± SD (n=6) in which the same letters in each column reflect a non-significant difference across varieties, whereas different letters reflect a significant difference at $P \leq 0.05$. Food efficiency=Body Gain / Total Food Intake

Table (11): The effects of orange oil (F) flavor pure and microencapsulated on different biochemical parameters

Groups / Parameters	Normal Control(negative)	orange oil(F)	F_1	F_2	F_3	F_4
Glucose (mg/dL)	98.3^{a}±2.93	98.1^{a}±2.43	97.6^{a}±2.33	99.2^{a}±2.45	101.3^{a}± 2.01	105.2^{a}± 2.34
GHb %	5.5^{a}±1.25	5.4^{a}±1.22	5.3^{a}±1.16	5.2^{a}±2.03	5.1^{a}±1.46	6.2^{a}±1.55
Cholesterol (mg/dL)	89.8^{a}±4.23	88.4^{a}±5.11	87.2^{a}±4.39	87.1^{a}±5.27	89.5^{a}±4.32	90.4^{a}±3.22
HDL (mg/dL)	26.8^{a}±1.91	25.2^{a}±2.93	25.4^{a}±2.32	26.7^{a}±1.85	25.7^{a}±1.79	26.7^{a}±1.99
LDL (mg/dL)	41.8^{a}±2.03	44.5^{a}±1.32	42.5^{a}±1.25	41.4^{a}±1.35	41.3^{a}±1.37	42.8^{a}±1.55
Triglycerides (mg/dL)	49.5^{a}±5.16	48.1^{a}±5.43	43.1^{a}±4.89	46.3^{a}±5.73	47.3^{a}±4.26	51.2^{a}±2.36
Creatinine (mg/dL)	0.89^{a}±0.21	0.88^{a}±0.62	0.86^{a}±0.35	0.86^{a}±0.24	0.85^{a}±0.31	0.92^{a}±0.28
Urea (mg/dL)	36.5^{a}±3.03	35.7^{a}±2.36	35.1^{a}±2.46	34.3^{a}±3.78	33.3^{a}±4.34	37.5^{a}±1.12
Uric Acid (mg/dL)	2.97^{a}±0.28	2.88^{a}±0.35	2.77^{a}±0.14	2.83^{a}±0.28	2.67^{a}±0.42	2.94^{a}±0.33
AST (IU/L)	135.2^{a}±2.79	134.1^{a}± 2.37	131.1^{a}± 3.35	131.2^{a}±3.56	129.2^{a}± 3.48	137.4^{a}± 1.87
ALT (IU/L)	96.9^{a}±2.71	96.2^{a}±2.39	95.1^{a}±2.36	94.3^{a}±2.25	92.9^{a}±2.56	99.8^{a}±2.73
ALP (IU/L)	69.8^{a}±12.46	68.3^{a}±10.46	69.2^{a}±12.54	67.1^{a}±11.42	65.1^{a}±11.67	70.3^{a}±11.72
Albumin (g/dL)	2.75^{a}±0.32	2.45^{a}±0.41	2.32^{a}±0.34	2.16^{a}±0.28	2.19^{a}±0.24	2.36^{a}±0.46
Total Protein (µmol/L)	12.17^{a}± 0.83	12.09^{a}± 0.62	11.79^{a}±0.09	11.84^{a}±0.38	12.13^{a}± 0.54	13.32^{a}± 0.62

Values are represented as Mean ± SD (n=6) in which the same letters in each column reflect a non-significant difference across varieties, whereas different letters reflect a significant difference at $P \leq 0.05$. Food efficiency=Body Gain / Total Food Intake

There is no doubt that the results of this study differed and agreed with the results of many other studies where little research had been done in this area. In the last years, numerous studies have found that limonene and orange oil possess powerful antioxidative properties and protect organisms from oxidative damage [67-68]. In particular, they're rapidly and nearly all absorbed in the gastrointestinal tract, both humans and animals [69], and also an effective anti-carcinogen [70] hepatoprotective, immunomodulatory, and anti-inflammatory properties [71]. Antimicrobial and antifungal effects of orange peel essential oil [72] may come from limonene content. Our study was performed for 30 days to assess the potential impact of limonene with a daily dose (600 mg/Kg BW /day) and orange oil with a daily dose (600 mg/Kg BW /day) on rats' health. Through our results, we noticed that no abnormalities were seen in the appearance or behavior of the rats at any time during the study. They appeared to reduce the symptoms of anxiety and depression. Also, there were no signs of irritation and acute toxicity during the study, such as diarrhea, alterations of skin and fur, mucous membranes, eyes, circulation, breadth, functions of the nervous system, salivation, diarrhea, and convulsions. The nutritional data of limonene and orange oil used in natural and microencapsulated forms represented in tables (8, 9) showed non-significant initial body weight, final body weight, total food intake, and food efficiency in all groups when compared with normal control. A significant reduction was observed in body gain in all groups compared with normal control, and these results agreed with other studies [73]. Although the decrease in body gain observed in all groups (natural and microencapsulated) is unknown, it was probably associated with the strong taste of the materials [74]. Also, different biochemical parameters were used in the current study to measure characteristics that presented as an indicator of some biological state or condition [75-76]. Generally, biomarkers are applied for clinical diagnostic purposes and as tools to evaluate the effectiveness of a nutrition or drug intervention. According to the biochemical parameters results in both natural and microencapsulated forms represented in tables (10, 11), there were non-significant in all parameters when comparing limonene and orange oil in all different groups with normal control results agreed with many studies [76-77]. Based on the *in vivo* results which included the nutritional and biochemical parameters, it is safe to conclude that there is no effect or any signs of acute toxicity or any significant impact on weight noticed by the use of limonene and orange oil in both natural and microencapsulated scale and they can be applying as feed additives.

Conclusion

The use of encapsulation by spray-drying technique to load the orange peel oil has not affected the nutritional or the biochemical parameters in all groups supplemented with the spray-dried flavorings compared to normal control. Compared to control samples, better sensory attributes were shown in sponge cake and jelly candies samples fortified with spray-dried flavorings. According to the present study's findings, applying such spray-dried flavorings in food products proved to be safe.

Ethics approval and consent to participate

The animal experiment was conducted in compliance with the U.K. Animals (Scientific Procedures) Act, 1986 and related guidelines; the Medical Research Ethics Committee (MREC) had reviewed and approved EU Directive 2010/63/EU on animal experiments and the use of animals. It was carried out for studies involving animals with the registration number (12912012022), complying with the World Medical Association code of ethics. National regulations accompanied it on the treatment and use of laboratory animals.

Competing interests

All authors declare no competing interests.

References

1- Nedovic,V.; Kalusevic, A.; Manojlovic,V.; Levic, S. and Bugarski, B. (2011). An overview of encapsulation technologies for food applications. Procedia food science, 1:1806-1815.

2- Silva, D.F.; Favaro-Trindade, C.S.; Rocha, G.A. and Thomazini, M. (2012). Microencapsulation of lycopene by gelatin–pectin complex coacervation. J. Food Process. Preserv., 36 (2):185–190.

3- Jyothi, N.V.N.; Prasanna, P.M.; Sakarkar, S.N.; Prabha,K.S.; Ramaiah, P.S. and Srawan, G.Y. (2010). Microencapsulation techniques, factors influencing encapsulation efficiency. J. Microencapsul., 27(3):187–197.

4- Özkan, G. and Ersus, S. (2014). Microencapsulation of natural food colorants. Int. J. Nutr. Food Sci., 3(3):145–156.

5- Zhang, Y.; Zhou, Y.; Cao, S.; Li, S.; Jin, S. and Zhang, S. (2015). Preparation, release and physicochemical characterisation of ethyl butyrateand hexanal inclusion complexes with beta- and gamma-cyclodextrin. Journal of Microencapsulation, 32(7): 711–718. https://doi.org/10.3109/02652048.2015.1073391.

6- de Lima Petito, N.; da Silva Dias, D.; Costa, V.G.; Falcao, D. Q. and de Lima Araujo, K. G. (2016). Increasing solubility of red bell pepper carotenoids by complexation with 2-hydroxypropyl-betacyclodextrin. Food Chemistry,

208, 124–131. https://doi.org/10.1016/j.foodchem.2016.03.122

7- Wang, M.; Doi, T. and McClements, D.J. (2019). Encapsulation and controlled release of hydrophobic flavors using biopolymer-based microgel delivery systems: Sustained release of garlic flavor during simulated cooking. Food Research International, 119:6–14. https://doi.org/10.1016/j.foodres.2019.01.042

8- Gharsallaoui, A.; Roudaut, G.; Chambin, O.; Voilley, A. and Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Res. Int., 40 (9):1107–1121.

9- Farouk, A.; Hathout, A.S.; Amer, M.M.; Hussain, O.A. and Fouzy, A.S.M. (2022). The Impact of Nanoencapsulation on Volatile Constituents of *Citrus sinesis* L. Essential Oil and their Antifungal Activity. Egypt. J. Chem. 65: (3): 527–538. DOI: 10.21608/ejchem.2021.95579.4485.

10- DeMedina-Salas, L.; Giraldi-Díaz, M.R.; Castillo-González, E. and Morales-Mendoza, L.E. (2020). Valorization of Orange Peel Waste Using Precomposting and Vermicomposting Processes.Sustainability, 12, 7626. https://doi.org/10.3390/su12187626.

11- Magalhães, M.L.; Ionta, M.; Ferreira, G.Á.; Campidelli, M.L.L.; Nelson, D.L.; Ferreira, V.R.F.; Rezende, D.A.D.C.S. and Cardoso, M.D.G. (2020). Biological activities of the essential oil from the Moro orange peel (*Citrus sinensis* (L.) Osbeck). Flavour and Fragrance Journal, 35(3): 294-301. https://doi.org/10.1002/ffj.3561.

12- Farouk, A.; El-Kalyoubi, M.; Ali, H.; Abd ElMageed, M.; Khallaf, M. and Moawad, S. (2020). Effects of carriers on spray-dried flavors and their functional characteristics. Pakistan Journal of Biological Sciences, 23 (3): 257-263. DOI:10.3923/pjbs.2020.257.263

13- Xin, X.; Essien, S.; Dell, K.; Woo, M.W. and Baroutian S. (2022). Effects of Spray-Drying and Freeze-Drying on Bioactive and Volatile Compounds of Smoke Powder Food Flavouring. Food Bioprocess Technol. ,https://doi.org/10.1007/s11947-022-02779-3

14- Kausadikar, S.; Gadhave, A.D. and Waghmare, J. (2015). Microencapsulation of lemon oil by spraydrying and its application in flavor tea. Advances in Applied Science Research, 6(4):69-78. www.pelagiaresearchlibrary.com

15- Edris, A.E.; Kalemba, D.; Adamiec, J. and Piatkowski, M. (2016). Microencapsulation of *Nigella sativa* oleoresin by spray drying for food and nutraceutical applications. Food Chem., 204:326-333. http://dx.doi.org/10.1016/j.foodchem.2016.02.143

16- Klinkesorn, U.; Sophanodora, P.; Chinachoti, P. and McClements, D.J. (2004). Stability and rheology of corn oil-in-water emulsions containing maltodextrin. Food Res. Int., 37:851-859.

17- Adamiec, J. and D. Kalemba, (2006). Analysis of microencapsulation ability of essential oils during spray drying. Drying Technology, 24(9):1127-1132. http://dx.doi.org/10.1080/07373930600778288

18- AOAC (2012). Official Methods of Analysis of AOAC international, 17th Ed. Edited by Dr. William Horwitz. Publish by AOAC international Suite 500.Gaithersburg,Maryland 20877-2417 U.S.A. Association of Official Analytical Chemists.

19- Quek, Y.; Chok, K. and Swedlund, P. (2007). The physicochemical properties of spraydried watermelon powder. Chemical Engineering and Processing: Process Intensification, 46:386–392.

20- Fernandes, R.V.d.B.; Borges, S.V. and Botrel, D.A. (2014).Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. Carbohydrate Polymers, 101:524–532. http://dx.doi.org/10.1016/j.carbpol.2013.09.083

21- Varavinit, S.; Chaokasem, N. and Shobsngob, S. (2001). Studies of flavor encapsulation by agents produced from modified sago and tapioca starches. *Starch*, 53, (6):281-287. http://dx.doi.org/10.1002/1521379X(200106)53:6<281::AIDSTAR281>3.0.CO;2-R.

22- Rubén, O.; Bustos, C.; Francesca, V.; Alberti, R.; Silvia, B. and Matiacevich. (2016). Edible antimicrobial films based on microencapsulated lemon grass oil. J Food Sci Technol (January 2016) 53(1):832–839. DOI10.1007/s13197-015-2027-5.

23- Ezzat, A.; Abdelhamid, A.O.; El Awady, M.K.; Abd El Azeem, A.S.; and Mohammed, D.M. (2017). The biochemical effects of nano tamoxifen and some bioactive components in experimental breast cancer. Bimed Pharmacother. 95: 571-576.

24- AOAC (1990). Official methods of analysis of the Association of Official Analytical Chemists, 12th ed.Washington D.C

25- Reeves, P.G.; Nielsen, F.H. and Fahey, G.C.J.R. (1993). AIN-93 purified diets for laboratory rodents: final report of the american institute of nutrition ad. Hoc writing committee on the reformulation of the AIN-76A rodent diet, J. Nutr.123:1939–1951.

26- Serota, D. (1990a). 28-Day Toxicity Study in Rats. Unpublished Report. Hazelton Laboratories America.

27- Serota, D.G. (1990b). 28-Day Oral Toxicity Study in Rats B72 (Orange Oil, Sweet). Unpublished Report. Hazelton Laboratories AmericaInc., Vienna.

28- Trinder, P. (1969). Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Annals of Clinical Biochemistry, 6(1):24-27.
29- Drabkin, D.L. and Austin, J.H. (1932). Spectrophotometric studies I. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J Biol Chem., 98:719–733
30- Richmond, W. (1973). Preparation and Properties of a Cholesterol Oxidase from *Nocardia* sp. And Its Application to the Enzymatic Assay of Total Cholesterol in Serum, *Clinical Chemistry*. 19 (12):1350–1356.
31- Allain, C.C.; Poon, L.S.; Chan, C.S.G.; Richmond, W. and Fu, P.C. (1974). Enzymatic Determination of Total Serum Cholesterol, Clinical Chemistry. 20 (4):470–475.
32- Wieland, H. and Seidel, D. (1983). A simple specific method for precipitation of low density lipoproteins, Journal of lipid Research, 24(7): 904-909.
33- Lopes-Virella, M.F.; Stone, P.; Ellis, S. and Colwell, J.A. (1977). Cholesterol determination inhigh-density lipoproteins separated by three different methods, Clin Chem., 23: 882-884.
34- Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, Am J Clin Pathol., 28(1):56-63.
35- Babson, A.L.; Greeley, S.J.; Coleman, C.M. and Phillips, G.E. (1966). Phenol phthaleinm on ophosphate as a substrate for serum alkaline phosphatase. Clin Chem., 12(8):482-90.
36- Rheinhold, J.G. (1953). Total protein, albumin and globulin in standard methods of clinical chemistry. D. Seligron, Ed. Vol. I. NewYork: Academic press, Inc., p. 88. and 21.
37- Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green, Clin Chim Acta., 31(1):87-96.
38- Schirmeister, J. (1964). Determination of creatininein serum, Dtsch Med Wschr.,89:1940.
39- Fawcett, J.K. and Scott, J.E. (1960). A rapid and precise method for the determination of urea, J Clin Pathol., 13(2):156.
40- Barham, D. and Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst, 97:142-145.
41- Rizk, I.R.S.; Abd El-Razik, M.M.M.; Abd El-Rahim, E. a. And Yousif, M.R.G. (2006). Effect of selected cereal flours on 95 chemical composition, rheological properties and cake quality. Egypt. J. Food Sci.,(34):59-80.
42- Burdock, G.A.(2005). Fenaroli's handbook of flavor ingredients. 5th ed.
43- Cano-Lamadrid, M.; Calín-Sánchez, Á.; Clemente-Villalba,J.;Hernández,F.;Carbonell-Barrachina, Á.A.; Sendra, E.; Wojdyło, A. (2020). Quality Parameters and Consumer Acceptance of Jelly Candies Based on Pomegranate Juice *"Mollar de Elche"*. Foods, *9*:516. https://doi.org/10.3390/foods9040516
44- Lee, J.H. (2015). Physicochemical and Sensory Characteristics of Sponge Cakes with Rubuscoreanus Powder. Prev. Nutr. Food Sci.,20(3):204-209. http://dx.doi.org/10.3746/pnf.2015.20.3.204
45- Bednarska, M.A. and Janiszewska-Turak,E. (2020). The influence of spray drying parameters and carrier material on the physico-chemical properties and quality of chokeberry juice powder. J. Food Sci. Technol., 57: 564–577. https://doi.org/10.1007/s13197-019-04088-8
46- Carneiro, H.C.F.; Tonon, R.V.; Grosso, C.R.F.; Hubinger, M.D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials, Journal of Food Engineering, 115 (4): 443-451, https://doi.org/10.1016/j.jfoodeng.2012.03.033
47- Botrel, D.A.; Borges, S.V.; Fernandes, R.V.B.; Viana, A.D.; daCosta, J.M.G. and Marques, G.R. (2012). Evaluation of spray drying conditions on properties of microencapsulated oregano essential oil. International Journal of Food Science&Technology, 47: 2289-2296. https://doi.org/10.1111/j.1365-2621.2012.03100.x
48- Pieczykolan, E. and Kurek, M.A. (2019). Use of guargum, gum arabic, pectin, beta-glucan and inulin for microencapsulation of anthocyanins fromchokeberry.Int.J.Biol.Macromol., 129: 665–671.
49- Aragüez-Fortes, Y.; Robaina-Morales, L.M.; Pino, J.A. (2019). Optimization of the spray-drying parameters for developing guava powder. J. Food Process Eng., 42,e13230.
50- Chew, S.C.; Tan, C.P. and Nyam, K.L.(2018). Microencapsulation of refined kenaf (*Hibiscus cannabinus* L.) seed oil by spray drying using β-cyclodextrin/gum arabic/sodium caseinate. J. Food Eng., 237:78–85.
51- Fernandes, R.V.B.; Borges, S.V. and Botrel, D.A. (2013). Influence of spray drying operating conditions on microencapsulated rosemary essential oil properties. FoodSci. Technol., 33 (suppl1). https://doi.org/10.1590/S0101-20612013000500025
52- Jafari, S. M.; HE, Y. and Bhandari, B. (2007). Encapsulation of nanoparticles of d-Limonene by spray drying: role of emulsifiers and emulsifying

techniques. Drying Technology, 25(6): 1079-1089. http://dx.doi.org/10.1080/07373930701396758
53- Rodríguez, J.F.C.; Uresti-Marín, R.M.; Gómez, M.G.S.; Santiago-Adame, R. and Ortiz-Basurto, R.I. (2020). Evaluation of spray-drying´soperable condition for obtaining orange juice powder: effects on physicochemical properties, CyTA-Journal of Food, 18(1):195-202. DOI:10.1080/19476337.2020.1728388
54- Reineccius, G.A. (2004). The Spray Drying of Food Flavors, DryingTechnology,22(6): 1289-1324. DOI:10.1081/DRT-120038731
55- Charve, J. and Reineccius, G.A. (2009). Encapsulation performance of proteins and traditional materials for spray dried flavours, J.Agric.Food Chem., 57(6):2486-2492. DOI:10.1021/jf803365t
56- Pratiwi, I.Y.; Darmadji, P. and Hastuti, P. (2016). Effect of storage temperature on the stability of microencapsulated essential oil from cinnamon (*Cinnamomum burmanii*). Citation: AIP ConferenceProceedings,1755:130014;https://doi.org/10.1063/1.4958558
57- King, C.J. (1995). Spray drying: retention of volatile compounds revisited. Drying Technol., 13(5–7):1221–1240.
58- Prince, M.V.; Thangavel, K.; Meda,V.; Visvanathan, R. and Ananthakrishnan, D. (2014). Effect of carrier blend proportion and flavor load on physical characteristics of nut meg (Myristica frangrans Houtt.) oleores in microencapsulated by spray drying. International Food Research Journal, 21(5):2039-2044.
59- Wang, W. and Zhou, W. (2012). Characterization of spray-dried soy sauce powders using maltodextrins as carrier. J. Food Eng., 109:399–405.
60- Farouk, A.; Pudil, F.; Janda, V. and Pokorny, J. (2001). Changes during the extrusion of semolina in mixture with sugars. Czech J. FoodSci., 19:24-30.
61- Mongenot, N.; Charrier, S. and Chalier, P. (2000). Effect of ultrasound emulsification on cheese aroma encapsulation by carbohydrates. J Agric Food Chem., 48(3):861-7. doi:10.1021/jf990494n.
62- Cano-Chauca, M.; Stringheta, P.C.; Ramos, A.M. and Cal-Vidal, J. (2005). Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. Innovative Food Science & Emerging Technologies, 5 (4):420–428.
63- Tonon, R.V.; Brabet, C. and Hubinger, M.D. (2009). Influence of drying air temperature and carrier agent concentration on the physicochemical properties of açaijuice powder. Food Sci. Technol., 29(2): 444-450. https://doi.org/10.1590/S0101-20612009000200034
64- Kalab, M.; Caric, M.; Zaher, M. and Harwalkar, V. (1989). Composition and some properties of spray-dried retentates obtained by the ultrafiltration of milk. Food Structure, 8:225–233.
65- Jones, J.; Prime, D.; Leaper, M.; Richardson, D.; Rielly, C. and Stapley, A. (2013). Effect of processing variables and bulk composition on the surface composition of spray dried powders of a model food system. Journal of Food Engineering, 118:19–30.
66- Subtil, S.F.; Rocha-Selmi, G.A.; Thomazini, M.; Trindade, M.A.; Netto, F.M. and Favaro-Trindade, C.S. (2014). Effect of spray drying on the sensory and physical properties of hydrolysed casein using gum arabic as the carrier. J Food Sci Technol., 51(9): 2014-21. doi:10.1007/s13197-012-0722-z.
67- Chaudhary, S.C.; Siddiqui, M.S.; Athar, M. and Alam, M.S. (2012). D-Limonene modulates inflammation,oxidative stress and Ras-ERK pathway to inhibit murine skin tumorigenesis. Hum Exp Toxicol., 31:798-811.
68- Tounsi, M.S.; Wannes, W.A.; Ouerghemmi, I.; Jegham, S.; Ben Njima,Y.; Hamdaoui, G.; Zemni, H. and Marzouk, B. (2011). Juice components and antioxidant capacity off our Tunisian Citrus varieties. J. Sci. Food Agric., 91:142-151.
69- Gültepe, N. (2018). How the use of orange (*Citrus sinensis*) peel essential oil affected the growth performance of rainbow trout (Oncorhynchusmykiss) Bull. UASVM Anim.Sci.Biotech., 75(1):16–20.
70- Russin, W.A.; Hoesly, J.D.; Elson, C.E.; Tanner, M.A. and Gould, M.N. (1989). Inhibition of rat mammary carcinogenesis by monoterpenoids.Carcinogenesis, 10(11): 2161–2164.
71- Roberto, D.; Micucci, P.; Sebastian, T.; Graciela, F. and Anesini, C. (2010). Antioxidant activity of limonene on normal murine lymphocytes: Relation to H2O2 modulation and cell proliferation. Basic Clin Pharmacol Toxicol., 106:38-44.
72- Chee, H.Y.; Kim, H. and Lee, M.H. (2009). In Vitro antifungal activity of limonene against. Trichophytonrubrum. Mycobiology, 37(3):243.
73- El-Messery, T.M.; El-Said, M.M.; Salama, H.H.; Mohammed, D.M. and Ros, G. (2021). Bioaccessibility of Encapsulated Mango Peel Phenolic Extract and its Applicationin Milk Beverage. International Journal of Dairy Science, 16 (1):29-40.
74- Gaunt, I.F.; Mason, P.L.; Hardy, J.; Lansdown, A.B.G. and Gangolli, S.D. (1974). Short-term toxicity of methyl phenyl carbonyl acetate in rats. Fd Cosmet. Toxicol., 12:185.
75- Gültepe, N.; Kesbiç, O.S.; Acar, Ü.; Gökkuş, K.; Gültepe, M.İ.; Sönmez, A.Y.; Bilen, S. and

Aydın, S. (2015). Effects of prebiotic mannan oligosaccharides (MOS) on histology and biochemical blood parameters of giltheadseabream, Sparusaurata. Isr. J. Aquac.-Bamidgeh,67:1–6.

76- Yilmaz, S.; Ergün, S. and Yigit, M. (2018). Effects of dietary FARMARIN® XP supplement on immunological responses and disease resistance of rainbow trout (*Oncorhynchusmykiss*). Aquaculture, 496:211–220.

77- Kesbiç, O.S. (2019). Effects of juniper berry oil on growth performance and blood parameters in common carp (*Cyprinus carpio*). Aquac. Res., 50(1): 342–349.