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Microencapsulation of juniper and black pepper essential oil using the coacervation method and its properties after freeze-drying

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Abstract

Essential oils are a mixture of chemical compounds very susceptible to the effects of the external environment. Hence, more attention is drawn to their preservation methods. The aim of the study was to test the possibility of using complex coacervation for the microencapsulation of essential oils. Black pepper (*Piper nigrum*) and juniper (*Juniperus communis*) essential oils dissolved in grape seed (GSO) and soybean (SBO) oil formed the core material, while gelatin (G) and gum Arabic (GA) formed the wall materials. Various mixing ratios of polymers were tested: 1:1; 1:2, and 2:1. The oil content was 10%, and the essential oil content was 1%. The prepared coacervates were lyophilized and then screened to obtain a powder. For all samples, among others, the following were determined: encapsulation efficiency (EE), Carr Index (CI), Hausner Ratio (HR), solubility, hygroscopicity, moisture content, and particle size. The highest encapsulation efficiency achieved was within the range of 64.09-59.89%. The mixing ratio G/GA=2:1 allowed to obtain powders that were characterized by the lowest solubility (6.55-11.20%). The smallest particle sizes, that did not exceed 6 µm, characterized the powders obtained by mixing G/GA=1:1. All powder samples were characterized by high cohesiveness and thus poor or very poor flow (CI=30.58-50.27, HR=1.45-2.01).

Keywords: essential oils, complex coacervation, gum Arabic, gelatin

1. Introduction

In response to consumer expectations, food producers focus on producing high-quality and low-processed products, which also come with the longest shelf life. In addition, care for the natural environment causes manufacturers to focus on reducing the amount or eliminating synthetic preservatives and stabilizers [1]. Plant-based substances such as essential oils can respond to these expectations. This is because essential oils have a strong antimicrobial effect, and their use minimizes the risk of foodborne pathogens acquiring resistance to antibiotics. In addition, essential oils have several health-promoting properties [1-3].

The main components of juniper essential oil (*Juniperus communis* L.) are α -pinene, limonene, and myrcene [4, 5], while the main ingredients of black pepper essential oil (*Piper nigrum* L.)

are α -pinene, sabinene, β -pinene, δ -3-carene, limonene, and β -caryophyllene [6-8]. Both plants are known worldwide as spices but also as pharmaceutical raw materials [9, 10]. They have strong antioxidant and antimicrobial properties [7, 11], but also have anti-inflammatory and antispasmodic properties [5].

The potential benefits of essential oils and the increased demand for natural additives in the food industry has led to research on their powdered forms as potential food ingredient [12]. The encapsulation of essential oil is necessary because these substances are sensitive to the effects of the external environment (light, temperature, pH, oxygen). However, these are not the only limitations. Their lipophilic nature and, therefore, low water solubility, low bio-accessibility, and bio-availability do not favor their wide application [13-15]. Applying the microencapsulation technique can protect their active ingredients. It is important to select the composition of the wall material for each system because the encapsulating material determines many physicochemical properties of the powdered product and its storage behavior [2]. An interesting method that allows microencapsulation is complex coacervation. The unique advantage of this process is that it can take place at room temperature, which is important with essential oils [16].

Complex coacervation is one of the most important microencapsulation methods used to protect sensitive substances such as aromas, omega-3 fatty acids, vegetable oils, antioxidants, and essential oils [9, 10, 15, 17, 18]. This process occurs when two oppositely charged polymers (in an aqueous solution) are attracted to each other by electrostatic forces, followed by phase separation resulting in two phases: a rich coacervated phase called "continuous phase" and a weakly coacervated liquid phase called "equilibrium solution" [11, 19, 20]. The coacervated phase consists of wall material deposited in a thin layer of the core material. The mixtures of proteins and polysaccharides are commonly used, and the most widely studied coacervation system is gelatin (G): gum Arabic (GA) [11, 21]. This technique allows for high encapsulation efficiency (up to 99%) [14], and enables the encapsulation of more core material per unit mass of wall material compared to other microencapsulation techniques [17, 2012, 19, 16]. This process has been used to microencapsulate essential oils [14, 9, 10, 16, 19]. However, so far, without first dissolving EO in the oil. Complex coacervation allows for obtaining high efficiency of oil encapsulation [22]. But essential oils, as highly volatile compounds, may suffer significant losses during the spray-drying or freeze-drying process [9, 10, 12, 23].

The objective of this research was to study the influence of the different mixing ratios of wall materials (gelatin, gum Arabic) on encapsulation efficiency and to investigate physiochemical properties of the produced powders.

2. Materials and methods

2.1. Materials

Gelatin (Agnex, Białystok, Poland), and Arabic gum (Warchem, Warsaw, Poland) were used as wall materials. Juniper berry (*Juniperus communis*) essential oil and black pepper peppercorns (*Piper nigrum*) essential oil (Ancient Wisdom, Sheffield, Great Britain) are firstly dissolved in soybean oil (Dary Natury, Koryciny, Poland) or grape seed oil (Basso Fedele e figli s.r.l., Avellino, Italy) were used as core material. Essential oils were dissolved in oil at a concentration of 1% v/v to reduce the risk of their evaporation during the freeze-drying process.

2.2. Preparation of coacervates

As a wall material, 5% gelatin solution with 5% gum Arabic solution was used. Gelatin (G) (Agnex, Białystok, Poland) and gum Arabic (GA) (Warchem, Warsaw, Poland) were dissolved at the temperature of 50 °C. The solutions were mixed in different mixing ratios: 1:1; 1:2, and 2:1. The volume of each system was 300g. After mixing the solutions together, they were subjected to high shear homogenization using Ultra turrax (IKA T18 basic, Germany) for 5 min at 15,000 rpm/min at room temperature. During homogenization, each variant was supplemented with 10% (30 g) of soybean oil (SBO) or grape seed oil (GSO), in which juniper (JEO) or black pepper essential oil (BPEO) had been dissolved for 1% of the system volume (3 g). After emulsification, the pH was adjusted to 4,0 (under the isoelectric point) using 1 mol HCl. All emulsions were stored at 4°C for 24h, then transferred to -20°C for 24h and then again transferred at -60°C for the next 24h. The frozen samples were lyophilized for 72h at -80°C. After this time, the lyophilizates were screened on a laboratory sieve with a mesh size of 710 µm, then vacuum packed and stored at 4°C for further determinations.

The samples were coded for easier identification (Tab. 1). Mixing ratio was marked as 1 - 1:1, 2 - 1:2, 3 - 2:1, juniper essential oil - J, black pepper oil - B, soybean oil - S, grape seed oil - G. For example, sample where G/GA = 1:1, containing juniper essential oil dissolved in soybean oil has been coded as SJ1.

Tab. 1: Coding of samples

Oil	Essential oil	Mixing ratio G/GA	Code
Grape seed	Juniper	1:1	GJ1
		1:2	GJ2
		2:1	GJ3
	Black pepper	1:1	GB1
		1:2	GB2
		2:1	GB3
Soybean	Juniper	1:1	SJ1
		1:2	SJ2
		2:1	SJ3
	Black pepper	1:1	SB1
		1:2	SB2
		2:1	SB3

2.3. Complex coacervation yield, solid yield, and encapsulation efficiency

To determine the efficiency of the complex coacervation yield (CY) was calculated according to the equation [24]:

$$CY = \frac{CM}{SM} * 100\%$$

CM – coacervate mass collected after the process

106 SM – total mass of the freeze-dried powder

107 To determine the losses during the freeze-drying process, the solid yield (SY %) was
108 calculated according to the equation [24]:

109
$$SY = \frac{PM}{LCM} * 100\%$$

110 Where:

111 PM – powder mass collected after freeze-drying

112 LCM – liquid coacervate mass, before the freeze drying process

113 Encapsulation efficiency (EE) was measured according to the method described by Hernandez-
114 Nava [25] with slight modifications. Briefly, it was calculated by measuring the surface oil (S_O)
115 and total oil (T_O) of the freeze-dried powders. All measurements were done in triplicates.

116 S_O was determined by weighing 1g of sample and dispersion in 30 ml of n-hexane with constant
117 stirring (60 rpm) for 15 min. The oil phase with n-hexane was filtered into the pre-weighted
118 round bottom flask and evaporated on a rotary evaporator (R-100 Büchi, Switzerland). After
119 that, the sample was stored in the oven at 105°C for 30 minutes to ensure all n-hexane had
120 evaporated. After this, the sample was left in the desiccator until it was cooled down (1h). The
121 round bottom flask was weighed, and the S_O was calculated as:

122
$$S_O = OM_1 - OM_2$$

123 Where:

124 OM₁ – oil mass after extraction and evaporation of the solvent

125 OM₂ - the theoretical weight of oil from the sample

126 T_O was determined by weighing 1.5 g of the sample and spreading it in 4 ml of KCl. In the next
127 step, 4 ml of acetone and 8 ml of chloroform were added to the sample. The sample prepared
128 was constantly stirred (60 rpm) for 15 min and then centrifuged (5000 rpm, 5 min). The sample
129 was separated during centrifugation. The top layer was poured, and the bottom layer containing
130 chloroform and the oil phase was passed through a filter paper with anhydrous sodium sulfate
131 into a weighed round-bottom flask, so the test sample remained in the falcon test tube. After
132 that, 4 ml of double-distilled water, 4 ml of acetone, and 8 ml of chloroform were added to the
133 residue. The sample was centrifuged again, the top layer was poured, and the bottom layer was
134 soaked through filter paper. Then the solvent was evaporated on a rotary evaporator (R-100
135 Büchi, Switzerland). After that, the sample was stored in the oven at 105°C for 30 minutes to
136 evaporate the excess chloroform. After this, the sample was left in the desiccator until it was
137 cooled down (1h). The round bottom flask was weighed, and the T_O was calculated as:

138
$$T_O = OM_1 - OM_2$$

139 Where:

140 OM₁ – oil mass after extraction and evaporation of the solvent

141 OM₂ - the theoretical weight of oil from the sample

142 Having the results for S_O and T_O, EE (%) was calculated according to the formula:

$$EE = \frac{T_o - S_o}{T_o} * 100\%$$

144

145 2.4. Bulk and tapped density, Carr index (CI), and Hausner ratio (HR)

146 The bulk density (ρ_{bulk}) was determined by measuring the volume occupied by a known quantity
 147 of powder in a 10 mL measuring cylinder, measuring its weight before and after adding the
 148 powder [25]. Similarly, the tapped density (ρ_{tap}) was determined in a 10 ml measuring cylinder
 149 by repeatedly tapping manually by lifting and dropping the cylinder under its own weight at a
 150 vertical distance of 14 ± 2 mm high for one minute (one tap per second) [26]. Determinations
 151 were made in triplicate. Bulk and tapped densities were expressed in g/cm^3 .

152 The compressibility index (CI) and Hausner ratio (HR) are measures of the tendency of a
 153 powder to be compressed. Determinations were made in triplicate. To calculate the CI and HR,
 154 ρ_{bulk} and ρ_{tap} were used in these equations [27]:

$$CI = \frac{\rho_{\text{tap}} - \rho_{\text{bulk}}}{\rho_{\text{tap}}} * 100\%$$

$$HR = \frac{\rho_{\text{tap}}}{\rho_{\text{bulk}}}$$

157 2.5. Color measurement

158 The color of the microcapsules was examined using a Minolta CR-400 colorimeter (Konica
 159 Minolta Inc., Japan) (D65 illuminate, measuring surface – 8 mm, standard 2° observers). The
 160 measurement results were expressed following the system of the International Commission on
 161 Lighting (Comission Internationale de L'Eclairage) in the CIELab color space. These parameters
 162 were determined and tested: L * (L = 0 (black), L = 100 (white)), a * (–a = green, + a = red),
 163 and b * (–b = blue, + b = yellow) [28]. Determinations were made in triplicate immediately
 164 after production.

165 2.6. Solubility, hygroscopicity, and moisture content

166 The solubility of samples was measured according to the method described by Shaddel et al.
 167 [29]. Briefly, 0,5g of freeze-dried sample was weighed into a falcon test tube containing 50 ml
 168 of double distilled H_2O . The sample prepared in this way was homogenized for 30 min and then
 169 centrifuged (5000 rpm, 5 min). After centrifugation, 25 mL aliquot of the supernatant was
 170 transferred into a dried and pre-weighed Petri dish and immediately oven-dried at 105°C for 6h.
 171 Then the solubility (%) was calculated by weight difference. Determinations were made in
 172 triplicate.

173 The hygroscopicity of samples was measured according to the method described by Shaddel
 174 et al. [29]. Briefly, 0,2g of each freeze-dried sample was placed in a Petrie dish and stored in a
 175 container containing the saturated solution of Na_2SO_4 for one week. Hygroscopicity was
 176 expressed as g of water absorbed per 100g sample (%). Determinations were made in
 177 triplicate.

178 The moisture content of microcapsules was determined gravimetrically by drying in a Binder
 179 FP 115 drying oven (Binder, Tuttlingen, Germany) at 70°C for at least 24 h, then cooling to

room temperature in desiccators until constant weight. Determinations were made in triplicate.

2.7. Particle size distribution

The measurement was carried out with the Morphologi® G3SE apparatus (Malvern Instruments Ltd., Malvern, UK) equipped with a dispersion unit for dry samples. The particle size distribution was calculated as the relative volume of molecules in the band shown as size distribution curves (Malvern Microsoft ware v.5.40, Malvern Instruments Ltd.). The examined parameters of the size distribution contained the largest particle size (D [v, 0.9]), mean particle volume (D [v, 0.5]), and the smallest particle size (D [v, 0.1]) [30]. The particle size distribution (Span index – SI) was estimated using the following formula [31]:

$$SI = \frac{D_{90} - D_{10}}{D_{50}}$$

2.8. Statistical analysis

For statistical analysis, the STATISTICA computer program was used. To check significant differences between the results, one-way and the multivariate analysis of variance ANOVA and the Fisher LSD test (p-value < 0.05, $\alpha = 95\%$) were used.

3. Results and discussion

3.1. Complex coacervation yield, solid yield, and encapsulation efficiency

The important factor affecting the complex coacervation process is the protein-polysaccharide mass ratio – different ratios affect the intensity of interaction and complexation, due to the charge balance between protein and polysaccharide [25, 32, 33]. Statistical analysis showed that the obtained results were influenced by the mixing ratio, the essential oil, the oil used, and the interactions between them. However, the mixing ratio had the greatest impact on obtained CY and SY values; right behind it, the EO was the most influential (Tab. 2). The mixing ratio had a strong positive effect on the CY, while it was a negative on the SY. Same for oil, although the effect of this factor was small. On the other hand, in the case of EO, the effect was the opposite - the content of EO caused a slight decrease in CY and a slight increase in SY values. In this study, both the coacervation yield (CY) and the solid yield (SY) were low and did not exceed 50%. Samples GJ3, GB3, SJ3, and SB3 presented the highest CY value and presented significant differences (p<0.05) in the CY. The samples had the same mixing ratios of G/GA (2:1), and the EOs was dissolved in different oil. Despite the highest CY value, it did not reflect the highest SY. These samples had the lowest SY values, while the highest SY was obtained for samples SB2, GB2, GJ2, and SB1, which were characterized by the lowest values of CY.

Gelatin consists primarily of glycine, proline, hydroxyproline, alanine, and lysine [34]. These amino acids, therefore, supply positively charged NH^{3+} ions to the coacervation process. Gum Arabic is primarily negatively charged COO^- ions derived from arabinic acid [35]. This process depends on the electrostatic attraction between these oppositely charged ions [36]. For every protein-polysaccharide mixture, there is always a specific molar mixing ratio to obtain a maximum coacervate yield – when protein and polysaccharide have the exact opposite charge density leading to charge neutralization [14, 36, 37]. Probably this state was achieved for a mixing ratio of 2:1, where the ratio of gelatin to gum Arabic was the highest. Therefore, it resulted in the highest CY. Gelatin has a high ability to bind water in its structure [38, 39].

Nevertheless, these polymer bind water with a weak hydrogen bond, which disintegrates and releases water during the freeze-drying process [40]. Hernandez-Nava [25] used G and GA as wall material (mixing ratio 2:1) and obtained an SY in the 72-88% range. These differences may be caused primarily by the difference in pH during the coacervation process and the difference in the selection of the drying method.

The encapsulation efficiency is the percentage of the core material enclosed in the powder particles; it is a highly important parameter for essential oils because they are volatile compounds very prone to loss during the drying process. Statistical analysis showed that the results were influenced by the interaction between the mixing ratio of polymers and oil (Tab. 2). The type of oil had a clear positive effect on the EE, causing it to increase. The highest EE value was obtained for sample SJ1 ($64.09\% \pm 0.09$). High encapsulation efficiency was also obtained for samples SB3 and GJ2 (61.92 ± 0.04 and 59.89 ± 0.01 , respectively), and it presented significant differences ($p < 0.05$) in the EE. The GB2, SB1, and SJ2 samples did not present significant differences ($p < 0.05$) in the EE – the values were in the middle of the obtained range (53.4-55.25%). However, similarities can be found in samples SB1 and GB2, which contained the same oil and essential oil, and in samples GB2 and SJ2 which had the same mixing ratio (1:2). The influence of interaction between mixing ratio and oil on the EE value is clearly visible in those cases. GJ1 and GJ3 samples were also in the same statistical group (49.30 ± 0.07 and 49.65 ± 0.02 , respectively) and contained the same EO. The lowest EE value was achieved by the GB1 sample (Fig. 1).

Similar studies were conducted by Rajnibhas et al. [41]. Various concentrations of wall and core material were tested by these researchers. A complex coacervation was performed between G and GA at pH = 4.0 with a mixing ratio of 1:1; 1:2, and 2:1. It is well known that encapsulation efficiency increases with increasing polymer concentration. The results confirmed this - the lowest EE was obtained with a mixing ratio of 1:1 and a core material content of 10% (38.56%). The highest with a mixing ratio of 2:1 and the content of core material 5% (95.15%). However, the core material also affects encapsulation efficiency - the more, the lower the EE is. Perhaps using less than 10% w/w of core material or increasing the concentration of polymers would result in higher EE values. This approach was successfully applied by Manaf et al. 2018 [42]. Their used complex coacervation between the G:GA to microencapsulate citronella EO. The mixing ratio was 1:1, but polymer concentration was higher (12.5% v/w), and the core material was lower (approx. 1%). The encapsulation efficiency of the system prepared in this way was 94.42%. It is worth noting that Manaf et al. [42] used cross-linking agents and higher pH (4,5).

Tab. 2: Complex coacervation yield (CY), solid yield (SY), and encapsulation efficiency (EE) values [%].

Sample	CY %	SY %	EE %
GJ1	30.93 ± 0.44^b	29.79 ± 1.21^c	49.3 ± 0.07^{abe}
GB1	30.49 ± 0.56^b	29.25 ± 1.29^c	42.7 ± 0.11^e
GJ2	28.01 ± 0.16^a	37.11 ± 0.49^e	59.89 ± 0.01^{cdf}
GB2	29.45 ± 0.08^d	36.23 ± 0.08^{de}	55.25 ± 0.01^{abcd}
GJ3	39.22 ± 0.16^g	21.32 ± 0.25^a	49.65 ± 0.02^{abe}
GB3	41.64 ± 0.15^i	23.01 ± 0.36^b	47.21 ± 0.04^{ae}
SJ1	33.78 ± 0.46^f	26.58 ± 0.36^g	64.09 ± 0.09^f

Effect	SB1	23.95±0.16 ^c	35.44±0.47 ^d	54.14 ±0.05 ^{abcd}
	SJ2	31.91±0.12 ^e	33.8±0.02 ^h	53.4 ±0.04 ^{abcd}
	SB2	28.34±0.12 ^a	38.45±0.34 ⁱ	52.33 ±0.01 ^{abc}
	SJ3	46.58±0.19 ^j	22.02±0.18 ^{ab}	56.8 ±0.01 ^{bcd^f}
	SB3	41.04±0.51 ^h	20.21±0.61 ^f	61.92 ±0.04 ^{df}
	S.E.M.	0.369	0.096	26.524
	Oil	**	NS	**
	Essential oil	**	**	NS
	Mixing ratio	**	**	NS
	Oil x Essential oil	**	**	NS
	Essential oil x mixing ratio	**	**	**
	Oil x mixing ratio	**	**	NS
	Oil x essential oil x mixing ratio	**	**	NS

Results in this table are expressed as mean ± standard deviation. Mean values with the same superscript letters within a row are not significantly different at $p \leq 0.05$.

S.E.M. – standard error of the mean

**= $p \leq 0.001$; NS – non – significant effect = $p > 0.05$

3.2. Bulk density, tapped density, Carr Index (CI), and Hausner Ratio (HR)

Bulk density (ρ_{bulk}) is defined as the ratio of the mass to the volume (including the inter-particle void volume) of an untapped (loose) powder sample. If the bulk density is high, the volume of the powder is lower; when the bulk density is low, the same powder mass takes up a larger volume. The tapped density (ρ_{tap}) is defined as an increased bulk density attained after mechanically tapping a cylinder containing the powder sample. This property influences the appropriate selection of the size of the containers for packing the powder (e.g., barrels, bags). Analysis of bulk and tapped density of powder gives a possibility to calculate the compressibility index (Carr index) and Hausner Ratio of the powder [27].

Carr Index (CI) is a measure of powder bridge strength and stability, and the Hausner Ratio (HR) is a measure of interparticulate friction [43]. Based on them, the flow character is rated. Bridging of powders occurs when materials interlock or bond to build an arch above the outlet of a container they are in. Cohesive strength and the internal friction of the individual particles will determine the potency of the arch itself. When this occurs, the arch holds the rest of the container's contents, preventing the remaining powder's discharge. A powder's lower CI or lower HR indicates better flow properties than higher ones. A CI of <10 or HR of <1.11 is

considered 'excellent' flow, whereas CI >38 or HR >1.60 is considered 'very very poor' flow [43].

Tab. 3: bulk density, tapped density, Carr Index, and Hausner Ratio [g/cm³].

	Sample	Bulk density g/cm ³	Tapped density g/cm ³	Carr Index g/cm ³	Hausner Ratio g/cm ³
	GJ1	0.12±0.01 ^c	0.24±0.00 ^{abc}	50.27±2.53 ^d	2.01±0.11 ^d
	GB1	0.11±0.01 ^c	0.22±0.02 ^{ab}	48.84±7.73 ^d	1.98±0.28 ^d
	GJ2	0.16±0.01 ^a	0.23±0.01 ^{ab}	30.58±4.93 ^a	1.45±0.10 ^a
	GB2	0.17±0.01 ^b	0.26±0.00 ^{de}	33.56±3.46 ^{ab}	1.51±0.08 ^{ab}
	GJ3	0.16±0.01 ^{ab}	0.24±0.01 ^{abc}	31.81±4.45 ^a	1.47±0.09 ^{ab}
	GB3	0.16±0.01 ^a	0.24±0.01 ^{abc}	34.64±2.13 ^{ab}	1.53±0.05 ^{ab}
	SJ1	0.12±0.00 ^c	0.22±0.03 ^a	44.35±6.21 ^{cd}	1.81±0.21 ^{cd}
	SB1	0.14±0.02 ^d	0.23±0.01 ^{ab}	38.05±8.82 ^{abc}	1.64±0.22 ^{abc}
	SJ2	0.16±0.00 ^{ab}	0.25±0.00 ^{cd}	34.74±0.64 ^{ab}	1.53±0.02 ^{ab}
	SB2	0.16±0.00 ^{ab}	0.25±0.01 ^{bcd}	34.33±2.05 ^{ab}	1.52±0.05 ^{ab}
	SJ3	0.17±0.01 ^{ab}	0.28±0.00 ^e	40.28±4.6 ^{bc}	1.68±0.13 ^{bc}
	SB3	0.15±0.01 ^{ad}	0.24±0.01 ^{abcd}	37.75±2.98 ^{abc}	1.61±0.08 ^{abc}
	S.E.M.	0.000	0.000	23.152	0.020
Effect	Oil	NS	NS	NS	NS
	Essential oil	NS	NS	NS	NS
	Mixing ratio	*	**	**	**
	Oil x Essential oil	NS	*	NS	NS
	Essential oil x mixing ratio	*	*	*	*
	Oil x mixing ratio	NS	*	NS	NS
	Oil x essential oil x mixing ratio	*	*	NS	NS

Results in this table are expressed as mean ± standard deviation. Mean values with the same superscript letters within a row are not significantly different at $p \leq 0.05$

S.E.M. – standard error of the mean

* = $p \leq 0.05$; ** = $p \leq 0.001$; NS – non – significant effect = $p > 0.05$

The powders obtained in this study were characterized by a very low bulk density in the range of 0.11-0.17 and a tapped density from 0.22 to 0.28 (Tab. 3). Tapped density was higher than bulk density since smaller particles occupy the voids between larger particles and attain a dense packing condition because of the tapping [44]. The greatest influence on the obtained bulk

density was the mixing ratio and the interaction between the mixing ratio, oil, and essential oil. All factors caused an increase in bulk density. The samples showed statistically significant differences among themselves ($p < 0.05$). Similarly, in the case of tapped density, the mixing ratio and interactions between it and the oil and essential oil had the greatest impact on the obtained results. The influence of the mixing ratio was so positive that it caused the tapped density value to increase as it increased. Oil also had a slight positive effect while EO had a slight negative effect. The samples differed statistically significantly ($p < 0.05$). The results correlate with those obtained by Hernandez-Nava et al. [25], who used the complex coacervation between gelatin and gum Arabic and gelatin and chia mucilage (CM) to microencapsulate oregano EO. Depending on the spray drying parameters, the bulk density in their study was in the range for G:GA 0.157-0.202 and 0.234-0.282 for G:CM. In contrast, the tapped density for G:GA was 0.0.314-0.404, and for G:CM 0.335-0.403.

In CI and HR, the obtained values were influenced by the mixing ratio and its interaction with the oil. The increase in the mixing ratio resulted in a decrease in the cohesiveness of the obtained powders, and thus a decrease in the CI and HR values. The samples differed statistically significantly ($p < 0.05$). The GB2, GB3, SJ2, and SB2 samples were in the same statistical group (Tab. 3), presenting the CI in the range of 31.81-34.74 and the HR in the range of 1.47-1.53, thus having high cohesiveness and very poor flow [43]. These were also the lowest CI and HR values obtained in this study. The highest values were recorded for the GJ1 and GB1 samples, which were also together in the same statistical group ($p < 0.05$). CI values were 50.27 ± 2.53 and 48.84 ± 7.73 , respectively, and HR values were 2.01 ± 0.11 and 2.98 ± 0.28 , respectively. These powders had the highest cohesiveness and virtually no flow [43]. The results are again in line with the work of Hernandez-Nava et al. [25], who report that the powders obtained by spray drying G:GA coacervates were characterized by a Carr Index of approx. 50, while the HR was approx. 2. Similarly, for the G:CM system, CI was approx. 30 and HR approx. 1.43. Therefore, it can be concluded that the powders obtained because of complex coacervation are characterized by very high cohesiveness and virtually no flow.

3.3. Color measurement

The color of obtained powders was affected mainly by the oil used to dissolve EO – grape seed oil is slightly greenish, while soybean oil has an intense yellow color. But the powder color was also influenced by the mixing ratio and essential oil as well as interactions between all factors.

The samples differed statistically significantly for each of the color components ($p < 0.05$) (Tab. 4). In terms of the brightness of the samples (L^*), the differences were not considerable. The L^* parameter values ranged from 78.72 ± 0.34 to 88.52 ± 0.25 for the samples containing soybean oil and grape seed oil. The same statistical homogenous group was determined for GJ3, GB3, and SJ1 samples and for SJ2 and SB2. The a^* parameter assumed negative values for all samples, corresponding to the green color. For samples containing GSO, the value of a^* ranged from -1.19 ± 0.02 to -3.83 ± 0.15 . Undoubtedly, the a^* value was influenced by the mixing ratio - along with its increase, the proportion of green color also increased. For samples containing SBO, the value of a^* ranged from -7.06 ± 0.27 to -7.66 ± 0.05 . A homogeneous group was determined for samples SB1 and SB2. In the case of the b^* parameter, its value was positive for all samples, corresponding to the yellow color. For samples containing GSO b^* , it was in the range of 5.61 ± 0.04 - 12.3 ± 0.47 , while for those containing SBO 41.87 ± 1.05 - 51.71 ± 0.47 .

Syed et al. [45] conducted color measurements of, inter alia, unrefined soybean oil. The results obtained in the study correlate with those obtained in this study in terms of the b^* parameter - the researchers indicate the value of b^* as 40.0 ± 0.60 . There is some discrepancy in the case of the a^* parameter. The team indicated positive values (4.0 ± 0.65), while the results we obtained clearly indicate negative a^* values. Such a change in the a^* parameter should be attributed to the wall materials used for encapsulation and to the presence of EO. Bruhl and Unbehend [46] carried out measurements of the color of grape seed oil. The L^* parameter obtained by the researchers was similar to the results obtained in this study - $L^* = 99.44$. In turn, parameters a^* and b^* had higher values, although with the same sign: $a^* = -4.68$, $b^* = 12.49$. Again, the discrepancy in the results was largely caused by wall materials and essential oil.

Tab. 4: Color parameters of obtained powders

	Sample	L^*	a^*	b^*
	GJ1	86.99 ± 0.32^{cd}	-1.74 ± 0.14^h	6.3 ± 0.19^{bc}
	GB1	87.08 ± 0.23^c	-1.19 ± 0.02^i	5.61 ± 0.04^b
	GJ2	88.52 ± 0.25^g	-2.09 ± 0.02^g	6.56 ± 0.13^c
	GB2	87.00 ± 0.11^c	-2.91 ± 0.01^e	9.24 ± 0.02^d
	GJ3	84.46 ± 0.97^a	-3.05 ± 0.08^e	9.47 ± 0.37^d
	GB3	84.10 ± 0.87^a	-3.83 ± 0.15^f	12.30 ± 0.47^f
	SJ1	84.52 ± 0.05^a	-7.66 ± 0.05^c	46.52 ± 0.13^{ae}
	SB1	86.07 ± 1.03^{bc}	-7.37 ± 0.21^{ab}	41.87 ± 1.05^g
	SJ2	85.64 ± 0.33^b	-7.38 ± 0.14^{ab}	45.82 ± 0.31^e
	SB2	85.70 ± 0.30^b	-7.49 ± 0.11^{ac}	46.86 ± 0.38^a
	SJ3	78.72 ± 0.34^d	-7.06 ± 0.27^d	51.71 ± 0.47^h
	SB3	82.29 ± 0.56^f	-7.2 ± 0.07^{bd}	47.06 ± 0.64^a
	S.E.M.	0.302	0.017	0.201
Effect	Oil	**	**	**
	Essential oil	*	**	**
	Mixing ratio	**	**	**
	Oil x Essential oil	**	**	**
	Essential oil x mixing ratio	**	**	NS
	Oil x mixing ratio	**	**	**
	Oil x essential oil x mixing ratio	*	**	**

∴ Results in this table are expressed as mean $\pm$ standard deviation. Mean values with the same superscript letters within a row are not significantly different at $p \leq 0.05$

S.E.M. – standard error of the mean

*= $p \leq 0.05$; **= $p \leq 0.001$; NS – non – significant effect = $p > 0.05$

3.4. Solubility, hygroscopicity, and moisture content

In the food industry, powders are required to present good solubility in water to increase their range of applications. Nevertheless, the purpose of complex coacervation is to obtain water-insoluble microcapsules, making it possible to use a controlled release of the core material. In our study, all samples showed low (>26%) solubility in water (Tab.5). The solubility of the obtained powders was influenced mainly by the mixing ratio of polymers but also by the interaction between the mixing ratio and the oil, and the interaction between the mixing ratio and EO ($p < 0.05$). Those interactions caused decreasing of the powders solubility. The least soluble samples were GJ3, GB3, and SB3, for which a homogeneous statistical group was determined. The solubility was below 8%. The influence of the mixing ratio and the oil used to dissolve the EO is visible here. The SB2 sample was the most soluble ($25.86\% \pm 6.81$). The results correlate with those obtained by Shaddel et al. [29]. The powders obtained because of freeze-drying coacervates (5% v/v G/GA) were characterized by low water solubility, not exceeding 30%. Caparino et al. [26] investigated the water solubility of mango powders obtained from the emulsion by freeze-drying. The solubility of over 85% characterized these powders. Fernandez et al. [10] also investigated spray-dried powders containing EO ginger solubility. Depending on the wall material used (whey protein; whey protein: maltodextrin; whey protein: inulin), the solubility of the powders was in the range of 76-82%. The above indicates that the powders obtained by the method of complex coacervation show much lower solubility in water than those obtained from the classical emulsion.

Hygroscopicity plays a significant role concerning the stability of food products, storage conditions, and packaging materials [45]. Powders with hygroscopic values higher than 15.1% are highly hygroscopic [48]. The hygroscopicity of the powders in this study ranged from 1.49 to 7.29 % (Tab. 5). All showed low hygroscopicity. The examined factors had no statistically significant influence on the hygroscopicity of the obtained powders ($p < 0.05$). No statistical differences were found between samples.

Caparino et al. [26] researched the hygroscopicity of mango powders obtained by freeze-drying. Researchers indicate these powders were characterized by hygroscopicity at about 18%. In turn, Zotarelli et al. [48] report that mango powders obtained by spray drying were characterized by high hygroscopicity in the range of 23.9-26.9%. The higher hygroscopicity of the powders, in this case, could result from spray drying. On the other hand, Fernandez et al. [31] report that the hygroscopicity of the powders obtained by spray drying containing rosemary oil ranged from 43,6-46,8% depending on the core material (10% or 30%). The powder containing more essential oil was less hygroscopic. This correlates with the results obtained by Tavares et al. [49]. The coacervates with ginger EO showed lower hygroscopicity than coacervates without, mainly due to the hydrophobic nature of ginger EO compounds [31]. Hygroscopicity ranged from 6.64-9.65%, was low (below 15.1%), and was much more similar to the values obtained in this study. Thus, it can be concluded that both the addition of essential oil and the use of complex coacervation as a method of microencapsulation have a positive effect on the hygroscopicity of the obtained powders, reducing it.

387 Tab. 5: Solubility, hygroscopicity, and moisture content [g/100g].

		Solubility	Higroscopicity	Moisture content
		g/100g	g/100g	g/100g
	GJ1	19.4±2.92 ^{be}	2.53±0.32 ^a	0.27±0.04 ^a
	GB1	18.22±3.35 ^{bd}	3.77±2.51 ^a	0.29±0.42 ^a
	GJ2	12.54±3.76 ^{acd}	7.29±8.02 ^a	0.18±0.12 ^a
	GB2	20.7±4.15 ^{be}	6.98±3.85 ^a	0.05±0.05 ^a
	GJ3	7.85±3.54 ^a	2.87±2.15 ^a	0.56±0.62 ^a
	GB3	6.58±2.69 ^a	6.00±4.84 ^a	0.36±0.29 ^a
	SJ1	16.39±4.46 ^{bcd}	4.92±4.18 ^a	0.30±0.21 ^a
	SB1	10.74±2.41 ^{ac}	2.63±0.17 ^a	0.24±0.25 ^a
	SJ2	18.72±5.65 ^{bd}	4.32±3.06 ^a	0.44±0.66 ^a
	SB2	25.86±6.81 ^e	2.86±0.82 ^a	0.28±0.24 ^a
	SJ3	11.14±3.01 ^{ac}	2.71±1.97 ^a	0.36±0.30 ^a
	SB3	7.47±1.34 ^a	1.48±0.48 ^a	0.49±0.08 ^a
	S.E.M.	15.466	12.105	0.113
Effect	Oil	NS	NS	NS
	Essential oil	NS	NS	NS
	Mixing ratio	**	NS	NS
	Oil x Essential oil	NS	NS	NS
	Essential oil x mixing ratio	*	NS	NS
	Oil x mixing ratio	*	NS	NS
	Oil x essential oil x mixing ratio	NS	NS	NS

388 Results in this table are expressed as mean ± standard deviation. Mean values with the same superscript letters
 389 within a row are not significantly different at $p \leq 0.05$

390 S.E.M. – standard error of the mean

391 $* = p \leq 0.05$; $** = p \leq 0.001$; *NS* – non – significant effect = $p > 0.05$

392 The moisture content is an important property of the powders, related to storage stability and
 393 shelf life. Moisture content was low and ranged from 0.05% to 0.56% (Tab. 5). The examined
 394 factors had no statistically significant influence on the hygroscopicity of the obtained powders
 395 ($p < 0.05$). No statistical differences were found between samples. Fernandez et al. [10] showed
 396 that EO ginger powders obtained by spray drying had a moisture content of 1.05-1.90%
 397 depending on the wall material used. Similar results were obtained by Zotarelli et al. [48], where
 398 moisture content was in the range of 1.2-4.7%. In turn, the powders obtained by freeze-drying

coacervates containing ginger EO, depending on the used wall material (gum Arabic:chitosan; whey protein:gum Arabic), were characterized by the moisture content of approx. 3.04% and 3.10% respectively. It was observed that the addition of essential oil significantly reduced these values. Without adding EO, the coacervates produced between gum Arabic and chitosan and whey protein and gum Arabic were characterized by the moisture content of approx. 6.01% and 8.02%, respectively. The selection and concentration of wall materials significantly impact moisture content. The results obtained in this study correlate with those obtained by Rajnibhas et al. [41], who by mixing gelatin and gum Arabic in a 1:1 ratio, obtained powders with a moisture content of 0.32% (3% w/v and 5% of core material), 0.7% (7% w/v and 5% of core material), 0.37% (3% w/v and 10% of core material), and 0.72% (7% w/v and 10% of core material).

3.5. Particle size distribution

The size of microcapsules should be in the range of 0.1 μm to 100 μm [12]. The particle sizes in all tested trials met these assumptions. The obtained results depended on all examined factors individually and their interactions. However, the mixing ratio of polymers had the greatest influence ($p < 0.05$). The highest values of the SI characterized the GJ3, GB3, SB3, and SJ3 samples. This means they had the highest diversity in terms of particle size (Tab. 6) [50]. In sample GJ3, the SI was the closest to 1.0 ($\text{SI}=0.96$), so this powder contained particles with the most uniform size distribution in the tested ranges [50]. The smallest range of particle sizes and the smallest SI value was found for samples SB2 and GB2 ($D_{10} = 59.04 \pm 1.18 \mu\text{m}$, $D_{90} = 96.72 \pm 0.5 \mu\text{m}$, $\text{SI} = 0.43 \pm 0.01$ and $D_{10} = 60.13 \pm 0.74 \mu\text{m}$, $D_{90} = 96.86 \pm 0.41 \mu\text{m}$, $\text{SI} = 0.44 \pm 0.01$ respectively). The smallest powder particles were obtained for a mixing ratio of 1:1 (GB1, SJ1, SB1). However, no clear relationship was observed between the increase in the content of one polymer and the size of the obtained powder particles.

Hernandez-Nava et al. [25] conducted similar studies using complex coacervation between G and GA. In the case of this study, the differentiation of the samples concerned the temperature and duration of the spray-drying process. Depending on the parameters, the results differed from each other, however slightly. Researchers report D_{10} in the range of 6.08-8.11 μm , D_{50} in the range of 14.30-16.34 μm and D_{90} in the range of 25.12-30.31 μm . It is known, however, that spray drying allows to obtain smaller particles [24]. Measurements of the particle size of smoke powder food flavoring showed that the spray-dried preparation was characterized by much smaller particle sizes (6.3-6.9 μm) than in the case of freeze drying (134.7-580.4 μm).

Tab. 6: Particle size and particle size distribution index - span (SI) [μm].

Sample	Particle size μm	$D_{10} \mu\text{m}$	$D_{50} \mu\text{m}$	$D_{90} \mu\text{m}$	SI μm
GJ1	20.48 ± 0.16^h	56.79 ± 0.62^b	83.42 ± 0.36^b	96.84 ± 0.35^a	0.48 ± 0.01^b
GB1	5.55 ± 0.29^a	45.86 ± 0.33^f	82.79 ± 0.75^{ab}	95.7 ± 0.34^b	0.6 ± 0.00^e
GJ2	9.18 ± 0.14^f	53.66 ± 0.54^h	81.98 ± 0.64^a	95.55 ± 0.41^b	0.47 ± 0.01^b
GB2	10.5 ± 0.25^c	60.13 ± 0.74^c	85.82 ± 1.46^d	96.86 ± 0.41^a	0.44 ± 0.01^a
GJ3	4.51 ± 0.18^d	51.9 ± 0.4^g	81.7 ± 0.98^a	95.37 ± 0.36^b	0.96 ± 0.01^g
GB3	12.74 ± 0.34^g	43.14 ± 1.01^e	77.77 ± 0.18^i	95.38 ± 0.26^b	1.17 ± 0.01^h
SJ1	5.57 ± 0.35^a	22.2 ± 0.25^d	72.98 ± 0.79^b	92 ± 0.18^d	0.53 ± 0.01^d
SB1	5.71 ± 0.38^a	17.37 ± 0.46^a	68.25 ± 0.58^g	97.24 ± 0.1^a	0.67 ± 0.01^f
SJ2	6.67 ± 0.29^b	57.59 ± 0.88^b	84.11 ± 0.29^{bc}	96.78 ± 0.55^a	0.51 ± 0.01^c
SB2	10.61 ± 0.35^c	59.04 ± 1.18^c	84.82 ± 0.22^{cd}	96.72 ± 0.5^a	0.43 ± 0.01^a

	SJ3	6.48±0.46 ^b	17.42±0.5 ^a	51.6±1.16 ^e	90.01±0.14 ^b	1.41±0.02 ^j
	SB3	7.59±0.34 ^e	16.69±0.25 ^a	60.77±0.94 ^f	92.67±0.14 ^e	1.25±0.02 ⁱ
	S.E.M.	0.094	0.437	0.630	0.118	0.000
Effect	Oil	**	**	**	**	**
	Essential oil	NS	**	*	**	**
	Mixing ratio	**	**	**	**	**
	Oil x Essential oil	**	**	**	**	**
	Essential oil x mixing ratio	**	**	**	**	**
	Oil x mixing ratio	**	**	**	**	**
	Oil x essential oil x mixing ratio	**	**	**	**	**

: Results in this table are expressed as mean ± standard deviation. Mean values with the same superscript letters within a row are not significantly different at $p \leq 0.05$

S.E.M. – standard error of the mean

* = $p \leq 0.05$; ** = $p \leq 0.001$; NS – non – significant effect = $p > 0.05$

4. Conclusions

Complex coacervation between gelatin and gum Arabic can be used to microencapsulate essential oils. However, not all the combinations of mixing ratios were equally effective. The highest encapsulation efficiency characterized the SJ1 sample.

A relatively low EE was obtained, influenced by the interaction between the mixing ratio and the oil. A low efficiency was probably caused by a too high concentration of core material in relation to the wall material. The highest EE value was obtained for sample SJ1 (64.09%±0.09). Similarly, high encapsulation efficiency was obtained for samples SB3 and GJ2 (61.92±0.04 and 59.89±0.01, respectively). Thus, no clear influence of the mixing ratio between gelatin and gum Arabic was demonstrated on the obtained efficiency of the encapsulation process. The powders obtained because of lyophilization of the coacervates with a 1:1 mixing ratio were characterized by the smallest particle sizes. However, this did not reflect an increase in bulk and tapped density, as well as did not reflect an increase in their hygroscopicity. However, the powders containing G/GA = 1:1 were the most soluble among the powders tested. The least dissolving microcapsules were obtained with a mixing ratio of G/GA = 2:1.

Further research should be carried out to find the optimal mixing ratio between gelatin and gum Arabic that would allow for higher encapsulation efficiency.

5. Data availability

All data generated or analysed during this study are included in this published article

6. Authors statements

The authors declare they have no financial interests. The authors have no competing interests to declare that are relevant to the content of this article.

7. Authors contribution

Alicja Napiórkowska - did the research, wrote the main manuscript, prepared figures and tables

Marcin Andrzej Kurek - supervised the content and introduced corrections to the text

8. References

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