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THE EFFECT OF CARRIER TYPE AND ANTIOXIDANT ON STABILITY OF B-CAROTENE MICROENCAPSULATED BY SPRAY DRYING

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Summary. The aim of the study was to determine influence of the type of microcapsule wall material and addition of selected antioxidants on retention of β-carotene microencapsulated by spray drying. Dye as an oil solution was microencapsulated. Rosemary or butylhydroxyanisole (BHA) extract was added to the dye solutions. The cell materials were gum arabic and mixtures of gum arabic and maltodextrin. It was found that the total content of β-carotene, regardless of the type of carrier used, decreased during storage (form initial ranged 228.2–243.5 mg · 100 g^{-1} to 170.3–183.7 mg · 100 g^{-1} after 60 days of storage). The type of carrier had a significant effect on stability of microencapsulated β-carotene. The highest dye retention at the time of drying was observed for gum arabic and maltodextrin sample (1:1), while gum arabic and maltodextrin sample (2:1) was more stable during storage. Use of natural antioxidant – rosemary extracts increased stability of the dye on the surface of the microcapsules to a greater extent than use of samples containing BHA. The decrease in β-carotene content on the surface for rosemary extracts ranged between 15–35% whereas for BHA between 38–48%.

Key words: gum arabic, β-carotene, maltodextrins, microencapsulation, rosemary

INTRODUCTION

Carotenoids are very important food components due to their health benefits as well as their role as food colorants and aroma compounds. β-carotene gives a yellow to orange colour to food, exhibits a provitamin A and antioxidant activity. During technological

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processing and storage β-carotene isomerization and degradation might occur. Colour intensity of *cis* β-carotene is lower than of all-trans isomers. As a result of the oxidation process β-carotene loses provitamin A activity, the colour may fade or even disappear and strange odours may be noted, characteristic for low molecular weight degradation products of colorants [Pesek et al. 1990, Chen et al. 1994, Cheng et al. 1995, Mínguez-Mosquera et al. 2002].

To extend food colorant stability, facilitate dosing and improve their solubility the microencapsulation process might be use. During microencapsulation a carrier material shells around core material (colorant) [Selim et al. 2000, Gouin 2004, Ersus and Yurdagel 2007]. Microencapsulation of colorants by spray-drying is achieved by emulsifying the colorants in an aqueous solution of carrier material, followed by atomization of the mixture. During this process, a film forms on the surface of a droplet, thereby retarding the larger active molecules and evaporating the smaller water molecules [Desobry et al. 1997, Re 1998].

Selection of carrier material for microencapsulation is important as it must have high water solubility, form solutions of low viscosity, have good emulsifying and film forming properties, provide a barrier to volatile components and oxygen [Kim et al. 1996]. Basing on the literature data regarding microencapsulation of colorants, it can be stated that gum arabic as carrier material has a high water solubility, its solutions have low viscosity with good emulsifying properties [Kim et al. 1996]. On the other hand, maltodextrins are cheaper, with higher barrier to oxygen, but also poorer emulsification properties [Anwar et al. 2010]. The combination of these two materials can provide a good protection of carotenoids' colorants.

In order to increase the stability of microencapsulated β-carotene, antioxidants might be added. The commonly used antioxidants are: L-ascorbic acid and tocopherols. Strong antioxidants are also products of chemical synthesis, e.g. BHA (butylhydroxyanisole) [Romano et al. 2009]. However, recently food producers have shown interest in using natural antioxidants such as extracts of herbs and spices like rosemary. Rosmarinic acid has a high antioxidant activity due to presence of the carboxyl group and four hydroxyl groups [Tepe et al. 2007]. The aim of this work was to determine effect of wall material and antioxidant type on stability of β-carotene microencapsulated by spray drying.

MATERIALS AND METHODS

Extraction of β-carotene from carrot

Carrots (*Karotka)* were ground and then juice was squeezed using a robot (Bauknecht, Germany). Coagulation of the carrot juice protein was carried out with 25% HCl. The juice was acidified to pH = 4.7 and heated to 89 ±1 ºC. Sedimentation of the obtained protein-carotene precipitate was performed at 8°C for 20 h, the precipitate was later centrifuged for 10 min at 25,000 × g (Sigma Centrifuge MPW-340, Poland). The coagulant was frozen and dehydrated (Christ Alpha 1-4 LSC Freeze Dryer, Germany). β-carotene was extracted from lyophilizate using n-hexane in a three-step extraction. The extract

was filtered under vacuum and the n-hexane was evaporated (Buchi Rotavapor R-210, Germany) and subjected to further lyophilization. The resulting powder was mixed with a refined rapeseed oil to get a 0.073% β-carotene oil solution. Obtained oil solution of the β-carotene was chemically characterized as a part of major project and the results are available in Przybysz et al. [2018].

Microencapsulation

Microcapsules were obtained by spray drying. The active substance of the microcapsules was a 0.073% carotene oil solution, while the capsule shell was a mixture of gum arabic (GA) Valcoat WM 960 (Valmar, Slovenia) and maltodextrine (MD) Glucidex IT 12 (DE 12) (Jar-Jaskulski Aromaty, Poland). The carriers were mixed at a ratio of 1:1, 1:2, 2:1. The spray dried emulsions consisted of 5% β-carotene oil solutions (with or without antioxidant), 30% carrier and 65% distilled water. For the samples with the addition of antioxidant, 0.04% of rosemary extract (AR-POL S.C. Sikorscy, Poland) or 200 mg of BHA (Kemin, USA) per 1 kg of emulsion was added.

Preparation of O/W emulsion

The continuous phase of the carrier solution was obtained by two steps dispersing gum arabic and maltodextrin in distilled water. At first arabic gum was added to the distilled water at 40°C and stirred (Janke & Kunkel RW 20 DZM, Germany) at 380 rpm, maltodextrin was added to the solution after 30 min and stirring continued using the same parameters. For complete hydration of the continuous phase it was left at 20 ±2°C for 24 hours. A dispersed phase (β-carotene solution) was added to the continuous phase and stirred for 10 min at 380 rpm. The emulsions were prepared using two-stage high pressure homogenization (APV-1000, Denmark): pressure at first stage was 55 MPa and 18 MPa at the second.

Drying

Drying of the obtained emulsion was carried out in a laboratory type spray dryer (A/S Niro Atomizer, Denmark). Counter-flow drying was used, the inlet air temperature was 190 ±5ºC, and the outlet air was 80 ±5ºC. Microcapsules were obtained in 3 parallel replicates. Microencapsulated α- and β-carotene were stored for 2 months at 20 ±2ºC without daylight.

Determination of total β-carotene content in and on microcapsules

Determination of β-carotene content in microencapsulated was performed by the spectrophotometric method. Extraction of the dye from microcapsules and from their surface was carried out by the modified Wagner and Warthesen [1995] method. The modification concerned the type of extraction solvent used. In the case of extraction of β-carotene from microcapsules, powder was dissolved in water, and then extracted by acetone chloroform

mixture in 1:1 ratio during 5 min stirring on magnetic stirrer (Polamed MM6, Poland). The organic phase was filtered and diluted with 1.5 cm^3 of chloroform. The absorbance was measured at 454 nm for chloroform as a control sample (Helios β-Thermo Spectronic spectrophotometer, United Kingdom). In the case of extraction of β-carotene from the surface of the microcapsules, the powder was stirred with acetone for 5 min. The organic phase was filtered and absorbance was measured with acetone as a control sample. Based on the determined content of β-carotene in microcapsules and on their surface, the microcapsule efficiency was calculated according to the equation proposed by McNamee et al. [2007]:

$$ME = (c_\beta - p_\beta) / c_\beta \cdot 100\%$$

where: ME – microcapsule efficiency,
c_β – initial total content of β-carotene in microcapsules,
p_β – initial content of β-carotene on the surface of microcapsules.

Determination of colour of microencapsulated β-carotene powders

The L* and a* colour components were determined with use of CIEL*a*b* at the surface of microcapsules, using a Minolta CR-200 colorimeter (Minolta, Japan; light source D65, observer 2°, a measuring head hole of 8 mm).

Statistical analysis

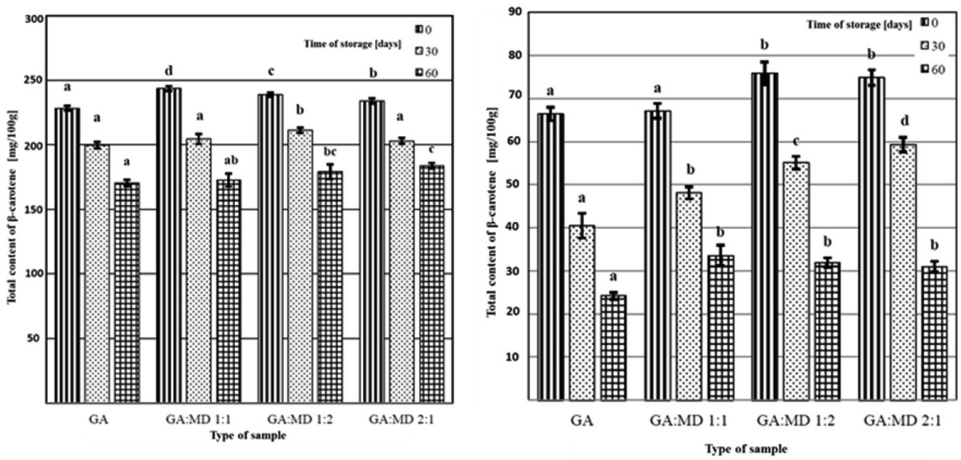
Two-way ANOVA and three-way ANOVA were applied to determine statistical significance of the type of carrier, time of storage, and type of antioxidant used. The Tukey post hoc test was performed to see which groups differ from each other. The statistical analysis was carried out using IBM SPSS Statistics v.24.

RESULTS AND DISCUSION

Effect of storage time on total β-carotene content

The initial content of β-carotene in microcapsules to which no antioxidant was added was 228.2 to 243.5 mg · 100 g^{-1}. However, after 60 days of storage (temp. 20°C, HR 40%), it ranged from 170.3 to 183.7 mg · 100 g^{-1} depending on the type of sample (Fig. 1a).

The decrease of β-carotene content ranged from 21 to 29%. The statistical analysis showed that the sample retention time had a significant effect on the total β-carotene content irrespective of the type of cell material used (Table 1). The results are confirmed by literature data, according to which the dye content decreases during storage despite the microencapsulation process. Debory et al. [1999] showed a reduction in β-carotene content during storage of maltodextrin microcapsules. Similar results were obtained by Elizalde et al. [2002], who used a mixture of trehalose and gelatine as the wall material for microcapsules. The authors have shown that the greatest decrease in β-carotene con-



a b

Fig. 1. Total β-carotene content in microcapsules (a) and content of β-carotene on the surface of microcapsules (b) without the addition of antioxidants

Rys. 1. Całkowita zawartość β-karotenu w mikrokapsułkach (a) i zawartość β-karotenu na powierzchni mikrokapsułek (b) bez dodatku przeciwutleniaczy

tent occurs in the initial storage period. Also Rodríguez-Huezo et al. [2004] stated that the initial period of carotene degradation is more violent at the early stage and the later on the process tends to be slowing down. The results are confirmed also by Liang et al. [2013].

Table 1. Multi-way ANOVA for models, were dependent variable was β-carotene content on the beginning and its decrease after 60 days of storage

Tabela 1. Wieloczynnikowa ANOVA dla modeli, gdzie zmienną zależną była zawartość β-karotenu na początku i jej spadek po 60 dniach przechowywania

Total β-carotene content Całkowita zawartość β-karotenu					
Source of variation Źródło zmienności	Degree deflation Stopień deflacji	Sum of squares Suma kwadratów	Mean squares Średnie kwadratów	*F* statistic Statystyka *F*	*p*-value
Type of sample Rodzaj próbki	3	2 322.60	774.20	6.85	0.0002***
Storage time Czas przechowywania	2	60 572.29	30286.15	267.98	0.0000***
Type of antioxidant Rodzaj przeciwutleniacza	2	5 062.70	2531.35	22.40	0.0000***
Random errors Błędy losowe	280	31 645.13	113.02		

cont. Table 1 / cd. tab. 1

β-carotene content on the surface of microcapsules Zawartość β-karotenu na powierzchni mikrokapsułek					
Type of sample Rodzaj próbki	3	14 321.74	4773.91	96.27	0.0000***
Storage time Czas przechowywania	2	57 667.26	28833.63	581.46	0.0000***
Type of antioxidant Rodzaj przeciwutleniacza	2	58 429.79	29214.89	589.15	0.0000***
Random errors Błędy losowe	280	13 884.67	49.59		
Decrease of total β-carotene content Spadek całkowitej zawartości β-karotenu					
Type of sample Rodzaj próbki	3	352.04	117.35	1.76	0.1613
Type of antioxidant Rodzaj przeciwutleniacza	2	28 003.26	14001.63	209.54	0.0000***
Random errors Błędy losowe	90	6 014.00	66.82		
Decrease of β-carotene content on the surface of microcapsules Spadek zawartości β-karotenu na powierzchni mikrokapsułek					
Type of sample Rodzaj próbki	3	943.97	314.66	6.20	0.0007***
Type of antioxidant Rodzaj przeciwutleniacza	2	5 852.61	2926.30	57.63	0.0000***
Random errors Błędy losowe	90	4 569.60	50.77		

*** significant differences

Effect of storage time on β-carotene content on the surface of microcapsules

A part of the core which is unlocked in the microcapsules remains on the surface of the microcapsules during the process of microencapsulation by spray-drying method. The microencapsulation efficiency reflects the real amount of the pigments enclosed within a matrix [Anwar and Kunz 2011]. β-carotene on the surface is subjected to oxidation, therefore, microcapsules dies obtained with greater efficiency are more stable.

The initial content of β-carotene on the surface of microcapsules, produced without the addition of antioxidant, was 66.4 to 75.9 mg · 100 g^{-1}. After 60 days of storage, it was from 24.2 to 33.6 mg · 100 g^{-1}, depending on the type of carrier used (Fig. 1b).

The decrease in β-carotene content on the surface was significantly higher than microencapsulated dye and accounted for 60 to 64% relative to the initial amount. Significant effects of storage time on the content of β-carotene on the surface of microcapsules have been shown (Table 1). Similarly, Przybysz et al. [2012] demonstrated that storage time affects β-carotene content on the surface of microcapsules. For microcapsules made from

a mixture of maltodextrin and gum arabic, it was observed that after 90 days of storage, up to 100% of initial amount of β-carotene on the surface of the microcapsules can be degraded.

Effect of carrier type on total β-carotene content

The type of carrier has a significant effect on the content of β-carotene in microcapsules. However, the decrease of β-carotene level after 60 days of storage did not depend on the type of carrier used (Table 1). The type of carrier determined the pigment retention during spray drying more than during storage of the powder. GA-based microcapsules were characterized by the lowest total β-carotene content. Among samples containing mixtures of GA and MD, ones containing GA and MD in a 1:2 ratio had the highest content of β-carotene immediately after drying while after 60 days of storage a sample containing the same carriers but mixed in ratio of 2:1 had the maximum content (Fig. 1a). Guadarrama-Lezama et al. [2012] have shown that the ratio of 1:2 (GA:MD) allowed to retain 90% antioxidant activity of microencapsulated chilli extract (β-carotene), whereas the ratio of 2:1 (GA:MD) only about 77% of the activity. It can therefore be concluded that a mixture of carriers (GA:MD) increases retention of β-carotene more effectively than a single carrier material. The efficiency depends also on the ratio of carrier materials used. Similarly Perez-Alonso et al. [2003], Krishnan et al. [2005] and Kanakdande et al. [2007] showed that the better carrier martial of microcapsule walls is a mixture of carriers than a single one.

Effect of carrier type on β-carotene content on the surface

The content of β-carotene on the surface of microcapsules and the decrease of its level after 60 days of storage significantly depended on the type of sample (Table 1). Less dye on the surface of the microcapsules was observed for samples containing GA than on those containing GA and MD. The efficacy of microencapsulation process, in the case of samples without antioxidant, was 70.9% for GA and 68.2 and 68.0% for GA:MD (1:2) and GA:MD (2:1), respectively. For samples containing antioxidant, similar relationships were observed. It was probably due to the lower emulsifying ability of GA and MD mixtures than of GA, as maltodextrins have very weak emulsifying properties [Desobry et al. 1999].

Effect of antioxidant type on total and surface β-carotene content

Basing on the statistical analysis, significant differences can be observed between average values of total and surface β-carotene content depending on the type of antioxidant used (Table 1 and 2). The highest concentration of total β-carotene was found, among samples with BHA, in samples made with GA:MD (1:2), among samples with rosemary and samples made with GA (Fig. 2). No difference was found between the mean values of total β-carotene decrease in BHA and rosemary samples. However, such differences were found between the samples with and without antioxidant additive. When analysing

the decrease in β-carotene content on the surface of microcapsules, it was found that there were no significant differences in mean values between samples without antioxidant and with BHA additions.

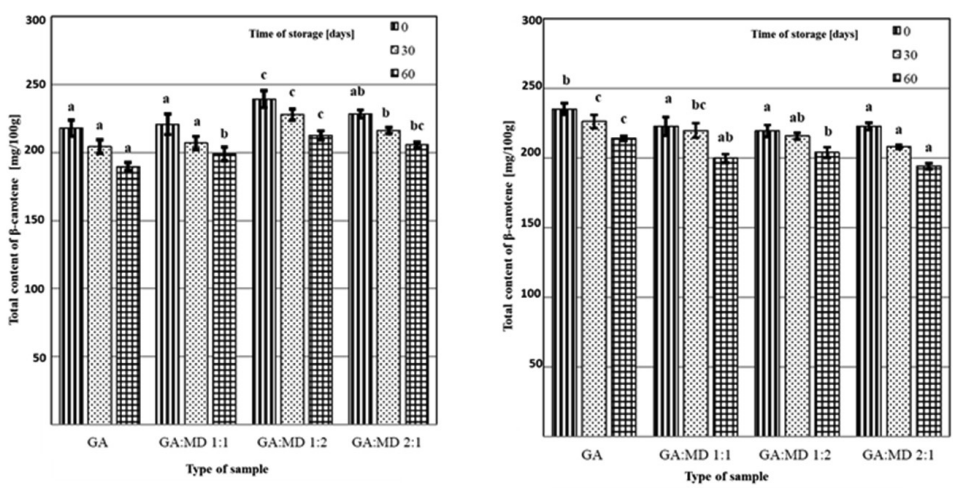
Table 2. The mean values of brightness (L*) and redness (a*) colour coefficient on the beginning and after 60 days of storage

Tabela 2. Średnie wartości parametru jasności (L*) i udział barwy czerwonej (a*) barwy na początku i po 60 dniach przechowywania

Type of sample Rodzaj próbki	L*		a*		Absolute difference Różnica bezwzględna
	Storage time [days] Czas przechowywania [dni]		Storage time [days] Czas przechowywania [dni]		
	0	60	0	60	**% Δa^2**
GA	86.63 ±0.94^{ab}	86.91 ±0.23^{b}	–1.31 ±0.31^{ef}	–0.91 ±0.18^{g}	31
GA:MD (1:1)	87.66 ±0.97^{bc}	87.97 ±0.37^{cd}	–1.70 ±0.22^{abcd}	–1.59 ±0.21^{cd}	6
GA:MD (1:2)	89.21 ±0.96^{f}	89.68 ±0.34^{g}	–1.92 ±17^{a}	–1.79 ±0.05^{a}	7
GA:MD (2:1)	87.85 ±0.12^{cde}	87.99 ±0.20^{cd}	–1.59 ±0.05^{cd}	1.42 ±0.06^{e}	11
GA + BHA	85.81 ±0.20^{a}	85.93 ±0.14^{a}	–1.29 ±0.08^{f}	–1.26 ±0.02^{f}	5
GA:MD (1:1) + BHA	88.54 ±0.32^{def}	88.60 ±0.23^{ef}	–1.61 ±0.07^{bcd}	–1.57 ±0.07^{cde}	2
GA:MD (1:2) + BHA	89.26 ±0.63^{f}	89.44 ±0.29^{g}	–1.84 ±0.07^{ab}	–1.72 ±0.04^{abc}	6
GA:MD (2:1) + BHA	87.52 ±0.40^{bc}	87.69 ±0.16^{c}	–1.53 ±0.10^{de}	–1.53 ±0.05^{cd}	0
GA + R*	85.71 ±0.28^{a}	85.80 ±0.45^{a}	–0.92 ±0.06^{g}	–0.92 ±0.08^{g}	0
GA:MD (1:1) + R[1]	88.22 ±0.24^{cde}	88.33 ±0.66^{de}	–1.77 ±0.08^{abc}	–1.77 ±0.17^{ab}	0
GA:MD (1:2) + R[1]	88.61 ±0.14^{ef}	88.80 ±0.38^{f}	–1.65 ±0.05^{bcd}	–1.60 ±0.04^{bcd}	3
GA:MD (2:1) + R[1]	88.12 ±0.35^{cde}	88.35 ±0.33^{de}	–1.63 ±0.08^{cd}	–1.56 ±0.5^{cde}	4

[1]R – rosemary; [2] – Different letters in the same column indicate significant differences (p <0.05)

[1]R – rozmaryn; [2] – Różne litery w tej samej kolumnie oznaczają istotne różnice (p <0,05)

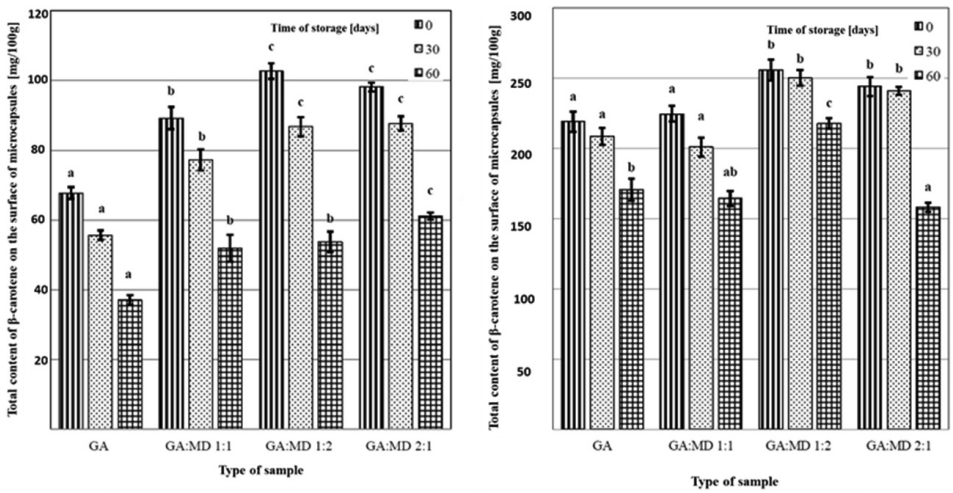


a b

Fig. 2. Total β-carotene content in microcapsules with BHA (a), and with rosemary (b)

Rys. 2. Całkowita zawartość β-karotenu w mikrokapsułkach z BHA (a) i z rozmarynem (b)

There were significant differences between the mean values for samples with rosemary and BHA additive and those with rosemary and no antioxidant (Fig. 3). Thus, the addition of rosemary extract allowed for greater retention of β-carotene on the surface of the microcapsules than for the other samples. It can be concluded that rosemary extract is a good additive to delay the degradation of β-carotene on the surface of microcapsules.



a b

Fig. 3. Content of β-carotene on the surface of microcapsules for samples with BHA (a) and with rosemary (b)

Rys. 3. Zawartość β-karotenu na powierzchni mikrokapsułek dla próbek z BHA (a) i rozmarynem (b)

Effect of carrier type and antioxidant on colour parameters of microcapsules

As far as the results for microencapsulated carotenoid colour are concerned (Table 2), it was found that brightness coefficients depended on the type of carrier used. The MD addition increased brightness of the powder in proportion to its added amount. This was probably due to the fact that the gum arabic is much darker than maltodextrins. Addition and type of antioxidants had no significant effect on the brightness coefficient L*. During storage, L* did not change significantly. Whereas Desobry et al. [1997] and Elizalde et al. [2002] showed an increase of brightness during storage of microencapsulated β-carotene. The red colour coefficient was negative, which indicated a greater share of green. Comparing changes of a* value during storage, for samples with antioxidant and without antioxidant, the changes were founded to be minimum for the samples with the addition of rosemary extract. This observation confirms the fact that rosemary extract protects the carotene remaining on the surface of microcapsules from oxidation.

CONCLUSIONS

The stability of β-carotene microencapsulated by spray drying method depended on the type of carrier used. It was greater when the GA and MD mixtures were used as carriers rather than the single GA. The type of carrier determined dye retention during spray drying more than during storage of the obtained powder. In addition, β-carotene retention and microencapsulation efficiency depended on a ratio of carriers (70.9% for single GA, 68.2% for GA:MD – 1:2 and 68.0% for GA:MD – 2:1).

β-carotene remaining on the surface of microcapsules degraded faster than the one inside microcapsules. The antioxidant addition had no effect on retention of β-carotene encapsulated in microcapsules, but it reduced the rate of degradation of the dye on the surface of microcapsules. Rosemary extract was more effective as an antioxidant than BHA. The decrease in β-carotene content on the surface for rosemary extracts ranged between 15–35% whereas for BHA between 38–48%.

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WPŁYW RODZAJU NOŚNIKA I ZASTOSOWANEGO PRZECIWUTLENIACZA NA STABILNOŚĆ B-KAROTENU MIKROKAPSUŁOWANEGO METODĄ SUSZENIA ROZPYŁOWEGO

Streszczenie. Celem pracy było określenie wpływu nośnika oraz dodatku wybranych przeciwutleniaczy na retencję β-karotenu mikrokapsułkowanego metodą suszenia rozpyłowego. Mikrokapsułkowany barwnik był w postaci roztworu olejowego, nośnikiem była guma arabska oraz mieszaniny gumy arabskiej i maltodekstryny. Do roztworów barwników dodano ekstrakt z rozmarynu lub butylohydroksyanizolu (BHA). Stwierdzono, że całkowita zawartość β-karotenu, niezależnie od rodzaju użytego nośnika, zmniejszała się podczas

przechowywania (początkowo 228,2–243,5 mg · 100 g–1 do 170,3–183,7 mg · 100 g–1 po 60 dniach przechowywania). Rodzaj nośnika miał istotny wpływ na stabilność mikrokapsułkowanego β-karotenu. Najwyższą retencję barwnika w czasie suszenia zaobserwowano dla mieszaniny gumy arabskiej i maltodekstryny (1:1), kapsułki zawierające gumę arabską i maltodekstrynę (2:1) natomiast były bardziej stabilne podczas przechowywania. Zastosowanie naturalnego przeciwutleniacza – ekstraktu rozmarynu zwiększyło stabilność barwnika na powierzchni mikrokapsułek w większym stopniu niż użycie próbek zawierających BHA. Spadek zawartości β-karotenu na powierzchni dla ekstraktów z rozmarynu wahał się w granicach 15–35%, a dla BHA 38–48%.

Słowa kluczowe: guma arabska, β-karoten, maltodekstryny, mikrokapsułkowanie, rozmaryn