BLEND OF NATURAL WAXES AS A MATRIX FOR AROMA ENCAPSULATION

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Abstract. *In this study, the possibility of using a blend of natural waxes (bees and carnauba) for encapsulation of some aroma compounds was investigated. Melt dispersion/melt solidification technique was applied for microbeads production. Since one of the most important characteristics of the particles are the size and shape, particle size distribution as well as morphological properties are tested. Thermal characteristics are also examined as significant properties for thermal behavior at elevated temperatures, important for application of encapsulated particles in food production processes. Different contents of the carnauba wax in the mixture with beeswax are investigated, from 10% to 50% (w/w). Since one of the potential applications of the encapsulated aroma is in feed additives production, the targeted particle size range was under 300 µm to be suitable for handling and mixing with other powder substances. According to the obtained results, a higher carnauba wax content in the wax blend had an impact on particle size distribution. Also, it had an impact on the surface morphology and thermal properties. The obtained results may contribute to the development of methods of encapsulation of hydrophobic aromas in the natural wax matrix.*

Key words: *beeswax, carnauba wax, encapsulation*

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INTRODUCTION

Waxes are of interest for the preparation of controlled release systems by means of melt granulation or extrusion techniques (for granules and beads) and melt dispersion or spray congealing techniques (for microparticles) which do not require the use of solvents (Passerini et al. 2003). Carnauba wax is one of the classic materials used in pharmaceutical formulation processes, e.g. as a glossing agent. It has also been suggested as a potential matrix material or even as a solvent for solid dispersions for sustained-release forms or microspheres manufactured by coacervation spray congealing or extrusion (Emas et al., 2000). Carnauba wax has been used in food formulations as a formulation aid, an anticaking agent and a surface finishing agent in baked foods and mixes and it is the hardest natural wax. It is significantly less viscous then other waxes and thus it is easier to manipulate during capsule processing (Milanovic et al. 2010).

 Matrix type formulations are prepared from either swellable hydrophilic polymers or non-swellable lipophilic excipients, like waxes and lipids (Ozyazıcı et al. 2006). Commonly used materials in lipid carriers, prepared by various melt dispersion techniques, are beeswax and carnauba wax. These waxes contain a wide group of chemicals such as glycerides, fatty acids, fatty alcohols and their esters. These are widely used as release retardants in the design of sustained release beads, tablets, suspensions, implants, and microcapsules. The advantages of waxes include good stability at various pH and moisture levels, a well-established safe oral application in humans due to their non-swellable and water insoluble nature, a minimal effect on food in the gastrointestinal tract, and no dose dumping (Kamble et al., 2004; Maheshwari et al., 2003). The oil-in-water meltable disperse-phase encapsulation method is a simple and useful procedure avoiding harmful organic solvents (Bodmeier et al., 1992, Uddin et al., 2001). Recently, Shaki H. et al. developed a sustained drug delivery system for KCl, based on beeswax and carnauba wax. The melt dispersion technique was used to produce KCl loaded waxy microparticles and the effective variables were determined (Shaki et al., 2014). Menthol/beeswax particles with size in the range of 2–50 μm were produced by modified particles from gas-saturated solutions (PGSS) process with controlling the gas-saturated solution flow rate (Linjing et al., 2010).

In this study carnauba and bees wax were used, due to the aforementioned advantages, to investigate the most suitable ratio in the bland, as a carrier for ethyl vanillin encapsulation, on the basis of particle size distribution. Flavors are considered valuable ingredients in any food formulation. They are usually expensive, delicate and volatile. Therefore, food manufacturers are usually concerned with the preservation of aromatic additives. Encapsulation offers an effective approach to cover an active compound with a protective wall material and to impart some degree of protection against evaporation, chemical reactions (such as flavor-flavor interactions, light-induced reactions, oxidation) or migration in a food (Madene et al., 2006, de Ross, 2003). The melt dispersion technique was applied as a simple non-solvent and low-cost method. This method basically involves the emulsification of the molten mass in the aqueous phase, followed by its solidification by chilling. The process variables developed for ethyl vanillin encapsulation by Milanovic et al. are used in this study (Milanovic et al. 2010).

MATERIALS AND METHODS

Feed grade carnauba wax and beeswax were purchased from Carl Roth GmbH (Karlsruhe, Germany), ethyl vanillin (Boregard Synthesis, Sarpsborg, Norway) were obtained as a kind gift from Aroma (Krusevac, Serbia), emulsifiers (Tween 20, Span 40, Span 60) were supplied by Sigma Aldrich (Germany).

PREPARATION OF THE MICROPARTICLES

The microbeads entrapping ethyl vanillin were produced by the method which is basically described in the literature (Bodmeier, 2007; Gowda, Shivakumar, 2007; Kambel et al., 2004; Singh et al., 2007; Paradkar et al., 200), and optimized for encapsulation in carnauba wax as a matrix material (Milanovic et al. 2011). Experiments were run with different bees wax to carnauba wax ratio, from 90:10 to 50:50.

PARTICLE CHARACTERIZATION

Size distribution of the particles was evaluated by a sieve analysis using a set of five standard sieves (U.S. Standard Sieve Series No. 16, 35, 50, 75 and 120).

The physical appearance and surface structure of microparticles were determined using scanning electron microscopy (SEM). Micrographs were obtained using a SEM-Jeol JSM 6460LV (SEM, 30 XL Phillips, and the Netherlands). The samples were prepared on aluminum stabs and coated with gold using a Spater coater device Baltec SCD 005(30 mA, the distance 50 mm, duration 90 s), prior to examination by SEM.

The thermal behavior of the particles was investigated by the DSC technique using a TA Instruments model SDT Q-600 (New Castle, Delaware, US). The samples (mass approx. 10 mg) were heated in a standard alumina sample pan. All experiments were performed out under dynamic air of a flow rate of 0.1 dm^3/ min using a heating rate of 10 °C/min.

RESULT AND DISCUSSION

Carnauba wax and beeswax are used extensively as inert lipophilic carriers both for water-insoluble and water-soluble materials (Milanovic et al. 2010; Shaki et al., 2014; Linjing et al., 2010). The conditions optimized for aroma encapsulation using carnauba wax as a carrier were applied in this study (Milanovic et al. 2010). The main goal was to select the most suitable mixture of two matrix materials to produce particles with narrow size distribution. The carrier on the beeswax basis may fulfill the criterion (considered as one of crucial importance by food manufacturers) of a cheap preparation procedure. Microparticles were produced in the size range suitable for food and feed applications. Namely, powders of wax micro particles entrapping aromas can be used as ingredients of food premixes. Constituents of food premixes have particle size in the range of 150– 300 μm (Card et al., 1960), therefore, this size range was the target fraction in our experiments.

Starting experimental conditions were set at 1100 rpm and 4 minutes stirring time aiming to produce particles in the range of 150-300 μm, as given in Table 1. During the experimental procedure it was concluded that the impeller speed 1100 rpm would produce

an undesirable coarse particle with broad size distribution. The frequency of particle size distribution for particles produced from carnauba wax and beeswax in different blend ratios is given in Table 1.

Table 1 Frequency of particle size distribution, F (%), obtained at 1100 rpm stirring speed and 4 min stirring time

Range (μm)	Beeswax to carnauba wax blend ratio (%)					
	90:10	80:20	75:25	70:30	60:40	50:50
< 100	5.18	7.84	5.7	11.88	10.6	13.5
100-200	43.73	49.95	68.9	72.51	75.5	75.98
200-300	14.58	20.27	14.8	7.57	8.5	5.79
300-500	10.61	14.82	6.1	3.38	3.8	3.42
500-1190	11.5	5.14	3.3	4.12	1.2	0.98
>1190	14.39	1.43	1,1	0.54	0.4	0.31

The applied experimental parameters resulted in the production of high prevalence particles larger than 300 μm, primarily for samples with a high beeswax content (90 and 80%) in a wax bland, 36.5% and 21.39%, respectively.

In order to reach the desired uniform particle size distribution, experiments were set with slightly changed conditions (stirring time 1.5 min, stirring speed 1400 rpm). The frequency of particle size distribution obtained under those experimental conditions is presented in the Table 2.

Table 2 Frequency of particle size distribution, F (%), obtained at 1400 rpm stirring speed and 1.5 min stirring time

Range (μm)	Beeswax to carnauba wax blend ratio (%)					
	90:10	80:20	75:25	70:30	60:40	50:50
< 100	3.9	6.2	13.88	10.6	9.9	11.2
100-200	65.2	64.0	76.51	76.7	81.3	81.9
200-300	10.8	15.2	3.57	4.9	5.1	5.0
300-500	7.7	7.5	3.38	3.6	2.5	1.4
500-1190	9.2	3.8	2.12	3.4	0.7	0.3
>1190	3.1	3.2	0.54	0.7	0.5	0.1

The results obtained from the experimental procedure (Table 2) show a decrease in the size of the particles, which leads to a conclusion that a decrease in the stirring time and an increase in the stirring speed lead to the production of smaller particles (< 300 μm).

In the experimental procedures applied under different conditions in regard to the stirring speed and the stirring time, the content of the used materials (carnauba and beeswax)varied in the mixture. Table 1 and Table 2 show the impact of the mixture content on the size of the produced particles. When the carnauba wax content was increased up to 50% (w/w), the prevailing fraction increased from 79.9 to 98.2% (for 1400 rpm and 1.5 minutes) and from 62.49% to 95.17% for the starting experimental set of conditions. From the particle size analysis   it can also be concluded that since there were as much as 20.0% and 14.5% (Table 2) fractions with large particles (size diameter

above 300 μm) blends with carnauba wax contents below 25% (bland ratio 90:10 and 80:20) are not suitable as an aroma carrier for application in food premixes.

Figure1 shows distribution of the particle size as a function of the wax content in the mixture for an improved set of experimental conditions (stirring time 1.5 min, stirring speed 1400 rpm).
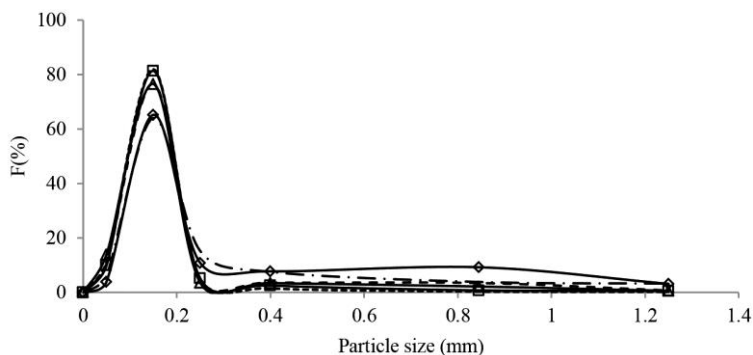

Fig. 1 Frequency distribution curves obtained for different wax blend ratios, beeswax: carnauba wax ◊ 90:10 −· −80:20 Δ 75:25 - - - - 70:30 □ 60:40 ---- 50:50, (stirring time 1.5 min, stirring speed 1400 rpm)

In all cases the size distribution is bi-modal, with the main fraction in the range 0.0–300 μm. There exists a small second shoulder representing large microparticles of sizes above 300 μm obtained for 90:10 beeswax to carnauba wax ratio.

Figure 2 shows frequency distribution curves obtained for pure waxes at stirring time 1.5 min and stirring speed 1400 rpm. Beeswax with a significantly lower melting point (56-60 °C) from carnauba wax (82-86 °C) congeals more slowly due to a smaller difference between the molten and the congealing point and as a result undergoes a subsequent agglomeration. This might explain larger particles production from pure beeswax (Figure 2, obtained at 1400 rpm stirring speed and 4 minutes stirring time) and, at the same time, larger particle production with a higher beeswax content in the bland (Table 1 and Table 2)
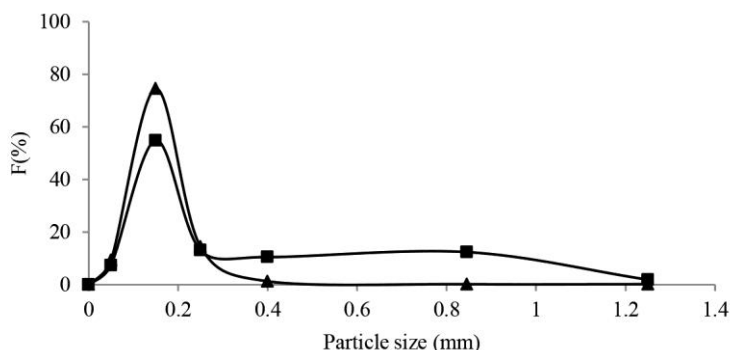

Fig. 2 Frequency distribution curves obtained for pure waxes ■ beeswax ▲ carnauba wax (stirring time 1.5 min, stirring speed 1400 rpm)

Figure 3 shows the SEM photos of the wax blend microparticles obtained at 1400 rpm steering speed and 1.5 minutes stirring time, (3a, 3b) and pure carnauba wax and beeswax microparticles (3c, 3d) with a low magnification (x 200).
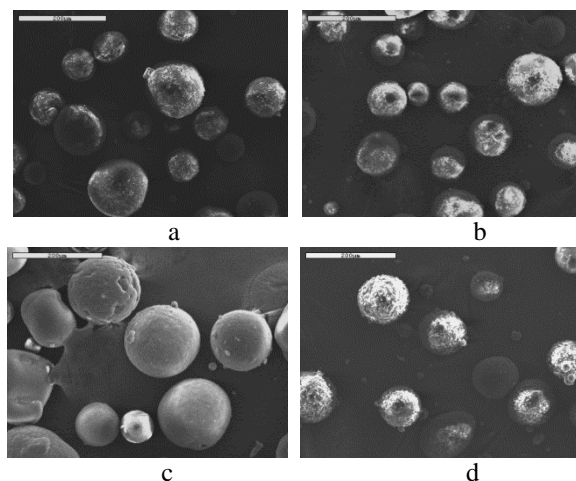

Fig. 3 SEM images of wax blend microcapsules (stirring time 1.5 min, stirring speed 1400 rpm) with low magnification (x 200) ; (a) beeswax:carnauba-50:50; (b) beeswax:carnauba-90:10; (c)pure carnauba wax; (d) pure beeswax

The SEM images show well separated microparticles with a regular spherical shape. SEM at higher magnification (x 2000), (Figure 4, stirring time 1.5 min, stirring speed 1400 rpm) reveals that the surface of microparticles depends on the beeswax content in the blend.
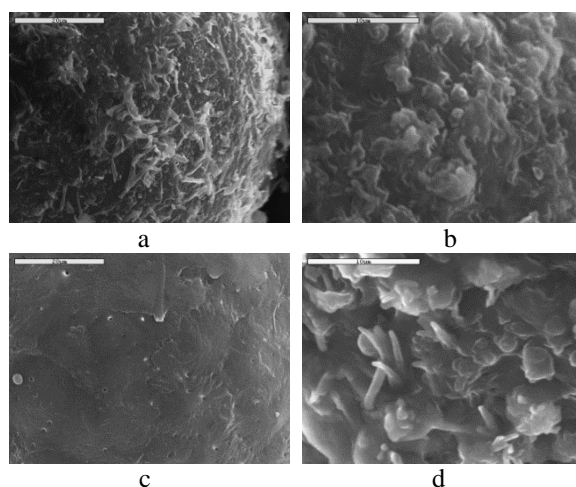

Fig. 4 SEM images of wax blend microcapsules (stirring time 1.5 min, stirring speed 1400 rpm) with high magnification (x 2000); (a) beeswax:carnauba-50:50; (b) beeswax:carnauba-90:10; (c) pure carnauba wax; (d) pure beeswax

Namely, the surface of the particles of pure beeswax (4d) is with many irregularities compared to the carnauba wax surface which seems smooth at the same magnification. This is confirmation of the fact that the particles are not solidified rapidly under experimental conditions, which implies a rough surface. The more beeswax content, the rougher surface is formed during congealing (4a, 4b). Figure 5 shows the thermograms of pure carnauba and pure bees waxes.
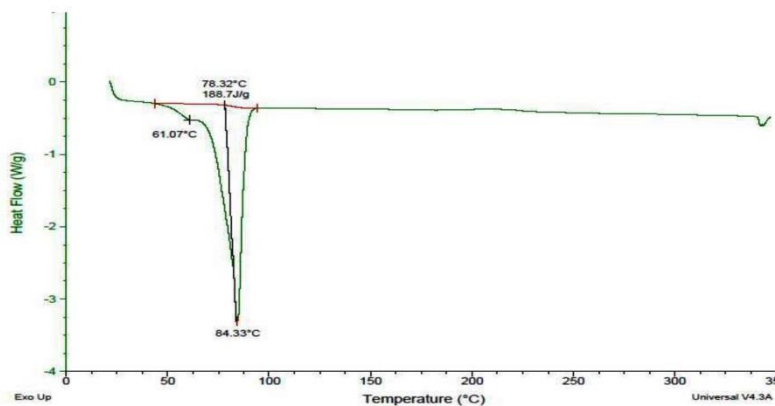
a)
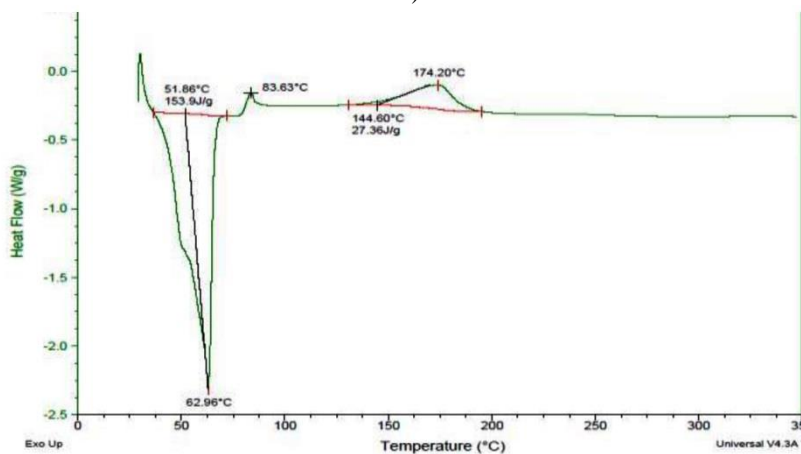
b)

Fig. 5 DSC scans of carnauba wax (a) and beeswax (b)

The DSC thermograms of pure waxes had one main prominent sharp endothermic peak with a maximum at 84.33 °C and at 62.96 °C, respectively, which corresponds to the competition of the melting process. The broad exothermic peak on the beeswax thermogram at 174.2 °C contributes to wax decomposition. Characteristic values obtained by DSC analysis are shown in Table 3.

Table 3 Characteristic values from DSC thermogram
for pure waxes and blend in different ratios

Bw:Cw ratio (%)	0:100	100:0	90:10	75:25	60:40	50:50
Beginning of melting (°C)	61.07	51.86	42.23	42.28	43.99	44.33
End of melting (°C)	84.33	62.96	63.78	63.9	65.57	63.85
Melting range (°C)	23.26	11.1	21.55	21.62	21.58	19.52
Fusion enthalpy (J/g)	188.7	27.36	162.2	161.2	161.9	149.2

According to the results, carnauba wax has a wide melting temperature range, 23.26 °C, compared to beeswax whose melting temperature range is as narrow as 11.1 °C. Also, all samples of wax blends under temperature exposition show a different behavior, compared to pure waxes. Namely, melting starts at a temperature lower than than for pure waxes, the ending of the melting process occurs at temperatures closer to the melting end of pure beeswax. The result of this shift towards lower temperature values is that the melting of all blends has a wider range. By increasing carnauba wax content from 10% to 50% in the wax mixture only a slight change in the melting process occurs. Thermal behavior of the wax blend might be of interest for the congealing process. Further investigation is needed to define the most suitable congealing conditions, temperature and time to obtain particles with a smooth surface.

CONCLUSION

In this study, mixtures of carnauba wax and beeswax in different weight ratios are investigated, as a potential carrier for the matrix type microcapsules production. The melt dispersion technique is used as a low-cost and no solvent method, which was recently optimized for ethyl vanillin encapsulation in carnauba wax as a carrier (3). At the stirring speed of 1400 rpm and stirring time of 1.5 min, predominantly spherical particles with narrow size distribution are obtained. The target fraction with a size below 300 μm was reached in the portion of 98.2% for the mixture with 50% carnauba wax in the blend. Analyzing the surface morphology it was concluded that the particles have a rather rough surface, and the DSC scans showed a different behavior for blends compared to pure waxes. The results indicate that carnauba wax and bees wax blend might be an attractive material for use as a matrix for encapsulation. The results achieved may contribute to the development of matrix materials for the encapsulation of aromas and other substances into micro particles based on natural waxes.

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SMEŠA PRIRODNIH VOSKOVA KAO NOSAČ ZA INKAPSULACIJU AROMA

U radu je ispitivana mogućnost upotrebe smeše prirodnih voskova (pčelinjeg i katnauba voska) za inkapsulaciju nekih aromatičnih jedinjenja. Primenjena je metoda disperzije/očvršćavanja rastopa za dobijanje mikročestica. Budući da su veličina i oblik čestica jedne od važnijih osobina, analizirana je raspodela veličine čestica, kao i morfološke osobine čestica. Termičke osobine su takođe analizirane, zbog važnosti poznavanja termičkog ponašanja na povišenim temperaturama, što je značajno u proizvodnji hrane. Ispitane su smeše pčelinjeg i karnauba voska u različitim masenim odnosima, od 10% to 50% (w/w). Jedna od mogućih primena inkapsuliranih čestica je u proizvodnji hrane za životinje, pa je ciljni opseg veličine čestica bio ispod 300 μm, kako bi bile pogodne za rukovanje i mešanje sa drugim praškastim supstancama u smeši. Prema dobijenim rezultatima, veći sadržaj karnauba voska u smeši ima uticaja na raspodelu veličina čestica. Takođe ima uticaj na površinsku morfologiju i termičke osiobine. Dobijeni rezultati mogu doprineti razvoju metoda inkapsulacije hidrofobnih aroma u nosače od prirodnih voskova.

Ključne reči: pčelinji vosak, karnauba vosak, inkapsulacija