

ENCAPSULATION AND STABILITY OF YANANG LEAF (*Tiliacora triandra*) EXTRACT

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Abstract

Yanang (*Tiliacora triandra*) is known as vegetable and herbal in the northeast of Thailand. This research was aimed to propose an experimental method for optimizing the encapsulation efficiency and bioactive compounds stability in Yanang leaf extract. The optimized conditions for the encapsulation was indicated as a ratio of core to wall material of 1.60:8.40 with an alginate concentration of 1% (w/v) and 1% (w/v) CaCl₂ solution. The results showed that the encapsulation efficiency and bioactivity were mainly effected by the ratio of Yanang leaf extract concentration to alginate concentration. The results revealed that the bioactive compounds were not lost in the encapsulation process. The study of bioactive compounds in encapsulated Yanang leaf extract showed only a slow reduction during storage at 4-10°C for three months (>50% residual). Moreover, encapsulated Yanang leaf extract was released in the small intestine (pH 7.4) more than in stomach (pH 1.2) at 24 h and in the soft drink model system, the encapsulated Yanang leaf extract showed the highest amount of bioactive compounds at pH 4 and 77°C for 1 min (high temperature short time: HTST).

Keywords: Yanang, *Tiliacora triandra*, encapsulation, hydrogel beads, alginate, bioactive compounds

Introduction

Herbal-related products are used as food or food additives, food supplements and traditional medicines. The World Bank has projected the global market for herbal products to grow from USD 200 billion in 2008 to USD 5 trillion in 2050 (Chan *et al.*, 2010). A key factor for the

rapid growth of this market is the growing knowledge and confidence of consumers in natural products or medicines. Bioactive compounds of herbal plants, such as antioxidants, phenolic compounds, carotenoids and chlorophyll, have been shown to have multiple functional

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and remedial properties (Bae and Suh, 2007). However, bioactive compounds are often unstable in certain pH, light, oxygen, temperature and enzymatic conditions (Saénz *et al.*, 2009). The stability of bioactive compounds can be improved using encapsulation technologies.

Yanang (*Tiliacora triandra*) is an herb found in northeast Thailand. Yanang root extract is used as a medicinal herb for treating fever in the local people due to its richness in alkaloids (Mahidol *et al.*, 1994). Some profile reports showed this plant had high antioxidant content, high vitamin content and moderate levels of calcium and iron (Bunluepuech and Tewtrakul, 2009).

Encapsulation is a process in which thin films, generally of polymeric materials, are applied to small solid particles, liquids or gas droplets. This method is used to trap bioactive compounds and release them under controlled conditions. Several materials have been encapsulated in the food industry, such as amino acids, vitamins, minerals, antioxidants, colorants, enzymes and sweeteners (Deladino *et al.*, 2008). In addition, encapsulation may promote better product stability by isolating the bioactive compounds from the detrimental effects of oxygen or moisture (Chan *et al.*, 2010).

Hydrogel is a commonly used material for encapsulation due to its ability to absorb a large amount of water or biological fluids. Calcium-alginate hydrogel is the most widely used due to several advantageous features, such as non-toxicity, biocompatibility, ease of production and thermal and chemical stability (Chan *et al.*, 2009). Alginate is a polysaccharide isolated from seaweed and is composed of two uronic acid salts β -D-mannuronate (M) and α -L-guluronate (G). Solutions of alginate can be cross-linked by a mild gelling reaction with calcium ions to form a hydrogel (Hunt *et al.*, 2010). The alginate gel structure is relatively stable at acidic pH, but it is easily swollen and disintegrated under mild alkali conditions (Yoo *et al.*, 2006).

The goal of our research was to develop an effective bioactive compound delivery system using an encapsulation technique to

achieve controlled release and improve the bioavailability of a model nutrient. Therefore, the aim of this research was to optimize the encapsulation process of Yanang leaf extract and to study the stability of bioactive compounds in encapsulation during storage at different temperatures. Furthermore, the bioactive compounds releasing were investigated in vitro study. Finally, the release of bioactive compounds in a soft drink model system was measured to different pH, temperature and time.

Materials and Methods

Materials

Yanang leaf, procured from a local market in northeast Thailand (Ubon Ratchathani Province) were cleaned with water to remove dust, and infected leaves were separated. The prepared leaves were then dried at 60°C for 3 h. The dried leaves were ground and stored at room temperature in a vacuum-packed container before use (Singthong *et al.*, 2009). The encapsulation matrix polymer was calcium alginate and calcium chloride (CaCl₂). Sodium alginate was obtained from International Speciality Product (ISP) Technologies Inc., Thailand.

Preparation of Yanang leaf extract

Yanang leaf extract was prepared using a method by Singthong *et al.* (2014). Approximately 100 mg Yanang leaf powder was extracted with three 12 mL portions of boiling water in a shaking water bath at 25°C for 15 min. The sample were centrifuged at 3,000×g for 3 min. The filtrates were combined, and the final volume was adjusted to 50 mL. Aliquots of 2 mL were dried by freeze-drying (FD 8, Heto Lab Equipment, Denmark), and the dried extract was stored in a desiccator at room temperature until use.

Encapsulation Optimization

The experimental design of encapsulation was performed under various conditions, according to a central composite design (CCD). The effect of three independent

variables: the ratio of core material (Yanang leaf extract) to wall material (alginate), X_1 (1:9-5:5), alginate concentration, X_2 (1-3%) and concentration of CaCl_2 solution, X_3 (1-3%), was determined, using the detailed experimental design shown in Table 1. The independent variables were coded to three levels of -1, 0 and +1. The optimization condition for encapsulation of Yanang leaf extract was determined using response surface methodology. All experimental data were statistically analysed with Design-Expert® software (Version 6.0.10, Stat-Ease, Inc., Minneapolis, MN). The encapsulation process was conducted using the method of Chan *et al.* (2010) with slight modifications. Sodium alginate solution was prepared to the desired concentration with deionized water. The solution (1%) was extracted through a flat tip and allowed to drip into a gelation bath containing calcium chloride. The Ca-alginate beads were then hardened for 1 h and dried by freeze-drying (FD 8, Heto Lab Equipment, Denmark), and the dried extract was stored in a desiccator at room temperature for further analysis.

The overall encapsulation efficiency was determined using the method of Chan *et al.* (2010) with slight modifications. It was calculated based on the mass of the extract absorbed by the blank Ca-alginate beads, as shown in Equation (1).

$$\text{Overall Encapsulation Efficiency} = \frac{m_{\text{Eb}} (\text{g extract})}{m_{\text{b}} (\text{g beads})} \quad (1)$$

where m_{Eb} is the mass of extract absorbed by beads (g) and m_{b} is the mass of beads (g).

Stability of Encapsulation

The stability of encapsulation was determined using the method of Nori, *et al.* (2011) with slight modifications. Encapsulated of Yanang extract was stored at cold temperature (4-10°C) and room temperature (25-30°C) for 3 months. The bioactive compounds were analysed every week for 3 months.

In Vitro Releasing Properties of the Bioactive Compounds

The in vitro bioactive compound releasing properties were evaluated using the encapsulation produced under the optimal conditions of Yanang extract encapsulation with calcium-alginate hydrogel. To mimic the digestive tract in a human body, 0.05 M HCl (pH 1.2) with 0.2% NaCl was used as a stimulated gastric fluid, and 0.05 M phosphate buffer (pH 7.4) was used as a stimulated intestinal fluid. This experiment was adjusted pH to be same as condition in stomach (pH 1.2) and intestinal (pH 7.4). However, this simulation system did not contain digestive enzymes (Yoo, *et al.*, 2006). An aliquot of the capsule (150 mg) was dispersed in 25 mL of the simulated fluid, and the releasing patterns of the bioactive compounds were monitored for 24 h in a shaking incubator at 100 rpm and 37°C.

Application of Encapsulation in a Soft Drink-Related Model System

Effect of pH

The study of the pH change effects on the bioactive compound release was performed following the method of Hansson *et al.* (2001) with slight modifications. First, citric acid (1.0 g) was added to a mixture of water (10 ml) and the Yanang encapsulation (0.1% w/v) to obtain pH values of 2. Sec, as a complementary study, 1.0 g of citric acid (pH 2) was added to the same encapsulation mixture, and sodium hydroxide was then added to adjust the pH values to 3, 4, 5, 6, and 7. All pH values were measured using a pH meter.

Effect of Temperature/Time

The effect of temperature and time changes on bioactive compound release was studied following the method of Pernice *et al.* (2009) with slight modifications. Low temperature long time (LTLT), as 65°C for 30 min, and high temperature short time (HTST), as 77°C for 1 min, were applied to soft drink-related model system. The bioactive compounds were analysed after cooling to room temperature.

Analysis of Bioactive Compounds

Total Phenolic

The measurement of the total soluble phenolic constituents of the encapsulation of Yanang leaves extract used the Folin-Ciocalteu reagent with gallic acid as standard. Twenty μL of a solution of encapsulated Yanang leaf extract (0.5 g encapsulated Yanang/50 mL water) was added into a 1.5 mL cuvette, to which 1.58 mL of water and 100 μL Folin-Ciocalteu reagent were added. The sample was thoroughly mixed and incubated for 5 min at room temperature. Following incubation, 300 μL of the Na_2CO_3 (2% w/v) solution was added, and the mixture was allowed to stand at room temperature for 2 h. The absorbance was measured at 765 nm, and the results were expressed as gallic acid equivalents (Oonsivilai *et al.*, 2007).

Total Antioxidant

The antioxidant activity was evaluated in accordance with the DPPH method. The free radical scavenging activity was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to a slightly modified procedure described by Brand-Williams *et al.* (1995). Briefly, 50 μL for encapsulation solution (0.5 g encapsulated Yanang/50 mL water) was added to 950 μL DPPH methanolic solution (12 mg/50 mL). The reaction mixture was covered and left in the dark at room temperature. After 15 min, the absorption was measured at 515 nm. Ascorbic acid was used as a standard control.

Total Carotenoids

The total carotenoids measurement was conducted using the methods of Wongsu *et al.* (2010) and Oliveira *et al.* (2010) with slight modifications. The encapsulation of Yanang leaf extract solution, 10 mL (0.5 g encapsulated Yanang/50 mL water), was extracted with 10 mL of acetone:ethanol (1:1 v/v). The extract was centrifuged at $1,500\times g$ for 15 min at $4-5^\circ\text{C}$. The supernatant final volume was adjusted to 100 mL. The absorbance was measured at 450 nm and calculated.

Total Chlorophyll

The total chlorophyll measurement was performed using the method of Ramesh and Devasenapathy (2006) with slight modifications. Ten mL of encapsulated Yanang leaf extract solution (0.5 g encapsulated Yanang/50 mL water) was extracted with 10 mL acetone. The supernatant final volume was adjusted to 50 mL. The absorbance was measured at 652 nm and calculated.

Vitamin C

Vitamin C was determined using the 2, 6-dichloroindophenol titrimetric method (AOAC, 1995). Five mL of Yanang encapsulated solution (0.5 g encapsulated Yanang/50 mL water) was extracted with 10 mL of trichloroacetic acid solution (5% w/v). The solution was titrated against a standard indophenol solution, and the result was expressed in μg of ascorbic acid.

Structural Analysis

The outer structures of the capsules obtained at optimal conditions were studied by SEM. The samples were coated with gold using a sputter coater, model SPI-MODULETM Sputter Coat and examined by a JEOL, JSM-5410LV scanning microscope.

Data Analysis

All experiments and analytical measurements were run in triplicate. The means of each parameter were analysed by analysis of variance (ANOVA). Differences between treatments at the 5% ($p\leq 0.05$) level were considered significant.

Results and Discussion

Encapsulation Process

The effect of the process variables on the overall encapsulation efficiency (OEE) is core material was protected against shown in Table 1. When the ratio of core to wall and alginate concentration increased, the oxidation by the wall material (Gharsallaoui *et al.*, 2007). The

Table 1. Central composite design (CCD) for the optimization of Yanang leaves extract encapsulation

Treatment	Coded variable ^a			Process variable ^b			OEE ^c
	X ₁	X ₂	X ₃	X ₁	X ₂	X ₃	
1	-1	-1	-1	2:8	1.5	1.5	0.16
2	-1	-1	+1	2:8	1.5	2.5	0.25
3	-1	+1	-1	2:8	2.5	1.5	0.26
4	-1	+1	+1	2:8	2.5	2.5	0.26
5	+1	-1	-1	4:6	1.5	1.5	0.29
6	+1	-1	+1	4:6	1.5	2.5	0.30
7	+1	+1	-1	4:6	2.5	1.5	0.36
8	+1	+1	+1	4:6	2.5	2.5	0.37
9	0	0	0	3:7	2	2	0.23
10	0	0	0	3:7	2	2	0.23
11	0	0	0	3:7	2	2	0.23
12	0	0	0	3:7	2	2	0.22
13	-2	0	0	1:9	2	2	0.36
14	+2	0	0	5:5	2	2	0.51
15	0	-2	0	3:7	1	2	0.11
16	0	+2	0	3:7	3	2	0.46
17	0	0	-2	3:7	2	1	0.38
18	0	0	+2	3:7	2	3	0.45

^a Coded level of variables: + = upper level, - = lower level and 0 = center points

^b X₁, X₂ and X₃ are the ratios of the core material to wall material (w/w), alginate concentration (%) and the concentration of the CaCl₂ solution (%), respectively.

^c The overall encapsulation efficiency for Yanang leaves encapsulation

ratio of core material (Yanang leaf extract) to wall material (alginate) and alginate concentration affected the overall encapsulation efficiency. Treatment 14 (the ratio of core material to wall material 5:5, 2% alginate concentration, 2% CaCl₂ concentration) showed higher encapsulation efficiency (OEE 0.51) than the others.

Alginate hydrogels are an import class of ionic biopolymers for medical and biotechnological applications. They are produced by the combination of alginate with a proper cross-linking agent and form three-dimensional gel structures. (Bartolo *et al.*, 1999). The effect of the process variables on the overall encapsulation efficiency is shown in Figure 1(a-b).

Bioactive compounds in many plants and fruits are important, such as phenolic compounds and vitamin C. The ratio of core material to wall material, alginate concentration and concentration of CaCl₂ solution affect the bioactive compounds in the Yanang leaf extract encapsulation, as shown in Figure 1-2. Figure 1(c-d) shows the response surface plots illustrating the effects of the ratio of the core

material to wall material, the alginate concentration and the concentration of CaCl₂ solution on total phenolic compounds. High ratios of core material to wall material, alginate concentrations and concentrations of CaCl₂ solution gave rise to the maximum total phenolic compounds. This indicated that high alginate concentration and CaCl₂ concentration promoted a conformational transition of the gel to a more compact three-dimensional structure (Bartolo *et al.*, 2003).

The total antioxidant value and vitamin C value showed results similar to the total phenolic compounds, as shown in Figure 1(e-f) and Figure 2(a-b), respectively. However, the ratio of the core material to wall material and alginate concentration had a greater effect than the concentration of the CaCl₂ solution. Increasing the ratio of the core material to wall material resulted in increased bioactive compounds of the encapsulation system. According to similar results used in the determination of total phenolic compounds, the result from Yerba mate (*Ilex paraguariensis*) extract encapsulation was high in the total phenolic compounds when increasing in the

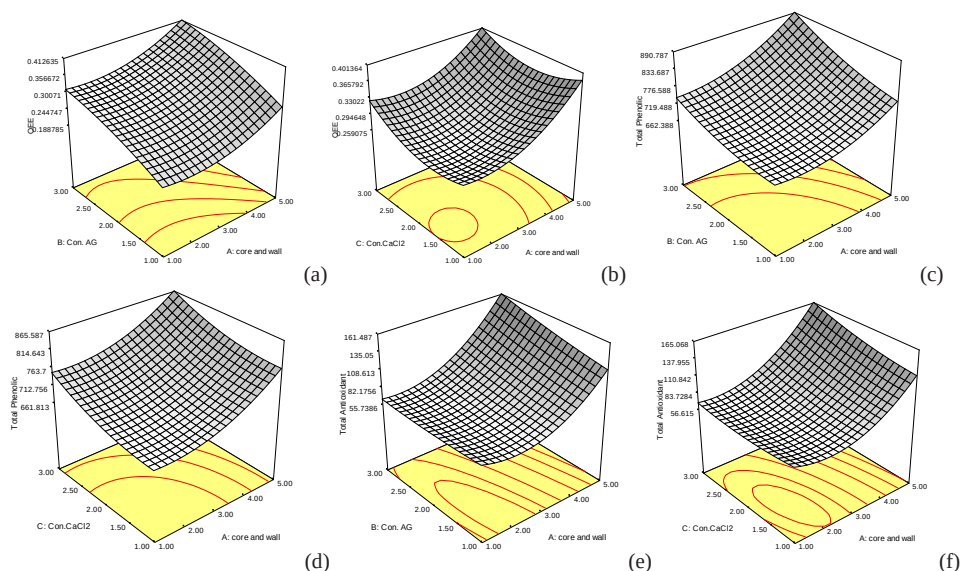


Figure 1. Response surface plots of the effect of the encapsulation conditions on the overall encapsulation efficiency, total phenolic and antioxidant value.

- The effects of the ratio of the core material to wall material and alginate concentration on the overall encapsulation efficiency.
- The effects of the ratio of the core material to wall material and concentration of CaCl_2 solution on the overall encapsulation efficiency.
- The effects of the ratio of the core material to wall material and alginate concentration on the total phenolic value.
- The effects of the ratio of the core material to wall material and the concentration of the CaCl_2 solution on the total phenolic value.
- The effects of the ratio of the core material to wall material and the alginate concentration on the total antioxidant value.
- The effects of the ratio of the core material to wall material and the concentration of CaCl_2 solution on the total antioxidant value

ratio of the core material to the wall material (Deladino *et al.*, 2008). In addition, Bakowska-Barczak and Kolodziejczyk (2011) reported that encapsulation of black currant (*Ribes nigrum*) by the spray-dry method showed high phenolic compounds when the ratio of the core material to wall material and alginate concentration increased. The ratio of the core material to wall material promoted gelling by alginate. When alginate and CaCl_2 are increased, they can be assigned to conjugated alginate and calcium (Chan *et al.*, 2010).

Figure 2(c-d) displays the response surface plots of the ratio effects of the core material to wall material, alginate concentration and concentration of CaCl_2 solution on the total

carotenoids. The maximum total carotenoid content was at high ratios of core to wall material and a wide range of alginate concentrations and CaCl_2 concentrations. In addition, the total chlorophyll value showed results similar to the total carotenoids (Figure 2(e-f)). As reported by Li *et al.* (2002), the chlorophyll value increased when the ratio of core material to wall material and the CaCl_2 concentration increased, causing the formation of the Ca-alginate gel structure.

Normally, carotenoids can be changed to pro-vitamin A by breaking double bonds (Packer *et al.*, 1999; Oliveira *et al.*, 2010). The free Mg^{2+} of chlorophyll can be changed to pheophytin (Ferruzzi and Blakeslee, 2007).

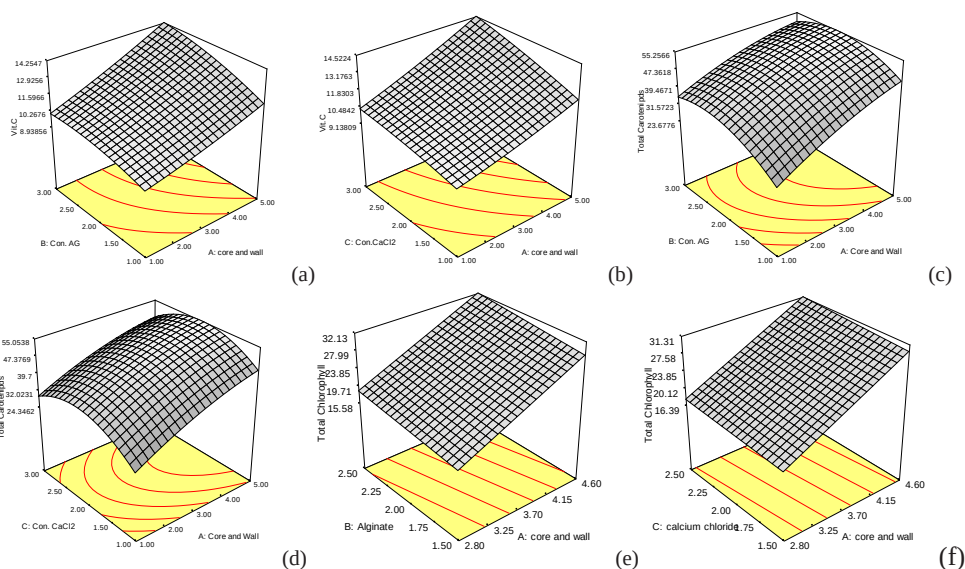


Figure 2. Response surface plots of the effect of the encapsulation conditions on the vitamin C, total carotenoids and chlorophyll value.

- (a) The effects of the ratio of the core material to wall material and the alginate concentration on the vitamin C value.
- (b) The effects of the ratio of the core material to wall material and the concentration of CaCl_2 solution on the vitamin C value.
- (c) The effect of the ratio of the core material to wall material and the alginate concentration on the total carotenoids value.
- (d) The effect of the ratio of the core material to wall material and the concentration of CaCl_2 solution on the total carotenoids value.
- (e) The effect of the ratio of the core material to wall material and the alginate concentration on the total chlorophyll value.
- (f) The effect of the ratio of the core material to wall material and the concentration of the CaCl_2 solution on the total chlorophyll value

Both pro-vitamin A and pheophytin are antioxidants. The pigment of the Yanang leaves extract, such as carotenoids and chlorophyll, should be bioactive compounds, especially as antioxidants. The statistical analysis indicated the proposed model was adequate, demonstrating no significant lack of fit and with satisfactory R^2 values of 0.85 ($\text{OEE} = 1.14 - 0.37X_1 + 0.01X_2 - 0.49X_3 + 0.06X_1^2 + 0.04X_2^2 + 0.17X_3^2 + 8.33 \times 10^{-3}X_1X_2 - 0.02X_1X_3 - 0.05X_2X_3$; X_1 = The ratio of core to wall material, X_2 = Alginate concentration, X_3 = CaCl_2 concentration). The optimized condition was 1.6:8.4, 1% and 1% for the ratio of core material to wall material, alginate concentration and CaCl_2 concentration, respectively. Under

microscopic imaging, the Yanang encapsulation using hydrogel beads was observed in Figure 3. The results showed that the surfaces of the hydrogel beads was attributed to the shrinkage during the drying process, which resulted in microspheres with smoother surfaces and fewer roughness (Saénz *et al.*, 2009).

Storage Stability Evaluation

Generally, the bioactive compounds of the Yanang leaves extract decreased almost 100% after three months (data not shown). Encapsulation may protect better product stability by isolating the bioactive compounds from the detrimental effects of oxygen and moisture (Chan *et al.*, 2010). Then, the stability

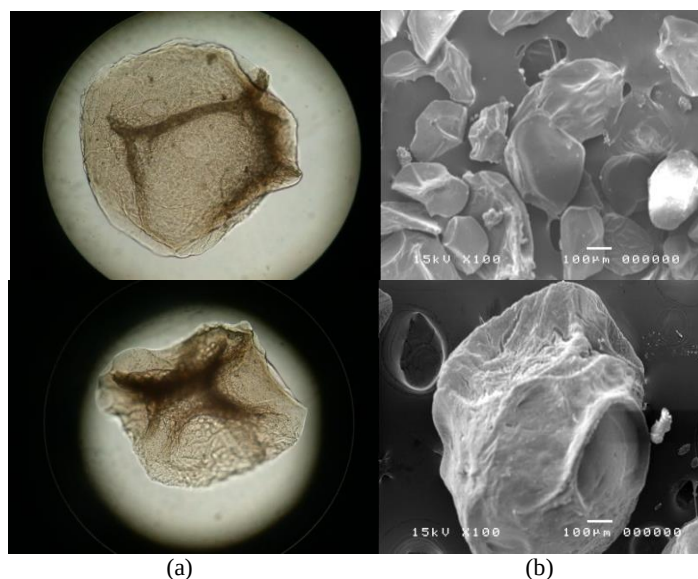


Figure 3. Structural analysis of the Yanang leaves extract encapsulations. (a) Microscopic images, (b) Electron microscope photograph

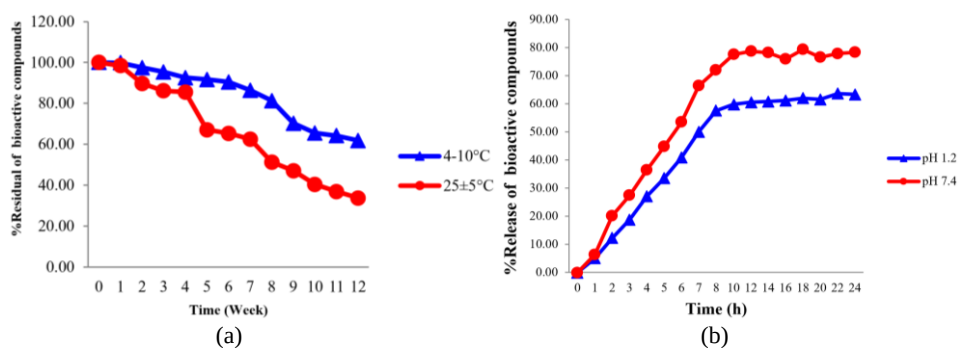


Figure 4. %Residual bioactive compound contents of the Yanang leaves extract encapsulations. (a) Storage temperature 25±5°C and 4-10°C for 3 months, (b) pH 1.2 and 7.4 at 37°C for 24 h

of bioactive compounds in encapsulated Yanang leaf extract was evaluated under various conditions of storage temperature. Figure 4(a) shows that an increase in storage time led to a decrease in bioactive compounds. The total chlorophyll and carotenoids contents of the Yanang leaf extract encapsulations decreased to less than 50% (% residual more than 50%) at the end of the three months storage period. For example, the total chlorophyll and carotenoids contents of the Yanang leaf

extract encapsulation decreased to 20% after one month at 4-10°C, 40% of the chlorophyll and carotenoids contents were lost by the end of the storage experiment. (3 months). Whereas at 25-30°C, 50% of chlorophyll and carotenoids contents were lost over the same period of storage (3 months). The total antioxidant contents of the Yanang leaf extract encapsulations were decreased by 62% at the end of 3 months storage at 25-30°C. At 4-10°C, the loss of total antioxidant content was determined as 34%.

An increase in the storage temperature led to a decrease in bioactive compounds. Storage at 4-10°C increased the stability of bioactive compounds 2 times compared to the 25-30°C storage temperature (Figure 4(a)).

A similar effect was observed by Candan and Tarhan (2003) for the chlorophyll and carotenoids contents of *Mentha pulegium* leaves. An increase in the storage time at a storage temperature of 25°C led to a decrease in the chlorophyll and carotenoids contents.

According to the results of Ersus and Yurdagel (2007), storage at 4°C increased the half-life of spray-dried anthocyanin pigments from black carrot (*Daucus carota* L.) 3 times compared to a 25°C storage temperature. Fang and Bhandari (2011) obtained similar results for the stability of bayberry polyphenols. At a storage temperature of 5°C, the total polyphenol and anthocyanin in bayberry powders decreased from 6% to 8% and 7 to 27%, respectively at the end of six months storage, whereas at 25°C they decreased from 6% to 9% and 9% to 37%, respectively. Bayberry powders decreased from 7% to 37% and 9% to 94%, respectively for the total polyphenol and anthocyanin at 40°C. The results suggest that bayberry powder should be stored at 5°C due to the greater polyphenol and anthocyanin stability under such conditions.

In vitro Releasing Properties of Bioactive Compounds

The pH-sensitivity of hydrogel is an important factor in designing polymers for controlled nutrient release in the gastrointestinal tract (GI tract), which has a variation of pH from the stomach to the intestine (Abd-El-Ghaffar *et al.*, 2012). The desirable capsule for a nutrient delivery system should protect the core material without acid-damaged destruction under a strong acidic environment, which means that the coating material should be protect acid damage. Once the capsule reaches the small intestine, it should initially release the core material rapidly and continue the releasing rate to maintain the effective concentration of core material as the absorption rate decreases (Yoo *et al.*, 2006).

For oral dosage forms, the formulator must consider that the natural pH environment of the GI tract varies from acidic (pH ~1.2) in the stomach to slightly alkaline in the intestine (pH ~7.4) (George and Abraham, 2007).

The Yanang extract capsule produced under the optimal yield and bioactive compounds conditions was used in an in vitro bioactive compounds releasing experiment in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) for 24 h (Figure 4(b)). The encapsulated Yanang leaf extract was more stable in acid condition (pH 1.2) same as in the stomach than pH 7.4 same as in small intestine at 37°C for 24 h. This may be due to the pH sensitive behaviour of alginate. Generally, nutrients are digested in the stomach for 3 h and absorbed in the intestine for 6-8 h (George and Abraham, 2007). The amount of released bioactive compounds at pH 1.2 was less than 20% within 3 h. At pH 7.4, the amount of bioactive compounds released increased significantly (~80% within 8 h). The bioactive compounds can be retained in the calcium-cross-linked alginate encapsulation process. Alginate is matrix that has pH sensitive properties suitable for the intestinal delivery of drugs and nutrients. However, it has a drawback that it is rapidly dissolved in at the higher pH values prevailing in the intestine (George and Abraham, 2007).

Application of Encapsulation in a Soft Drink-Related Model System

Acids are important ingredients in soft drinks because they are necessary for balancing sweetener. They are used as flavour enhancers and as protectors against microbiological spoilage (Hansson *et al.*, 2001). Citric acid is the acid most often used in the soft drink industry. It lowers the pH to enhance the taste and to provide microbiological stability. To study their effect on bioactive compounds release, citric acid was added in variable concentrations to a soft drink model system containing water and Yanang leaf extract capsules. The bioactive compounds from the Yanang leaf extract capsules at low pH were found to be maintained more than at high pH, and this may

Table 2. Effect of pH, temperature and time on the bioactive compounds of the Yanang leaves extract encapsulations

pH	Total carotenoids (mg/g)	Total chlorophyll (mg/g)	Total phenolics (mg GAE/g)	Total antioxidant (AEAC mg/g)	IC ₅₀ (mg/g)
2	51.27±0.47 ^{ci}	15.99±0.20 ^d	57.03±1.39 ^d	191.99±1.55 ^c	8.13±0.01 ^c
3	56.20±0.66 ^b	17.49±0.27 ^c	72.76±1.39 ^b	224.77±1.03 ^b	8.00±0.00 ^d
4	61.00±0.09 ^a	28.07±0.48 ^a	90.46±5.56 ^a	263.39±5.16 ^a	7.89±0.02 ^e
5	50.53±1.51 ^{cd}	19.42±0.41 ^b	79.64±2.73 ^b	225.50±7.73 ^b	8.08±0.03 ^c
6	49.87±3.39 ^{cd}	15.99±0.48 ^d	64.90±1.39 ^c	180.70±7.73 ^c	8.86±0.03 ^b
7	47.07±0.94 ^d	9.66±0.55 ^e	27.04±2.09 ^e	103.10±2.58 ^d	9.66±0.01 ^a
Temp/time	Total carotenoids (mg/g)	Total chlorophyll (mg/g)	Total phenolics (mg GAE/g)	Total antioxidant (AEAC mg/g)	IC ₅₀ (mg/g)
65°C: 30 min	11.56±0.56 ^b	11.18±1.33 ^b	24.58±1.97 ^b	159.32±28.50 ^b	8.58±0.06 ^a
77°C: 1 min	68.67±2.92 ^{a/i}	48.63±5.49 ^a	77.68±8.73 ^a	341.59±41.49 ^a	7.57±0.17 ^b

^{i/} Values with different letters in the same column are significantly different ($p \leq 0.05$).

Means $\pm$ SD, each value in the table is the mean of three replications.

be due to the pH sensitive behaviour of alginate (Chen *et al.*, 2004). These results indicate that pH 4 showed the highest amount of bioactive compounds (Table 2). The bioactive compound capacity is shown in Table 2. Using high temperature short time (HTST) at 77°C for 1 min., the bioactive compound activity was 2-6 times greater than at low temperature long time (LTLT) at 65°C for 30 min.

Conclusions

The optimized Yanang leaf extract encapsulation was identified as a ratio of core material to wall material of 1.60:8.40, an alginate concentration of 1% and a concentration of CaCl₂ solution of 1%. The strongest effect for the encapsulation of Yanang leaf extract was the ratio of the core material to wall material. The hydrogel beads method can give high overall encapsulation efficiency and stabilize bioactive compounds. Storage at 4-10°C increased the stability of bioactive compounds two times compared with a 25°C storage temperature. In the simulated intestinal fluid (pH 7.4), the amount of released bioactive compounds was greater than in the simulated gastric fluid (pH 1.2). The encapsulated Yanang leaf extract maintained more of the bioactive compounds at pH 4 and 77°C for 1 min in the soft drink model system. In conclusion, this study demonstrates the potential of using hydrogel

beads process in optimizing the encapsulation of herbal extract, and it represents an interesting food additive option for incorporation into functional foods.

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