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## Evaluation of Turkish Delight Prepared With Pigments And Essential Oils Extracted From Clementine (Citrus Clementine) Peels As Natural Antioxidants

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### Abstract:

This experiment presented essential oils by GC/MS, pigment content, and their antioxidant activities as well as sensory evaluation of delight samples. Limonene (66.88%) was the most prevalent yield. The peels of clementine had DPPH and ABT Scavenging activity. All levels of pigment extract had better scores for all sensory values and recorded acceptable scores in terms of appearance, color, aroma, and overall acceptability compared to control delight. Besides, delight samples containing 15 mg astaxanthin pigment extract showed maximum sensory scores compared to other samples and control delight. On the other hand, the product was less acceptable to the panelists compared to control in the case of the addition of 3.75 mg astaxanthin pigment extract. All the analyzed samples showed that delight fortified with astaxanthin pigment which has high contents of essential oils has good antioxidant activities. As a general conclusion, Clementine peels are recommended as a useful value-added functional ingredient for the food industry as natural additives that will be healthier than artificial additives.

**Key words:** Antioxidant, Astaxanthin, Clementine peels, Delight, Essential oils, GC/MS.

### Introduction:

The Turkish delight (malban) is consisting of sugar, starch, and water. Some ingredients, flavor enhancer, food coloring, and fruits are used in malban production. Color and odor additives are imported and have to be convenient to “Food Additives Regulation”. This pattern of food additives is consumed concerning the consumer’s desires.

Therefore, it was returned to nature using natural products, one of them is Citrus fruits which are considered essential crops with high economic and medicinal value <sup>1,2</sup>. Approximately 50% of the juice extracted from peels, seeds, and pulps can be used as a potential source of secondary plant metabolites and essential oils according to Loizzo *et al.*; Hsouna *et al.* and Pfukwa *et al.* <sup>3,4,5</sup>. The essential oils and carotenoids are known to have numerous health benefits, predominately attributed to their antioxidant activity. Clementine has nutritional prominence as other citrus fruits due to its

flavonoid’s composition especially poly methyl flavones and flavones (hesperidin, naringin, and naringin) according to Barreca *et al.* and Hamdan *et al.* <sup>6,7</sup>. Astaxanthin and vitamin E are the best antioxidant compounds and they have been used for cosmetic properties <sup>8</sup>.

So far, clementine peels have been rarely investigated, and limited data are available on their composition of the bioactive compound. Therefore, the main purpose is the utilization of citrus fruit wastes that demonstrate prospective environmental benefits combined with increased economic gains as well as the production of healthy food that will ameliorate the lifestyle of consumers.

### Materials and Methods:

Fresh fruits of Clementine were obtained from the local market in Cairo, Egypt. The fruits were peeled by using a sharp knife to avoid any damage to the oil of glands. The peels were dried in

the shaded area and ground into a particle by a laboratory blender. Corn starch, sucrose, and gelatin

were obtained from local markets in Cairo, Egypt.

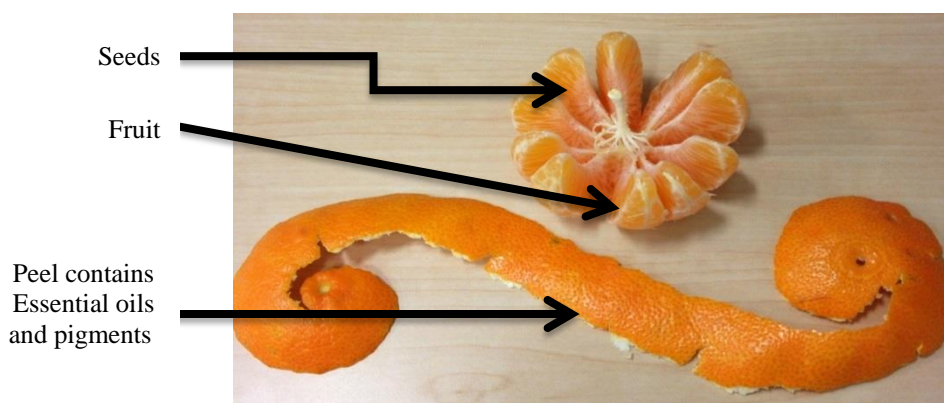


Figure 1. Fruit parts of *CITRUS CLEMENTINE*

### Chemicals and reagents

ABTS<sup>•+</sup> (2, 2'-Azinobis (3-Ethylbenzothiazoline- 6-Sulfonic Acid)), DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) and BHT (Butyl Hydroxytoluene) and Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

### Preparation and extraction of citrus clementine peels.

One hundred grams of citrus clementine peels (dried outer layer) were blended for 60 sec. with 100 ml of ethanol/Hexane (1:1, v/v). The slurry was filtered using Whatman No. 1 filter paper under reduced pressure. The combined filtrates were shaken. The extract evaporated to dryness under reduced pressure at 40°C.

### Analysis of essential oils

#### GC/MS analysis of essential oils Clementine peels

The essential oils were analyzed by GC/MS a Perkin Elmer Auto System XL model equipped with a flame ionization detector (FID) with a fused silica capillary column ZB-5(60m x 0.32mm) . The oven temperature was programmed from 50 to 240°C at a rate of 3°C/min. the Carrier gas Helium was used, at a flow rate of 1.1ml/min. The temperatures of the injector and detector were 230 and 250°C. Peak area was used to calculate essential oils by the data analysis of instrument software based on the standards and the expressed area percentage is :

**Area percentage = Area of peak /Total area peak x100.**

### Measurement of pigments content

The pigment from the fresh peels of clementine was extracted with 80% acetone and measured with LKB spectrophotometer at 663,645,480 nm. The

pigments concentration (mg/g f.wt.) were calculated according to Lichtenthaler *et al*<sup>9</sup>.

### Determination of Astaxanthin content

The astaxanthin content of the crude extract of clementine peel was assayed by the Technical report<sup>10</sup>. Powder of peel (0.5mg) was a mixture with 2mL DMSO (dimethyl sulfoxide). Then, the sample was incubated at 70 °C after 5 minutes. The absorbance was measured at 492nm .

### Determination of total anthocyanin content

The pH-differential spectrophotometric was used for measuring the anthocyanin content according to Safdar *et al*<sup>11</sup>. Each sample was diluted with potassium chloride (0.025 M) at pH 1.0 and sodium acetate (0.4 M) at pH 4.5. They were allowed for 15 min to equilibrate before detection by spectrophotometer. The absorbance was measured at 520 nm and 700 nm. The difference in the absorbance at differing pH values and wavelengths was calculated as  $A = (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH}1.0} - (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH}4.5}$ . Total anthocyanin content (mg/l) =  $(A \times MW \times DF \times 1000) / \epsilon \times l$ , where MW(449.2g mol<sup>-1</sup>) is the molecular weight, DF is the dilution factor,  $\epsilon$  is the molar absorptivity ( $\epsilon = 26,900 \text{ Lcm}^{-1}\text{mol}^{-1}$ ) of cyanidin-3-glucoside were used) and  $l$  is for 1 cm path length.

### Antioxidant activities

#### DPPH• radical scavenging assay:

The measurement of DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts. In the DPPH assay, violet color DPPH solution is reduced to yellow colored product, diphenylpicryl hydrazine, by the addition of the extract at different concentrations (25, 50, 75, 100µg/ml) in a concentration dependent manner. (Unicum UV 300) at 515 nm according to Ye *et al*<sup>12</sup>. The capacity to scavenge the DPPH• radical was calculated using

the following equation: DPPH· scavenging activity % =  $[(A_c - A_s) / A_c] \times 100$  Where: ( $A_c$ ) was the absorbance of the control reaction and ( $A_s$ ) the absorbance in the presence of the extracts according to Ye *et al*<sup>12</sup>.

#### ABTS•+ scavenging activity

To estimate the total antioxidant activity of plant extracts by assay of radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS•+). Percent activity was calculated using the equation: % Inhibition =  $[(A_0 - A_1) / A_0] \times 100$  % Where:  $A_0$  is the ABTS•+ absorbance of the control reaction.  $A_1$  is the ABTS•+ absorbance in the presence of the sample according to Armao *et al*<sup>13</sup>.

#### Delight preparation

A stock solution of clementine peels was prepared by dissolving 1500 mg of crude extract in 10 ml of corn oil. The delight was prepared by using a formula consisting of 100g sucrose, 11.7 g corn starch, 12 g gelatin, 103 ml of water, and the crude extract was added at the end of cooking at levels 25, 50, 75, and 100  $\mu$ l (based on preliminary experiments) to obtain 3.75, 7.50, 11.25 and 15 mg astaxanthin. 100 g of granulated sucrose was poured into the cooker with 37 ml cold water while stirring and the temperature was raised to boiling. Then, starch (11.7 g) was dissolved in approximately 35 ml of cold water and added to the previous sugar mixture. Moreover, gelatin was dissolved in 31 ml warm water and added to the mixture. At the end of cooking (10-15min) the crude extract was added as mentioned before. All of the delights were spread on trays covered with corn starch, left sitting for 24 h at room temperature and samples were cut by a knife and then delight samples were packed in polyethylene bags and stored at room temperature until further analysis (Fig.2).



Figure 2. Functional delight supplemented with different levels of 15 mg extract

#### Sensory evaluation of Turkish delight

The ten panelists from the Food Technology Research Institute evaluated the sensory analysis of delight samples by using a 5-point hedonic scale. For sensory evaluation, four delight samples with 3.75, 7.50, 11.25, and 15mg pigment extract and control delight were presented to the panelists using a sensory evaluation form consisting of six parameters, *i.e.*, appearance, color, aroma, and overall acceptability according to Meilgaard *et al*<sup>14</sup>.

#### Statistical analysis

For the analytical data, mean values and standard deviation are reported. The obtained data were subjected to one-way analysis of variance (ANOVA) at  $p < 0.05$  followed by Duncan's new multiple range tests to assess differences between samples mean using Costas statistical software according to the method of Gomez *et al*<sup>15</sup>. All measurements were carried out at least in triplicate except for sensory evaluation which was out of 10 replicates.

#### Results and Discussion:

##### Essential oils composition of clementine peel extracts (*Citrus clementine*)

The health and nutritional importance of both the pulp as well as the skin of clementine are due to the presence of many bioactive compounds. Essential oils, pigments, especially astaxanthin, Flavonoids, especially poly ethoxy flavones, and flavones (hesperidin, naringin, and naringin) which are identified according to Hamdan *et al*<sup>7</sup>. The major compounds of clementine essential oils were D-Limonene 66.88%, while the dominant components clementine essential oils were Diethyl malate 6.79 % and Linalool 5.25%, Geranial and  $\alpha$ -Terpinene (1.66 and 1.62%) were identified by the GC/MS analyses (Table 1). It has been found by Zhang *et al* and Chaouch *et al*<sup>16,8</sup> that the yield of citrus essential oils in most of the cases ranged from 0.2-2.0% which is following our results. It has been found by Chen *et al*<sup>17</sup> that the total oil yield from sweet orange 0.80%, eureka lemon 0.90%, and mandarin 0.80% which were lower than our results. On the other hand, the results obtained by Bozkurt *et al*<sup>18</sup>, clarified that the essential oil of *Citrus clementine* contained *d*-limonene (66-93%),  $\alpha$ -pinene, sabinene,  $\beta$ -pinene,  $\beta$ -myrcene, linalool, *m*-cymene, and 4-terpineol (8) reported that limonene (56.8–93.3%) and  $\alpha$ -terpinene (0.1–36.4%) were the major components of volatile fraction in clementine juice and peels.

**Table 1. Essential oils composition of clementine peels extracts (*Citrus Clementine*).**

Compounds	% Area	Compounds	% Area
$\alpha$ - Pinene	1.34	Citronyl acetate	0.80
Octanal	0.57	Gernayl acetate	0.31
D-Limonene	66.88	$\beta$ -Cubenene	0.13
Octanol	0.17	Dimethylantranthranilate	1.27
Linalool	5.25	$\alpha$ -Humulene	0.54
Camphene	0.20	(E)-2-Dodecanol	0.37
Citronellol	0.58	Valencene	1.07
$\alpha$ - Terpinene	1.62	$\alpha$ -Selinene	1.28
Decanal	2.18	$\delta$ - Cadinene	1.31
Citronellal	0.60	Elemol	1.11
Geraniol	0.26	$\delta$ - Elemene	0.67
Peryldaldehyde	0.27	Caryophyllene oxid	0.59
Geranial	1.66	Diethylmalate	6.79
Undcanal	0.97	Hexadecanone	0.20
E, E)- 2,4-Decadienal	0.61		0.40
Unknown compounds			0.40
		Total	100

**Pigment composition of clementine peels (*Citrus clementine*)**

The pigment content of clementine peels was measured by spectrophotometer as shown in (Table 2). Chlorophyll b (24.2  $\mu\text{g/ml}$ ) was the most abundant pigment in peels followed by Chlorophyll a (12.75  $\mu\text{g/ml}$ ). Carotenoids were 8.01  $\mu\text{g/ml}$  followed by anthocyanin (3.17  $\mu\text{g/ml}$ ) and astaxanthin (0.4  $\mu\text{g/ml}$ ) from clementine peels. According to Chen *et al*<sup>19</sup>, it has been found that the carotenoids content in *C. Rocalmora* and *C. Mandarin* from Cheylard was  $76.0 \pm 0.7 \text{mg}/100 \text{g DW}$   $75.3 \pm 1.3 \text{mg}/100 \text{g DW}$ , respectively which were higher than our results. Anthocyanin is an important pigment that plays role in the prevention of cancer, cardiovascular and inflammatory diseases<sup>20</sup>. Also, anthocyanins prevent the cell from DNA cleavage, lipid peroxidation, decreasing capillary permeability, and membrane strengthening<sup>21</sup>.

**Table 2. Pigment composition of clementine peels (*Citrus clementine*).**

Pigments	$\mu\text{g/ml}$
Astaxanthin	0.40
Anthocyanin	3.17
Chlorophyll a	12.75
Chlorophyll b	24.2
Carotenoids	8.01

**Antioxidant activity DPPH of functional delight**

Previous studies demonstrated that citrus fruits have received much attention because of their nutritional and antioxidant properties, and prevention of health problems<sup>22</sup>, due to mainly the contribution of antioxidant compounds including vitamin C, phenolic compounds, and carotenoids.

As shown in Table 3 the DPPH scavenging activity was lower than that formally reported by Gaafar *et al*<sup>23</sup> in tomato pomace. Previous studies reported that the different mandarin fruit tissues: pulp residues, juices, peel, seed of 19 citrus genotypes belonged to *C. Reticulata* Blanco are rich sources of phenolic compounds with antioxidant activity. While, the peel and juice are the main tissues with higher phenolic content and stronger scavenging free radical ability compared to the other tissues<sup>24, 25</sup>.

**Table 3. Antioxidant activity DPPH of functional Turkish delight enriched with 15 mg extract**

	DPPH				ATBS			
	Scavenging %				Scavenging %			
Concentration	25µg	50µg	75 µg	100 µg	25 µg	50 µg	75 µg	100 µg
Standard	29.54 <sup>g</sup>	43.41 <sup>d</sup>	47.57 <sup>c</sup>	57.08 <sup>b</sup>	30.11 <sup>i</sup>	45.37 <sup>f</sup>	59.27 <sup>d</sup>	81.03 <sup>bh</sup>
Peels extract (PE)	26.37 <sup>h</sup>	39.98 <sup>ef</sup>	46.22 <sup>cd</sup>	65.11 <sup>a</sup>	41.05 <sup>g</sup>	50.48 <sup>e</sup>	65.90 <sup>e</sup>	84.36 <sup>a</sup>
Malban without extract (M)	6.25 <sup>l</sup>	8.18 <sup>l</sup>	11.11 <sup>j</sup>	18.90 <sup>i</sup>	9.55 <sup>o</sup>	11.24 <sup>n</sup>	12.38 <sup>m</sup>	20.11 <sup>l</sup>
Malban with extract (MPE)	19.84 <sup>i</sup>	30.77 <sup>g</sup>	36.40 <sup>f</sup>	40.26 <sup>ef</sup>	25.67 <sup>j</sup>	39.40 <sup>h</sup>	45.31 <sup>f</sup>	50.55 <sup>e</sup>

\* BHT was used as the standard for DPPH method and Trolox were used as a standard for the ABTS method. Results are mean values ± standard deviations (n=3). Means followed by the different letters in a column are significantly different (P ≤ 0.05).

The considerable differences in antioxidant activities may be due to the diversity of methods used, plant species, cultivation practices, and environmental conditions as previously reported by Asikin *et al* and Gaafar *et al*<sup>26,27</sup>. However, the difference in extraction methods used in previous studies should be taken into account in generalizing the results. The results demonstrate that all these samples significantly (P < 0.05) show antioxidant activities. The multiple health benefits of citrus fruits by preventing and curing certain diseases are attributed to contain high contents of vitamin C contents and phenolic compounds as reported by Suleria *et al*; Azuma *et al* and Deng *et al*<sup>28, 29, 30</sup> and pigments mainly astaxanthin and essential oils<sup>31</sup>. Besides, the presence of phenols, included numerous flavanones, flavone glycosides, polymethoxylated flavones, hydroxycinnamate, and other miscellaneous phenolic glycosides and amines<sup>32</sup>.

#### ABTS scavenging activity of functional delight enriched with extract

Considerably different ABTS•+ radical scavenging activities of clementine, as well as the positive control Trolox, are shown in (Table 3). In the ABTS•+ scavenging activity, the inhibition values are varied significantly which ranged from 9.55 to 84.36 %. The peel extract showed the highest value 84.36% at 100 µg/ml, while the lowest value was noticed in malban extract 20.11µg/ml. As shown in Table 3 ,significant ABTS scavenging activity is obtained when the sweet malban was supplemented with astaxanthin 50.55 µg/ml more than the standard. This result confirmed that essential oils and astaxanthin had a remarkable effect on scavenging ABTS radicals<sup>33, 34</sup>. The previous studies specified that the phytochemical profile and antioxidant scavenging activity may significantly diverge among citrus species and cultivars as presented by Asikin *et al*<sup>26</sup>. Our findings may be attributed to the high antioxidant activity of

peels extract. The antioxidant activity of peel makes it useful for utilization as an antioxidant in food<sup>22</sup>.

#### Sensory evaluation of functional delight enriched with different levels of extract

To enhance the bio-functional and nutritional quality of food products and improve their stability against auto-oxidative and improve the nutritional quality of their food products, the results of the sensory evaluation are tabulated in (Table 4). Therefore, different levels of clementine crude peels extract (25, 50, 75, and 100 µl) were used as an ingredient delight preparation to obtained 3.75, 7.50, 11.25, and 15 mg pigment extract. Delight (malban) without any pigment addition was used as control. All sensory values had better scores for all pigment extract levels and recorded acceptable scores in terms of appearance, color, aroma, and overall acceptability compared to control delight. 15 mg of pigment extract (100 µl crude extract) recorded the higher acceptable scores in terms of appearance, color, aroma, and overall acceptability compared to control delight. The less acceptable delight sample according to the panelists was with the addition of 3.75 mg pigment extract (25 µl crude extract) pigment extract. It could be concluded that using natural pigments from fruit wastes as food additives will be healthier than artificial additives<sup>35</sup>. Generally, sensory evaluation is an important step to consider the possibility of an industrial and commercial approach. The results of the sensory evaluation confirm the success of the natural pigments studied in obtaining results close or similar to the artificial pigments used in pigments (betalain) instead of using Carmine dye as a coloring agent in ice cream processing. The results also confirm the possibility of using plant wastes of neglected vegetable industrial purposes, as rich sources of plant chemicals, in addition to using them in the production of environmentally friendly dyes Eco-friendly<sup>36</sup>. Therefore, agro-food by-

products are considered as a new source of natural food colorant<sup>36, 37, 38</sup>.

**Table 4. Sensory evaluation of functional delight supplemented with different levels of 15 mg crude extract**

Samples	Appearance	Color	Aroma	Overall acceptability
Control delight (without pigment)	4.85± 0.24 <sup>a</sup>	4.65± 0.41 <sup>a</sup>	4.70±0.48 <sup>a</sup>	4.70±0.48 <sup>ab</sup>
25 µl (3.75 mg)	4.25±0.26 <sup>c</sup>	4.15±0.24 <sup>b</sup>	4.15±0.82 <sup>b</sup>	3.95±0.69 <sup>c</sup>
50µl (7.50mg)	4.55±0.36 <sup>b</sup>	4.45±0.44 <sup>ab</sup>	4.60±0.21 <sup>ab</sup>	4.50±0.53 <sup>b</sup>
75µl (11.25mg)	4.80±0.42 <sup>a,b</sup>	4.75±0.26 <sup>a</sup>	4.60±0.46 <sup>ab</sup>	4.85±0.24 <sup>ab</sup>
100 µl (15mg)	4.90±0.21 <sup>a</sup>	4.70±0.33 <sup>a</sup>	4.80±0.42 <sup>a</sup>	4.99±0.01 <sup>a</sup>

Results are mean values ± standard deviations (n=3). Means followed by the different letters in a column are significantly different (P ≤ 0.05).

### Conclusion:

Due to the increase in consumer's concern over the safety of artificial food colorants, the demand for natural food colorants has been increased. The results of our research reveal that the clementine peels are a good source of essential oils and astaxanthin which can be used as a functional ingredient and natural food colorant in different food products and at the same time assist in the utilization of food wastes and the protection from environmental hazards.

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### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in National Research Centre (NRC), Egypt.

### Authors' contributions:

E.A.I., A.A.O. and Z.A.S. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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## تقييم الملبن المعد من الصبغات والزيوت الأساسية المستخلصة من قشور الكلمينتين كمضادات أكسدة طبيعية *CITRUS CLEMENTINE*

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### الخلاصة:

مستخلص قشر الكلمينتين غني بالمركبات الفعالة كالزيوت الطيارة والصبغات النشطة بيولوجياً. وقد يؤدي خلط الملبن بمستخلص قشور الكلمينتين الى ان يصبح أكثر فائدة من الناحية الصحية بالإضافة الى انه قد يؤثر على صفات الجودة الفيزيائية والكيميائية والحسية للمنتج. وقد تم في هذه الدراسة تقدير محتوى الزيوت الأساسية بواسطة ال GC/MS والصبغات والنشاط المضاد للأكسدة لقشور الكلمينتين بالإضافة الى التقييم الحسي لعينات الملبن. اظهرت النتائج ان الليمونين (66.88%) كان هو المركب الأعلى في المحتوى مقارنة بالزيوت الأساسية الأخرى. أظهرت قشور الكلمينتين نشاط مضاد للأكسدة باستخدام طرق DPPH و ABTS. أظهرت جميع تركيزات المستخلص قيم حسية مقبولة من حيث المظهر واللون والرائحة والقبول العام مقارنة بالملبن الكونترول. إلى جانب ذلك، أظهرت عينات الملبن المحتوية على 15 مللجرام من مستخلص صبغة الأستازانثين أعلى درجات التقييم الحسي مقارنة بعينات الملبن الأخرى والكونترول. وكان المنتج الأقل قبولاً لدى المحكمين هو المحتوى على تركيز 3.75 مللجرام من مستخلص صبغة الأستازانثين مقارنة بالكونترول. كما أظهرت جميع العينات التي تم تحليلها أن الملبن المدعم من صبغة الأستازانثين والتي تحتوي على نسبة عالية من الزيوت الأساسية لها نشاط مضادات للأكسدة جيد. و توصى الدراسة باستخدام قشر الكلمينتين كمكون وظيفي مفيد ذو قيمة غذائية وصحية يمكن استخدامه في الصناعات الغذائية كإضافات.

**الكلمات المفتاحية:** مضادات أكسدة، استازانثين، قشور الكلمينتين، ملبن، الزيوت الأساسية، تحليل كروماتوگرافي.