Agronomy Research **17**(5), 1829–1838, 2019
https://doi.org/10.15159/AR.19.155

Development of encapsulated extracts on the basis of meadowsweet (*Filipendula ulmaria*) in the composition of functional foods with oncoprotective properties

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Abstract. Meadowsweet *(Filipendula ulmaria)* is a quite common plant throughout the European countries, including Russia. Therapeutic and prophylactic properties of the meadowsweet are mainly associated with the action of biologically active substances (BAS), in particularly tannins, phenolic compounds, phenolcarboxylic acids, catechins, flavonoids, essential oils etc. The main substances with proven clinical effects are salicylates and flavonoids, what allows to consider meadowsweet as an anti-inflammatory, immunostimulating, antioxidant, hepatoprotective, nootropic, adaptogenic and antihypoxic agent.
The aim of this study was to analyze the content of BAS in water and 70% ethyl alcohol extract of *F. ulmaria* flowers from different regions of Russia and develop their encapsulated forms for further use as an ingredient for functional food products.
To increase the shelf life of meadowsweet extracts and create a stable form for their delivery to the human body with various food products, encapsulated forms of extracts in the form of micro- and nanosized capsules were developed. The method of encapsulation was carried out using a spray dryer. It was shown that encapsulated meadowsweet BAS can be added to a chicken pate without negative effect on the organoleptic properties of the finished product. The calculation of the cost of the meat product with the complex functional dry mixture showed a slight increase in the cost of the final product compared to the traditional analogue. This study shows that encapsulated meadowsweet BAS can be used for inclusion in various food products, to ensure the functional properties of food and optimize the population's rations.

Key words: meadowsweet *(Filipendula ulmaria)*, flavonoids, encapsulated extracts, biologically active substances.

INTRODUCTION

Meadowsweet *(Filipendula ulmaria* L. Maxim.) is a perennial herbaceous plant of the family Rosaceae, growing in the wet meadows of Europe and Western Asia (Katanicet al., 2015; Shaldayeva et al., 2018). Over the years, various parts of the plant – aerial parts and roots were used in traditional medicine as a drug with antibacterial, anti-inflammatory and other properties (Hasler et al., 1989; Vysochina et al., 2016; Shaldayeva et al., 2018). In previous studies extensive data on the antioxidant properties of the components of meadowsweet were obtained, which is associated with the chemical composition of the plant, in particular with the presence of a number of phenolic compounds: flavonoids, phenolic acids, tannins, salicylate aglycons and glycosides (Katanic et al., 2015; Vysochina et al., 2016; Shaldayeva et al., 2018). Meadowsweet extracts also have a pronounced antimicrobial effect, which is confirmed by a study against 11 human pathogens (Denev et al., 2014).

Biologically active substances (BAS) of meadowsweet are studied for preventive effect on cancer, vascular heart disease, atherosclerosis, hypertension, diabetes, neurodegenerative diseases, rheumatoid arthritis and aging (Bruneton, 1995; Schulz et al., 1998; Duke, 2001). Alcohol and water extracts of plants, including meadowsweet, as effective means in the prevention of cancer are reflected in the studies of Korsun et al., 2015 and Bespalov, et al., 2017; Bespalov, et al., 2018.

A variety of biologically active substances (BAS) of meadowsweet is of interest to the research of this plant in order to obtain new highly effective preparations of a wide action on its basis. There is also longtime growing interest in developing methods for preserving biologically active substances of plants (Popescu, 2000). Stabilization of the extracted forms of biologically active substances can be achieved by means of encapsulation (Dziezak, 1988; Shahidi & Han, 1993). Currently technological methods of micro- and nanoencapsulation are widely used to ensure the transformation of BAS in a stable form (Zabodalova et al., 2014). Sensitive ingredients can retain their properties against the adverse reaction of the environment while preventing the loss of BAS by encapsulation. Modern technologies are also able to control the release of encapsulated ingredients. Different techniques are wide employed to microencapsulate food ingredients, the most often used technique in the food industry is spray-drying (Gibbs al., 1999; Loksuwan, 2007). Selection of encapsulating method and materials for the capsule shell are determining factors in the encapsulated biologically active substances development and directly influence the final product characteristics.

Up to the present time, no studies have been conducted on the encapsulation of meadowsweet biologically active substances for further use as a part of the formulations for functional food products. It is assumed that the encapsulated forms of meadowsweet with onco-protective properties will help providing the food product with the same effect.

Thus, the purpose of the study was to evaluate the content of flavonoids in extracts of the aerial part of *Filipendula ulmaria* L. Maxim. from different regions of the Russian Federation and to develop encapsulated forms of its biologically active substances for further use as a part of a complex dry mixture for functional food products.

MATERIALS AND METHODS

Aerial parts (flowers) of *Filipendula ulmaria* L. Maxim. were carefully selected in the phase of mass flowering of plants from nature populations in three regions of the Russian Federation: in the territory of the Leningrad and Yaroslavl regions and in the Republic of Bashkortostan in 2018. Currently, in these regions, *F. ulmaria* is being collected in commercial volumes, so they can be considered as its suppliers for possible industrial production. The plant samples of *Filipendula ulmaria* were confirmed and deposited in the Herbarium at the Botanical Institute of the Russian Academy of Sciences, St. Petersburg. Flowers were subjected to convection drying to a moisture content of 8.1 ± 0.5%.

Preparation of the meadowsweet extracts

Dried meadowsweet flowers were ground in a laboratory mill to the size of particles passing through a sieve with holes of 0.2 cm in diameter, then 1g of the crushed sample was placed in a conical flask with a capacity of 100 cm^3, where 100 cm^3 of ethyl alcohol with a volume fraction of 70% or 100 cm^3 of distilled water was added.

Encapsulation of biologically active ingredients of meadowsweet

Production of encapsulated BAS of meadowsweet included some main stages: preparation of the extracts according to the scheme described above; mixing the extracts with a solution of maltodextrin; obtaining encapsulated BAS; drying of encapsulated biologically active substances and obtaining stable micro- and nanoscale capsules; packing and storage.

The prepared alcoholic extract of meadowsweet BAS was mixed with maltodextrin solution in a certain proportion 3:1, respectively, then the resulting solution was intensively mixed on a magnetic stirrer US-1550A (Ulab) at 1,000 rpm and kept for 30 minutes in a closed flask at room temperature. To achieve the smallest dispersion (with a nanoparticle size of not more than 500 nm), the prepared solution was subjected to mechanical treatment using the ultra-powerful ULTRATURREX dispersant at 30,000 rpm and the TwinPanda 600 high-pressure homogenizer at 60 MPa. The resulting dispersion was placed in a closed flask at a temperature of 4 ± 2 °C for at least 24 hours for stabilization. At the next step the dispersion was sent to the Eyela SD-1000 spray dryer (Japan), where the encapsulation process took place at input temperature of 150 ± 20 °C and output temperature of 50 ± 20 °C. The air flow rate was equal to 0.7–0.8 $m^3 h^{-1}$, the fluid flow rate was 500–700 mL h^{-1} and the inlet pressure was 8–10 atm. The obtained encapsulated meadowsweet BAS were removed from the receiving flask of the spray dryer and sent for further technological operations. The above presented modes and parameters are based on experimental data obtained in previous studies (Yoshiia et al., 2001; Ahmed et al., 2010; Choi et al., 2019) in modification to specific technological characteristics of the raw materials used.

Production of pate with the addition of a complex functional dry mixture

To ensure that encapsulated meadowsweet BAS can be used as a part of functional food products its was added to a complex functional dry mixture for emulsified meat products Optilad Plus from the manufacturer Nordena LLC (St. Petersburg, Russia). The original Optilad Plus dry mixture contains skim milk powder, mono- and diglycerides of fatty acids, wheat fiber, modified starch, salt. Encapsulated meadowsweet BAS were added in the amount of 4% to the dry mixture.

A chicken pate made according to the recipe developed by the authors was chosen as an example of a food product for encapsulated meadowsweet BAS introduction. The following broiler chicken raw materials were obtained from a local producer and used as ingredients: femur meat, mechanically deboned meat, raw fat and skin. Only browned onion was used as a flavor component in addition to the complex functional dry mixture, so it was easier to study encapsulated meadowsweet influence on the products characteristics. Production of the pate was carried out by a standard hot technology with using a broth.

The total flavonoids content in extracts and encapsulated forms of meadowsweet

The total flavonoids content in extracts and encapsulated forms of meadowsweet was studied as follows: 1 cm^3 of extract or water solution of capsules were placed in a 25 mL volumetric flask, where a 5 cm^3 of an aluminum chloride solution was added with a mass fraction of 2% in ethyl alcohol, then the volume of the solution was adjusted to the mark by adding ethyl alcohol. The reference solution was prepared in the same way by mixing 1 cm^3 solution of capsule with alcohol, but without introducing aluminum chloride with a mass fraction of 2% in ethanol. The resultant solution optical density was analyzed using Shimadzu UV-2600 spectrophotometer at a wavelength of 410 nm.

The calculation of the total content of flavonoids was carried out according to the calibration curve (Fig. 1). The calibration curve is a graph of the dependence of optical density on the concentration of rutin in solution. The above presented methods were previously used in the researches of other authors (Katanic et al., 2015; Bespalov et al., 2017).

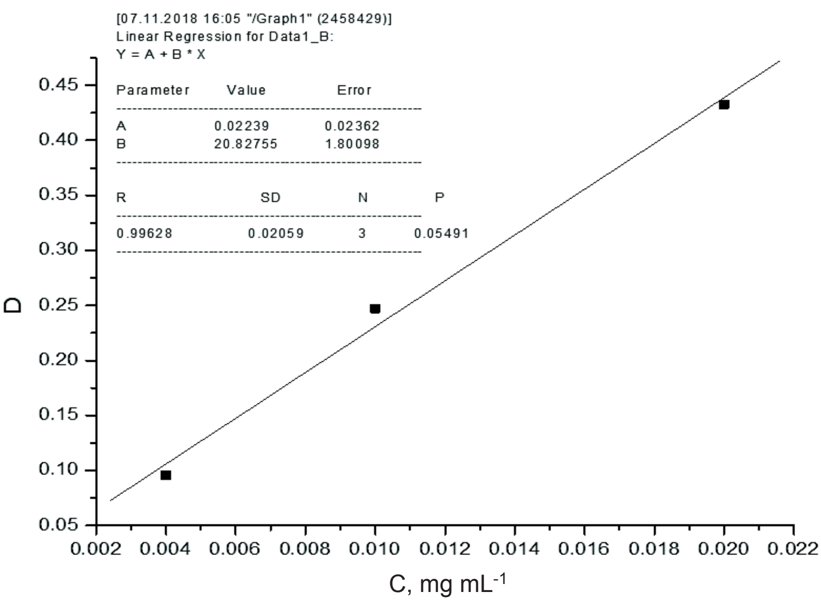

Figure 1. Calibration curve for determining the total flavonoid content.

Determination of the size of micro- and nanoparticles of meadowsweet BAS

Scanning electron microscope (SEM) of the Carl Zeiss brand Cross-Beam, model Neon40EsB was used to determine the size of developed micro- and nanocapsules of meadowsweet BAS.

Sensory evaluation of meat product (pate)

The organoleptic quality assessment of the developed functional meat product was carried out according to State Russian Standard 55334-2012 'Meat and meat containing pate. Technical conditions'.

Statistical analysis

The experiments were performed in triplicate, the data were processed by the methods of mathematical statistics with finding confidence intervals with a probability of 0.95.

RESULTS AND DISCUSSION

In this study, flavonoids isolated from the meadowsweet flowers from different regions of the Russian Federation were the objects of particular interest. The results of the study of the flavonoids quantitative content in water and alcoholic meadowsweet flowers extracts are shown in Table 1.

Table 1. The flavonoids content of meadowsweet flowers extracts from three regions of the Russia

Samples	Flavonoids (mean ± confidence interval)					
	mg / 100 mL extract			mg / g dry substance meadowsweet		
	Leningrad Region	Yaroslavl Region	Bashkir Republic	Leningrad Region	Yaroslavl Region	Bashkir Republic
Water extract	403 ± 32	309 ± 33	287 ± 36	80.6 ± 2.9	61.8 ± 1.8	56.4 ± 1.9
Alcohol extract	529 ± 34	390 ± 50	360 ± 40	105.8 ± 3.1	78.8 ± 2.5	69.3 ± 2.2

Based on the Table 1 data, it can be concluded that the content of flavonoids in alcoholic extracts is higher than in water extracts of meadowsweet for all the samples studied. Moreover, the content of flavonoids in the studied samples of the meadowsweet extracts from the Leningrad region exceeds the studied indicator in other samples studied. The highest value of the content of flavonoids is observed in alcoholic extract of meadowsweet from the Leningrad region, which averaged 529 mg / 100 mL of extract or 105.8 mg / g of dry matter. The lowest content of flavonoids in the studied samples was found in the water extract of meadowsweet from the Bashkir Republic and the Yaroslavl Region and averaged 360 and 390 mg / 100 mL of extract or 69.3 and 78.8 mg / g of dry matter respectively. It should be noted that the content of flavonoids in the water extract of meadowsweet from the Leningrad region is comparable to the content of flavonoids in the alcohol extract of meadowsweet from the Yaroslavl region. This variation in the content of flavonoids in extracts of meadowsweet in various regions can be associated with regional climatic conditions of plants growth, for example, with the soil composition of the studied regions of Russia (Shamshev et al., 2003; Zhdanov et al., 2016; Vysochina et al., 2016; Shaldayeva et al., 2018).

Thus, the analysis of the chemical composition of meadowsweet extracts (water and alcohol extraction) showed high content of total flavonoids of meadowsweet from various regions of the Russian Federation. This data corresponds to the results of the other authors (Denev et al., 2014; Katanic et al., 2015; Bespalov et al., 2017).

The above-mentioned alcoholic and water extracts were taken as the basis for obtaining encapsulated meadowsweet BAS. Table 2 shows the results of the flavonoids content in them.

Table 2. Content of flavonoids in meadowsweet capsules

Samples	Content of total flavonoids, mg / g capsules (mean ± confidence interval)		
	Leningrad Region	Yaroslavl Region	Bashkir Republic
Capsules of water extract	37.1 ± 2.2	28.3 ± 1.9	21.5 ± 2.2
Capsules of alcohol extract	48.7 ± 1.9	35.2 ± 2.2	29.5 ± 2.8

The data presented in Table 2 confirmed the results of the previous study and showed the highest content of flavonoids in capsules produced on the basis of alcoholic extracts of meadowsweet from the Leningrad region, which averaged 48.7 mg / g capsules. The lowest content of flavonoids in the samples was found in capsules made on the basis of water extracts of meadowsweet from the Bashkir Republic, which averaged 21.5 mg / g capsules.

The loss of flavonoids from meadowsweet extracts during the encapsulation was 40–60%, depending on the raw materials and the extractant used, which is supposedly due to the substance's oxidation during the process. The obtained results of the flavonoids content are correlated with the antioxidant activity of encapsulated preparations of meadowsweet and can exhibit various biological effects, including anticancer properties (Denev et al., 2014; Katanic et al., 2015; Bespalov et al., 2017).

The obtained results on the chemical composition of meadowsweet extracts are important because flavonoids have antioxidant activity and can exhibit various biological effects, including anti-carcinogenic properties (Oktyabrsky et al., 2009; Denev et al., 2014; Katanić et al., 2015; Bespalov et al., 2018). Pronounced antioxidant properties of meadowsweet extracts are shown on different models (Samardžić et al., 2018). In this study, extracts and encapsulated forms of meadowsweet contained concentrations of total flavonoids that suggest a possible oncoprotective effect.

Extractive BAS of meadowsweet were obtained in the form of micro- and nanosized capsules (Fig. 2) for the production of different functional foods.

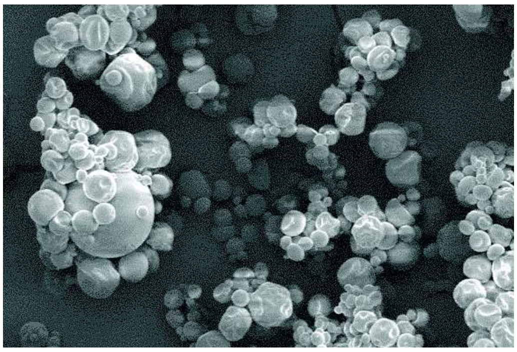

Figure 2. Micro- and nanosized capsules of meadowsweet BAS.

Evaluation of the structure of nano- and microcapsules of the meadowsweet BAS using a scanning electron microscope confirmed the particles sizes from 90 nm to 5 µm. The developed encapsulated form of meadowsweet extract for meat functional foods is presented in Fig. 3.

In this study, the possibility of producing a functional food product with the addition of the developed dry encapsulated meadowsweet BAS was considered on example of meat pate. Even though the physiological functionality of the developed encapsulated extracts is possible in the range from 1 to 4%, the decision was made to introduce the maximum dosage. Organoleptic evaluation of the pate with the addition of the encapsulated meadowsweet BAS in order to identify possible effects on the properties of the finished product was carried out. Table 3 shows the results of the organoleptic evaluation of the experimental pate and a control pate sample without adding the meadowsweet BAS.

Figure 3. Encapsulated form of meadowsweet extract for meat functional foods.

Table 3. Organoleptic characteristics of the chicken pate

Descriptors	Control sample	Sample with the complex functional dry mixture
Appearance	pate with a clean, dry, evenly baked surface	the same
Consistency	easily smeared	the same
View on the cut	homogeneous, uniformly mixed mass of light beige	the same
Smell and taste	peculiar to this type of product, moderately salty and fatty with a creamy aftertaste, without foreign taste and smell, with a pronounced aroma	the same and a slight aroma of meadowsweet

Thus, the functional complex dry mixture as an ingredient in recipes of pate did not lead to a decrease in organoleptic characteristics and contributed to the enrichment with high content of meadowsweet BAS. Even though the encapsulated extracts have a yellow color, in the dosages used they did not have a strong coloring ability. Even with a physiologically high dosage of encapsulated extracts of 4%, their application did not change the color of the product as compared with the control sample without extracts. Perhaps this is due to the original beige color of the meat pate, in white milk and yogurt without fillers, the color could change more significantly.

To assess the industrial production of the developed product, an analysis of the cost of the meat product with the addition of the encapsulated meadowsweet BAS in comparison with the traditional pate (control sample) per a portion of the product (100 g of pate) was carried out. According to the results of a comprehensive calculation, it was established that the cost of a portion of the meat product with the addition of a complex functional dry mixture was 0.53 euros, which is 0.06 euros and 12.8% higher than the cost of a portion of traditional pate.

Improving the bioactive compound profile of food products is believed to elevate their cancer fighting properties (Vanamala, 2017). Fruit and vegetables are key components in the Mediterranean diet that provide health promoting effects, which mostly connected with flavonoids (Ortega, 2006). While it is not easy to increase fruit and vegetable consumption in all vulnerable populations, developing functional food products and ingredients for their production can be one of innovative strategies to support increased consumption of bioactive compounds for cancer prevention. Flavonoids are often associated with the prevention of various forms of cancer with the help of food components (Terahara, 2015; Aghajanpour et al., 2017). Flavonoids are found in different types of common plant products such as citrus, parsley, broccoli, garlic, onion, blueberries, apples, tea, nuts, artichokes (Yao et al., 2004; Lattanzio et al., 2009). Thus, it is important to enrich the diet with flavonoids, including from more rare sources, such as red clover and meadowsweet (Kroyer, 2004; Vysochina et al., 2011). The found high content of flavonoids in the raw materials and encapsulated forms of extracts allow us to consider the developed ingredients as physiologically functional. The obtained results correspond with previous data on oncoprotective effect of plant flavonoids and extracts of this species (Lima et al., 2014; Ghasemi & Lorigooini, 2016; Bespalov et al., 2019). Thus, the developed encapsulated forms of meadowsweet extract are a promising ingredient for the creation of functional food products.

CONCLUSIONS

The phytochemical composition of alcoholic and water extracts of *Filipendula ulmaria* L. Maxim. from various regions of the Russian Federation showed that the flowers of the plant are rich in flavonoids. In addition, F. ulmaria exhibits antioxidant activity and anticancer properties according to present studies. This can be the basis for the assumption that the encapsulated extracted forms of meadowsweet can retain their properties in the composition of food. This study has shown that encapsulated extracts of meadowsweet can be incorporated into complex functional dry mixtures for food production with some loss in the content of flavonoids in the process of encapsulation. The experimental complex functional dry mixture is considered as an alternative to the existing one with some additional biological value. An assessment of the possibility of using the developed complex functional dry mixture in the composition of the meat product (pate), which proves the absence of any negative effect on the organoleptic properties of the finished product, was carried out. The calculation of the cost of the meat product with the complex functional dry mixture showed a slight increase in the cost of the final product compared to the traditional analogue. This study shows that encapsulated meadowsweet BAS can be used for inclusion in various food products, to ensure the functional properties of food and optimize the population's rations. However, the future studies are necessary on the content of meadowsweet BAS in food products during storage, as well as on their functional or health promoting properties in certain foods.

ACKNOWLEDGEMENTS. This research work was financially supported by the government of the Russian Federation, Grant RFMEFI58117X0020.

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