

Usage of encapsulated *Hypericum scabrum* in ayran and determination of antioxidant, phenolic and sensory properties

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Abstract

Hypericum scabrum (HS) widely used in traditional medicine due to its bioactive compounds was extracted by using ethanol-water (3:7, v/v). The extract was encapsulated with maltodextrin and gum arabic in a spray dryer in order to protect the phenolic compounds in its structure. Different amounts of microcapsules were added to our traditional drink, i.e. ayran (drinking yoghurt). The total phenolic content (TPC) and DPPH radical scavenging activity of the microcapsules in extract of HS and ayran samples were determined. The amount of total phenolic compounds in the microcapsule provided a superior effect than the extract. The ayran samples were supplemented with 2%, 3%, 4%, 5% and 6% of *Hypericum scabrum* microcapsules and it is observed that total phenolic content and DPPH radical scavenging activity indicated an increase with concentration. TPC and DPPH activity were determined as 268.86 mg GAE100 mL⁻¹ and 78.05% for 6% supplemented samples. As a result of the sensory analysis, ayran samples supplemented with 4% of HS microcapsule gained the highest scores by the panelists and received more appreciation than the control group. It is concluded that HS4 (ayran produced by 4% HS supplemented microcapsule) sample was determined as the best sample according to the sensory analyses while the HS6 (ayran produced by 6% HS supplemented microcapsule) sample had the highest value in terms of DPPH scavenging activity and TPC results. The overall results of the present study revealed that 4% HS supplemented ayran can be produced with its enhanced health beneficial and desirable properties.

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1. Introduction

In recent years, natural products have gained interest due to their diverse pharmacological properties and minimum side effects (Aras et al., 2018; Farhan et al., 2019; Farooqi and Ahmad, 2019; Farooqi et al., 2019; Qureshi et al., 2019). Some medicinal plants include diverse natural antioxidants, such as phenolic acids, flavonoids, and tannins, which exhibit stronger antioxidant activities. It is known that these compounds are of important in terms of preventing the beginning or progressing of many diseases (Kızıll et al., 2011). Studies have shown that antioxidant activities of natural products are significantly effective on different diseases. Studies are focused on natural antioxidants in order to prevent the negative effects of the synthetic antioxidants in human life. It has been proved that compounds such as polyphenols, anthraquinones, phenolic terpenes and flavonoids are responsible for the antioxidant activity of plants (Uysal et al., 2018; Zengin et al., 2018a, b; Lazarova et al., 2019).

The *Hypericum* genus belongs to the Hypericaceae family and it has been used as a traditional medicine for a long time due to its biological activities. In addition, it is widely used especially in the field of health for its bioactive phytochemicals (Llorent-Martinez et al., 2018; Keser et al., 2020). It consists of approximately 500 flowering plant species. Although there are lots of studies on *Hypericum perforatum* (HP) belonging to genus *Hypericum*, studies on *Hypericum scabrum* also known as ‘Yeastherb, Kepir herb’ are insufficient. This plant has also antiseptic, anthelmintic, antimicrobial and antifungal properties (Unal, 2008; Ebrahimzadeh, 2013; Pirbalouti, 2014). Moreover, secondary metabolites of *Hypericum* species are hypericin, flavonoids, tannins, phenolic acids, hyperoside, quercitrine, isooctine, routine and chlorogenic acid (Barnes et al., 2001; Dall'Agnol et al., 2003).

Chemical preservation techniques are highly effective in the prevention of antimicrobial abilities, enzyme inhibitors and antioxidant activities of the extracts as well (Hasanuddin, 2019). Encapsulation is a method that protects the plant extract from undesirable reactions such as temperature, oxygen and heat during processing and storage (Koç et al., 2015; Rosa et al., 2019). There are several methods for encapsulation of materials such as spray, cooling/freezing, extrusion coating, spray drying, liposome binding, coacervation and centrifugation, fluid bed coating (Madene et al., 2006). Among these methods, spray drying is the most preferred method due to the easy use of equipment, constant production, wide selection of carrier materials, good retention of volatile compounds and low cost (Calvo et al., 2012; Mahdavi et al., 2016). The

choice of coating material in the encapsulation method is one of the most important factors affecting the success of the process. The coating material should protect the core material against external effects, prevent any reaction, and have a high emulsion stability and a structure that allows film formation (Madene et al., 2006). In the food industry, maltodextrin, whey protein isolate, and gum arabic are used for coating materials, respectively (Chew et al., 2018; Korma et al., 2019). Maltodextrin has some alternatives such as low cost, neutral taste, low viscosity at high solids concentration, and providing good protection against oxidation (Barros Fernandes et al., 2014; Korma et al., 2019). Coating materials are often used in combinations in order to obtain enhanced properties. It is combined with maltodextrin, gum arabic and other coating materials to provide the desired properties in encapsulation (Korma et al., 2019).

In this study, the bioactive compounds of the HS plant were encapsulated with the combination of maltodextrin and gum arabic coating materials. In addition, the usage of HS microcapsules in the production of ayran was investigated and the results were evaluated in terms of TPC, DPPH, and sensory analyses, respectively. Although there are studies on the chemical structure, volatile compounds, and medicinal use of the HS, to the best of our knowledge, this is the first report for encapsulation of HS and use of HS microcapsules in foods.

2. Materials and Methods

2.1. Material

Hypericum scabrum (Figure 1) was collected from Amasya region in 2018 between June and August. Identification of the plant was performed by Dr. Cengiz Yıldırım and specimen was deposited at the Herbarium of Ondokuz Mayıs University, (OMUB 0527). The aerial parts of the plant were dried in the shade at room temperature and then grounded to a fine powder. A combination of maltodextrin (DE 16.5-19.5, Sigma, St. Louis, MO, USA) and gum arabic (Merck, Darmstadt, Germany) were used as coating material for the encapsulation of HS extract. Ayran products were produced in OTAT Provisions Industry and Trade LLC, Samsun/Havza according to the process of the factory and supplied from the factory.



Figure 1. *Hypericum scabrum*

2.2. Extraction Procedure

Ultrasonic assisted extraction (UAE) technique was used to extract the bioactive compounds of HS. Extraction of the plant was performed at 30°C for 40 min with ethanol-water (3:7, v/v) solvent mixture, keeping the material to liquor (M:L) ratio as 1:30 (v/v) based on the previous results (Seyrekoğlu and Temiz, 2019).

2.3. Encapsulation of the Extract

Encapsulation of HS extract was carried out at 180°C in a spray dryer (B-290, Buchi Corporation, Flawil, Switzerland). The aspirator speed of the spray dryer was set as 100% (35 m³h⁻¹), the air flow rate was 50% (601 Lh⁻¹), and the feed rate was 30% (9 mL min⁻¹) as well. Spray drying was applied using 10% of coating concentration and same ratio of coating material and core, respectively. Maltodextrin and gum arabic were used as coating materials in equal proportions. Drying process was given in Figure 2. TPC and DPPH radical scavenging activity of the samples were determined for HS microcapsules. Analyses of the microcapsules were performed according to the method of Robert et al. (2010) with slight modifications. For this purpose, 10 mL of ethanol-acetic acid-water (50:8:42, v/v/v) mixture was added on 1 g of microcapsule sample and vortexed for 2 mi. It was centrifuged at 1000 rpm for 5 min and then filtered through 0.45 µm porous filter paper (Robert et al., 2010).

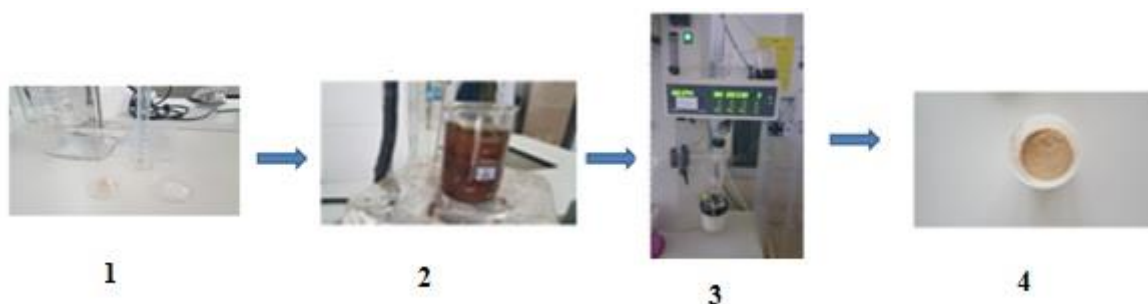


Figure 2. Production of *Hypericum scabrum* microcapsule; 1, Preparing of coating materials; 2, Mixing of *Hypericum scabrum* extract and coating materials; 3, Drying process; 4, *Hypericum scabrum* microcapsule

2.4. Microcapsule Supplemented Ayran Production

The HS microcapsules were used during the ayran production process of OTAT Food Industry and Trade CLL. Different amounts of microcapsules (1%, 2%, 3%, 4%, and 6%) were added to the drinking cup of ayran after the ayran production process (Figure 3). Produced microcapsule supplemented ayran samples were subjected to TPC and DPPH analyses, respectively.

Acidified ethanol (30 mL) was added to 20 mL of ayran sample and kept at 4°C for 16 h in order to extract the bioactive compounds from microcapsule supplemented with ayran. Afterwards, the sample was centrifuged at 6000 rpm for 3 min. The experiments were carried out according to the method of Singleton and Rossi (1965) and as it specified in the method, the best result has been obtained by applying centrifugation at the specified time and rpm since there is turbidity and proper reading is not performed. The solution was filtered through Whatman No: 1 filter paper. TPC and DPPH were analysed in the filtrate (Singleton and Rossi, 1965; Koca et al., 2008).

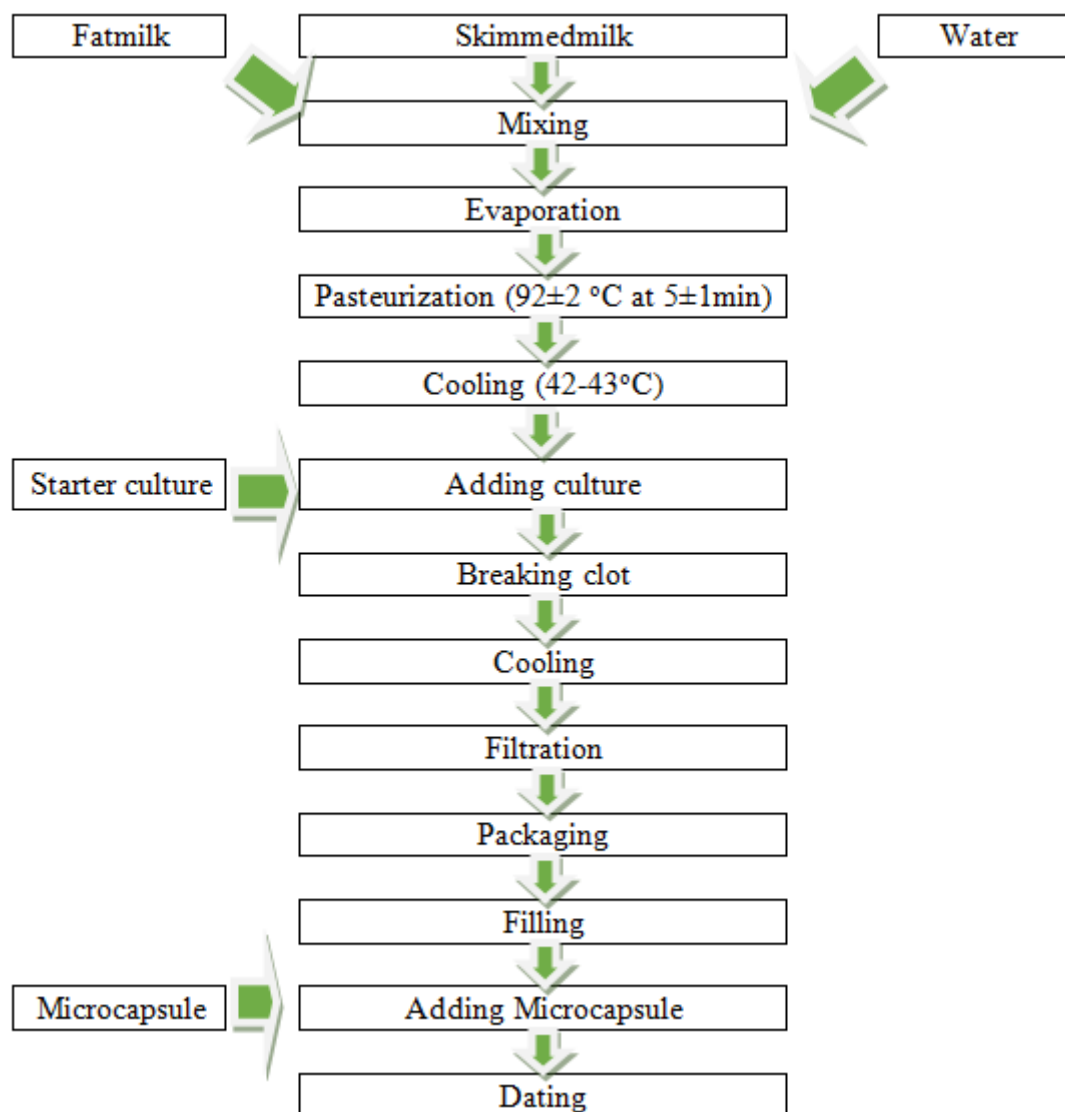


Figure 3. Production of drinking yoghurt

2.5. Total Phenolic Compound Analysis

The total phenolic compound analysis of the samples was carried out according to Singleton and Rossi (1965). Distilled water (2.4 mL) and Folin-Ciocalteu solution (200 μL) were added to 40 μL of HS extract (1 mgmL^{-1}). Then, 600 μL of saturated sodium carbonate and 760 μL of distilled water were put into the mixture. The absorbance values were measured at 760 nm (using Standard gallic acid solution) in a spectrophotometer (UV-1601 Shimadzu). The results were expressed as $\text{mg GA equivalentg}^{-1}$ of extract.

2.6. DPPH Radical Scavenging Activity

Diluted DPPH solution (3.8 mL) was added to 200 μ L of filtrate and then it was vortexed for 15s and left in the dark for 60 min. Phase separation was observed at the end of the period. Supernatant was carefully taken from the mixture, and the absorbance values were recorded at 515 nm (Aksoylu, 2012). DPPH scavenging activity was calculated as percent inhibition for the extract, microcapsules and ayran samples as well.

2.7. Sensory Analysis

Color–appearance, texture–consistency, taste–smell, and general taste characteristics of the samples were evaluated by panelist group. HS microcapsule supplemented with ayran samples were evaluated by a panelist group consisting of 10 people. The evaluation was made in a range of points from 1 (quite bad) to 5 (quite nice) (Anonym, 1982).

2.8. Statistical Analysis

All the analyses were performed at least two technical experiments and mean standard deviations were calculated. The results were analysed with SPSS 16.0 package program and Tukey Test was used for as post-hoc test. The significance levels of the groups were evaluated at $p < 0.05$ (SPSS, 2011).

3. Results and Discussion

Total phenolic content was calculated as 149.67 mgg^{-1} GAE for HS extract and 435.89 mgg^{-1} GAE for its microcapsule (Table 1), respectively. The complete removal of water by the encapsulation process and the fact that the coating core ratio was 1/1 caused an increase in the amount of extract in the microcapsule and thus an increase in the total amount of phenolic substances viceversa. The experimental results showed that HS ethanol-water extract contains 149.67 mgg^{-1} GAE of phenolics, a lower value compared with those reported for aqueous (186.94 mg GAEg^{-1} extract), methanol (171.00 mg GAEg^{-1} extract), and ethanol (262.00 $\mu\text{g GAEmg}^{-1}$ extract) extracts of HS (Barış et al., 2011; Karatoprak, 2019). The phenolic content of the plant depends on several factors such as the parts used, collection region, collection time, extraction conditions, etc.

Table 1. TPC results and DPPH radical scavenging activity of HS extract and microcapsule

Sample	TPC (mg_{GAE}/g_{extract})	DPPH (% Inhibition)
HS extract	149.67 ± 3.42	79.17± 0.27
HS microcapsule	435.89 ± 3.43	77.07±2.21

Data are expressed as mean ± Standard deviation (n = 3)

Phenolics are important compounds that have the ability of scavenge free radicals. DPPH is an easy and fast method to evaluate the antioxidant activity. The reduction of DPPH free radicals is based on the capacity of some hydrogen-donating compounds including phenolics such as flavonoids, phenolic acids and tannins by donating hydrogen atom (Barış et al., 2011). DPPH scavenging activity was found as 79.17% in HS extract and 77.07% in encapsulated form, respectively. Several studies revealed that DPPH activity is correlated with TPC (Yeo and Shahidi, 2015; Parikh and Patel, 2017; Parikh and Patel, 2018). Although HS microcapsule contain higher amounts of TPC than HS extract, both of them exhibit similar DPPH radical scavenging activities (Table 1). It may be due to the fact that antioxidant compounds are adversely affected by the heat applied during encapsulation. Application of 180°C temperature during the encapsulation process may damage the chemical structure of the compounds that provide DPPH activity and therefore cause a decrease in % inhibition. Especially hyperforin, one of the bioactive compounds, is a very sensitive compound to heat and temperature, and the increase in temperature to high degrees causes degradation of the active compounds and decrease in inhibition (%).

DPPH activity results of the present study are approximately similar as obtained by Unal et al. (2008). Unal et al., (2008) determined the DPPH activity of the ethanol extract of HS as 78% while we found 79% and 77% for HS extract and microcapsule, respectively. On the other hand, Baris et al. (2011) obtained higher DPPH scavenging activity (90%) for the ethanol extract of HS. HS extract is a powerful source of antioxidant on the account of its composition. Omidi et al. (2020) determined that HS contains flavonoids such as quercitrin and quercetin that can scavenge the free radicals. Previous studies revealed that TPC amount of microcapsule is quite high when compared with its extract form. It is also reported that air inlet temperature of encapsulation process, coating material, the core composition, and ultrasonic power effect the TPC of the samples (Franceschinis et al., 2014; Peanparkdee et al., 2016; Tatar, 2016).

Ayran samples supplemented with different concentrations of HS microcapsule were examined in terms of their TPC and DPPH values. The results were found statistically significant ($p < 0.05$) (Table 2). TPC was increased with the increasing concentration of HS microcapsule. The control group has the lowest TPC value ($10.58 \text{ mg GAE}100\text{mL}^{-1}$), while HS6 contains the highest TPC value ($268.86 \text{ mg GAE}100\text{mL}^{-1}$). It is also observed that, DPPH activity of the ayran samples were increased with increasing concentration of HS microcapsule. In literature, there is no study related with the addition of HS microcapsule to ayran. However, similar to our results, the studies related with the comparison of TPC values of different plant/food extract with its supplemented form in food revealed that TPC was increased with the increase of supplemented ratio (Aydemir, 2015; Jalal, 2018). Encapsulation of plant extract provides high inhibition values for the final product by protecting the antioxidant compounds (Barretto et al., 2020).

Table 2. TPC and DPPH radical scavenging activity of ayran samples.

Sample	TPC (mg GAE/100mL)	DPPH (%Inhibition)
C	$10.58^f \pm 0.33$	$5.75^f \pm 0.14$
HS2	$77.58^e \pm 0.37$	$35.48^e \pm 0.31$
HS3	$130.58^d \pm 0.22$	$56.49^d \pm 0.30$
HS4	$202.41^c \pm 0.49$	$67.48^c \pm 0.18$
HS5	$230.53^b \pm 0.26$	$75.49^b \pm 0.32$
HS6	$268.86^a \pm 0.58$	$78.05^a \pm 0.66$

*: mean of standard \pm deviation.

a-f: The lower case letters in the same column are the comparison of the ayran samples and the same letters show that there is no statistical difference between the samples. ($P > 0.05$)

C: Control ayran, HS2: ayran produced by 2 % HS supplemented microcapsule, HS3: ayran produced by 3 % HS supplemented microcapsule, HS4: ayran produced by 4 % HS supplemented microcapsule, HS5: ayran produced by 5 % HS supplemented microcapsule, HS6: ayran produced by 6 % HS supplemented microcapsule

TPC amount of HS microcapsule (435.89 ± 3.43) was higher than all HS microcapsule supplemented with ayran samples (Table 1 and Table 2). This result can be explain by the fact that ayran is a fermented product, it is acidic and thus, causes the amount of phenolic compounds to be lower in the final product by causing dissolution in the microcapsule. Tseng and Zhao (2013) stated that oxygen, pH, temperature, light, metal ions, enzymes and moisture content are among the main factors affecting the stability of polyphenols which are more stable at lower pH values. This reduction can be prevented by changing the coating material and coating material ratios used or by using different methods such as encapsulation method.

Table 3. The sensory properties of ayran samples.

Sample	Color and appearance	Texture – consistency	Taste – aroma	General taste
C	3.60 ^a ± 0.54	3.00 ^b ± 0.00	3.40 ^{ab} ± 0.89	3.60 ^{ab} ± 0.89
HS2	3.60 ^a ± 0.89	4.40 ^a ± 0.89	4.00 ^a ± 0.00	4.00 ^a ± 0.00
HS3	3.60 ^a ± 0.54	4.00 ^{ab} ± 0.70	3.60 ^{ab} ± 0.54	3.20 ^b ± 0.44
HS4	3.60 ^a ± 0.54	3.80 ^{ab} ± 0.83	3.20 ^b ± 0.44	3.20 ^b ± 0.44
HS5	3.60 ^a ± 0.54	3.60 ^{ab} ± 0.54	2.40 ^c ± 0.54	2.20 ^c ± 0.44
HS6	3.60 ^a ± 1.14	3.80 ^{ab} ± 1.09	2.00 ^c ± 0.00	2.00 ^c ± 0.00

*: mean standard ± deviation.

a-f: The lower caseletters in the same column are the comparison of the ayran samples and the same letters show that there is no statistical difference between the samples. ($P > 0.05$)

C: Control ayran, HS2:ayran produced by 2 % HS supplemented microcapsule, HS3: ayran produced by 3 % HS supplemented microcapsule, HS4:ayran produced by 4 % HS supplemented microcapsule, HS5: ayran produced by 5 % HS supplemented microcapsule, HS6: ayran produced by 6 % HS supplemented microcapsule.

The sensory properties of microcapsule supplemented ayran samples are given in Table 3. There is no statistical difference in the color-appearance scores of the ayran samples. It is observed that the increase in the amount of microcapsule did not have a negative effect on the color-appearance. Thus, the encapsulation process prevented the passage of the dark yellow color of the HS extract into the product, ensuring high scores. The highest scores for texture-consistency and taste-aroma were gained with HS2 and HS3, respectively. All HS microcapsule supplemented ayran samples had higher texture-consistency values than control. On the other hand, lower taste-aroma scores were achieved for HS4, HS5 and HS6, respectively. The highest general taste value was obtained with HS2 while HS6 gave the lowest value.

4. Conclusion

In recent years, oxidative stress and its effects on human health are important issues that have been emphasised. The increase in diseases such as obesity, cancer, immune system and the desire of people to have more healthier life canalized researcher stop reduce functional and healthy foods (Essa et al., 2021). Therefore, the interest in natural additives and healthy foods is increasing day by day. In this study, *H. scabrum* plant, which is used in the field of health but has not been studied enough, was investigated in terms of its usage in the production of healthy functional foods. It is suggested that HS microcapsule which was coated with maltodextrin and gum arabic can be used as a new additive food supplement with high TPC. The amount of TPC and antioxidant capacity of the final product can be increased with different coating materials or different encapsulation methods.

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