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Evaluation of Fortification Sponge Cakes with Microencapsulation Natural Antioxidants of Citrus Clementine Peel Extract

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Abstract

The bioactive compounds, such as total carotenoids and total phenols, of Citrus clementine peels are frequently utilized in food items as antioxidant agents. Such compounds were, also, used in encapsulation to protect natural antioxidants during backing and storage. This study aimed to extract bioactive components from the leftover citrus clementine peels. The prepared Microcapsules from Citrus clementine peels extracted were added to the sponge cake at concentrations of 1, 2, 4, and 6% of T3, T4, T5, and T6 treatments, respectively. Negative control preservative free, coded as T1 and positive control (was incorporated with 200 ppm butylated hydroxyl toluene (BHT), coded as T2 treatments were run at the same time for comparison. Results indicated that the chemical composition of fortified cakes with microencapsulation was not significantly higher in protein, fat, and ash, except carbohydrate. The color parameters were significantly higher in T6 than the control sample (T1). In sensory terms, the microencapsulated cake sample T6 had the best color, taste, flavor, and general acceptance when contrasted with the control sample and the other formulations. The data showed that antioxidant activity increased with increasing concentrations of carotenoid and polyphenol. The rate of antioxidant loss during storage decreased with an increase in bioactivity in the microcapsules of clementine peels compared with control (T1 and T2). The rate of stalling for all treatments was less than control within sixty days of storage. These results demonstrated that clementine microencapsulation effectively protected the bioactive compounds from loss and extended of food goods' shelf life. The microbial quality criteria of all sponge cake samples were within the permissible counts during storage up to 60 days, while the control sample lasted only for 15 days. It was, also, found that microencapsulated carotenoid and phenol clementine peel extracts can be successfully used in food applications. Therefore, to increase the stability and improve the quality of natural derived from clementine peel extract, encapsulation procedures are commonly employed. We concluded that microencapsulated carotenoid and phenol clementine peel extract can be successfully used in food applications.

Key words: **Citrus Clementine Peel, Microencapsulation, antioxidant agent, bioactive compounds, carotenoid, phenolic, sponge cake**.

1. Introduction

The Citrus fruits manufactories worldwide produce approximately15 million tons of by-product waste each year [1]. Approximately 45% of the weight of the fruit is made up of peels; sadly, they are a waste product from processing citrus that pollutes the environment **[2].** In order to lessen their influence on the environment, advance the circular economy, and produce value-added products through financially advantageous procedures, they must reuse their output. [**3].** It found that citrus byproduct is composed of highly bioactive substances and phytochemicals **[4],** and the high concentration of phenolic compounds in citrus peels and natural flavonoids. Citrus clementine, which is a member of the Rutaceae family, is rich in bioactive compounds and possesses powerful antiviral, analgesic, antioxidant, and antibacterial properties. **[5].**

It was reported that the Clementine is a sweet and seedless variety of tangerines **[6]**. It is also known as the *Algerian Tangerine*. The skin that covers the inner fruit is thinner than what you will find with regular tangerines. Improving the stability and availability of total carotene in food products is crucial for the food business. Because of its unsaturated molecular structure, carotene easily undergoes isomerization and oxidative destruction in the presence of heat, light, and oxygen. **[7]**

Encapsulation technologies are widely used to improve the stability of natural compounds extracted from citrus peels **[8]**. Encapsulation is an effective method of enhancing the stability of carotene by encasing the substrate within a protective layer, often referred to as a wall material. It is worth noting that various encapsulation techniques have been proposed to make carotene feasible in the food industry, protect carotene from oxidative degradation and enhance its stability. Encapsulation is a useful method for increasing the stability of carotenoids by covering the core material with a barrier that is also known a wall material. Notably, a number of encapsulation methods have been put forth to preserve carotene from oxidative destruction and improve its stability, making it suitable for use in the food sector **[7].**

Different forms of bioactivities, such as antioxidative, antibacterial, antihypertensive, and ant obesity activities, are possessed by the natural bioactive substances. The most popular technique for creating functional meals is to fortify food products with naturally occurring bioactive compounds. However, many of these natural bioactive compounds are heat-labile and less

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stable. It was reported that the creation of functional food items with improved quality qualities that are health-oriented requires the use of natural bioactive ingredients**[9]**.Consequently, experts in the industry suggested microencapsulating naturally occurring bioactive substances to increase their stability throughout processing and storage. Due to its cheap cost, variety, and nutritional content, cake is one of the most widely consumed baked goods in the world. Cake production is hampered by lipid oxidation and the formation of mold, which shortens the product's shelf life. When bakery goods start to get rancid, it significantly affects their texture, color, and other organoleptic characteristics. It also reduces their nutritional content **[10].** Because of the encapsulation protective action prior to baking, cakes integrated with microcapsules showed enhanced qualitative qualities and increased polyphenol content more than those integrated with extracts **[11].** In comparison to commercial cake, cake fortified with micro-encapsulated oils has greater nutritional value, sensory appeal, and antioxidant qualities. This enhances the quality and nutritional characteristics of the new product, influencing customer acceptance over time and lengthening its shelf life before being sold **[12].**

The purpose of the current research was to microencapsulate natural bioactive compounds such as carotenoid and polyphenol extracts from clementine peel. The evaluation of the polyphenols, carotenoids, shelf life, and quality characteristics during storage of sponge cake incorporation with microencapsulated natural antioxidants.

MATERIALS AND METHODS

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Raw materials

Raw materials such as wheat flour (72% extraction rate), sugar, butter, eggs, baking powder, vanilla, salt and fresh milk (ml) were obtained from local super markets in Giza, Egypt. Micro-encapsulate citrus Clementine peels extract has difference concentration (1, 2, 4 and 6%) of microcapsule that equivalent (0.804, 1.608, 3.216 and 5.824) % carotenoids. 1130.59, 2261.18, 4522.36 and 6783.45 mg Gallic acid/100gm of total phenols respectively **[13].**

Chemicals

Chemical reagents of 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ABTS•+ [2, 2 - azinobis3ethyl benzothiazoline - 6-sulfonic acid] and potassium persulfate ($K_2S_2O_8$). Folin-Ciocalteu, Butylated hydroxyl toluene (BHT), gallic acid, linoleic fatty acid, isopropanol, and thiobarbituric acid (TBA) were obtained from Sigma-Aldrich, Chime, Steinheim, Germany. potassium hydroxide, chloroform-glacial acetic acid, sodium thiosulphate, potassium iodide, hydraulic acid Ethyl alcohol, methyl alcohol, hexane Copper sulphate ,Phenolphthalein and starch reagents from El-Nasr Pharmaceutical Chemicals Co., Egypt, Yeast and molt extracts agar medium, MacConkey agar medium, beef extract, sodium chloride and Distilled water.

Methods

Preparation of microencapsulate

Preparation of microencapsulate was done by using encapsulation agents 15 g of Arabic gum (GA) and 15 g of maltodextrin (MD) to obtain a 1:1 GA: MD (w/w) ratio. Then concentrations of Clementine peel extract 7% w/v were added to generate an emulsion. The concentration of 7% was chosen because it was the best efficiency of encapsulating fine particles [**13]**. Then Freeze drying was carried out at the National Research Centre by using Catalog number 7754030, Serial number 100931482 D, U.S.A., 12L, Condition: -50C 0.1mbar Time 48h, Labconco Freeze Dryer, Console, 12L, -50ºC, Stoppering Tray Dryer, Freeze, 240V. [**14]**

Preparation of Sponge cakes

Ingredients used in the preparation of sponge cakes are tabulated in Table (1). With a few changes, sponge cakes were made in accordance with Lu et al. [15]. The formula is shown in Table (1). The micro encapsulated clementine peel bioactive extract was added to milk during preparation of cakes.

Table (1) the formula for the produced sponge cake.

Ingredients in (gm)	T1	T2	T3	T4	T5	T6
Wheat flour	200	200	200	200	200	200
Sugar	165	165	165	165	165	165
Egg	108	108	108	108	108	108
Butter	110	110	110	110	110	110
Baking powder	4	4	4	4	4	4
Vanilla	1	1	1	1	1	1
Salt	1	1	1	1	1	1
Fresh milk (ml)	30	30	30	30	30	30
MCCPE	-----	----	2	4	8	12
BHT (ppm)	---	200	----	----	---	----

MCCPE: micro-encapsulate citrus Clementine peel extricates. BHT: Sponge cake with synthetic antioxidant (200ppm).T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% micro-capsulate clementine peel extract, T4: sponge cake with 2% microencapsulate Clementine peel extract, T5: sponge cake with 4% microencapsulates clementine peel extract, and T6: sponge cake with 6% microencapsulates Clementine peel extract.

Storage sponge cake

After an hour, the cooked sponge cakes were cooled at rom temperature and taken out of the pan. After cooling down, the cake was wrapped in a polyethylene plastic sheet and stored at 4±1 ºC for 60 days, with an analysis performed every 15 days to assess the sponge cake's microbiological and physiochemical properties.

Chemical composition of sponge cake

Moisture, fat and ash contents of sponge cakes were determined using AOAC standard methods 925.10, 920.85, and 923.03, respectively (AOAC, 2006) **[16]**. The

total protein content of the sponge cakes was estimated by Kjeldahl procedure (N, 6.25) as described in AOAC official method 981.10. Available carbohydrates (A.C) were calculated by difference according to the following equation: On a dry basis =100 – (% protein + % fat + % ash).

Physical properties of sponge cake

Within an hour of baking, each sponge cake's weight (in grams) was measured, and the average was computed. **The AACC (2010) [17]** approach was used to calculate both volume (cm^3) and specific volume [17]. The displacement of clover seeds was used to calculate the volume (cm^3) of the various cake varieties produced.

Sensory evaluation of sponge cake by panel test

Twenty experts in baking technology assisted in the sensory evaluation at the Food Technology Research Institute Agriculture Research Center, Giza. At the first day of baking, the sponge cake samples were sensory evaluated for texture, color, taste, and appearance. In a testing area, the panelists assessed the samples. According to **Lawless and Heymann et al. [18**], the sensory profile of a sponge cake, which is made up of sensory qualities, describes the cake's objective sensory quality.

Determination of Bioactive Compounds

1) Determination of Total Carotenoids in sponge cake

The UV/V (Shimadzu UV 1800, Kyoto, Japan device) spectrophotometric technique was applied according to **Ogawa, et al. [19]**, to determine the total carotenoid, contents.

2) Determination of Total Phenolic Contents in sponge cake

The Folin Ciocalteu micro-method, according to **Skerget et al. [20],** was used to determine the sponge cake's total phenolic content.

3) Assessing Antioxidant Activity of the sponge cake

Using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) test, the antioxidant activity of many sponge cakes was assessed according to **Cuendet *et al.* [21]** for total phenol. Also, antioxidant activity of different sponge cakes ware determined using Azinobis-3-ethylbenzothia-zoline-6- sulfonic acid (ABTS) according to **Olszowy** and **Dawidowicz [22]** for carotenoid.

Determining the rancidity and quality of the Cake

Oil extraction from sponge cake

Oil was extracted from the sample (sponge cake) using the cold percolation technique and hexane. A conical flask containing around 3 grams of sponge cake and 25 mL of hexane was shaken at 120 rpm for two hours. Following a two-hour period, the extract was filtered and the oil was obtained by utilizing a rotary vacuum evaporator. The oil was then preserved in airtight bottles at 4ºC in 4ºC according with **[23** and **24].**

1) Acid value

The acid value was calculated using the industry standard methods outlined in **AOCS (2006) [25].** The amount of potassium hydroxide milligrams needed to neutralize one gram of oil was used to express the acid value.

2) Peroxide Value (PV)

The iodine emitted from potassium iodide is measured to get the peroxide value, which is a measure of the peroxides in the oil. Peroxide value calculation was carried out using the **AOCS (1990)** technique **[**26**].** The oil sample (5±0.01 g) was dissolved in a 30 mL acetic acid-chloroform (3:2) solution. After that, distilled water and saturated KI solution were added, and the flask was briskly agitated to release the iodine from the chloroform layer. Using starch solution as an indication, 0.01 N sodium thiosulphate was added to the mixture to titrate it. The PV (meq kgG1) calculation was performed in Eq.

$$PV\ (meq\ kgG1) = C\times (V-V0)\times 12.69\times 78.8/m$$

Where C is the sodium thiosulphate concentration (mol LG1), V and V0 represent the volumes of sodium thiosulphate exhausted by the samples and the blank, respectively (mL) and m is the mass of Navel orange and Clementine peel encapsulated extract (mg).

3) Thiobarbituric acid (TBA)

Thiobarbituric acid values (TBA) of the sponge cake samples were measured after 0, 15, 30, 45 and 60 days of storage at 4±1ºC room according to the **AOCS (2006)** method **[25]**.

Microbiological assessment

Samples were examined for coagulase-positive staphylococci, yeast and molds, coliforms, and total aerobic plate counts using, with slight changes, the protocols described in the Compendium of Techniques for the Microbiological Examination of Foods [**27].** Cake samples (5 g) were diluted up to 10−10 dilutions with peptone water (0.1%) and blended with 45 mL of 0.1% peptone water (10−1 dilution). The proper dilutions were then utilized for plating. The total number of bacteria was determined by pour-plating one milliliter from each dilution in duplicate on plate count agar and incubating it for 48 hours at 37°C.

Yeast and mold: The determination of yeast and mold counts was carried out using malt agar medium according to the methods outlined in the **Jay et al. [28].**

Coliform group: Using MacConkey agar medium and Staphylococci, the coloniform group was ascertained as suggested by **Atlas, [29].**

Proteolytic bacterial count The method outlined by **[30] and [28]** was followed while counting proteolytic bacteria.

Lipolytic bacterial count: The counting of lipolytic bacteria was done using the techniques outlined by **[30].**

Psychrophilicbacteria: Psychotropic bacteria were determined as recommended by **APHA [31] and [28**].The plates were incubated at +8ºC for 5 days.

Texture profile analysis (TPA) of sponge cake

The texture profile analysis was conducted by the BROOKFILED CT3 TEXTURE ANALYZER (version2.1, 1000gram unit). Parameters were automatically recorded by computer software (TA-CTPRO software). According to AACC **(2010)** method **[17]** the samples (2.5 cm height and 4 cm diameter) were compressed twice to 40% deformation using Prope-36 mm cylindrical, a trigger load of 5N, and a test speed- 2 mm/s. The experiments were conducted under ambient conditions. Rate of a staling [**RI= (Hardness after (N) time - Hardness at zero time / Hardness at zero time) X100**] was calculated from the differences in firmness

after 15, 30, 45, and 60 days of storage. The measurements were performed in triplicate**.**

Determination of water activity (a_w)

Water activity was determined by Rotronic Grindelstrasse 6 CH-830 Bassersdorf According to **Vuong et al [32].** The ratio of a food's (P) vapor pressure to the vapor pressure of water (P0) is known as water activity (a_w).

Measurement of color

In accordance with **Pathare et al. [33],** sponge cake samples' color characteristics (*L*, a*,* and *b**) were measured using a spectrocolorimeter (Konica Minolta, INC. Color Machine) and the CIE lab color scale. The instrument was standardized *L** (lightness/darkness that ranges from 0-100), *a** (redness/greenness that ranges from -120 to 120), *b** (yellowness/blueness that ranges from -120 to 120), and total color difference (ΔE value).

Statistical Analysis

The General Linear Model (GLM) process was used to evaluate the data using Analysis of Variance, in accordance with the methodology described by **Snedecor and Cochran [34].** To determine if a one-way analysis of variance (ANOVA) with multiple ranges of significant difference ($p < 0.05$) had been performed in SPSS version 26.0 software (IBM Corp. Armonk, NY), means were separated using Duncan's multiple range tests. The three replicates' mean value and standard deviation (±SD) are used to report the data.

Result

Chemical composition

The effect of the microencapsulated Clementine peel extract at different concentrations (1, 2, 4 and 6%) on the chemical composition of sponge cake was identified and is shown in Table (2). These findings reported that no significant differences have been observed in protein, fat and ash contents. The total carbohydrate content showed an increase by the increasing in the added microencapsulates clementine peel extract. These outcomes concur with Ahmed et al. [35] who reported that no significant difference was observed in the chemical composition of the sponge cake that was mixed with encapsulated orange peel compared with the control sample. On the other hand, there was a significant difference in total carbohydrate in microencapsulate peel extract compared with the control and BHT treatments. The total carbohydrate content increased due to the cover of bioactive (wall material) capsulation by maltodextrin and arabic gum, which are characterized by a high proportion of sugar polysaccharides [36].

Table (2) Effect of microencapsulated clementine tangerine peels extract on chemical composition of sponge cake form (on dry weight basis).

TC*: Total carbohydrates were calculated by difference.T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with 2 % microencapsulate Clementine peel extract, T5: sponge cakes with 4% microencapsulate clementine peel extract, and T6: sponge cakes with 6% microencapsulate Clementine peel extract. The results were expressed as mean ± standard deviation (n = 3). Values followed by different letters in the same row are significantly different at $P < 0.05$, according to Duncan's test.

Parameter (%)	T1	T2	T3	T4	T5	T6
Moisture	**24.33±0.21^{a}**	**24.57±0.03^{a}**	**23.77±0.15^{ab}**	**23.52±0.08^{ab}**	**23.13±0.15^{ab}**	**23.10±0.10^{ab}**
Protein	**7.40±0.17^{a}**	**7.38±0.18^{a}**	**7.23±0.06^{ab}**	**7.25±0.05^{ab}**	**7.26±0.06^{ab}**	**7.26±0.07^{ab}**
Fat	**17.45 ±0.18^{a}**	**17.44±0.01^{a}**	**17.60±0.07^{a}**	**17.63+0.03^{a}**	**17.64±0.18^{a}**	**17.66±0.02^{a}**
Ash	**1.24±0.43^{a}**	**0.99±0.00^{a}**	**0.99±0.00^{a}**	**0.98±0.01^{a}**	**0.99±0.00^{a}**	**0.99±0.00^{a}**
TC*	**49.58±0.01^{e}**	**49.63±0.002^{d}**	**50.41±0.28^{c}**	**50.61±0.17^{b}**	**50.98±0.39^{a}**	**51.99±0.19^{a}**

Baking quality of sponge

A cake's specific volume may be used to gauge volume development and, in turn, the product's porous structure. The quality of the sponge cake is also indicated by a certain volume **[37]** and **[38]**. The effect of the addition of microencapsulated clementine peel extract in Table (3) displays the addition on the baking quality of sponge cake. Although there were significant differences in weight and volume, the specific volume non-significant. This indicates that incorporating capsules didn't negatively impact the sponge cake cakes' standards for quality. The physical and sensory properties were the best up until a concentration of 6%.

Table (3) Effect the addition of microencapsulated on baking quality of sponge cakes.

Parameter	T1	T2	T3	T4	T5	T6
Weight (gm)	**186.67±0.01^{a}**	**177.89±1.7^{b}**	**166.87±0.02^{f}**	**168.4±0.20^{e}**	**170.76±0.01^{d}**	**176.26±0.02^{c}**
Volume(cm^3)	**476.57±0.02^{a}**	**455.08±0.02^{b}**	**420±0.05^{f}**	**424.07±0.06^{e}**	**430.33±0.01^{d}**	**439.33±0.01^{c}**
Specific volume (cm^3/gm)	**2.553±0.001^{a}**	**2.558±0.01^{a}**	**2.517±0.00^{a}**	**2.518±0.01^{a}**	**2.520±0.01^{a}**	**2.499±0.00^{a}**

T1: sponge cake control, T2: sponge cake with BHT as an antioxidant, T3: sponge cake with 1% micro-capsulate clementine peel extract, T4: sponge cake with % microencapsulate Clementine peel extract, T5: sponge cake with 4% microencapsulates clementine peel extract, and T6: sponge cake with 6% microencapsulates Clementine peel extract. The results were expressed as mean ± standard deviation (n = 3). Values followed by different letters in the same row are significantly different at $P < 0.05$, according to Duncan's test.

The rate of stalling (RI*%) of sponge cake during storage time at refrigerator (4±1ºC).

In Figure (1) it showed that, during storage time, the rate of stalling (RI* %) was increased for the control and BHT was higher than cakes with microencapsulated citrus clementine peel extract during storage. The sponge cake content microcapsules of clementine peel extract had less increment stalling than control and BHT. It could be noted that storage in the refrigerator, led to a little increase in the rate of stalling, with increasing addition levels of microcapsules of clementine peel extract. This concluded that the rate of hardness decreased as the

microcapsules concentrations were increased during storage. The rate of stalling decreased with the increase in microcapsules due to the cover (maltodextrin and Arabic gum) compounds. This agrees with **Sladana et al. [39].**

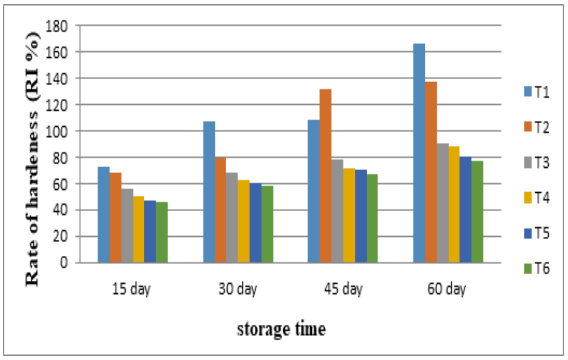

Figure (1): rate of hardness (*RI %) of sponge cake during storage time in the refrigerator. T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with2% micro-capsulate Clementine peel extract, T5: sponge cake with 4% microencapsulate clementine peel extract,, and T6: sponge cake with 6% microencapsulate Clementine peel extract.

Determination of water activity (a_w)

Water activity (a_w) is an appropriate factor for measuring the microbiological stability and durability of food items, **[40]**. Lower a_w causes the baked cakes to take longer to mold. Mold development, which regulates shelf life, is the most prevalent form of microbial deterioration in bread operations, according to **Earl and Pull [41].** In Figure (2), it showed that water activity decreased with an increase in the bioactive concentration of microcapsules from T3, T4, T5 and T6 sponge cake micro-capsulate clementine peels. It showed that range of $\mathbf{a_w}$ during storage time at (4±1ºC) in all treatments during storage for 60 days was ranged of 0.797 – 0.857 these range was safe. concurrent with **Maino et al. [42]** who confirmed that the water activity ranges between (0.75 -0.90) is safe**.** The lowering of a_w is related to the ability of microcapsule powder which has well water-binding properties to lock up any free water that is available this agrees with **Sladana et al. [39]**. Consequently, the lowering of $\mathbf{a_w}$ could help preserve the food as well as maintain it's quality **[43].**

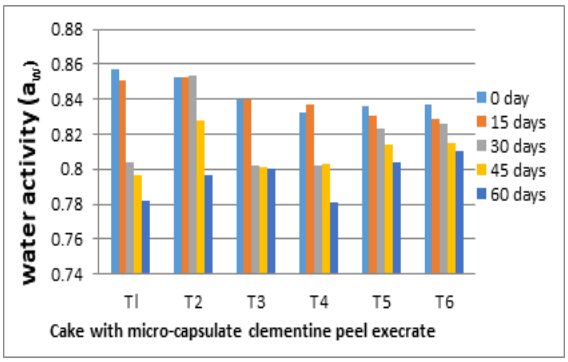

Figure (2) water activity (a_w) of sponge cake during storage. T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with 2 % microencapsulate Clementine peel extract, T5: sponge cake with 4% microencapsulate clementine peel extract, and T6: sponge cake with 6% microencapsulate Clementine peel extract,.

Sensory properties of sponge cake

The date presented in Table (4) showed the effect of adding of microencapsulate clementine peel extracted on sensory attributes of sponge cake samples. The obtained results indicated that sponge cake samples containing the microencapsulated extract gained higher scores of color, texture, taste and overall acceptability compared to control samples (either contains BHT as an antioxidant or not). The current data showed a high significant difference in T5 and T6 in color than the other treatments. Hence, the color effect began to appear with an increasing concentration of the added microencapsulated clementine peel extract. Regarding the taste and texture parameters, the results showed that T4, T5 and T6 gained higher significant values. Moreover, there were no significant differences in sponge cake between control samples (contains BHT or no) and samples containing different concentrations of the microencapsulated extract (T3, T4, T5 and T6). These results align with the findings published by **Manal et al. [12].**

Table (4): Sensory properties of sponge cakes containing microencapsulated functional extract of clementine peel.

Sample	Color	Texture	Flavor	Taste	Appearance	Overall acceptability
T1	**8.45±0.67^{c}**	**9.40±0.57 ^{ab}**	**9.45±0.78^{a}**	**9.25±0.42^{b}**	**9.30±0.42^{a}**	**46.05±1.18^{b}**
T2	**8.15±1.16^{c}**	**8.95±0.60^{b}**	**9.55±1.19^{a}**	**9.65±0.34^{ab}**	**9.50±0.33^{a}**	**45.80±1.77^{c}**
T3	**9.25±0.59^{b}**	**9.35±0.53^{b}**	**9.65±0.60^{a}**	**9.70±0.80^{ab}**	**9.45±0.50^{a}**	**47.40±2.13^{ab}**
T4	**9.40±0.52^{b}**	**9.65±0.59^{a}**	**9.70±0.52^{ab}**	**9.75±0.89^{a}**	**9.50±53^{a}**	**48.00±2.51^{ab}**
T5	**9.60±0.52^{ab}**	**9.32±0.24^{ab}**	**9.95±0.16^{a}**	**9.75±0.35^{a}**	**9.80±0.42^{a}**	**48.82±0.92^{a}**
T6	**10.00±0.00^{a}**	**9.45±0.44^{ab}**	**9.70±0.47^{a}**	**9.85±0.47^{a}**	**9.50±0.53^{a}**	**48.1±2.16^{ab}**

T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with % microencapsulate Clementine peel extract, T5: Sponge cakes with 4% microencapsulate clementine peel extract, and T6: sponge cake with 6% microencapsulate Clementine peel extract. The results were expressed as mean ± standard deviation (n = 3). Values followed by different letters in the same column are significantly different at P < 0.05, according to Duncan's test.

Color of sponge cake

The crust color of sponge cake in all treatment with microencapsulate clementine peel extract T3, T4, T5 and T6 had a non-significance lightness (*L**) value of crust compared with the control sample color (T1and T2).While it had a significant impact on the crumb. We noted that the microencapsulated didn't affect the yellow (*b**) of the crust, except crumb for the T6 content (6%) microencapsulate concentration. This result may be an interaction between raw ingrained material colors and their crumb. We noted that 6% of sponge cakes had higher significantly values (*b**) in the yellow color of the crumb but were non-significant in the crust color. It could be noted that crust color has a greater effect than crumb

due to being exposed to heat directly. These outcomes concur with the findings published by [44-46], who reported that color of sponge cake sample containing microencapsulated extract (Orange peel) was closer to that of control sample. By increasing the amount of the microencapsulated clementine peel extract in T6, it was observed that the sponge cake crust containing the extract had a considerable rise in redness (*a**). When the percentage of microencapsulated tangerine peel extract T6 (6%) was increased in sponge cake samples with crumb color, it was observed that the redness (*a**) more significantly increased than in the control sample. According to these results, sponge cakes with Clementine micro-capsulate carotenoids had a total color difference (ΔE*) that was appreciable by the human eye (ΔE*> 3) **[47].** From Table (5), the total color difference (Δ E) is higher and more significant in T6 micro-capsulate carotenoid but non-significant among T3, T4 and T5 compared with the control (T1, T2) of crust and crumb. The highest concentration of carotenoid-rich extract showed higher values of *a**, *b** and ΔE, as expected.

Table (5) the color characteristics of the cakes with different concentration of microencapsulate carotenoid of clementine peel extract.

	Crust				Crumb			
Samples	***L****	**a***	***b****	**Δ E***	***L****	***a****	***b****	**Δ E**
T1	**46.61±0.87 ^{abc}**	**9.20±0.10 ^{bc}**	**29.31±1.31^{a}**	**8.45± 1.18 ^{b}**	**66.07±0.77 ^{a}**	**0.71±0.10 ^{b}**	**20.57±6.01^{c}**	**15.57±0.23 ^{b}**
T2	**50.33±0.87 ^{a}**	**9.38±0.10b^{bc}**	**29.68±1.31^{a}**	**9.66± 1.18 ^{b}**	**66.09±0.77 ^{a}**	**-1.11±0.10 ^{c}**	**23.59±6.01^{b}**	**15.59±0.23 ^{b}**
T3	**44.52±0.81^{abc}**	**13.00±0.65^{ab}**	**27.27±2.53^{a}**	**9.78±2.35 ^{ab}**	**64.03±1.30 $^{b\,c}$**	**0.19±0.15 ^{b}**	**23.61±0.89$^{b\,c}$**	**15.28±1.20^{b}**
T4	**47.84±1.74 ^{ab}**	**8.65±0.49^{c}**	**26.24±5.48^{a}**	**8.86±0.59 ^{b}**	**62.94±0.78 ^{c}**	**1.50±0.92 ^{a}**	**23.82±0.97$^{ab\,c}$**	**13.64±0.99 ^{c}**
T5	**46.70±1.47^{abc}**	**11.41±0.75^{abc}**	**29.80±3.14^{a}**	**9.90±1.77 ^{ab}**	**65.01±0.26 ^{ab}**	**0.06±0.15^{b}**	**26.89±0.66$^{a\,b}$**	**16.73±0.34^{ab}**
T6	**46.68±2.48 ^{c}**	**14.08±2.07 ^{a}**	**30.48±0.60^{a}**	**12.28±0.06^{a}**	**65.14±0.50 ^{ab}**	**1.60±0.15 ^{a}**	**29.08±0.65 ^{a}**	**17.37±0.62^{a}**

T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with % microencapsulate Clementine peel extract, T5: Sponge cakes with 4% microencapsulate clementine peel extract, and T6: sponge cake with 6% microencapsulate Clementine peel extract. The results were expressed as mean ± standard deviation (n = 3). Values followed by different letters in the same column are significantly different at P < 0.05, according to Duncan's test.

Assessment of total carotenoid and antioxidant activity% by ABTS$^{\bullet+}$ assay in sponge cake during storage at the temperature refrigerator (4±1^{o}C).

The total carotenoid of sponge cake control (T1) and (T2) content of BHT as a synthetic antioxidant at zero days was 0.987and 0.973 mg/100g respectively, and after 60 days reached 0.803 and 0.81 mg/100g respectively. While the total carotenoid of micro-capsulate clementine peel at zero days were 1.797, 2.598, 4.207 and 5.819 mg/100g for T3, T4, T5 and T6 respectively. Total carotenoid after 60 days reaches 1.795, 2.596, 4.205 and 5.817 mg/100g for T3, T4, T5 and T6 respectively. Comparing the microencapsulated clementine to the control, these results showed that the bioactive components were shielded from the carotenoid loss. These results lead to a longer shelf life of food products of food products. We noted that T3, T4, T5 and T6 of sponge cakes were non-significant during storage for 60 days in clementine peel encapsulation. It was also noted that the carotenoid levels of microcapsules were unaffected by storage. This indicated that micro-capsulation was able to protect the carotenoid compounds. It was, also, we showed that the rate of loss in total carotenoids and antioxidant contents during storage of sponge cake with microencapsulate clementine was lower than total carotenoid percentage cake without micro-capsulate control (T1) and (T2) loss with an increase in the concentration of micro-capsulate from (T3, T4, T5 and T6). These results explained that the carotenoid compound of the coating material during microencapsulation had an additional layer of defense against heat deterioration. These findings concur with those of **Khaled et al. [48]** who said that the micro-capsulate improves the quality and stability of carotenoid and antioxidant (carotenoid) mechanism in food products.

Table (6): Total carotenoid mg/100g and antioxidant activity % of sponge cake with microencapsulate clementine peel extracted during storage at refrigerator (4±1^{o}C) on dry weight.

Storage day	**Total carotenoid of Cake with micro-capsulate clementine peels extracted mg/100g DW**					
	T1	T2	T3	T4	T5	T6
0	**0.987±0.005^{a}**	**0.973±0.004^{a}**	**1.797±0.001^{a}**	**2.598±0.001^{a}**	**4.207±0.001^{a}**	**5.819±0.0005a**
15	**0.923±0.005^{b}**	**0.946±0.006^{b}**	**1.797±0.001^{a}**	**2.598±0.001^{a}**	**4.207±0.001^{a}**	**5.819±0.0005a**
30	**0.883±0.006^{c}**	**0.907±0.003^{c}**	**1.796±0.002^{a}**	**2.597±0.002^{a}**	**4.206±0.001^{ab}**	**5.818±0.0005^{ab}**
45	**0.847±0.006^{d}**	**0.857±0.006^{d}**	**1.795±0.001^{a}**	**2.597±0.001^{b}**	**4.206±0.002^{bc}**	**5.818±0.0004^{b}**
60	**0.803±0.02^{e}**	**0.810±0.01^{e}**	**1.795±0.001^{a}**	**2.596±0.001^{b}**	**4.205±0.001^{c}**	**5.817±0.0004^{b}**
Antioxidant activity% by ABTS$^{\bullet+}$ assay						
0	**80.51±0.01 ^{a}**	**90.24±0.01 ^{a}**	**88.87±0.01 ^{a}**	**94.52±0.01 ^{a}**	**98.56±0.01 ^{a}**	**99.72±0.0 2^{a}**
15	**76.19±0.01^{b}**	**87.15±0.01^{b}**	**88.59±0.01**	**94.38±0.01^{b}**	**98.22±0.01^{b}**	**99.14±0.02^{b}**
30	**72.35±0.01 ^{c}**	**84.16±0.01 ^{c}**	**87.53±0.01^{c}**	**93.70±0.29^{c}**	**97.70±0.01^{c}**	**99.01±0.01^{c}**
45	**69.88±0.01^{d}**	**81.80±0.01^{d}**	**84.55±0.01^{d}**	**91.73±0.01^{d}**	**97.77±0.01^{d}**	**98.70±0.01^{d}**
60	**67.79±0.05^{e}**	**79.64±0.01^{e}**	**84.49±0.01^{e}**	**90.72±0.01^{e}**	**94.56±0.01^{e}**	**95.67±0.01^{e}**

T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with % microencapsulate clementine peel extract, T5: sponge cake with 4% microencapsulates clementine peel extract, and T6: sponge cakes with 6% microencapsulate clementine peel extract. The results were expressed as mean ± standard deviation (n = 3).. Duncan's test indicates that values in the same row. Individually for either total carotenoid or antioxidant activities at each storage day that are followed by different letters differ significantly at P < 0.05.

Table (6) presents the data of an evaluation conducted on the impact of adding microencapsulate clementine peel extract to all treatment forms on antioxidant activity as measured by the ABTS•+ test. The results showed that, in comparison to the untreated sponge cake (T1 and T2), all treatments had significantly greater antioxidant activity over storage periods. The results showed that, in comparison to untreated sponge cake, where ABTS•+ of control T1, T2, and microencapsulated clementine peel extract in sponge cake, antioxidant activity increased with concentration from (T3, T4, T5, and T6) addition and was maximal at zero time. Samples reached 88.87, 94.52, 98.56 and 99.72 for T3, T4, T5 and T6 respectively. It was 69.88and 81.80 % in untreated cake (T1and T2).

Furthermore, the ABTS•+ of micro-encapsulated clementine peel declined to 84.49, 90.72, 94.56 and 95.67% respectively after 60 days, respectively, Nevertheless, it dropped to 67.79 and 79.64 % in control(T1) and BHT(T2) respectively. This means that the rate of antioxidant loss during storage decreased with an increase in bioactivity components in the microcapsules of clementine peels compared with control (T1 and T2) in sponge cake during storage. These results indicated that the bioactive compounds (carotenoid) from the micro-encapsulation of clementine peel have been used as bioactive compounds due to an increase in antioxidant activity compared to T1 and T2. This result leads to a longer shelf life of food products. These findings concur with those of **Ahmed et al. [35]** who reported that the microencapsulation of orange peel has been able to protect the bioactive compounds due to an increase in antioxidant activity compared to the control compared to other samples, thus leading to an increase in the shelf-life of food products.

Antioxidant activity and total phenolic content using the DPPH assess

The data presented in Table (7) showed the total phenolic content of sponge cake samples cold storage period (4+1ºC). These findings revealed that adding the microencapsulated extract led to increasing the total phenolic content and there is a linear correlation between the increased added amount of this extract and the content of polyphenols in sponge cake samples. In this regard, T6 showed the highest phenolic content and control sample (T1) showed the lowest polyphenol content whether at zero, 15, 30, 45 or 60 days of cold storage (4±1ºC). The present results showed slight and insignificant changes in polyphenol content in T3, T4, T5, and T6 with the progress of cold storage period. On the contrary, T1 showed significant decline in its content of polyphenols cold storage period. This reflects the importance of microencapsulation process in keeping the stability, structure, and content of polyphenols and reducing the effect of processing and storage. Similar findings have been reported by **[48].** According to **[49] and [50],** the encapsulation through its wall materials has a substantial protective effect against the heat degradation of polyphenols during baking. These results were consistent with their findings. This may be due to the effect of shell substances as physical processes that cannot destroy encapsulated bioactive ingredients. Therefore, because wall materials function as a physical barrier to prevent bioactives from degrading, encapsulation of naturally occurring bioactive compounds (phenolic and carotenoid) can be used to increase their storage and processing stability. In addition, wall materials such as maltodextrin and gum Arabic can be explained by the fact that phenolic entrapped in the coating material have an extra protective barrier against thermal degradation **[51].**

Data presented in Table 7 displayed that the increased in added amount of the microencapsulated extract led to increasing the antioxidant activity. It is well-established that there is a linear correlation between polyphenols content and antioxidant activity. The higher the polyphenol content, the greater the antioxidant activity so, the current findings confirmed this hypothesis. T1 had the lowest antioxidant activity as it had the lowest polyphenol content, while T6 showed the highest antioxidant activity, which was mainly attributed to its higher polyphenol content. Generally, increasing the added amount of this extract led to increasing the polyphenol content, which in turn reflected on increasing the antioxidant activity. Rat of loss of polyphenol and antioxidant T3, T4 and T5 was less than T1 and T2 during storage. Enhancing the amount of polyphenols, carotenoids, and antioxidant activity is positively associated with prolonging food items' shelf life. **Ahmed et al. [35]** have achieved similar findings.

Table (7) Total phenol content mg Gallic acid /100g DW and antioxidant activity % of sponge cake with microencapsulate clementine peel extracted during storage at refrigerator (4±1ºC) on dry weight.

Storage day	TPC of Cake with microencapsulate clementine Peel extract (mg Gallic acid /100g DW)					
	T1	**T2**	**T3**	**T4**	**T5**	T6
0	**65.47±0.067^{a}**	**73.733±0.039^{a}**	**91.776±0.034^{a}**	**99.73±0.028^{a}**	**106.62±0.038 ^{a}**	**112.72±0.027^{a}**
15	**57.91±0.010^{b}**	**64.94±0.041^{b}**	**91.40±0.014^{a}**	**99.73±0.011 ^{a}**	**106.62±0.003^{a}**	**112.73±0.006^{a}**
30	**51.37±0.010 ^{c}**	**57.63±0.017^{e}**	**90.75±0.005^{b}**	**99.72±0.018 ^{a}**	**105.42±0.006^{b}**	**112.73±4.78^{a}**
45	**37.70±0.148^{d}**	**49.51±5.76^{d}**	**90.69±0.035^{b}**	**98.08±0.023^{b}**	**105.41±0.009^{b}**	**112.69±0.004^{ab}**
60	**35.55±0.028^{e}**	**38.54±0.61^{e}**	**90.12±0..32^{b}**	**98.074±0.017^{b}**	**104.39±0.014 ^{b}**	**112.69±0.021^{ab}**
	Antioxidant % of Cake with micro-capsulate clementine Peel extract by DPPH					
0	**6.90±0.01a**	**9.95±0.01^{a}**	**12.59±0.01^{a}**	**13.91±0.03^{a}**	**15.01±0.02^{a}**	**16.65±0.01^{a}**
15	**6.03±0.01^{b}**	**9.42±0.02^{b}**	**12.59±0.01^{a}**	**13.89±0.01^{a}**	**14.99±0.01^{a}**	**16.64±0.02^{a}**
30	**6.20±0.01^{c}**	**8.93±0.18 ^{C}**	**12.58±0.01^{a}**	**13.86±0.02^{a}**	**14.99±0.01^{a}**	**16.65±0.01^{a}**
45	**5.99±0.01f ^{d}**	**7.64±0.001^{d}**	**12.57±0.01^{ab}**	**13.69±0.59^{b}**	**14.99±0.02^{a}**	**16.49±0.02^{a}**
60	**5.21±0.01 ^{e}**	**7.37±0.01^{e}**	**12.55±0.01^{b}**	**13.69±0.01^{b}**	**14.98±0.02^{ab}**	**16.49±1.07^{a}**

T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with % microencapsulate Clementine peel extract, T5: sponge cake with 4% microencapsulates clementine peel extract, and T6: sponge cake with 6% microencapsulate Clementine peel extract. The results were expressed as mean ± standard deviation (n = 3). Duncan's test indicates that values in the same column that are followed by different letters are substantially different at $P < 0.05$.

Assessment of acid value and peroxide value

Hydroperoxidations are the main byproducts of lipid peroxidation. Following the addition of microencapsulated natural antioxidants of citrus Clementine peel at varying concentrations (1, 2, 4, and 6%), changes in the Acid Value (AV), Peroxide Value (PV), and thiobarbituric acid (TBA) of sponge cake samples were assessed during storage and are shown in Table (8). the acid value at zero time for sponge cake control (T1) and with Butylated hydroxyl toluene (T2) were 1.00 and 0.92 while the acid value at zero time for sponge cake with microencapsulate citrus clementine peel(T3, T4, T5 and T6) was 0.93, 0.53, 0.36 and 0.38 **meqO_2 /kg**, respectively. After 60 days of storage at

4±1º C they increased to 1.96, 1.88, 1.03, 0.89, 0.82 and 0.90 **meqO₂ /kg**, respectively. **Kim et al. [52]** state that one of the key elements that might lead to the oxidation of oils is the acidity of the surrounding environment. The peroxide values are used as an index of the degree of oxidative rancidity of lipids. From the statistical analysis of the data in Table (8) it could be noticed that there were significant differences in peroxide values among cake treatments at zero time and through storage periods. The peroxide values of all treatments ranged from 0.41to 3.86 **meqO₂ /kg** of fat immediately after processing (at zero time). The control samples had a significantly higher peroxide value in comparison with other treatments at any time in the storage period. This might be due to the treatments prepared with microencapsulated citrus clementine peel extracted which contains many phenolic and flavonoid compounds that have antioxidant activity as reported by **[47].** Cake (control and BHT) during 1-60days ranged between 3.86 - 4.96 and , 3.64 - 4.19 meqO_2 /kg oil respectively, while microencapsulated citrus clementine peel extracted sponge cakes for the same storage period little increased to 2.19 - 2.78, 2.03 - 2.14, 0.70 - 0.93, and 0.41- 0.82 meqO_2 /kg oil respectively. As concentration increased from T3 to T6%, the value's increment rate dropped. According to **Izzreen and Noriham [53],** food products with a PV of 10 to 20 **meqO2 / kg** oil are thought to be rancid but still acceptable, whereas those with a PV of more than 20 meqO2/kg oil are already regarded as unfit for consumption since they might not be edible. All of the samples used in this investigation were seemed non-rancid and suitable for storage for up to 60 days.

Thiobarbituric acid (TBA)

The obtained results in Table (8) indicated that the oxidation of oils or fats in the sponge cakes was effectively suppressed by clementine peel extract and its encapsulated forms. When compared to the control, the TBA values of all citrus Clementine peel extract sponge cakes or encapsulated samples were lower, suggesting that the Clementine peel extract displayed antioxidant qualities and inhibited lipid oxidation in cakes. Formation and delay oxidation during storage which means it will increase the shelf life of sponge cake. This finding is consistent with the findings of **[35],** who found that the control samples' thiobarbituric acid value (TBA) was greater than the examined samples of microencapsulated or nanoencapsulated orange peel sponge cakes. During the storage periods, TBA levels were lower in non-encapsulated and microencapsulated orange peel extract (EO) as compared to the control sample. All cake samples remained satisfactory at the conclusion of storage (sixty days); this finding is consistent with **Spalvins et al. [54]** Fats and oils are not rancid and are OK if their TBA levels are less than 1.5 mg MDA/kg. The acquired results indicated that the oxidation of oils or fats in the sponge cakes was effectively suppressed by the micro-encapsulated citrus clementine peel extracted forms. The **Egyptian Standardization** (**4037/ 2020) [55]** that the acidity of the extracted fat as oleic acid is 1%, and the peroxide value be less than 10 meqO_2 /kg oiloil. For many years, preservatives have been added to baked goods like cakes to extend their shelf life. They have also been useful in lowering the risk of severe nutritional deficiencies. They assist in making sure that a variety of tasty, reasonably priced goods that satisfy the needs of customers are available. Finally, the AV, TBA, and PV were in the safe line ($p \leq 0.05$) by the storage period and incorporation concentrations (1, 2, 4 and 6%) microencapsulated from Citrus clementine peel

Table (8) Effect of micro encapsulated carotenoid of citrus clementine peel extracted on Acid, peroxide and TBA values of sponge cake during storage.

Quality parameter	Storage time	Control T1	BHT T2	Cake with micro-capsulate clementine peel			
				T3	T4	T5	T6
Acid value (mg KOH/g oil)	**Zero time**	**1.00±0.12^{a}**	**0.92±0.03^{a}**	**0.93±0.01^{a}**	**0.53±0.07^{b}**	**0.36±0.02^{c}**	**0.38±0.001^{c}**
	15days	**1.13±0.08^{b}**	**0.98±0.02^{a}**	**0.93±0.00^{b}**	**0.59±0.00^{c}**	**0.38±0.02^{d}**	**0.40±0.001^{d}**
	30 days	**1.21±0.11^{a}**	**1.12±0.06^{a}**	**0.95±0.00^{b}**	**0.60±0.01^{c}**	**0.43±0.01^{d}**	**0.47±0.01^{d}**
	45 days	**1.58±0.11^{a}**	**1.39±0.10^{b}**	**1.00±0.01^{c}**	**0.66±0.002^{d}**	**0.65±0.01^{e}**	**0.88±0.05^{e}**
	60 days	**1.96±1.88^{a}**	**1.88±0.03^{b}**	**1.03±001^{c}**	**0.89±0.01^{d}**	**0.82±0.004^{f}**	**0.90±0.01^{d}**
Peroxide value (meqO_2 /kg oil)	**Zero time**	**3.86±0.01^{a}**	**3.64±0.01^{b}**	**2.19±0.005^{c}**	**2.03±0.03^{d}**	**0.70±0.001^{e}**	**0.41±0.001^{f}**
	15days	**3.90±0.001^{a}**	**3.75±0.04^{b}**	**2.46±0.45^{c}**	**2.04±0.01^{d}**	**0.71±0.001^{e}**	**0.42±0.001^{f}**
	30 days	**4.90±0.01^{a}**	**4.13±0.003^{b}**	**2.15±0.10^{c}**	**2.08±0.03^{d}**	**0.73±0.02^{e}**	**0.45±0.01^{f}**
	45 days	**4.95±0.02^{a}**	**4.15±0.02^{b}**	**2.78±0.06^{c}**	**2.09±0.01^{d}**	**0.80±0.005^{e}**	**0.69±0.01^{f}**
	60 days	**4.96±0.05^{a}**	**4.19±0.003^{b}**	**2.79±0.02^{c}**	**2.14±0.06^{d}**	**0.93±0.02^{e}**	**0.82±0.01^{f}**
Thiobarbituric Acid (Mg MDA /kg)	**Zero time**	**0.12±0.02^{d}**	**0.07±0.00^{c}**	**0.05±0.00^{b}**	**0.05±0.01^{c}**	**0.04±0.01^{e}**	**0.04±001^{c}**
	15days	**0.15±0.00^{c}**	**0.11±0.01^{c}**	**0.07±0.01^{b}**	**0.05±0.01^{c}**	**0.05±0.00^{d}**	**0.05±0.00^{b}**
	30 days	**0.8±0.01b^{c}**	**0.13±0.06^{c}**	**0.07±0.00^{b}**	**0.07±0.00^{b}**	**0.06±0.01^{c}**	**0.05±0.001^{b}**
	45 days	**1.20±0.01^{b}**	**0.19±0.01^{b}**	**0.09±0.01^{b}**	**0.07±0.00^{b}**	**0.07±0.00^{b}**	**0.05±0.00^{b}**
	60 days	**2.23±0.04^{a}**	**1.03±0.03^{a}**	**0.14±0.03^{a}**	**0.10±0.02^{a}**	**0.08±0.00^{a}**	**0.07±0.01^{a}**

T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with % microencapsulate Clementine peel extract, T5: sponge cake with 4% microencapsulates clementine peel extract, and T6: sponge cake with 6% microencapsulates Clementine peel extract. The results were expressed as mean ± standard deviation (n = 3). Duncan's test indicates that values in the same column that are followed by different letters are substantially different at $P < 0.05$.

Microbiological attributes of cakes during storage

Manufacturing is carried out under hygienic conditions. Data in Table (9) showed viable counts (CFU/g) in that all cake samples were stored at refrigerator (4±1ºC) conditions for 60 days. The total bacterial count of different cake treatments is affected by the difference in the concentration of microencapsulated citrus clementine peel extract. However, the total count for T1 (2×10^2 CFU/g) was detected after 15 days of storage in the refrigerator and reached a high total count of bacterial growth after 60 days. In the T2 sample, total bacterial growth appeared after 30 days (2×10 CFU/g) of storage in the refrigerator.In comparison to the control group without additional antioxidants. From these data, it

could be noticed that, total bacterial count of control samples reached 9×10^6 CFU/g in 60 days. On the other hand, for all treatments, it doesn't appear bacteria with these data indicated a longer shelf life. By using the microencapsulate, the total bacterial count in sponge cake of clementine tangerine peel extract ranged between 2×10 in concentration 6% and 6×10^4 CFU/g in concentration 1%. It was observed that the duration of storage had an impact on the overall number of bacteria. By increasing the concentration of microencapsulated peel extract.

From these results in Table (9), it could be noticed that yeasts and molds were not detected in all samples at the initial time of storage until 30 days of storage. Meanwhile, yeasts and molds appeared in T1 low count (ranging from 3×10 at 15 days to 4×10^3 CFU/g) at 60 days and gradually increased with increasing storage period. The yeast and molds of cake in (T2, T3, T4 and T5) started to appear in 45 days and increased with low counts by increasing the storage period in the refrigerator, except in T6 data was not detected. Our findings were consistent with those of **Arslan-Tontul et al. [56],** who stated that the use of double-layered microcapsules, which prolong the shelf life of cakes during storage, might result in acceptable numbers in bakery products. Meanwhile, in all treatments, the coliform group is not detected as obtained from the data in Table (9).

Psychrophilic bacteria

From un-tableted data, it could be observed that the pyschrophilic bacterial counts of the control cake ranged from 2 x 10 in 15 days to 9 x 10^2 CFU /g after 60 days. The T1 had the highest count in 60 days and also appeared in T2 to 4 x 10 CFU /g in 60 days and T3 was 5 x 10 CFU /g in 60 days but in other treatments the count wasn't detected. **Bradford et al. [57]** who reported that psychrophilic populations were increased during refrigerated storage.

Proteolytic and lipolytic bacteria

Microorganisms in food can hydrolyze proteins, resulting in a range of taste and smell abnormalities. After extended periods of cold storage, several common psychrotrophic spoilage bacteria become highly proteolytic and alter items in ways that are not desired. **Yuan et al. [58]**. Un tableted data it could be observed that T1 sample had proteolytic and lipolytic bacterial counts appearing in 30 days (10 colony) and 5 x 10 CFU /g in 60 days of storage but not detected in all other treatments. Finally, the microbial quality criteria of all cake samples were within the permissible counts reported by **Egyptian Standards (4037 / 2020) [55].**

Tundis et al. [58] conducted a research on the antibacterial activity of citrus clementine against Gram (+) and Gram (-) bacteria as well as fungus. Citrus fruits, which are natural and safe for human health, should be used as a substitute for artificial preservatives in order to reduce their negative effects **[60].** The result agrees with **Khaki et al. [61]** who reported that natural antioxidants and antimicrobial agents can increase the shelf-life of cake to 75 days. Cake that has been fortified with microencapsulated natural antioxidants has more nutritional value, sensory appeal, and antioxidant qualities than commercial cake. This enhances the nutritional value and quality of the new product, which influences customer acceptance over time. It also extends the shelf life of the products and allows them to be sold **Martinez et al. [62].**

Table (9) Microbiological counts (CFU /g) of cake samples as affected by addition of different levels of cake microencapsulated clementine tangerine peel during storage at refrigerator (4 ±1ºC) conditions for 60 days.

Storage day	Control T1	BHT T2	Total count bacteria T3	T4	T5	T6
0	ND	ND	ND	ND	ND	ND
15	2×10^2	ND	ND	ND	ND	N D
30	4×10^4	2 × 10	4 × 10	ND	ND	ND
45	3×10^5	3×10^2	2×10^3	3×10^2	ND	ND
60	9×10^6	5×10^5	6×10^4	5×10^4	1×10^2	2 × 10
Yeast and molds						
0	ND	ND	ND	ND	ND	ND
15	3 × 10	ND	ND	ND	ND	ND
30	2×10^2	ND	ND	ND	ND	ND
45	8×10^2	1×10^2	3 ×10	3 × 10	ND	ND
60	4×10^3	2×10^3	5×10^2	2×10^2	4 × 10	ND
Coliforms						
0	ND	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND	ND
30	ND	ND	ND	ND	ND	ND
45	ND	ND	ND	ND	ND	ND
60	ND	ND	ND	ND	ND	ND

T1: sponge cake control, T2: sponge cake with BHT antioxidant, T3: sponge cake with 1% microencapsulate clementine peel extract, T4: sponge cake with % microencapsulate Clementine peel extract, T5: Sponge cakes with 4% microencapsulate clementine peel extract, and T6: sponge cake with 6% microencapsulate Clementine peel extract. ND= Not detected.

CONCLUSION

This study found that the natural antioxidant found in citrus clementine peel extract may be utilized to baking products (sponge cakes) without causing rancidity, baking effects, or changes to the finished product's physical or chemical qualities after storage because of encapsulating methods. Innovative goods with improved antibacterial qualities and longer shelf life might be produced using encapsulation techniques, which would be advantageous to both consumers and businesses.

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