



## Production of functional beverage based on cross-linked bioactive compounds of green tea and green coffee in chitosome structure

Moradi Kashksara, K. <sup>1</sup>, Tavakolipour, H. <sup>2\*</sup>, Mokhtarian, M. <sup>3</sup>

1. Department of Food Science and Technology, North Tehran Branch, Islamic Azad University, Tehran, Iran

2. Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran

3. Department of Food Science and Technology, Roudehen Branch, Islamic Azad University, Roudehen, Iran

### ABSTRACT

Aiming to improve nutritional value (reduce sugar), bioactive compounds stability, producing of novel products and based on up-to-date knowledge (applying the encapsulation technology) & reducing of potential pomace in the agricultural part (like green tea & green coffee), a functional beverage based on Stevia sweetener was enriched by adding 84 g/L of cross-linked bioactive compounds of green tea and green coffee in chitosome microcapsule and physicochemical properties (pH, acidity, turbidity, total phenolic compounds & antioxidant power) and organoleptic characteristics (flavor, color, aroma, mouth feel and overall acceptance) of product were evaluated during storage time (1, 9, 18, 27, 36 & 45 days) and at different temperatures (5, 25 & 45°C). While the release rate of beverage phenolic compounds at temperature of 45°C, were significantly ( $p < 0.05$ ) increased to ~50% after 27 days of storage, the noted parameters increased at the rates of ~36 and ~46% at 5 and 25°C respectively at the same conditions. As well, the results show that, the parameter of beverage antioxidant productivity ratio (TPC/EC<sub>50</sub>) was obtained 0.06 and 5.71 in control and enriched samples, respectively after 27 days of storage (at 25°C). Generally, the chitosomal structure due to biodegradability, biocompatibility and non-toxicity is recommended as a suitable option for stability of bioactive compounds and design of effective systems for drug delivery.

### ARTICLE INFO

#### Article History:

Received 2022/ 07/ 01

Accepted 2022/ 10/ 29

#### Keywords:

Functional beverage,  
Chitosome,  
Bioactive compounds release,  
Phenolic compounds,  
Physicochemical properties and  
sensory evaluation.

**DOI:** 10.22034/FSCT.19.133.79

**DOR:** 20.1001.1.20088787.1401.19.133.7.2

\*Corresponding Author E-Mail:  
[h.tavakolipour@gmail.com](mailto:h.tavakolipour@gmail.com)

## 1. Introduction

In recent years, there have been significant changes in the understanding of the role of food in improving the quality of life, increasing life expectancy, and preventing diseases such as gastrointestinal infections, atherosclerosis, cholesterol, cancer, and inflammation of the digestive tract. In this regard, the food pattern of advanced and developing societies has tended towards the production of extra-beneficial foods (especially beverages). In addition to their nutritional value, multipurpose foods have at least one health-giving feature [1]. These products are usually enriched with vitamins, minerals and natural antioxidants including polyphenols [2].

Today, the isolation and application of natural compounds, including herbal medicines, for the treatment and prevention of common diseases has become the basis of extensive research. Outstanding bio-providing features<sup>1</sup>, bioavailability, toxicity reduction, high compatibility with the body environment of living organisms that exist in compounds of plant origin, are examples of countless reasons for the direction of extensive research in this direction. Polyphenols are a group of these natural compounds that are abundant in fruits and spices [3]. For example, the wastes from the processing of green tea leaves and green coffee beans, which are used as raw materials in this research, are rich in polyphenolic compounds.

Tea from the young leaves of *Camellia sinensis* (*Camellia sinensis*) prepared in different parts of the world in green, black and oolong<sup>2</sup> It is consumed. Among the types of tea, green tea

has been reported to have the most beneficial effects on human health. The important health effects (including reducing the risk of cancer and cardiovascular diseases, anti-inflammatory, anti-arthritic, antibacterial, antioxidant, antiviral, nervous system protection and cholesterol-lowering effects) of green tea mainly depend on the amount of phenolic compounds present in it. Especially flavonol and flavonol, which make up 30% of the weight of freshly dried leaves. Catechin is also one of the polyphenolic compounds, and its various types in green tea include (-) epicatechin.<sup>3</sup> (EC), (-) epicatechin-3-gallate<sup>4</sup> (ECG), (-) epigallocatechin<sup>5</sup> (EGC), (-) epigallocatechin-3-gallate<sup>6</sup> (EGCG), (+) catechin<sup>7</sup> and (+) galocatechin<sup>8</sup> (GC) has been identified. It should be noted that the most health-promoting effects of green tea are related to "epi" group catechins, especially (-) epigallocatechin-3-gallate (65% of the total catechin content). In addition, catechins in green tea have more antioxidant properties than vitamin C and E, and their free radical scavenging power is in the order of ECG>EGCG>EGC>EC>catechin [4].

Coffee beans are unroasted seeds from evergreen bushes *Rubiaceae* is obtained [5]. Coffee is known as a beneficial food due to its high content of antioxidant compounds and health-giving bioactive properties, rich in antioxidants (especially phenolic compounds and caffeine) and free radical scavengers. Four important bioactive compounds found in coffee include caffeine, chlorogenic acid (CGA), diterpene, and trigonelline. The main compound found in coffee beans is chlorogenic acid. The health effects of chlorogenic acid for humans include antioxidant, antiviral, and liver protection activities<sup>9</sup> and help with antispasmodic activities. In addition,

<sup>1</sup>. Bioavailability

<sup>2</sup>. Oolong tea

<sup>3</sup>. (-) Epicatechin (EC)

<sup>4</sup>. (-) Epicatechin-3-gallate (ECG)

<sup>5</sup>. (-) Epigallocatechin (EGC)

<sup>6</sup>. (-) Epigallocatechin-3-gallate (EGCG)

<sup>7</sup>. (+) Catechin

<sup>8</sup>. (+) Galocatechin (GC)

<sup>9</sup>. Hepatoprotective

chlorogenic acid is an important compound that plays a role in the production of taste and flavor during coffee roasting and also affects its quality [6].

Polyphenols have antioxidant properties and in addition to being anti-diseases such as cancer, smallpox, diabetes, rheumatism, viral, heart and lung diseases, they also increase the stability and durability of food [3]. In addition, polyphenols have a bitter taste and are highly sensitive to environmental factors such as light, moisture and oxygen. Despite having hydrophilic properties due to the presence of hydroxyl groups (-OH), these compounds have low solubility in polar solvents including water (due to the presence of aromatic groups and glycosidic rings). The set of these factors limits the use of these compounds in the food industry as bioactive compounds in food enrichment and the production of extra-beneficial products. One of the ways to overcome these problems is to be placed in a polymeric coating (such as a liposome-chitosan or chitosome hybrid system).

Liposomes are spherical particles (vesicles) with one or more double-layered membranes, with amphipathic (biphilic) characteristics, which are formed by the accumulation of fat or phospholipid molecules (especially phosphatidylcholine) in aqueous medium. Liposomes can be in suspension, semi-solid and powder forms. Despite the many advantages of liposomes (especially the possibility of production from healthy and harmless compounds for human health), only a few unmodified liposomes are stable, and most of them are sensitive to tension and have a great tendency to aggregate and fuse, which over time It may also lead to leakage of trapped content [7]. To overcome these defects and for more stability, modification of the surface of liposomal carriers using biopolymers (such as pectin or chitosan) has been done to design more effective drug delivery systems. Biopolymers are of special

importance due to their biocompatibility and biodegradability [8-10] and among them, chitosan has aroused many motivations for the healthy and effective development of drug delivery system due to its unique physicochemical (presence of hydroxyl and amine groups of the first type) and biological characteristics. .

Encapsulation protects polyphenols from degradation, adverse effects of light, moisture and oxygen by reducing the reaction with the external environment, and as a result, helps to increase their useful life [11]. In addition, encapsulation will result in reduced core vaporization, controlled release of the core, dilution of the core (in cases where only small amounts of phenolic compounds are required), and increased half-life [12]. The process of encapsulation of polyphenols in polymeric structures will be associated with increased interactions with biological environments on a molecular scale, along with the stability of loaded effective substances, due to easy adhesion to the surface of the intestine, with targeted and controlled release of bioactive compounds [13]. On the other hand, depending on the type of shell or surface active layer (surfactant) surrounding the final shell, due to the increase of surface to volume and increase of intermolecular interactions, solubility in different environments (hydrophobic or hydrophilic) increases [14].

So far, various researches have been conducted regarding the use of bioactive compounds in polymer coatings. Seyedabadi et al. (2021) investigated the production process and characteristics of nanoliposome intertwined with chitosan for caffeine microencapsulation. The results showed that the microencapsulation efficiency of nanoliposomes containing caffeine was in the range of 72-95%. The results of the device showed that approximately 50% of the

particle diameter<sup>10</sup> The prepared chitosome was less than 90 nm (PD>90 nm). The images obtained from SEM, TEM and AFM tests confirmed the appearance and spherical morphology of liposome and chitosome. In general, caffeine coating in chitosome structure decreased the release rate. The chitosome nanostructure showed a controlled release in the conditions of the simulated system of the digestive system and food models (simulating systems of milk and juice) [15]. In addition, several reports have been presented in the field of micro (nano) coating of ginger essential oil [16], grape polyphenols [17], curcumin [18] and quercetin [19] for use in specific applications, whose details are prevented due to the length of the introduction. .

According to the studies conducted, this research will develop a suitable formulation for the production of practical drinks based on the structure of chitosome in order to microencapsulate the bioactive compounds of green tea and green coffee. Then, the physicochemical characteristics of the produced beverage will be monitored and evaluated during the storage period.

## 2- Materials and methods

### 2-1- Preparation of raw materials

The required quantities of green tea leaves and green coffee beans were purchased from the local market (Tehran province). According to the common method of AOAC No. 931/04, the moisture content of each sample at temperature<sup>0</sup>C105 was measured for 24 h [20]. The moisture content of green tea leaves and green coffee beans was 9.5% and 11.5% (moist basis), respectively. Also, the chemical compounds used in this research were purchased

from Sigma Aldrich (USA) and Merck (Germany).

### 2-2- Extraction of bioactive compounds of green tea and green coffee beans

The extraction of bioactive compounds of green tea and green coffee beans was done using an ultrasound probe system (Sonopuls ultrasonic homogenizer, 3200HD, W150, 20 kHz, Germany) [22-21]. For this purpose, first extracting the bioactive compounds of green tea and green coffee respectively under operational conditions (<sup>0</sup>C53~ and min 26) and (<sup>0</sup>~C50 and min 35) (the range of ultrasound waves was considered constant and equivalent to 100% in both cases). A ratio of 1 to 30 dry matter to solvent (distilled water) was used for the extraction process [4, 22]. The extracted extract at temperature and pressure (respectively<sup>0</sup>C40 and 0.1 MPa) was desolvated by rotary evaporator (IK, Germany) until constant weight was reached. Then, the concentrated extract was dried by a freeze dryer (Dena-Sanat, Tehran, Iran) at the appropriate temperature and pressure.<sup>0</sup>It was dried at -C40 and 0.001 mbar [23].

### 2-3- Microencapsulation of bioactive compounds in the structure of chitosome

Microencapsulation of bioactive compounds obtained from green tea and green coffee extracts was done by the method of Tan et al. (2016) [24]. For this purpose, first, powdered soy lecithin was dissolved in ethanol solvent (1% w/v). In the next step by rotary evaporator under vacuum (at temperature and pressure respectively<sup>0</sup>C40 and 0.1 MPa), the solvent was removed to form a thin layer (liposome lipid multilayer vesicle formation) at the bottom of the balloon. Then the

<sup>10</sup>. Particle diameter (PD)

balloon was placed in a desiccator to remove the remaining solvent. Next, using a magnetic heater, the formed lipid layer was dissolved in a phosphate buffer containing 152 ppm of bioactive compounds (the mentioned concentration was obtained after mixing two bioactive compounds of green tea and green coffee in a ratio of 30 to 70, which The ratio was also chosen due to the higher bioactive compounds of green coffee. Ultrasonic treatment was applied at 75% range for 10 min to improve the microcoating process. In the next step, chitosan solution with a concentration of 0.3% w/v was added to the previous solution drop by drop (injection was done using a syringe and at a very low speed).

#### **2-4- Preparing the base formulation of the drink and producing a practical drink containing chitosome microcapsules**

In order to produce a beneficial dietary drink based on stevia, the components of the basic formulation were first prepared. The components used in the production of the drink include: stevia glycosidic powder (*Stevia rebaudiana*) type of rhabdioside A (stovia leaf contains 1.9% stevioside and 3.8% rhabdioside A) (0.15 L/g), citric acid (0.10 L/g), sodium benzoate (0.3 L/g), ascorbic acid (0.08 L/g), sodium citrate (0.03 L/g), gum arabic (0.25 L/g) and water. In order to produce a drink containing chitosome microcapsules, first the optimal amount of chitosome microcapsules was determined (the amount of chitosome microcapsules added was determined based on preliminary sensory and taste tests and the optimal amount was reported as 84 g/L) and it was added to the base formulation of the beneficial drink [27- 25].

#### **5-2- Release test of bioactive compounds and storage conditions**

In order to check the release rate of bioactive compounds during the storage period (for a maximum of 45 days), drinks produced in different temperature conditions (5, 25 and 45°C) were kept statically. Then, the rate of release of bioactive compounds, during the storage period at 5-day intervals, by measuring total phenolic compounds (TPC) and antioxidant activity ( $_{50}EC$ ) was monitored. It should be noted that the mentioned tests are performed according to Fulin Ciocultiv's methods.<sup>11</sup> and free radical scavenging (DPPH<sup>O</sup>) was evaluated [28-29].

#### **6-2- Beverage quality control tests**

In order to check the quality characteristics of the drink during the storage period, quality control tests of pH (acidity) and turbidity were used. These tests were measured according to the methods of the Iranian national standard No. 2685 (1386) and Mokhtarian et al. (2013) respectively [4, 30].

#### **2-7- Sensory and taste evaluation**

Sensory-taste evaluation of the produced drink was done by a group of semi-trained sensory evaluators, consisting of 10 people in the age range of 20 to 50 years (male and female). All evaluations were done by five-point hedonic scoring method. In this way, questionnaires were prepared and distributed among the evaluation team (panellists). For each question, 5 options were considered as answers. In order to evaluate the sensory-taste properties of the produced drink (on the first day of production), about 100 ml of the test samples (the fresh sample without chitosome microcapsules and the fresh sample containing 84 g/L of chitosome microcapsules) were provided to the evaluators in separate

<sup>11</sup>. Folin-Ciocalteu

containers and then The evaluated sensory-taste characteristics (taste, aroma, color, mouthfeel and overall acceptance) were recorded in the relevant questionnaire [31].

## 8-2- Statistical analysis

Statistical analysis of data was done using factorial test in the form of completely randomized design. Comparison of mean data was done using Duncan's multi-range test at 95% error probability level. For statistical analysis, SAS software version 3.1.9 (2003) of SAS Institute of America was used. All qualitative tests were performed in 3 repetitions and the results were reported as (mean  $\pm$  standard deviation).

## 3. Results and Discussion

### 3-1- Acidity and pH

The average comparison results showed that the interaction of storage temperature (5, 25 and  $^{\circ}\text{C}45$ ) and storage time (zero, 9, 18, 27, 36 and 45 days) on the pH (acidity) of practical drink containing bioactive compounds (green tea and green coffee extracts) microencapsulated in chitosome structure, significant ( $>0.05$ )  $p$  Is. As can be seen in table (1), the highest pH number (corresponding to the lowest acidity) corresponds to the samples kept at the temperature  $^{\circ}\text{C}45$  and on the 45th day of storage (~7% increase in pH and ~53% decrease in acidity compared to the first day of storage). The results of Pearson's linear correlation model between the pH number and the acidity of practical drinks confirm the findings ( $r=0.9809$  and  $r=0.9621^2\text{R}$ ). In addition, the results showed that keeping the samples at temperatures of 5 and  $^{\circ}\text{C}25$  (in a similar storage time), respectively, caused a change in the pH value (acidity) by ~2.9% increase (~26% decrease) and ~4.8% increase (~42% decrease)

compared to the first day. This condition is probably due to the breaking of the chitosome wall and the release of amine-containing compounds in chitosan biopolymer, which has caused a slight increase in pH by neutralizing the acid in the beverage environment [32]. In a research, Seyedabadi et al. (2021) investigated the release rate of bioactive compounds (caffeine) intertwined in the structure of chitosome in different conditions (simulated digestive system and juice). However, the qualitative characteristics of juice have not been investigated in the research [15]. Also, the review of previous sources showed that so far no research has been done regarding the monitoring of pH (acidity) changes during the storage period in the food model due to the destruction of the chitosomal structure. However, Pavia et al. (2015) reported that the (moderate-strong) tensile vibrations observed at  $\text{cm frequency}^{-1}$  1087, the presence of the functional group C-N corresponding to amines (especially amines of the first and second type) present in the acetamide groups ( $-\text{NHCOCH}_3$ ) and amine ( $-\text{NH}_2$ ), is in the structure of chitosan; which is probably the cause of the increase in pH during the storage period, the destruction of the polymer structure, which is associated with the release of amines [33].

### 3-2- Turbidity

One of the important parameters in the production of beverages is turbidity. The results of the variance analysis of the changes in the turbidity index of practical drinks containing bioactive compounds (green tea and green coffee extracts) microencapsulated in the chitosome structure during approximately 7 weeks of storage at the investigated temperatures (5, 25 and  $^{\circ}\text{C}45$ ) is given in table (1). According to the obtained results, it was observed that the storage temperature and storage time have a significant

effect ( $>0.05$ ). $p$ ) on the mentioned index. The highest amount of turbidity index throughout the storage period in relation to the beverage stored at the temperature<sup>o</sup>C25 was observed (~22% increase compared to the first day of storage). In response to the cause of this phenomenon, the observed changes of turbidity during the storage period should be investigated in more detail (refer to Table 1). According to the presented findings, it was observed that on the ninth day of storage (approximately one week after the production of the drink), the highest amount of turbidity (a significant change) in temperature<sup>o</sup>C45 was reported and its intensity decreased with increasing storage time (up to day 45). This is probably due to the rapid release of phenolic compounds at higher temperatures. Mokhtarian et al. (2013) reported that the main components in the formation of green tea cream (as a measure of turbidity), are phenolic compounds (especially catechins) and caffeine. Also, they stated that the caffeine-polyphenol interaction is a main factor in the formation of green tea cream, which is associated with increasing the appearance of cloudiness in the extract [4]. Considering that the release rate of phenolic compounds from the chitosome structure to the beverage environment at temperature<sup>o</sup>C45 relative to the temperature<sup>o</sup>C25 is faster, so it will be associated with faster degradation of phenolic compounds during the storage period (corresponding to the reduction of turbidity of the drink). While, in the temperature<sup>o</sup>C25, due to the slower release of phenolic compounds, the amount of turbidity increased gradually. Correlation (positive and strong) between the release rate of phenolic compounds and the turbidity of the practical drink (at temp<sup>o</sup>C25), confirms the claimed reasons ( $r=0.9199+$  and  $0.846=^2R$ ).

### 3-3- Total phenolic compounds (TPC), antioxidant power (<sub>50</sub>EC)

### and release rate of phenolic compounds

Bioactive compounds (including carotenoids, essential oils, antioxidants, or flavors) are a group of chemicals with health-giving, medicinal, and nutritional properties that are widely used in food products to enhance sensory properties or develop pharmaceutical properties.<sup>12</sup> They are used [23]. The results of analysis of variance of total phenolic compounds index changes (antioxidant power or<sub>50</sub>EC) practical drink containing bioactive compounds (green tea and green coffee extracts) microencapsulated in the chitosome structure during about 7 weeks of storage at different temperatures (5, 25 and<sup>o</sup>C45) is presented in table (1). According to the reported results, it was observed that the storage temperature and storage time (zero, 9, 18, 27, 36, and 45 days) had a significant effect ( $>0.05$ ). $p$ ) on these nutritional indicators. The highest amount of total phenolic compounds (corresponding to the highest release rate of phenolic compounds and the lowest amount<sub>50</sub>EC) present in the beverage environment, in connection with the beverage stored at temperature<sup>o</sup>C45 was observed, which was associated with an increase of ~54% (corresponding to a ~17% decrease in antioxidant power) after 27 days of storage. This state is probably due to the decrease in the resistance of the cytosome wall (corresponding to the decrease in membrane fluidity) and as a result the release of phenolic compounds [34]. In order to study these changes in more detail, the Pearson correlation between the two mentioned parameters was tested using descriptive statistics (in the sample kept at temperature<sup>o</sup>C45 in all storage days) and high and negative correlation ( $r=0.9640$  and  $r=0.9292^2R$ ) between the traits of total phenolic compounds and<sub>50</sub>The EC of pragmatic drink was reported (which indicates a strong and non-coherent dependence of the two

<sup>12</sup>. Nutraceutical



traits), which confirms the above findings. Also, the release rate of total phenolic compounds during the same storage period (27 days), at temperatures of 5 and °C25 was determined as ~37% and ~51%, respectively, compared to the storage temperature °C45 is lower. In addition, the results showed that after 27 days of storage (at all studied temperatures), the amount of total phenolic compounds in the beverage environment started to decrease. This state is probably due to the degradation of polyphenolic compounds released in the drinking environment and the destructive effects of environmental storage conditions. Seyedabadi et al. (2021) in a research, the release rate of caffeine from chitosome in the simulated environment of fruit

juice at temperature °C37 checked. Their results showed that the sudden release of caffeine from the chitosome structure in the simulated environment of fruit juice is due to the swelling of the structure caused by the influence of the simulating environment and subsequently the release of caffeine on the surface of nanoparticles [15]. In the study conducted by Liu et al. (2017), the results of the release of chitosan nanoparticles containing tea polyphenols mixed with gelatin film in 50 and 95% ethanol fat simulator environments showed that the release of polyphenolic compounds in the 50% environment compared to the 95% environment due to the increase of two The equalization of the diffusion coefficient was faster [35].

**Table 1** The mean values (average of 3-replication) of quality control parameters of functional beverage containing bioactive compounds encapsulated in chitosome structure during storage period for 45-days at different temperatures.

Storage temperature (°C)	Storage time (day)	Product quality control parameters <sup>(*)</sup>					
		pH	Acidity	Turbidity	Total phenolic content (TPC)	Antioxidant activity (EC <sub>50</sub> )	Releasing rate of TPC
		(–)	(g/[100 g])	(NTU)	(mg GAE/[100 g DM])	(%)	(%)
5°C							
	0	5.066±0.006 <sup>k</sup>	0.3093±0.0037 <sup>a</sup>	1.2323±0.0011 <sup>m</sup>	65.000±0.979 <sup>m</sup>	26.614±0.381 <sup>a</sup>	22.916±0.345 <sup>m</sup>
	9	5.080±0.010 <sup>jk</sup>	0.2880±0.00 <sup>b</sup>	1.3260±0.00 <sup>j</sup>	77.222±0.979 <sup>l</sup>	25.984±0.181 <sup>b</sup>	27.225±0.345 <sup>l</sup>
	18	5.097±0.006 <sup>j</sup>	0.2731±0.0037 <sup>c</sup>	1.3413±0.010 <sup>i</sup>	82.407±1.335 <sup>k</sup>	24.869±0.129 <sup>d</sup>	29.053±0.471 <sup>k</sup>
	27	5.133±0.021 <sup>i</sup>	0.2581±0.0037 <sup>d</sup>	1.3743±0.008 <sup>h</sup>	103.518±0.979 <sup>i</sup>	23.933±0.155 <sup>lt</sup> <sub>is</sub>	36.496±0.345 <sup>i</sup>
	36	5.190±0.010 <sup>gh</sup>	0.2368±0.0064 <sup>if</sup>	1.3967±0.0042 <sup>g</sup>	119.074±1.111 <sup>f</sup>	23.235±0.159 <sup>f</sup>	41.980±0.392 <sup>f</sup>
	45	5.216±0.015 <sup>fg</sup>	0.2282±0.0037 <sup>f</sup>	1.4190±0.0060 <sup>f</sup>	124.753±0.566 <sup>d</sup>	22.862±0.182 <sup>f</sup> <sub>gh</sub>	43.982±0.199 <sup>d</sup>



25°C						
0	5.143±0.01 5 <sup>i</sup>	0.3008±0.006 4 <sup>a</sup>	1.2337±0.00 29 <sup>m</sup>	64.136±1.750 <sup>m</sup>	26.616±0.455 <sup>a</sup>	22.611±0.617 <sup>m</sup>
9	5.180±0.01 0 <sup>h</sup>	0.2858±0.003 7 <sup>b</sup>	1.3920±0.00 g	82.531±0.566 <sup>k</sup>	25.806±0.257 <sup>b</sup>	29.097±0.199 <sup>k</sup>
18	5.233±0.01 5 <sup>f</sup>	0.2581±0.003 7 <sup>d</sup>	1.4417±0.00 55 <sup>It is</sup>	107.592±1.614 <sup>h</sup>	23.912±0.069 <sup>It is</sup>	37.932±0.569 <sup>h</sup>
27	5.287±0.01 5 <sup>It is</sup>	0.2304±0.006 4 <sup>if</sup>	1.4933±0.00 58 <sup>d</sup>	132.160±0.932 <sup>b</sup>	23.124±0.083 <sup>f</sup> g	46.594±0.328 <sup>b</sup>
36	5.327±0.02 1 <sup>d</sup>	0.1984±0.006 4 <sup>g</sup>	1.5187±0.01 46 <sup>c</sup>	123.395±0.771 <sup>of</sup>	23.267±0.099 <sup>f</sup>	43.503±0.272 <sup>of</sup>
45	5.400±0.02 0 <sup>c</sup>	0.1749±0.009 7 <sup>h</sup>	1.5667±0.00 77 <sup>a</sup>	121.913±1.402 <sup>It is</sup>	23.251±0.124 <sup>f</sup> is	42.981±0.494 <sup>It is</sup>
45°C						
0	5.193±0.01 5 <sup>gh</sup>	0.2858±0.003 7 <sup>b</sup>	1.2333±0.00 32 <sup>m</sup>	64.629±2.312 <sup>m</sup>	26.663±0.637 <sup>a</sup>	22.785±0.815 <sup>m</sup>
9	5.233±0.01 5 <sup>f</sup>	0.2752±0.006 4 <sup>c</sup>	1.5520±0.00 90 <sup>b</sup>	86.358±1.497 <sup>j</sup>	25.377±0.176 <sup>c</sup>	30.446±0.528 <sup>j</sup>
18	5.283±0.01 5 <sup>It is</sup>	0.2389±0.009 7 <sup>It is</sup>	1.4090±0.01 20 <sup>f</sup>	112.654±1.496 <sup>g</sup>	22.752±0.219 <sup>g</sup> h	39.717±0.528 <sup>g</sup>
27	5.353±0.02 5 <sup>d</sup>	0.1984±0.006 4 <sup>g</sup>	1.3160±0.00 96 <sup>j</sup>	140.556±2.963 <sup>a</sup>	22.235±0.227 <sup>i</sup>	49.554±1.044 <sup>a</sup>
36	5.483±0.01 5 <sup>b</sup>	0.1728±0.006 4 <sup>h</sup>	1.2970±0.00 30 <sup>k</sup>	127.592±1.335 <sup>c</sup>	22.688±0.171 <sup>h</sup>	44.983±0.471 <sup>c</sup>
45	5.593±0.04 1 <sup>a</sup>	0.1344±0.006 4 <sup>i</sup>	1.2747±0.00 15 <sup>l</sup>	118.086±0.932 <sup>f</sup>	23.740±0.079 <sup>It is</sup> is	41.632±0.328 <sup>f</sup>

(\*)In each column, the mean values (Ave.±SD) with similar superscript letters had no significant difference ( $p<0.05$ ).

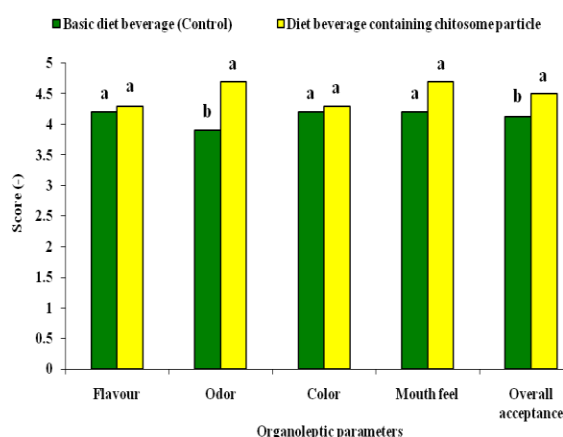
The results of the release of methyl cellulose film containing alpha-copherol nanocoated in polycaprolactone in a 95% fat simulating environment showed that after one day, the release rate had an upward slope due to the swelling caused by water infiltration into the film structure [36]. In another study, the release of limonene contained in chitosan film enriched with bergamot oil in food simulating

environments of zero to 95% ethanol showed that the release process was gradual and with the increase of alcohol ratio, the release rate was faster due to the non-polar nature of limonene [37].

### 3-5- Sensory-taste characteristics

The results of comparing the average effect of the type of drink (basic diet drink or control and

beneficial diet drink containing microparticles of chitosome) on the sensory and taste indicators (taste, aroma, color, mouthfeel and overall acceptance) of the product produced on the first day of production are presented in Figure (1) has been As can be seen, the type of drink has a significant effect ( $>0.05$ ). $p$ ) on sensory and taste parameters (aroma and overall acceptance) of the produced product. However, the highest sensory and taste scores obtained were related to the ultra-beneficial diet drink containing chitosome nanoparticles.



**Fig 1** The mean comparison (average of 10-replication) between organoleptic parameters of basic diet beverage (control) and functional beverage containing chitosome particle at 1<sup>st</sup>-day.

Mokhtarian et al. (2014) investigated the production of green tea super-beneficial soft drink based on sucralose sweetener, and their results showed that the super-beneficial drink had higher consumer satisfaction than the control drink, which is consistent with the findings of the present study [4].

## 4 - Conclusion

In this research, the practical drink based on the dietary sweetener stevia was enriched by microparticles of chitosome intertwined with bioactive compounds (green tea and green

coffee), and its physical (turbidity), chemical (pH, acidity, total phenolic compounds and antioxidant power) properties and Sensory and taste characteristics (taste, aroma, color, mouth feel and overall acceptance) of the product produced during 45 days of storage in different temperature conditions (5, 25 and 45°C) was monitored. Although, the addition of 84 g/L of chitosome microparticles to the active drink formulation caused a significant increase ( $<0.05$ ). $p$ ) parameter of total phenolic compounds (~2.6 times) compared to the control sample (sample without microparticles of chitosome) was produced per day, antioxidant power parameter showed the opposite behavior (0.16 times reduction) in the same conditions. In general, according to the findings, it can be concluded that the chitosomal structure is a potential packaging for microencapsulation of bioactive compounds, and its addition to food products (especially beverages) not only causes the stability of bioactive compounds, but also can improve the nutritional, sensory-tasting and pharmaceutical properties of the product. to improve and lead to the production of a new and knowledge-based product.

## 5- Resources

- [1] Jaddi, Z.S., Ahmadi-Dastgerdi, A., Sharafati-Chaloshtori, R. (2020). Production of Functional Beverage of Grape Juice and Lemon Skin Distillate Contained *Bacillus coagulans*. J. Food Hygiene, 9(36): 33-47. [In Persian]
- [2] Siro, I., Kápolna, E., Kápolna, B., Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance-A review. Appetite, 51(3): 456-67.
- [3] Safarzadeh-Matin, S., Shahbazi, M. (2013). Modeling and optimization of operation parameters in production of nanocapsules from

polyphenol extract from apple industry by-product. *Innovative Food Technol.*, 3(12): 1-13.

[4] Mokhtarian, M., Tavakolipour, H., Jafari-Savareh, S.H., Amiri, M. (2014). Determination of optimal parameters to extraction and formulation of functional drink from green tea and determining its physicochemical and rheological properties. *J. Res. Innovation Food Sci. Technol.*, 3(1): 51-66. [In Persian]

[5] Macheiner, L., Schmidt, A., Schreiner, M., Mayer, H.K. (2019). Green coffee infusion as a source of caffeine and chlorogenic acid. *J. Food Composition Analysis*, 84: 103307.

[6] Mangku, I.G.P., Wijaya, I.M.A.S., Ganda Putra, G.P., Permana, D.G.M. (2019). The bioactive compounds formation of "Kintamani" arabica coffee bean during dry fermentation. *J. Life Sci. Chem.*, 36 (2): 45-52.

[7] Alavi, S., Haeri, A., Dadashzadeh, S. (2017). Utilization of chitosan-caged liposomes to push the boundaries of therapeutic delivery. *Carbohydrate polymers*, 157: 991-1012.

[8] Mady, M.M., Darwish, M.M. (2010). Effect of chitosan coating on the characteristics of DPPC liposomes. *J. Advanced Res.*, 1(3): 187-191.

[9] Nag, O.K., Awasthi, V. (2013). Surface engineering of liposomes for stealth behavior. *Pharmaceutics*, 5(4): 542-69.

[10] Zhuang, J., Ping, Q., Song, Y., Qi, J., Cui, Z. (2010). Effects of chitosan coating on physical properties and pharmacokinetic behavior of mitoxantrone liposomes. *Int. J. Nanomedicine*, 5: 407.

[11] Mora-huertas, C.E., Fessi, H., Elaissari, A. (2010). Polymer-based nanocapsules for drug delivery. *Int. J. Pharm.*, 385: 113-142.

[12] Fang, Z., Bhandari, B. (2010). Encapsulation of polyphenols-A review. *Trends Food Sci. Technol.*, 21: 510-523.

[13] Laroui, H., Wilson, D.S., Dalmaso, G., Salaita, K., Murthy, N., Sitaraman, S.V., Merlin, D. (2011). Nanomedicine in GI. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 300: G371-G383.

[14] Fathi, M., Mozafari, M., Mohebbi, M. (2012). Nanoencapsulation of food ingredients using lipid based delivery systems. *Trends Food Sci. Technol.*, 23(1): 13-27.

[15] Seyedabadi, M.M., Rostami, H., Jafari, S.M., Fathi, M. (2021). Development and characterization of chitosan-coated nanoliposomes for encapsulation of caffeine. *Food Biosci.*, 40: 100857.

[16] Fathi, M., Samadi, M., Rostami, H., Parastouei, K. (2021). Encapsulation of ginger essential oil in chitosan-based microparticles with improved biological activity and controlled. *J. Food Proc. Preserv.*, 00: e15373.

[17] Gibis, M., Ruedt, C., Weiss, J. (2016). In vitro release of grape-seed polyphenols encapsulated from uncoated and chitosan-coated liposomes. *Food Res. Int.*, 88: 105-13.

[18] Shahgholian, N., Rajabzadeh, G. (2016). Fabrication and characterization of curcumin-loaded albumin/gum arabic coacervate. *Food Hydrocolloids*, 59: 17-25.

[19] Ni, S., Sun, R., Zhao, G., Xia, Q. (2015). Quercetin loaded nanostructured lipid carrier for food fortification: preparation, characterization and in vitro study. *J. Food Process Eng.*, 38(1): 93-106.

[20] AOAC. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists, (17<sup>th</sup> ed.); AOAC Press: Arlington, TX.

- [21] Bindes, M.M.M., Reis, M.H.M., Cardoso, V.L., Boffito, D.C. (2018). Ultrasound-assisted extraction of bioactive compounds from green tea leaves and clarification with natural coagulants (chitosan and Moringa oleífera seeds). *Ultrasonics Sonochemistry*, <https://doi.org/10.1016/j.ultsonch.2018.10.014>.
- [22] Guglielmetti, A., D'ignoti, V., Ghirardello, D., Belviso, S., Zeppa, G. (2017). Optimisation of ultrasound and microwave-assisted extraction of caffeoylquinic acids and caffeine from coffee silver skin using response surface methodology. *Italian J. Food Sci.*, 29: 409-423.
- [23] Shabani, M., Mokhtarian, M., Kalbasi-Ashtari, A., Kazempoor, R. (2021). Effects of extracted propolis (*Apis mellifera*) on physicochemical and microbial properties of rainbow-trout fish burger patties. *J. Food Proc. Pres.*, 00: e16027.
- [24] Tan, C., Feng, B., Zhang, X., Xia, W., Xia, S. (2016). Biopolymer-coated liposomes by electrostatic adsorption of chitosan (chitosomes) as novel delivery systems for carotenoids. *Food Hydrocolloids*, 52: 774-784.
- [25] Liang, J., Yan, H., Wang, X., Zhou, Y., Gao, X., Puligundla, P., Wan, X. (2017). Encapsulation of epigallocatechin gallate in zein/chitosan nanoparticles for controlled applications in food systems. *Food Chem.*, <http://dx.doi.org/10.1016/j.foodchem.2017.02.106>.
- [26] Meng, R., Wu, Z., Xie, Q., Cheng, J., Zhang, B. (2021). Preparation and characterization of zein/carboxymethyl dextrin nanoparticles to encapsulate curcumin: Physicochemical stability, antioxidant activity and controlled release properties. *Food Chem.*, 340: 127893.
- [27] Rajkumar, V., Gunasekaran, C., Amita Paul, C., Dharmaraj, J. (2020). Development of encapsulated peppermint essential oil in chitosan nanoparticles: characterization and biological efficacy against stored-grainpest control. *Pesticide Biochem. Physiology*, 170: 104679.
- [28] Moosazad, S., Ghajarbeigi, P., Mahmoudi, R., Shahsavari, S., Vahidi, R., Soltani, A. (2019). Antibacterial and antioxidant properties of colorant extracted from red onion skin. *J. Chem. Health Risks*. 9(3), 235-243. <https://doi.org/10.22034/jchr.2019.668188>.
- [29] Uribe, E., Marín, D., Vega-Galvez, A., Quispe Fuentes, I., Rodriguez, A. (2016). Assessment of vacuum dried peppermint (*mentha peppermint* L.) as a source of natural antioxidants. *Food Chem.* 90, 559-565. <https://doi.org/10.1016/j.foodchem.2015.05.108>.
- [30] Iranian National Standardization Organization (INSO). (1986). Fruit juices-Test methods. Iran: (number 2685).
- [31] Reis, A.S.D., Diedrich, C., Moura, C.D., Pereira, D., Almeida, J.D.F., Silva, L.D.D., Plata-Oviedo, M.S.V., Tavares, R.A.W., Carpes, S.T. (2016). Physico-chemical characteristics of microencapsulated propolis co-product extract and its effect on storage stability of burger meat during storage at -15°C. *LWT-Food Sci. Technol.*, 1-8.
- [32] Moradi Kashksara, K. (2022). Extraction of bioactive compounds of green tea and green coffee extracts by ultrasound and their microencapsulation in the structure of chitosome and investigation of release kinetics in the food system model (Beverage). Ph.D. Thesis on Food Science. Islamic Azad University, North Tehran Branch. [In Persian]
- [33] Pavia, D.L., Lampman, G.M., Kriz, G.S., Vyvyan, J.R. (2015). Introduction to Spectroscopy (Fifth Edition). Cengage Learning, USA.

[34] Tai, K., Rappolt, M., Mao, L., Gao, Y., Li, X., Yuan, F. (2020). The stabilization and release performances of curcumin-loaded liposomes coated by high and low molecular weight chitosan. *Food Hydrocolloids*, 99: 105355.

[35] Liu, F., Avena-Bustillos, R.J., Chiou, B-S., Li, Y., Ma, Y., Williams, T.G., et al. (2017). Controlled-release of tea polyphenol from gelatin films incorporated with different ratios of free/nanoencapsulated tea polyphenols into fatty food simulants. *Food Hydrocolloids*, 62: 212-21.

[36] Noronha, C.M., de Carvalho, S.M., Lino, R.C., Barreto, P.L.M. (2014). Characterization of antioxidant methylcellulose film incorporated with  $\alpha$ -tocopherol nanocapsules. *Food Chem.*, 159: 529-35.

[37] Sánchez-González, L., Cháfer, M., González-Martínez, C., Chiralt, A., Desobry, S. (2011). Study of the release of limonene present in chitosan films enriched with bergamot oil in food simulants. *J. Food Eng.*, 105(1): 138-43.



# مجله علوم و صنایع غذایی ایران

سایت مجله: [www.fsct.modares.ac.ir](http://www.fsct.modares.ac.ir)

مقاله علمی-پژوهشی

## تولید نوشیدنی عملگرا بر پایه ترکیبات زیست فعال چای سبز و قهوه سبز در هم تنیده در ساختار کیتوزوم

کریم مرادی کشکسرا<sup>۱</sup>، حمید توکلی پور<sup>۲\*</sup>، محسن مختاریان<sup>۳</sup>

۱- گروه علوم و صنایع غذایی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران.

۲- گروه علوم و صنایع غذایی، واحد سبزوار، دانشگاه آزاد اسلامی، سبزوار، ایران.

۳- گروه علوم و صنایع غذایی، واحد رودهن، دانشگاه آزاد اسلامی، رودهن، ایران.

### اطلاعات مقاله

### چکیده

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۱/۰۴/۱۰

تاریخ پذیرش: ۱۴۰۱/۰۸/۰۷

کلمات کلیدی:

نوشیدنی فراسودمند،

کیتوزوم،

رهایش ترکیبات زیست فعال،

ترکیبات فنولی،

خصوصیات فیزیکوشیمیایی و ارزیابی حسی.

با هدف بهبود ارزش تغذیه ای (کاهش شکر)، پایداری ترکیبات زیست فعال، تولید فرآورده نوین و مبتنی بر دانش روز (بکارگیری فناوری ریزپوشانی) و کاهش ضایعات بالقوه بخش کشاورزی (نظیر چای سبز و قهوه سبز)، نوشیدنی عملگرا بر پایه شیرین کننده استویا با افزودن ۸۴ g/L ریزذرات کیتوزوم در هم تنیده شده با ترکیبات زیست فعال چای سبز و قهوه سبز، غنی سازی شد و ویژگی های فیزیکوشیمیایی (pH، اسیدیته، کدورت، ترکیبات فنولی کل و قدرت آنتی اکسیدانی) و حسی و چشایی (طعم و مزه، رنگ، رایحه، احساس دهانی و پذیرش کلی) فرآورده در طول دوره نگهداری (۱، ۹، ۱۸، ۲۷، ۳۶ و ۴۵ روز) و در دماهای (۵، ۲۵ و ۴۵°C) ارزیابی گردید. با وجود اینکه، پارامتر نرخ رهایش ترکیبات فنولی نوشیدنی در درجه حرارت ۴۵°C، بعد از ۲۷ روز نگهداری، به طور معنی دار ( $p < 0.05$ )، ۵۰٪ افزایش یافت، پارامتر یاد شده به ترتیب با نرخ های ۳۶٪ و ۴۶٪ در درجه حرارت های ۵ و ۲۵°C و در شرایط مشابه افزایش یافت. همچنین نتایج نشان داد که پارامتر نسبت بهره وری آنتی اکسیدان (TPC/EC<sub>50</sub>) نوشیدنی بعد از ۲۷ روز نگهداری (در درجه حرارت ۲۵°C)، در نمونه های کنترل و غنی شده با ریزذرات کیتوزوم به ترتیب ۰/۰۶ و ۵/۷۱ بود. به طور کلی، ساختار کیتوزومی به دلایل زیست تخریب پذیری، زیست سازگاری و عدم سمیت، به عنوان گزینه ای مناسب برای پایداری ترکیبات زیست فعال و طراحی سیستم های مؤثر تحویل دارو پیشنهاد می شود.

DOI: 10.22034/FSCT.19.133.79

DOR: 20.1001.1.20088787.1401.19.133.7.2

\* مسئول مکاتبات:

h.tavakolipour@gmail.com