PAPER

THE STABILITY OF SPRAY-DRIED MICROENCAPSULATED β-CAROTENE IN THE MIXTURE OF GUM ARABIC, OSA-TYPE MODIFIED STARCH AND MALTODEXTRIN

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ABSTRACT

The study analyzed effects of mixtures of three carriers: gum Arabic, OSA-type modified starch and maltodextrin, applied in different ratios on the properties of β-carotene microencapsulated by spray drying. β-Carotene emulsions were prepared using high-pressure two-stage homogenization. Emulsion properties and microcapsule features were characterized. Correlations between the studied characteristics were determined with the use of Principal Component Analysis (PCA).
The study showed that the use of media mixed in equal proportions increased pigment retention during the storage of microcapsules to a lesser extent than the mixtures with different ratios of the carriers. The matrix which was the most effective in protecting the core was made of a mixture of modified starch, gum Arabic and maltodextrin in the ratio of 1:3:2. It was also found that the higher content of gum Arabic in the mixture of carriers was more effective in preventing β-carotene degradation than an increasing content of OSA-type modified starch.

Keywords: carotenoids, food colorant, storage stability, microstructure, principal component analysis (PCA)

1. INTRODUCTION

β-Carotene arouses interest among carotenoid colorants because it has the highest provitamin activity and is commonly used as a food colorant (FERREIRA and RODRIGUEZ-AMAYA, 2008). Furthermore, it is an antioxidant, which prevents aging (DESOBRY *et al*., 1997) and certain diseases such as heart disease and molecular degeneration (MELÉNDEZ-MARTÍNEZ *et al*., 2007). However, carotenoids are sensitive to thermal and chemical oxidation, and also isomerization influenced by oxygen, light and heating during technological processing and storage (FERREIRA and RODRIGUEZ-AMAYA, 2008). Degradation of β-carotene may also be induced by active prooxidative compounds (e.g. radicals) (CAO-HOANG *et al*., 2011). Moreover, high hydrophobicity of β-carotene makes it insoluble in water and slightly soluble in oil at room temperature (LIANG *et al*., 2013), which also limits its use as a food ingredient and in various medicines (YIN *et al*., 2009). The bioavailability of β-carotene depends on the type of food, factors influencing gastrointestinal absorption and the factors that control its metabolism and distribution in the body. The composition, freshness and content of fat in consumed food are of particular importance. Excessive intake of essential fatty acids causes an increase of β-carotene demand in the body. A low content and poor quality of fat reduce intestinal absorption of β-carotene and carotenes conversion into vitamin A. The intake of vegetable oils and natural antioxidants increases β-carotene bioavailability. Therefore, dietary β-carotene should be incorporated into lipid micelles to ensure effective absorption by intestinal cells (HORNERO-MÉNDEZ and MÍNGUEZ-MOSQUERA, 2007). The bioavailability of β-carotene is often limited and depends on food constituents (RIBEIRO *et al*., 2008).

In order not to prevent the loss of bioactive properties of β-carotene, it should be protected from adverse effects of the environment (BONNIE and CHOO, 1999). The process of spray-dried microencapsulation, based on the inclusion of β-carotene into oil-in-water emulsion, can be considered as an effective, inexpensive and convenient method for increasing stability of hydrophobic carotenoids (RIBEIRO *et al*., 2008). In this encapsulation method it is important to select a proper coating material. Usually gum Arabic, maltodextrin and modified starch are used as coatings. Their usability in the microencapsulation process has been described in literature (PRZYBYSZ *et al*., 2012), however none of them meets all requirements of coating materials, thus only their combination may provide a good protection of an active substance.

Literature data shows that the use of a mixture of modified starch, gum Arabic and maltodextrin is more effective than the application of only one of these carriers (BUFFO and REINECCIUS, 2000, KRISHNAN *et al*., 2005, KANAKDANDE *et al*., 2007, DONHOWE *et al*., 2013). Studies of BUFFO and REINECCIUS (2000) showed that a high content gum Arabic in spray-dried emulsions increased retention of microencapsulated substances. KANADANDE *et al*. (2007) found that a mixture of three carriers (gum Arabic, modified starch and maltodextrin) with the highest contribution of gum Arabic was more efficient in the microencapsulation of cumin oleoresin than other mixtures and better than gum Arabic applied as the carrier alone. A similar conclusion was made by KRISHNAN *et al*. (2005) who encapsulated cardamom oleoresin.

The aim of this study was to evaluate the effect of different ratios of gum Arabic, OSA-type modified starch and maltodextrin applied in mixtures used as microcapsules coating on storage stability of β-carotene microencapsulated by spray drying.

2. MATERIALS AND METHODS

2.1. Emulsion preparation

A commercial oil preparation of β-carotene E160a (ii) 1 % OS (Food Colours – Komponenty do żywności) containing 1 % of pigment was used in the study. According to the manufacturer's declaration it was a nature-identical colorant Oil-in-water type pigment emulsions were spray dried. They were made of mixtures of three media: instant gum Arabic E414 (GA), maltodextrin 150 LOW-DE 15.6 (M) and commercial OSA-type modified starch from tapioca "Capsule TA" (sodium starch octenylsuccinate) E1450 (MS), obtained by starch esterification with n-octenyl succinic anhydride acid (<3%). Eight types of emulsions were prepared with the following composition: oily pigment preparation: 10 %, carrier mixture: 25 % and distilled water: 65 %. The media were mixed in the following ratios: sample 1: MS:GA:M – 1:1:1, sample 2: MS:GA:M – 1:2:1, sample 3: MS:GA:M – 1:3:1, sample 4: MS:GA:M – 2:1:1, sample 5: MS:GA:M – 3:1:1, sample 6: MS:GA:M – 1:1:2, sample 7: MS:GA:M – 3:1:2, and sample 8: MS:GA:M – 1:3:2. Sample 1 was taken as a control.
Carrier solutions were prepared by dispersion of the matrix made of gum Arabic, maltodextrin and OSA-type modified starch in distilled water. The carriers were added to distilled water at 40 °C and stirred with a laboratory stirrer (IKA LABORTECHNIK, RW 20 DZM, Germany) at 380 rpm for 30 min. The continuous phase was left at room temperature (20 ± 2 °C) for about 24 h in order to fully hydrate the carrier. On the following day, the β-carotene oil solution of core was added to wall material solutions and the mixtures were stirred together using a laboratory stirrer at 380 rpm for 10 min. To prepare pre-emulsions, all mixtures were homogenized in a high-shear homogenizer (IKA LABORTECHNIK, Ultra Turrax Model T25, Germany) at 13,500 rpm for 10 min. The pre-emulsions were then subjected to two-stage homogenization using a high-pressure homogenizer (Model APV-1000, Albertslund, Denmark). In the first stage of homogenization, the pressure was 55 MPa and in the second stage 18 MPa, according to DŁUŻEWSKA and LESZCZYŃSKI (2005).

2.2. Emulsion analysis

Stability and color parameters of the obtained emulsions were determined. All experiments were done in triplicate.

2.2.1. Emulsion stability

Emulsion stability (SE) was determined by forced dissolution by centrifugation, which involved 24 h of storage at 37°C (thermostable conditions). Subsequently, the samples were centrifuged (centrifuge type MPW-340, Poland) at 3,500 rpm for 10 min. The volume of separated phases was measured and SE was calculated from the formula:

$$SE = \frac{(V_0 - V)}{V_0} \times 100 \qquad (1)$$

where: SE: emulsion stability, V_0: total volume after centrifugation [cm^3], V: volume of separated non-emulsified phase [cm^3].

2.2.2. Color measurements

Emulsions and solutions (10 % microencapsulated pigment solutions) were subjected to color analysis in CIE L*a*b* system using a colorimeter (Konica-Minolta, CM-3600d, Japan) at the wavelength of 450 nm. The assay was performed in reflected light using an observer 10° and illuminant D_{65}, in cuvettes with a thickness of 10 mm. The thickness of aperture was 25.4 mm. The color values represented brightness (+L/-L), redness/greenness (+a/-a) and yellowness/blueness (+b/-b). Based on the a* and b* parameters, color chromaticity (C*) and hue angle (h^0) were calculated using the following equations:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$h^0 = \arctan (b^*/a^*) \quad (3)$$

h^0 equal to 0 indicates red color, 90: yellow, 180: green and 270: blue.

2.3. Drying

The obtained emulsion was dried in a laboratory spray drier (type A/S Niro Atomizer, Copenhagen, Denmark; diameter of a drying chamber: 0.85 m, height: 1.3 m, spraying mechanism: disk). It was fed to spray disk using a peristaltic pump (Elpan, type 372.1, Poland) with a speed of 4 rpm. The emulsion was heated to a temperature 40±2°C before inserting it into a dryer in order to reduce its viscosity. It facilitates atomization and ensures more efficient microencapsulation. Countercurrent drying was used, the temperature of the inlet air was 190±5°C, that of the outlet air was 80±5°C and the pressure of air propelling the spray disk was at 3.0±0.2 kg cm-2 (PRZYBYSZ *et al*., 2012). Microcapsules were obtained in six parallel series.

2.4. Microcapsules analysis

200 g of microencapsulated pigment were stored in colorless glass jars at 20±2°C with the access of daylight for 64 days. The properties of microcapsules were studied immediately after spray drying and during storage by determining: the total content of pigment in microcapsules and on their surface, the color of microcapsules, the color of β-carotene solutions obtained after dissolution of microcapsules, apparent density and morphology of powders. All measurements were done in triplicate.

2.4.1. Determination of β-carotene content in microcapsules and on their surface

The spectrophotometric measurement of β-carotene content in microcapsules and their surface was performed according to POLISH STANDARD PN-A-75101-12:1990. Extraction of pigment from microcapsules and their surface was carried out according to the method of PRZYBYSZ *et al*. (2012) on the day of manufacturing the microcapsules, as well as after 28, 49 and 64 days of storage (the content of pigment in oily preparation was measured at the same time). The extraction was carried out 3 times. The extraction solution was a mixture of hexane and acetone (1:1).
Microencapsulation efficiency (ME) is the ratio of pigment content inside the microcapsules to the total pigment content (inside and on the surface) expressed as a percentage. ME was calculated according to the equation proposed by McNAMEE *et al*. (2001) based on the determined β-carotene content in microcapsules and on their surface:

$$ME = \frac{(c_b - p_b)}{c_b} \times 100 \quad (4)$$

where: ME: microencapsulation efficiency [%], c_b: total pigment content in microcapsules [mg (100 g)$^{-1}$], p_b: pigment content on microcapsule surface [mg (100 g)$^{-1}$].

2.4.2. Color of microcapsules

The color of microcapsules was determined with a colorimeter (Minolta, type CR-200, Japan), which defines L*, a*, b* color parameters. Light D_{65} was used for the study. Samples of microcapsules were placed in Petri dishes (the dishes were filled up to the full volume). A measuring head was applied to the surface of microcapsules. Measurements were taken at three different points and results were expressed as an average. Before the test, the camera was calibrated with a white pattern.

2.4.3. Apparent particle density

The apparent density ϱ_p was determined in a helium pycnometer (Stereopycnometer/Quantachrome Instruments, BoyntonBeach, USA) with specification as described by DOMIAN and BIALIK (2006). The apparent density is the ratio of powder mass and volume of powder particles. This volume does not include the volume of the air between particles.

2.4.4. Scanning electron microscopy (SEM)

The morphology of microencapsulated β-carotene was analyzed based on images taken with a scanning electron microscope (Hitachi Tabletop Microscope TM 3000, Tokyo, Japan) using a Multi Scan Base v. 18.03 software (computer scanning system, Warsaw, Poland) operating at 15 kV. Microcapsules were applied to a SEM platform with a double-sided adhesive tape. Samples were not sprayed with a coating material before observation. The paper presents selected images of microcapsules (magnification at 200x and 500x).

2.5. Statistical analysis

Statistical analyses were performed using Statistica 10.0 software (StatSoft). The significance of differences between mean values was evaluated with one-way ANOVA at a significance level of $p = 0.05$. The least significant difference was determined with the Tukey's test. The linear model was used for the random in the variance analysis.
Correlations between the evaluated properties and division of samples into groups were interpreted with a multivariate statistical method: principal component analysis (PCA). Results are presented in a two-dimensional plot.
Half-life and decay constant of β-carotene were calculated according to SZTERK and LEWICKI (2007). The half-life was calculated from the regression formula:

$$y = at + b \quad (5)$$

where: y: concentration of pigment [mg/100 g]; t: half-life [days].

The half-life of β-carotene ($T_{1/2}$) was determined by substituting "y" for half of the initial content of β-carotene. Decay reaction rate constant of β-carotene (K) was calculated from the equation:

$$K = (CA_0 - CA)/T \quad (6)$$

where: K: Decay reaction rate constant of β-carotene [(mg/100 g)/day]; CA_0 and CA: initial and final content of β-carotene [mg/100 g]; T: time [day].

3. RESULTS AND DISCUSSIONS

3.1. Emulsion stability

Oil-in-water emulsions may become instable which reduces the efficiency of the microencapsulation process. Stability of emulsions containing OSA-type modified starch, gum Arabic and maltodextrin ranged from 71.75 to 90.27 % and depended significantly on carrier ratios in the mixture. The emulsion containing the above-mentioned before carriers in the ratio of 1:1:1 was taken as a control sample. Its stability was 85.54 %. A higher content of gum Arabic and OSA-type modified starch in the mixture increased emulsion stability. However, emulsions with a higher content of modified starch than gum Arabic were more stable. The increase in emulsion stability results from a higher ratio of carriers with good emulsifying properties: gum Arabic and modified starch. Gum Arabic is a good emulsifier due to the presence of the arabinogalactan and protein complex. However based on the conducted study, the OSA-type modified starch is a better emulsifier than gum Arabic. In the emulsion systems with modified starch, crosslinking occurs in the aqueous phase. The starch forms a thick adsorptive layer on the surface of the oil droplet. Due to the polymeric stevic integration it prevents the release and migration of the oil phase (CHANAMAI and McCLEMENTS, 2000). The least stable emulsion obtained in this study was a mixture with the highest content of maltodextrin (sample 6), which is related to its very poor emulsifying properties (REINECCIUS, 1991). The low emulsifying ability of maltodextrin makes the dispersion of lipid components harder and reduces stability of the emulsion (DRURI and PAWLIK, 2001). Also, studies of LEWANDOWICZ *et al*. (2005) on the functional properties of maltodextrin in emulsion systems demonstrated that emulsions obtained from solutions of maltodextrin and oil were delaminated immediately. Based on the conducted study, it was found that the most stable emulsion system was obtained with the combination of MS, GA and M in the ratio of 3:1:2.

3.2. Apparent particle density

The apparent density of powders (i.e. the mass ratio of the particle to its reduced volume by open pore volume) depended significantly on matrix composition. In a preliminary study, the carriers were analyzed individually and process parameters were the same for all of them. The highest apparent density (1438 kg m^{-3}) was shown for the powders made of maltodextrin and the lowest one for the powders with modified starch (1107 kg m^{-3}), while that of the powders with gum Arabic was estimated at 1340 kg m^{-3}. It may, therefore, be hypothesized that the apparent density of obtained microcapsules, made of three carriers mixture, will have additive properties. Powders obtained from a stabilized emulsion of a mixture of carriers with the highest content of gum Arabic (sample 3 and 8) were characterized by the highest apparent density (1241 kg m^{-3}), which is indicative of the smallest internal porosity (Fig. 1).

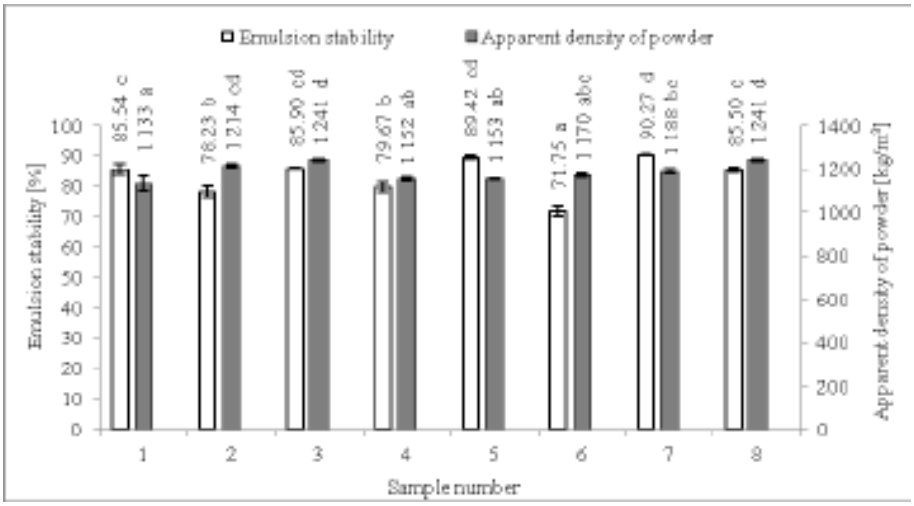

Figure 1: Selected physical properties of emulsions and powders.
Explanations:
Numbers represent variants of the microcapsules, in which the matrix is a mixture of: MS – modified starch, GA: gum Arabic, M: maltodextrin used in the following ratios: sample 1: MS:GA:M – 1:1:1, sample 2: MS:GA:M – 1:2:1, sample 3: MS:GA:M – 1:3:1, sample 4: MS:GA:M – 2:1:1, sample 5: MS:GA:M - 3:1:1, sample 6: MS:GA:M - 1:1:2, sample 7: MS:GA:M - 3:1:2, sample 8: MS:GA:M - 1:3:2.
Occurrence of at least one the same letter means no significant difference between the compared mean values in the group ($p<0,05$). Comparisons were made separately for each of the storage times.

It was found that the higher ratio of this carrier in the matrix (samples 1-3) tended to increase apparent density of the obtained powders. Increased apparent density of powders may result from a higher degree of structure condensation of these powders (DOMIAN, 2005). The obtained results shows that the powders with gum Arabic formed a more dense structure than modified starch. It may be explained by the fact that it forms emulsions with a higher viscosity compared to other carriers. The spraying of emulsion with a higher viscosity by a disk in the spray-drying chamber results in the formation of larger droplets, hence powder particles are larger as well. Many gas particles are not retained, which makes them more compact. The lower apparent density of powders produced from stabilized emulsions by a mixture of carriers with a higher content of modified starch than gum Arabic, may indicate greater amount of air spaces inside powder particles (i.e. internal pores, which are not connected with the surrounding atmosphere). Particularly, greater aeration of the sample occurred and a larger amount of foam was created during the mixing and homogenization of emulsion with an increased content of modified starch. The lower apparent density of powders may also be associated with their dustiness. Increasing the ratio of modified starch to two parts boosted the apparent density (sample 4). Further increase of modified starch addition in the mixture to three parts did not affect the value of this parameter. A higher content of maltodextrin in the carrier mixture (sample 6) resulted in an increase of apparent density due to the fact that among the applied carriers, it forms powders with the highest apparent density. Only in the case of sample 8 with the highest content of gum Arabic and the highest content of maltodextrin in the matrix, no influence of carrier on apparent density was observed and its value was the same as for sample 3, with the lower content of maltodextrin. According to CUPIAŁ *et al*. (2010), the addition of maltodextrin during spray drying to hydrolyzed protein resulted in a decrease of apparent density of the resultant powders compared to the hydrolyzed protein applied alone. The authors explain that maltodextrin has a lower

density and is a "skin-forming" material, which is characterized by the ability to form an impermeable film on the surface of droplets during evaporation. As a result, gas bubbles remain trapped inside particles, and powders have a lower apparent density because of the increased internal porosity. An important parameter that should be examined is the wettability of the powders.

3.3. Stability of microencapsulated β-carotene

The microencapsulated pigment as well as the control (i.e. oil β-carotene solution) underwent a 64-day storage test. The microencapsulation process increased pigment retention during storage. Pigment retention reached 44.8 % in the control (oil β-carotene solution) and ranged from 67.9 to 86.7 % in the microencapsulated β-carotene (Table 1).

Table 1: Comparison of the stability of β-carotene in the oil solution and stability of the microencapsulated pigment.

Analyzed factor	Oil β-carotene solution	Type of microcapsules							
		MS:GA:M 1:1:1	MS:GA:M 1:2:1	MS:GA:M 1:3:1	MS:GA:M 2:1:1	MS:GA:M 3:1:1	MS:GA:M 1:1:2	MS:GA:M 3:1:2	MS:GA:M 1:3:2
Microencapsulation efficiency [%]	-	88.6±0.4	81.2±0.2	81.0±5.3	79.3±9.1	66.9±2.7	81.5±4.4	75.6±5.5	81.5±9.5
Total β-carotene retention after 64 days of storage [%]	44.8±0.8	75.7±3.6	85.9±12.4	83.1±0.9	70.8±7.4	67.9±5.3	84.4±7.3	68.4±1.6	86.7±2.8
Retention of β-carotene on microcapsules surface after 64 days of storage [%]	-	23.7±7.1	14.0±0.3	21.8±5.6	16.4±6.1	14.6±0.1	15.8±2.5	14.2±2.5	14.0±6.6
Decay constant of total β-carotene [mg/100g/24 hours	8.82±0.11	1.37±0.23	0.71±0.60	0.88±0.05	1.41±0.37	1.57±0.35	0.77±0.42	1.55±0.12	0.70±0.14
Decay constant of β-carotene on microcapsules surface [mg/100g/24 hours]	-	0.49±0.02	0.84±0.04	0.79±0.28	0.85±0.44	1.37±0.04	0.74±0.13	1.03±0.24	0.85±0.48
The half-life [days]	53±0	123±2	230±14	189±1	105±1	95±5	187±17	97±3	288±5

β-Carotene content inside microcapsules depended on time and varied from 33.8 to 94.1 %, whereas on the microcapsule's surface it varied from 75.5 to 90.6 % for different variants (the percentage is based on the determination coefficient R^2). The loss of the pigment resulted from degradation of this labile compound caused by daylight, oxygen and temperature (RODRÍGUEZ-HUEZO *et al*., 2004). A decrease in the amount of microencapsulated pigment during storage test was also observed by ELIZALDE *et al*. (2002), who encapsulated β-carotene inside trehalose and gelatin shells. DESOBRY *et al*. (1999) reported on the loss of β-carotene enclosed within maltodextrin microcapsules during storage. The above findings were consistent with results of the study by DESOBRY *et al*. (1997).

The stability of β-carotene depended significantly on the content of different types of carriers in the emulsion. It was found that it is necessary to apply carriers mixed in

different ratios, because when evenly mixed the protection of microencapsulated pigment was weaker (the highest efficiency of microencapsulation was neither related to a high retention coefficient and a high decay constant of total β-carotene at a relatively short half-life, which additionally confirmed its short stability (Table 1).

The microcapsule's core was best protected when the matrix was made of a mixture of carriers, containing mainly gum Arabic reinforced with maltodextrin (sample 8). The pigment inside such a matrix had the highest retention and the longest half-life (it had the lowest decay constant of total β-carotene: [(0.70 mg / 100 g) / 24 hours]. This was also associated with the highest apparent matrix density due to the lowest pore number inside powder particles, which as a consequence led to the most effective protection of the core against oxygen. Gum Arabic is flexible and resistant both to microcapsules deformation and to cracking, which makes the loss of β-carotene much lower. Moreover, the high maltodextrin addition has reinforced the protective properties of the gum Arabic carrier. According to literature, maltodextrin is a strong oxygen barrier due to its ability to form a glassy structure (REINECCIUS, 1991; GOUIN, 2004). Maltodextrin combined with gum Arabic has a stronger protective effect on the microencapsulated substance. This relation was true for the sample 6, which contained an increased amount of maltodextrin (retention reached 84.4% and the decay constant of total β-carotene: [(0.77 mg / 100 g) / 24 hours].

In turn, the weakest protection of the core against oxidation was observed for the matrix containing the highest percentage of modified starch in the mixture of carriers (sample 5). In this case, the total microencapsulation efficiency as well as pigment retention were the lowest and the decay constant for β-carotene was the highest – both in total and on the microcapsule's surface.

Carotene degradation results from its oxidation initiated by isomerization. This process occurs easily at high temperature, in daylight, in acidic environment and in the presence of catalysts. In those conditions, a typical *trans* isomer of carotene is converted into *cis* isomer (RODRIGUEZ-AMAYA, 2001). *Cis* isomer is in fact much more susceptible to oxidation. As a result of this process, carotenyl peroxide is formed, which upon the activity of free radicals gives epoxy carotenoids or dioxetanes. The latter can decompose, thus producing aldehydes and ketones (MORDI *et al*., 1993). It is noteworthy that the emulsion used to prepare microcapsule 5 turned out to be the most stable. However, although the modified starch is capable of forming emulsion as a main component of the shell it does not provide sufficient protection against pigment oxidation. REINECCIUS (1991) came to a similar conclusion. Thus, the relatively high stability of the emulsion is not necessary to achieve high microencapsulation efficiency if dried emulsion formula has been properly chosen. When only starch content was increased in the wall material (samples 1, 4, 5), we have observed a decrease in microencapsulation efficiency as well as shortening of both β-carotene total retention and its retention on the microcapsule's surface. In the same time, an increase was observed in β-carotene decomposition rate leading to its degradation. Therefore it was concluded that the following dependency is true: the higher the amount of modified starch in the carrier mixture, the faster degradation of β-carotene is. Changes in the total content of microencapsulated pigment and pigment absorbed on the microcapsule's surface are shown in Fig. 2 and 3. For different ratios of the carriers, the total pigment content and pigment content on the surface of the microcapsules ranged from 308.3 to 361.6 mg $(100\ g)^{-1}$ and from 41.1 to 102.4 mg $(100\ g)^{-1}$ at the beginning of the storage period.

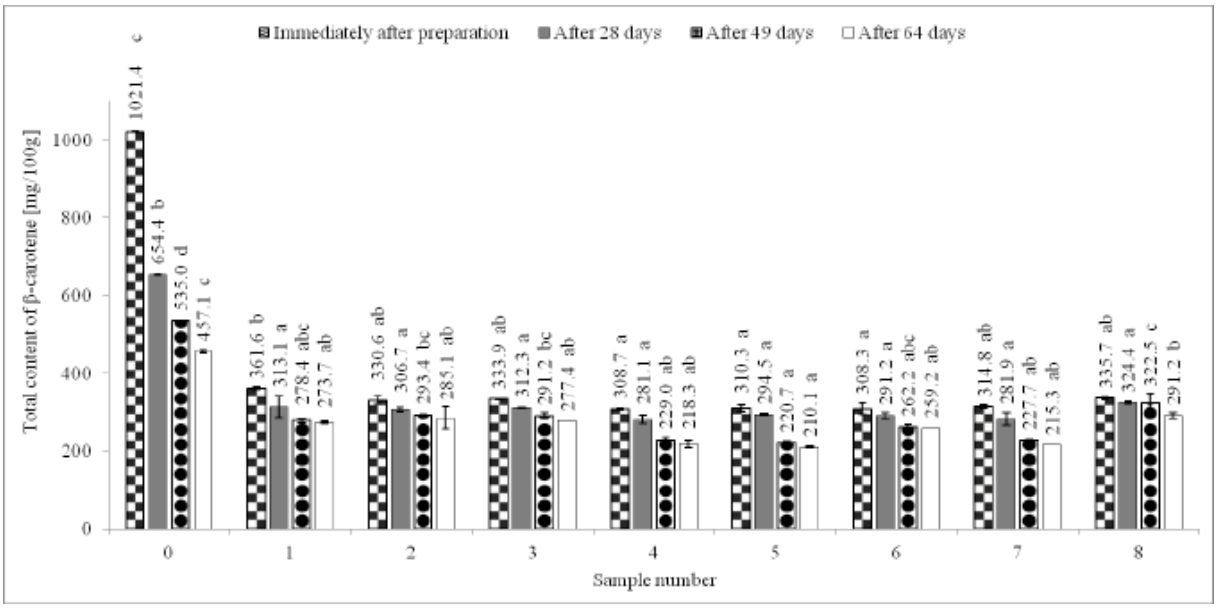

Figure 2: Contents of total β-carotene in microcapsules and in the oil formulation.
Explanations:
Numbers represent variants of the microcapsules, in which the matrix is a mixture of: MS: modified starch, GA: gum Arabic, M: maltodextrin used in the following ratios: sample 1: MS:GA:M – 1:1:1, sample 2: MS:GA:M – 1:2:1, sample 3: MS:GA:M – 1:3:1, sample 4: MS:GA:M – 2:1:1, sample 5: MS:GA:M - 3:1:1, sample 6: MS:GA:M - 1:1:2, sample 7: MS:GA:M - 3:1:2, sample 8: MS:GA:M - 1:3:2. Sample 0: oil β-carotene solution. Total amount of β-carotene for sample 0 [mg/100 g oil], for samples 1 - 8 [mg/100 g powder].
Occurrence of at least one the same letter means no significant difference between the compared mean values in the group ($p<0,05$). Comparisons were made separately for each of the storage times

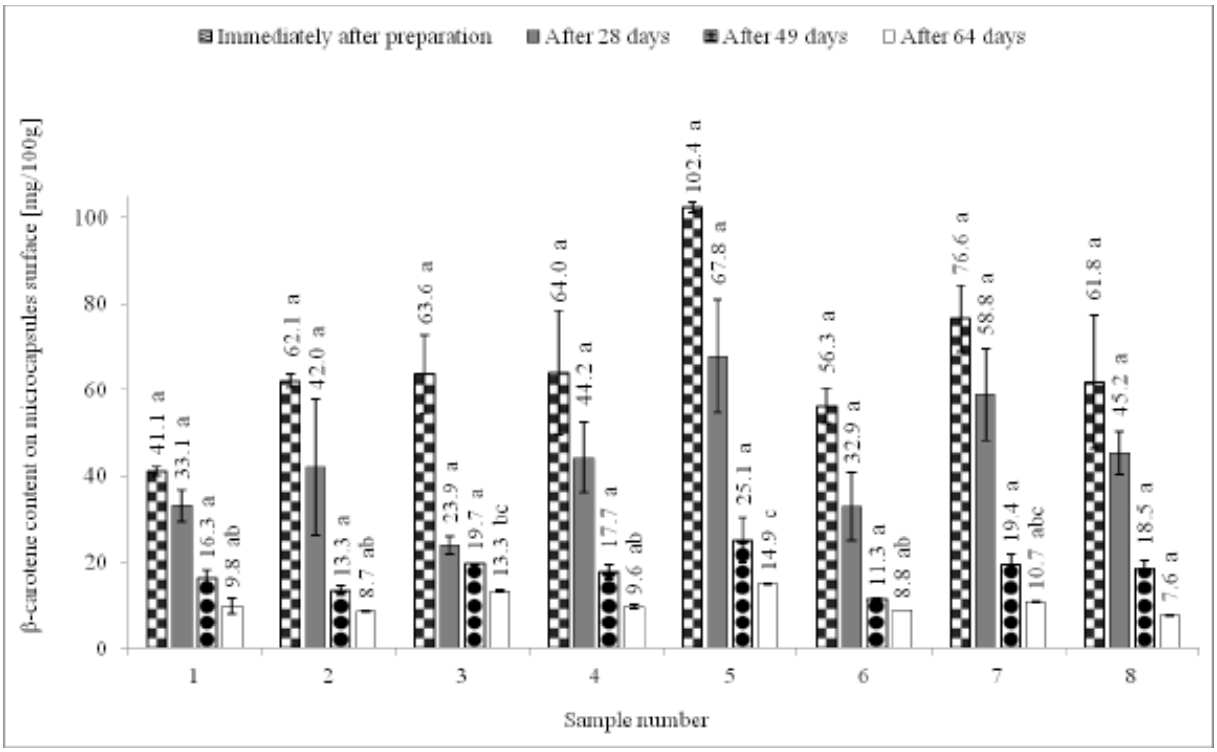

Figure 3: Content of β-carotene on microcapsules surface.
Explanations as under Fig. 1.

However, an in-depth analysis shows that the surface of microcapsules containing an increased amount of modified OSA starch had the largest content of the pigment. This has also been confirmed in studies by PRZYBYSZ *et al*. (2012) and by DŁUŻEWSKA *et al*. (2011). Thus, the poor β-carotene stability in those matrices might result from its high concentration on the microcapsule's surface, which leads to its more rapid degradation (DESOBRY *et al*., 1997). Both in the case of total β-carotene and surface β-carotene content changes, it was found that in all the samples the highest average loss of the pigment occurred between the 28th and the 49th day of storage and averaged 25.8 mg $(100\ g)^{-1}$. The minimal pigment level at the end of the storage period was on average 7.2 mg $(100\ g)^{-1}$. The above results are typical according to literature, which proves that the loss of β-carotene during the initial period of sample storage as microcapsules is higher than during the final period (RODRÍGUEZ-HUEZO *et al*., 2004).

3.4. Color changes during storage

β-Carotene has 11 conjugated double bonds and is an orange pigment (RODRIGUEZ-AMAYA, 2001). The color of emulsion and solutions containing the microencapsulated pigment may help to determine its stability (PESEK and WARTHESEN, 1990, SPADA *et al*., 2012). The color analysis should be preceded with a note that the color parameters of the emulsion as well as the colors of the solution of microcapsules are a sum of particular color parameters: both of β-carotene oil solution and of carriers dissolved in water. In this case, the color was largely determined by the ratio of all types of carriers, whereas in the case of the color parameters of the obtained microcapsules, color will be largely determined only by the color of β-carotene absorbed on their surface. The color of a carrier mixture will be developing in time. It cannot be determined whether color measurement of powders indicates the oxidation of pigment on the surface. During β-carotene degradation, the color of the carrier on the surface of microcapsules is revealed. Therefore color measurements were not performed during storage. Average values of brightness (L*) for microcapsules, emulsions and solutions of the dissolved microcapsules were: 74.54; 57.27 and 65.69, respectively (Fig. 4).
Regardless of the type of the tested material, the same tendencies were observed: the higher the ratio of modified starch, the higher brightness value (for samples 1, 4, 5). Similar results were obtained at a higher ratio of maltodextrin. Microcapsules with the highest luminescence (L* = 75.13) are those with an increased addition of maltodextrin only (sample 6), while the most bright emulsions (L* = 60.39) are those with an increased addition of both modified starch and maltodextrin (sample 7). The high brightness of microcapsules is caused by white color of the carriers, for both those made of modified starch and of maltodextrin, and by the fact that in the case of samples with a higher content of maltodextrin the quantity of pigment on microcapsule's surface was lower. It may also result from high brightness of maltodextrin solution itself. Chromatic parameters of microcapsule color were positive for both a* (indicating the dominant red hue) and for b* (indicating the dominant yellow hue).

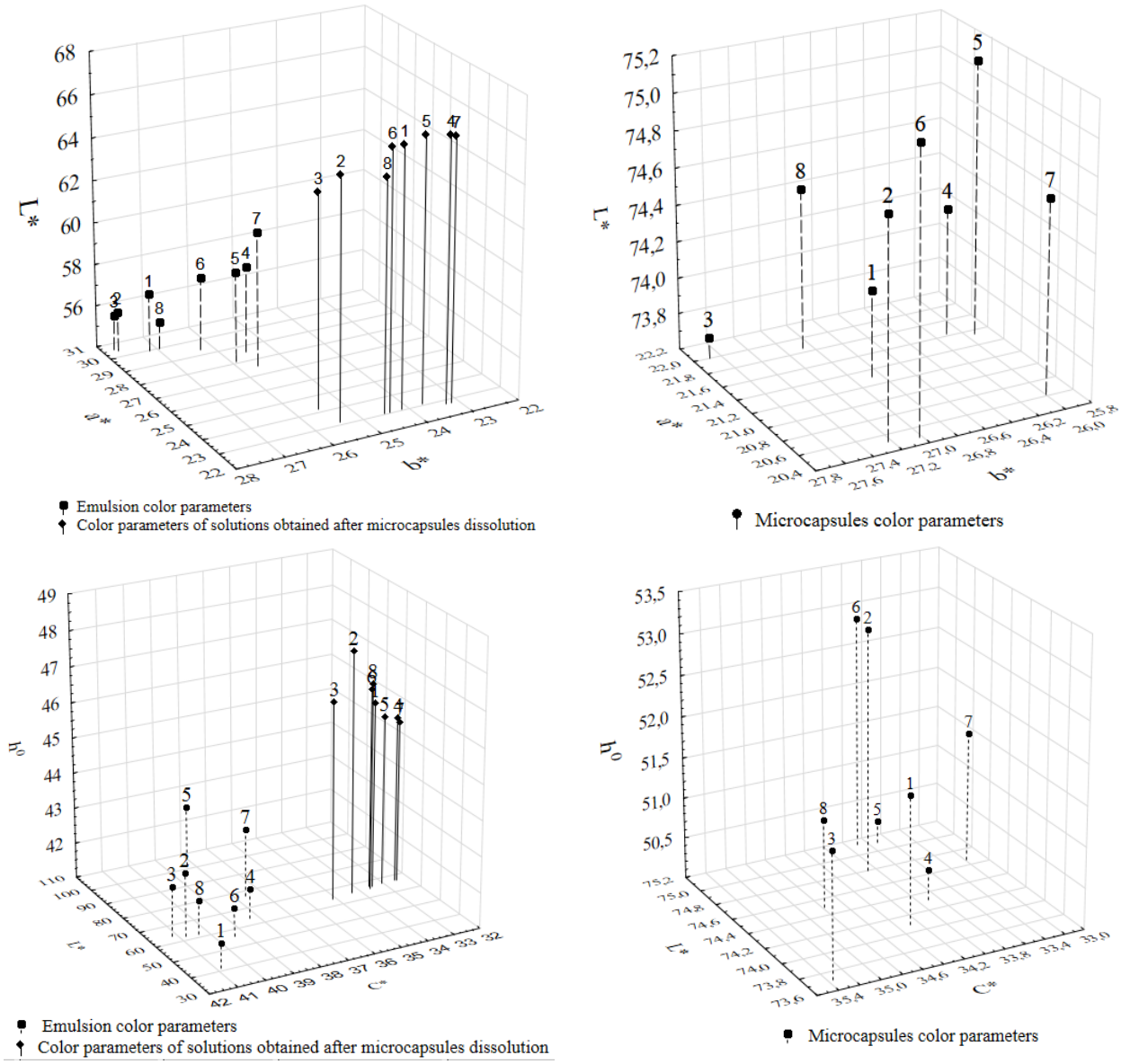

Figure 4: Color values of emulsions, microcapsules and solutions obtained from the dissolved microcapsules.
Explanations as under Fig. 1.

Average values of red parameter (a*) for microcapsules, emulsions and solutions prepared from dissolved microcapsules were 21.24; 29.30 and 23.12 respectively. The carrier mixture redness parameter (a*) of the analyzed samples increased as well with the increasing ratio of the gum Arabic. The same dependency was observed for the chromatic parameter b*. Sample 3 with an increased addition of gum Arabic had the highest values of both red parameter (a*) and yellow parameter (b*), regardless of the type of the material analyzed. However, gum Arabic could have caused differences in chromatic parameters due to its yellow color. Sample 3 (with the highest content of GA) showed the highest chroma, i.e. 41.19, 32.47, and 33.91 respectively for emulsions, powder solutions and powders. Sample 7 (with the highest content of MS and M) had the smallest chroma value. The hue angle for all samples ranged from 41.71° to 53°, which indicates that they were red to yellow in color. Sample 2 (high GA amount) was characterized by the largest tone hue angle, i.e.

47.80°and 53.00° respectively for powder solutions and powders, which shows that these samples were mostly yellow.

3.5. SEM analysis

Fig. 5 (a, b) shows some photomicrographs of β-carotene microencapsulated in a mixture of carriers GA: MS: M used in a ratio of 1: 1: 1.

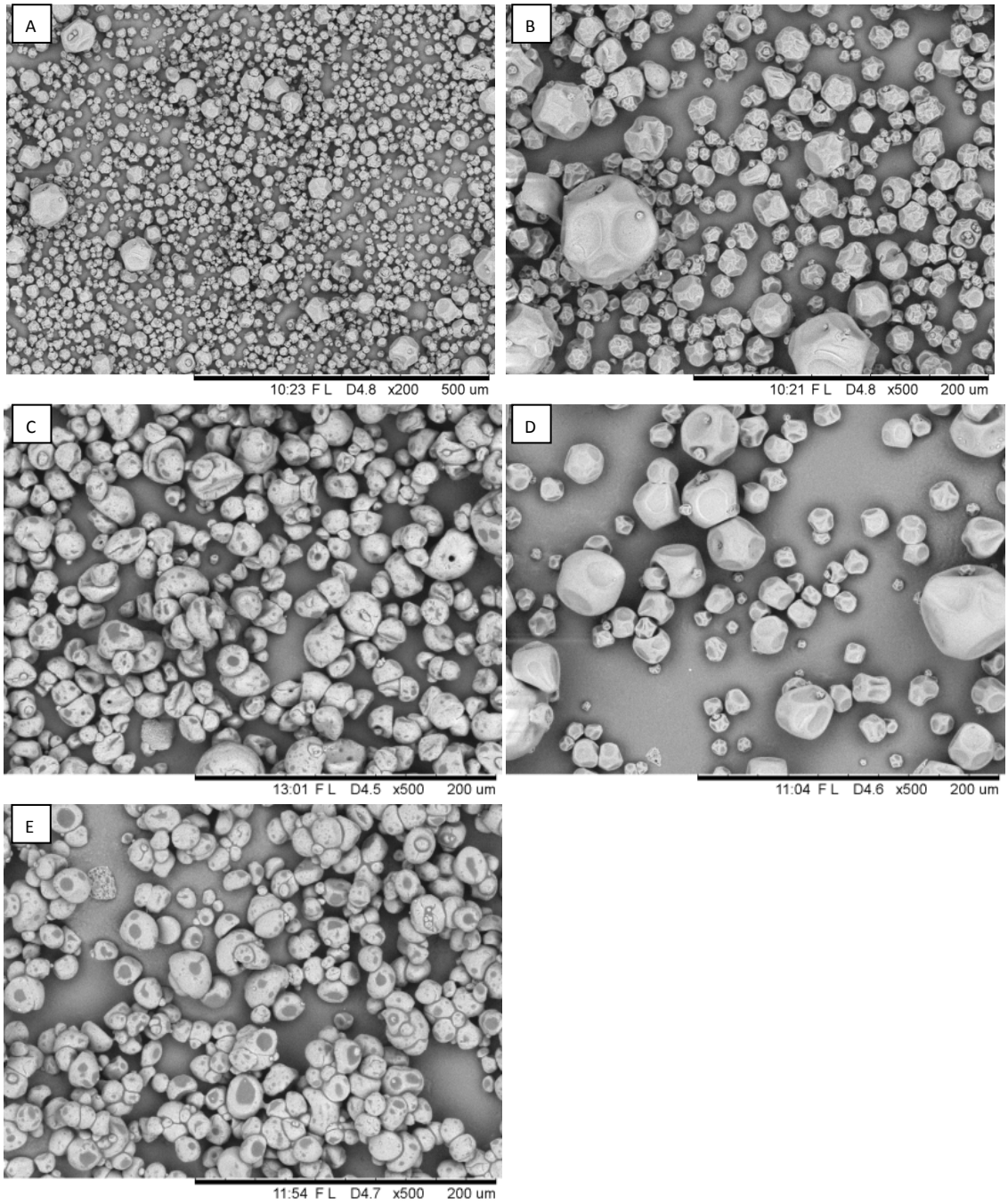

Figure 5: Morphology of microcapsules with GA:MS:M ratio of 1:1:1 (magnification at A: 200x, B: 500x), microcapsules morphology made of A) 25% gum Arabic, B) 25% modified starch, C) 25% maltodextrin (magnification at 500x), accelerating voltage 15 kV.

Observations of powder, performed with a scanning electron microscope (SEM), showed microparticles of powder of mean diameters reaching 15-20 μm and some larger ones as well, but never exceeding 60 μm. Microcapsule shape resembles a distorted sphere with characteristic holes and indentations on its entire surface. Noteworthy is the high degree of morphological similarity of microcapsules produced from a combination of gum Arabic, modified starch and maltodextrin applied in both identical and different proportions (hence this study presents only one variant). Preliminary drying experiments performed with β-carotene emulsion and single carriers (the SEM analysis is attached in Fig. 5 (c, d, e)) have confirmed that the nature and origin of the microencapsulating material strongly affect morphological properties of microcapsules produced with the use of spray-drying method, and in the case of different proportions of the three types of carriers used in the study the OSA modified starch had the key impact. Similar pictures of β-carotene microcapsules consisting solely of the OSA CAPSULTA modified starch were obtained by LIANG *et al*. (2013).

3.6. Principal component analysis (PCA)

Results obtained for carrier mixture properties were analyzed with the PCA method. Two main components explained 72.75 % of total variation: the first one (factor 1) accounted for 55.40 % and the second (factor 2) for 17.35 % (Fig. 6).
The PCA analysis proved that several expected correlations between the analyzed parameters were true. The apparent density of the particles was positively correlated with the total β-carotene content in microcapsules (measured immediately after microcapsules preparation and after 28 days of storage) and pigment retention on the surface of the microcapsules. They were negatively correlated with the brightness of solutions obtained from dissolved microcapsules (Fig. 6). Moreover, the microencapsulation efficiency was negatively correlated with pigment content measured on the surface of microcapsules immediately after their preparation and with the decay constant of β-carotene on their surface. A negative correlation was also found between total β-carotene retention in microcapsules and the decay constant of total β-carotene and pigment on the surface of microcapsules after 28 days of storage. Not all expected correlations were, however, confirmed, e.g. it had been expected the β-carotene content on the surface of the microcapsules would be correlated with chromatic parameters of their color, but it has not been confirmed in this study.
PCA classification of microcapsules allowed distinguishing two groups: the first for samples 1, 2 and 8, and the second for samples 4 and 7, which are similar to one another in terms of all examined traits. Moreover, there are some microcapsules that are distinctly different from others concerning both parameters analyzed (samples 3, 5 and 6). It was also observed that the increased content of gum Arabic (samples 2, 3, 8) caused a shift of the results to the left side of the X axis (factor 1). The first parameter was strongly negatively correlated with Rβ, T½, β28, β49, β64, aE, bE, bM, bR and positively correlated with Kβ, βp28, LE and LR. It indicates the positive effect of gum Arabic on the parameters negatively correlated with factor 2 and its negative effect on the variables correlated positively. On the contrary, microcapsules with matrices containing a higher amount of modified starch (samples 4, 5, 7) are shifted to the right part of the projection. This is related to the opposite effect of MS on the analyzed parameters compared to GA.

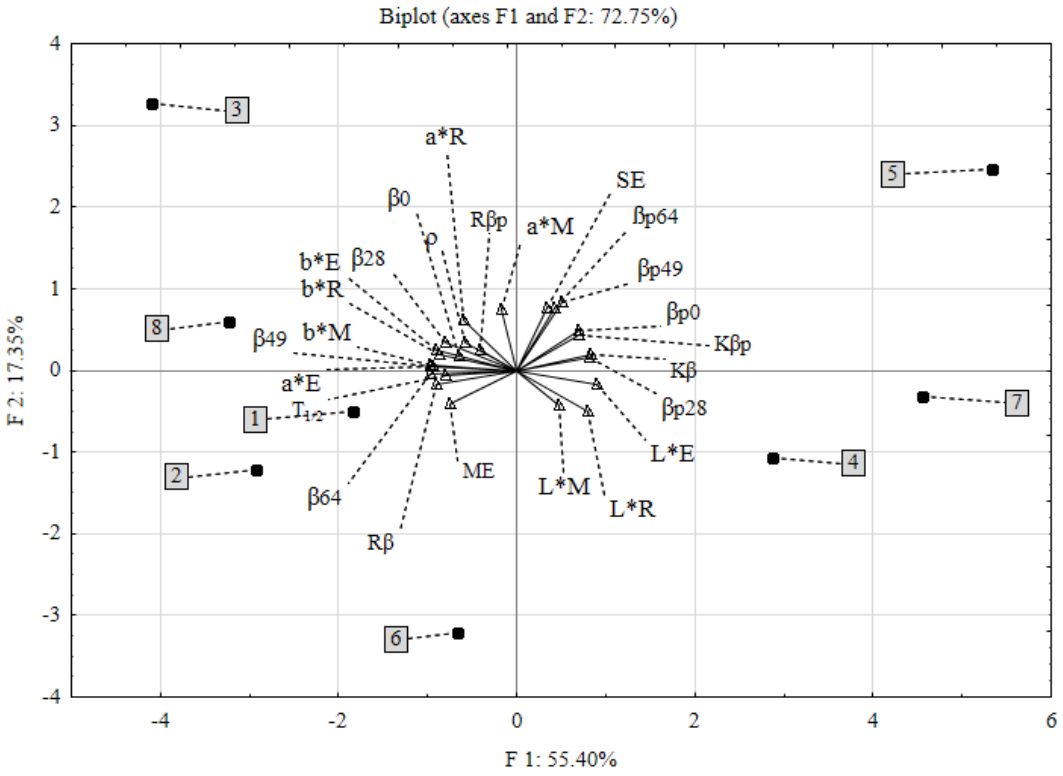

Figure 6: Examined characteristics and samples in the plot of the first two principal components.

Explanations:
SE: emulsion stability, ϱ: apparent density of powders, ME: microencapsulation efficiency, Rβ: overall β-carotene retention after 64 days of storage, Rβp: retention of β–carotene on microcapsules surface after 64 days of storage, Kβ: decay constant of total β-carotene, Kβp: decay constant of β-carotene on microcapsules surface, $T_{1/2}$: half-life, β0, β28, β49, β64: total content of β-carotene in microcapsules determined after 0, 28, 49 and 64 days of storage, βp0, βp28, βp49, βp64: content of β-carotene on the surface of microcapsules determined after 0, 28, 49 and 64 days of storage, L*E, a*E, b*E: emulsion color parameters, L*M, a*M, b*M: microcapsules color parameters, L*R, a*R, b*R: color parameters of solutions obtained after microcapsules dissolution.
Other explanations as under Fig. 1.

4. CONCLUSIONS

Stability of β-carotene emulsion depends on the type of carrier applied. Emulsion in the mixture of modified starch, gum Arabic and maltodextrins is stabilized by an increasing content of modified starch and is destabilized by an increasing content of maltodextrins.
The apparent density of β-carotene microcapsules obtained with the use of spray-drying method depended on the ratio of the carriers (modified starch, gum Arabic and maltodextrin) in the mixture. The higher content of gum Arabic in the emulsion allows obtaining a higher powder density, which results in an increase of β-carotene retention in microcapsules.
When the mixture consisted of modified starch, gum Arabic and maltodextrin in the ratio of 1: 3: 2, we obtained microcapsules that had the highest β-carotene retention, the lowest pigment degradation rate constant and the longest half-life in comparison to the other analyzed samples. Increased content of modified starch in the wall material of the microcapsules resulted in a decrease of their stability (retention reduction, increase of

microcapsule degradation rate constant - both total and on the surface) and reduced microencapsulation efficiency.
Regardless of carrier type microencapsulated, β-carotene was much more stable than in the form of oil solution.
The type of wall material has a significant effect on the value of brightness (L*) and redness parameters (a*) of microcapsules. With an increasing amount of modified starch in the carrier mixture, brightness (L*) of the emulsion, microcapsule, and solution obtained with the addition of microencapsulated β-carotene increases, while the higher content of gum Arabic causes an increase of the redness parameter (a*).

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