PREPARATION AND STABILITY OF ORGANIC CORE-SHELL PARTICLES WITH ENCAPSULATED COMPLEX NATURAL PLANT SOURCES OF PHENOLICS, CAFFEINE AND VITAMINS

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Abstract

Presented work is focused on possibilities of encapsulation some complex natural sources of phenolics, caffeine and vitamins in core-shell type organic micro- and nanoparticles. Encapsulation of these substances is suitable to controlled release of caffeine and for disguising of bitter taste of phenolics. Water extracts of guarana, ginseng and goji were prepared and the concentrations of phenolics, anthocyanins, caffeine and vitamin C were evaluated. Extracts were packed into liposomes and polysaccharide particles (alginate, chitosan) using encapsulator B-392P (Büchi) or manual procedures. The efficiency of encapsulation was determined by HPLC/UV-VIS (individual phenolics – rutin, morin, myricetin, luteolin; caffeine, vitamin C) and by spectrophotometry (phenolics). Stability of the particles was followed in model/real foods and in a model physiological environment. Size of prepared particles was determined by dynamic light scattering. In this work it was found that polysaccharide particles with higher activity and stability were obtained by encapsulator, while in the liposomes better results were obtained in manually prepared particles. Liposomes exhibited the smallest particle size (80 – 200 nm), the highest efficiency encapsulation, excellent stability and the best value of zeta potential (-40 mV). Polysaccharide particles prepared manually were smaller (500 – 3000 nm) and less stable than core-shell microparticles formed by encapsulator (diameter 200 and 300 mm). Prepared particles could be used to modern types of energy drinks, food supplements and also some cosmetics applications.

Keywords:

encapsulation, organic particles, caffeine, vitamins, phenolics

1. INTRODUCTION

1.1 Encapsulation

Encapsulation is a process to entrap active agents within a carrier material and it is a useful tool to improve delivery of bioactive molecules and living cells into foods. Materials used for design of protective shell of encapsulates must be food-grade, biodegradable and able to form a barrier between the internal phase and its surroundings [1]. Among all materials, the most widely used for encapsulation in food applications are polysaccharides. Proteins and lipids are also appropriate for encapsulation. There are number of reasons why to employ an encapsulation technology. This technology may provide barriers between sensitive bioactive materials and the environment, and thus, to allow taste and aroma differentiation, mask bad tasting or smelling, stabilize food ingredients or increase their bioavailability. One of the most important reasons for encapsulation of active ingredients is to provide improved stability in final products and during processing. Another benefit of encapsulation is less evaporation and degradation of volatile actives, such as aroma.

Furthermore, encapsulation is used to mask unpleasant feelings during eating, such as bitter taste and astringency of polyphenols [2]. Also, another goal of employing encapsulation is to prevent reaction with other components in food products such as oxygen or water. In addition to the above, encapsulation may be used to immobilize cells or enzymes in food processing applications, such as fermentation process and metabolite production processes. There is an increasing demand to find suitable solutions that provide high productivity and, at the same time, satisfy an adequate quality of the final food products [3].

Research on and the application of polyphenols, have recently attracted great interest in the functional foods, nutraceutical and pharmaceutical industries, due to their potential health benefits to humans. However, the effectiveness of polyphenols depends on preserving the stability, bioactivity and bioavailability of the active ingredients. The unpleasant taste of most phenolic compounds also limits their application. The utilization of encapsulated polyphenols, instead of free compounds, can effectively alleviate these deficiencies [2,3].

1.2 Natural plant complex sources of vitamins, phenolics and caffeine

Goji, goji berry or wolfberry is the fruit of *Lycium barbarum* and *Lycium chinense*, two closely related species of boxthorn in the family Solanaceae (which also includes the potato, tomato, eggplant, deadly nightshade, chilli pepper, and tobacco). Since the early 21st century, interest has increased for wolfberries for their novelty and nutrient value. They have been termed a super-fruit, which has led to a profusion of consumer products. In traditional medicine, the whole fruit or its extracts have numerous implied health effects, which remain scientifically unconfirmed as of 2014. As a food, dried wolfberries are traditionally cooked before consumption. Dried wolfberries are often added to rice congee and almond jelly, as well as used in Chinese tonic soups, in combination with chicken or pork, vegetables, and other herbs. The berries are also boiled as a herbal tea, often along with chrysanthemum flowers and/or red jujubes, or with tea. Various wines containing wolfberries are also produced, including some that are a blend of grape wine and wolfberries [4].

Ginseng is any one of 11 species of slow-growing perennial plants with fleshy roots, belonging to the genus *Panax* of the family Araliaceae. Ginseng is found in North America and in eastern Asia (mostly Korea, northeast China, Bhutan, eastern Siberia), typically in cooler climates. *Panax* ginseng is characterized by the presence of ginsenosides. The root is most often available in dried form, either whole or sliced. Ginseng leaf, although not as highly prized, is sometimes also used. Folk medicine attributes various benefits to oral use of American ginseng and Asian ginseng (*P. ginseng*) roots, including roles as an aphrodisiac, stimulant, type II diabetes treatment, or cure for sexual dysfunction in men. Ginseng may be included in small doses in energy drinks or herbal teas, such as ginseng coffee [5].

Guarana, *Paullinia cupana*, is a climbing plant in the maple family, Sapindaceae, native to the Amazon basin and especially common in Brazil. Guarana features large leaves and clusters of flowers, and is best known for its fruit, which is about the size of a coffee bean. As a dietary supplement, guarana is an effective stimulant: its seeds contain about twice the concentration of caffeine found in coffee beans (about 2–4.5% caffeine in guarana seeds compared to 1–2% for coffee beans). As with other plants producing caffeine, the high concentration of caffeine is a defensive toxin that repels herbivores from the berry and its seeds. According to the Biological Magnetic Resonance Data Bank, guaranine is defined as only the caffeine chemical in guarana, it is identical to the caffeine chemical derived from other sources, for example coffee, tea, and mate. Natural sources of caffeine contain widely varying mixtures of xanthine alkaloids other than caffeine, including the cardiac stimulants theophylline and theobromine and other substances such as polyphenols, which can form insoluble complexes with caffeine. The main natural phenols found in guarana are (+)-catechin and (-)-epicatechin. Guarana is used in sweetened or carbonated soft drinks and energy shots, an ingredient of herbal teas or contained in capsules [6].

All above mentioned plants are widely used in several food supplements and energy drinks as a source of phenolics, vitamins and provitamins, specific phenolics, and, some of them also as a source of caffeine. The aim of presented work is to test possibilities of encapsulation of complex plant extracts prepared from goji, guarana and ginseng and to evaluate stability of particles and rate of active substances release.

2. METHODS

Water and 10% lactic acid extracts of guarana, ginseng and goji were prepared and the concentrations of phenolics, anthocyanins, caffeine and vitamin C were evaluated. Extracts were packed into liposomes and polysaccharide particles (alginate, chitosan) using encapsulator B-392P (Büchi) or manual procedures. Liposomes were prepared manually from mixture of egg lecithin and cholesterol using ultrasonic homogenization and reverse phase evaporation. The size of resulting particles was of diameter ranged from 30 nm to 1 mm. The polysaccharide particles were prepared by the method based on the principle of gelation and cross-linking polymers. Alginate particles were prepared by mixing enzymes in sodium alginate solution and dropped this into a swirling solution of calcium chloride. A calcium alginate membrane was formed immediately on the surface of the droplet by ionic interaction.

The efficiency of encapsulation was determined by HPLC/UV-VIS (individual phenolics – rutin, morin, myricetin, luteolin; caffeine, vitamin C) and by spectrophotometry (phenolics). Stability of the particles was followed in model/real foods and in a model physiological environment. The physicochemical evaluation of micro- and nanoparticles (Zeta potential, size and distribution) was analyzed by dynamic light scattering and by analytical centrifugation. Stability of particles in artificial stomach and intestinal juice and in bile acid as well as in model foods (water, 3% acetic acid, 10% ethanol, oil/water emulsion) was tested too.

3. RESULTS AND DISCUSSION

In plant extracts prepared from solid guarana, dried goji berries and ginseng powder the total amount of phenolics, flavonoids and anthocyanins was detected. Extraction was proved by water and by 10% lactic acid, in which probably partial acid hydrolysis of glycoside bonds in phenolic compounds have been occurred. The highest amount of total phenolics was detected in acidic guarana extract (**Table 1**), while the lowest amount was found in goji. The reason could be partial destruction of phenolics during fruit drying or non-complete extraction.

Individual flavonoids detected by RP-HPLC/UV-VIS were found as follows: rutin, morin, myricetin and luteolin in guarana, rutin, myricetin and luteolin in ginseng and rutin only in goji (**Table 2**).

Table 1: Concentration of phenolics and flavonoids in plant extracts

	concentration in extract [mg/g of plant]			
plant	**phenolics**		**flavonoids**	
	water extract	**10% lactic acid extract**	**water extract**	**10% lactic acid extract**
Guarana	63,38± 1,26	111,84± 2,58	30,94± 5,12	33,67± 2,87
Goji	2,78± 0,09	2,96± 0,04	0,19± 0,06	0,26± 0,08
Ženšen	4,33± 0,15	4,75± 0,11	0,43± 0,03	0,44± 0,02

Table 2: Concentrations of individual phenolics detected in plant extracts by HPLC

plant	Rutin [µg/g]	Morin [µg/g]	Myricetin [µg/g]	Luteolin [µg/g]
Guarana	112,60 ± 3,58	2,52 ± 0,72	2,20 ± 0,12	ND
Goji	8,12 ± 0,09	ND	ND	ND
Ženšen	15,1 ± 1,56	ND	0,22 ± 0,02	1,19 ± 0,28

In **Fig.1** (sample preparation and description see **Table 3**) the comparison of encapsulation efficacies of individual techniques of particle preparation were compared. The best results were reached using 2% alginate and encapsulator as preparative technique. In general, the best encapsulation effectiveness was reached in ginseng extracts followed by guarana, while encapsulation efficacy of goji *vs* relatively low (**Fig.1**). Higher concentration of polysaccharide led to better effect. Use of encapsulator enables optimized preparation of core-shell particles of defined size. In this technique potential negative effects of natural extract components are lowered. It seems that in encapsulation of complex plant extracts microparticles are the better application form than nanoparticles.

Table 3: Encapsulation techniques used for particles preparation

	particle type		particle type
A	alginate particles - manual	**G**	1% alginate particles (encapsulator, diameter 300 µm)
B	chitosan particles - manual	**H**	1% alginate particles (encapsulator, diameter 450 µm)
C	alginate p. – ultrasonication	**I**	2% alginate particles (encapsulator, diameter 300 µm)
D	chitosan p. – ultrasonication	**J**	1% alginate core-shell particles (encapsulator, 300 µm)
E	Liposomes – ultrasonication	**K**	2% alginate core-shell particles (encapsulator, 300 µm)
F	Liposomes – reverse phase evap.	**L**	mixed alginate-CMC particles (encapsulator, 300 µm)

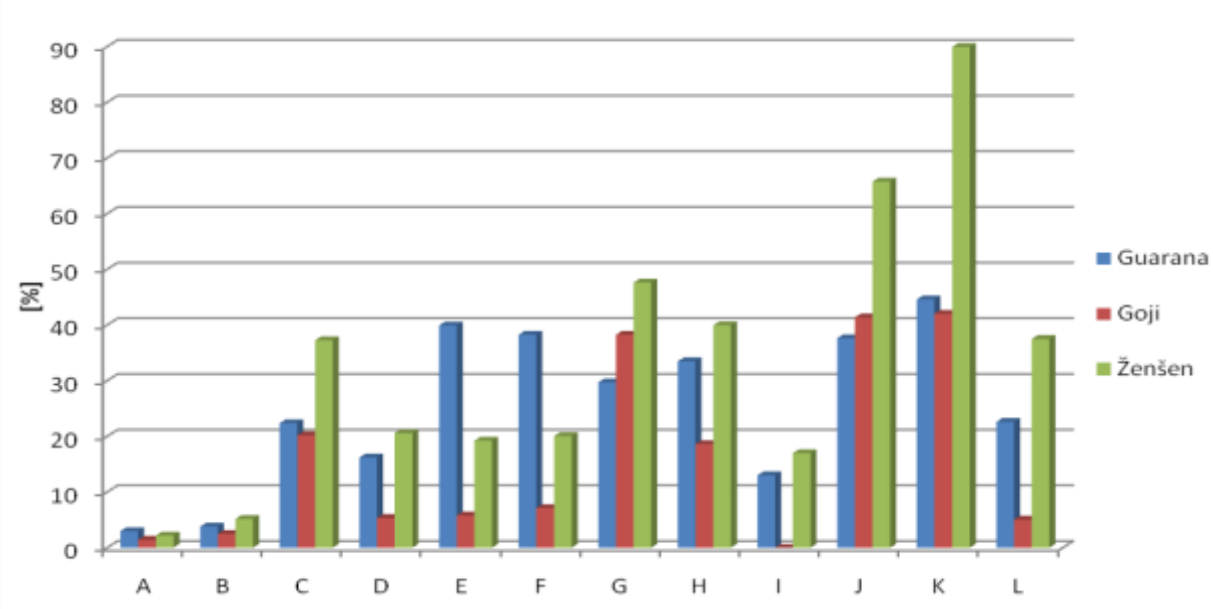

Fig. 1 Encapsulation efficiency in individual particles measured as % of released phenolics

Stability of prepared particles was tested in artificial stomach juice, pancreatic juice and in bile acids. The highest phenolics release was observed in pancreatic fluid, which could be caused by presence of enzymes hydrolyzing polysaccharides and lipids. In stomach juice the most stable were liposomes and alginate particles wit ginseng extract (**Fig.2**). As the most stable environment for mot of particles the juice with bile acids was found (no more than 60% of phenolics were released). In model foods as the most stable particles

containing ginseng were found, while particles with goji were the less stable. Some components of encapsulated extract probably might influence the particle stability from its internal environment.

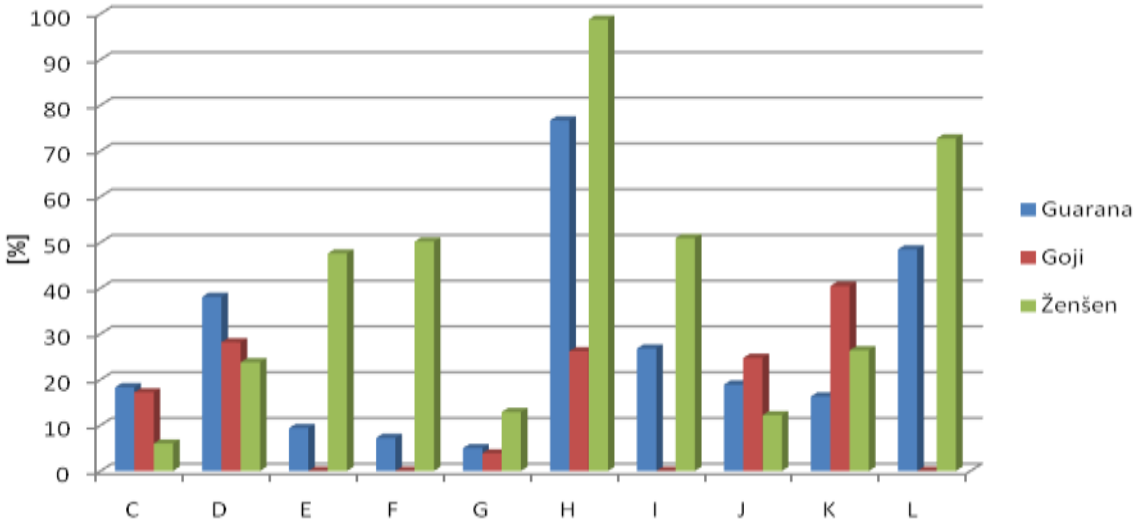

Fig. 2 Stability of individual particles (as described in Table 3) in artificial stomach juice; measured as % of released phenolics

The size and stability of prepared particles was analyzed by dynamic light scattering. The size of manually prepared polysaccharide particles was in range of 100 – 7 000 nm. The size of liposomes was found in range of 116 – 167 nm. The highest were chitosane particles with encapsulated ginseng. The values of Zeta potential were used for characterization of particle stability (**Table 4**). As the stable the particles with Zeta potential lower than -40 mV and higher as +40 mV can be considered [1, 3]. As the most stable liposomes and chitosan particles were found. The less stable were empty particles, after encapsulation of plant extracts the stability of particles increased substantially (**Table 4**).

Table 4: Stability of selected manually prepared particles evaluated by dynamic light scattering (Zeta potential)

particle type	empty particles [mV]	Guarana [mV]	Goji [mV]	Ženšen [mV]
alginate	-13,85	-14,95	-19,50	-25,70
chitosan	25,30	21,50	23,80	0,49
alginate - ultrasonication	-15,50	-18,50	-19,05	-19,20
chitosan - ultrasonication	47,10	44,40	39,60	14,50
Liposomes – ultrasonication	0,03	-43,25	-35,00	-42,15
Liposomes – RPE	-39,75	-38,15	-41,70	-50,00

We can conclude that in this work it was found that polysaccharide particles with higher activity and stability were obtained by encapsulator, while in the liposomes better results were obtained in manually prepared particles. Liposomes exhibited the smallest particle size (80 – 200 nm), the highest efficiency encapsulation, excellent stability and the best value of zeta potential (-40 mV). Polysaccharide particles prepared manually were smaller (500 – 3000 nm) and less stable than core-shell microparticles formed by encapsulator

(diameter 200 and 300 mm). As the most suitable form for encapsulation of complex plant extracts core-shell alginate particles prepared by encapsulator can be recommended.

4. CONCLUSION

Encapsulation could be a promising alternative for the enlargement of stability and controlled release of caffeine and other active substances from complex extracts. Capsules can maintain their integrity during food/beverage storage and during passage through the gastrointestinal tract until they reach their target destination. Encapsulated phenolics and caffeine can be used in modern types of energy drinks, food supplements and also some cosmetics applications.

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