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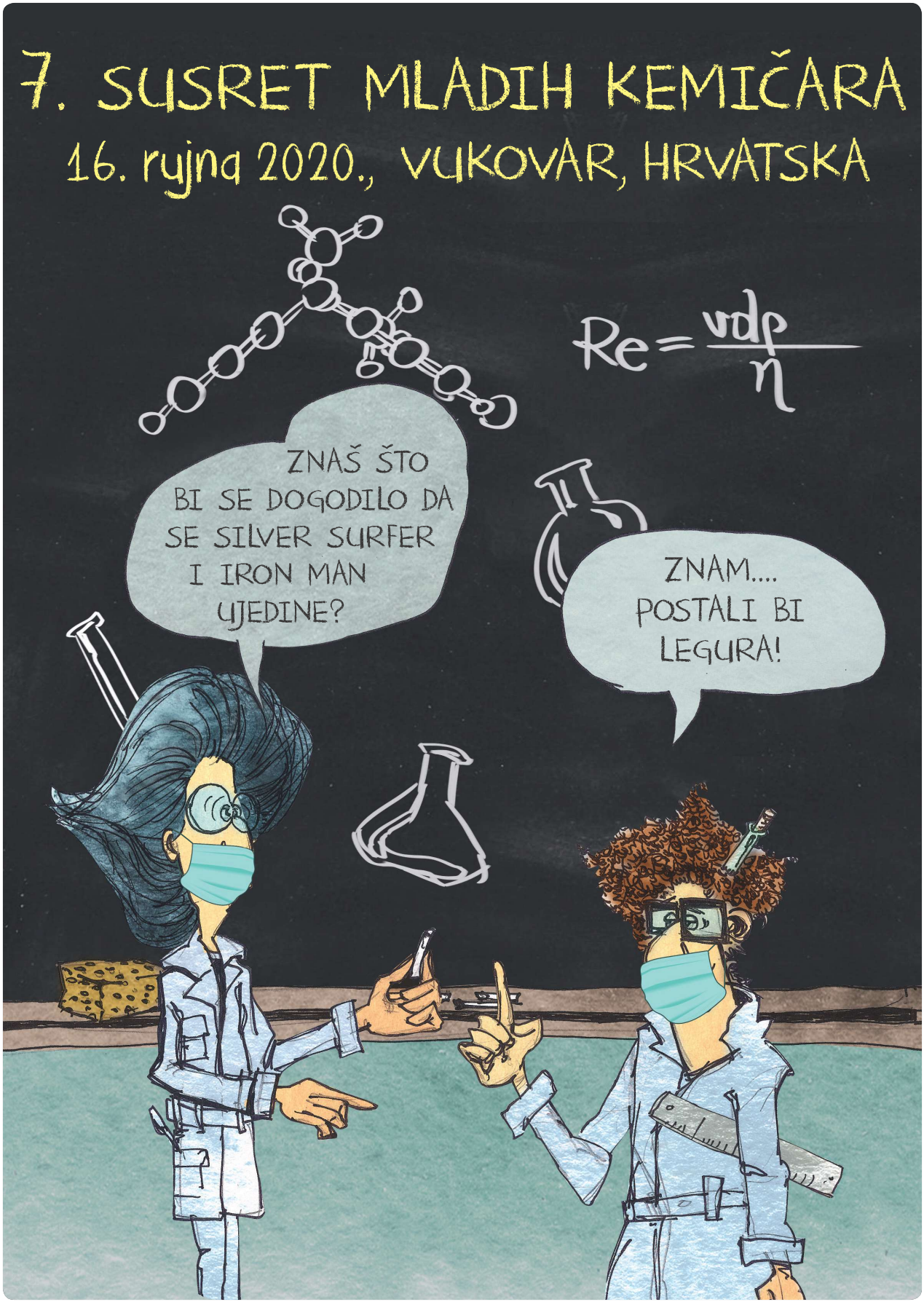
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Development and characterization of dopamine-loaded liposomes extracted from banana peel

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Summary

Banana (*Musaceae*) is the second most consumed fruit in the world, after tomato. Approximately 30 % of banana fruit is comprised of peel, resulting in a substantial amount of waste from banana processing. The peel is abundant in bioactive compounds, especially in dopamine. The aim of this study was to develop liposomes containing dopamine extracted from banana peel. Aqueous extract (80 °C, 30 min) of freeze-dried banana peel was characterized spectrophotometrically for total phenolic content (25.50 mg GAE/g dmb) and antioxidant capacity using DPPH and ABTS assays (0.156 and 0.160 mmol Trolox/g dmb), while dopamine content (9.94 mg/g dmb) was determined by HPLC-DAD. Formulated liposomes were characterized by encapsulation efficiency, as well as by size and surface charge evaluated by light scattering and zeta potential, respectively. Antioxidant capacity of encapsulated extract was investigated in terms of inhibitory action on liposome peroxidation using TBARS assays. The encapsulation efficiency of dopamine into liposomes was 41.12 % and measured values of size, polydispersity index and zeta potential indicate relative homogeneity and uniformity of the formulated liposomes.

Keywords: agro-industrial waste, banana peel, dopamine, liposomes

Introduction

The bioactive content of agro-industrial waste, especially the presence of polyphenolic compounds, is gaining attention in the food industry. The outer layers of plant fruits used in human nutrition, like husks, peels and shells, usually discarded as waste, contain a higher content of polyphenolic compounds than the inner edible parts (Manach et al., 2004). Banana, tropical fruit from *Musaceae* family, is the second most consumed fruit in the world, after tomato. Banana production is growing every year. In 2018 it was estimated to 115 million tons, with India as the biggest producer (FAO, 2019). Since 30 % of banana fruit is comprised of peel, it is obvious that a lot of peel waste is generated annually on a global scale in an industrial banana processing and in households. Similar as with other secondary plant materials, banana peel has been recognized as a valuable source of various bioactive compounds. Banana peel contains a high content of dopamine, a strong water-soluble antioxidant from the group of catecholamines (Kanazawa and Sakakibara, 2000).

Polyphenols are bioactive compounds with positive effects on human health and are of great interest to many scientists, nutritionists, food engineers, etc. However, the retention of their biological activity is closely related to their stability upon in extraction and further formulation conditions (Fangand Bhandari, 2010). In addition, polyphenols can negatively affect the sensory perception of a food product, especially its taste. Encapsulation technology is one mechanism to overcome these problems. Encapsulation can be defined as a process to entrap one substance within another substance, thereby producing particles with diameters of a few nanometres to a few millimetres (Đorđević et al., 2015). Many encapsulation techniques have been developed and many of them are already implemented on an industrial scale. Liposomes are a form of encapsulated systems in which spherical-shell capsules consist of one or more phospholipid layers enclosing a liquid core. Due to the amphipathic character, they can be used for encapsulation both hydrophobic and hydrophilic compounds (Emami et al., 2016). Liposomes have been initially developed for pharmaceutical applications, such as for the delivery of vaccines, enzymes, hormones and vitamins into the body (Gibbs et al., 1999) and recently they started to gaining attention in the field of food technology.
The aim of this study was to develop liposomes loaded with banana peel extract rich in dopamine and other water-soluble polyphenols. Different combinations of blanching and drying were explored in order to preserve the polyphenolic compounds at a maximum scale. Obtained extracts were characterised with the determination of total phenolic content, antioxidant capacity and dopamine content. Extract with the best bioactive properties was encapsulated into liposomes. The encapsulation efficiency and physico-chemical parameters (size, zeta potential and polydispersity index) of formulated liposomes were determined. Additionally, the antioxidant capacity of the encapsulated extract was investigated in terms of its inhibitory action on liposome peroxidation.

Materials and Methods

Materials

Banana (var. Cavendish, produced by Costanza Organic Bananas, Ecuador) was purchased at a local store. Folin–Ciocalteu's reagent was supplied from Kemika (Zagreb, Croatia). Dopamine hydrochloride, (S)-6-Methoxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,20-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, 2-thiobarbituric acid, perchloric acid and trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, USA). Methanol was supplied from Panreac (Barcelona, Spain) while ethanol (96 %) and acetonitrile from Carlo Erba (Val de Reuil, France). Chloroform was supplied from Gram-mol d.o.o. (Zagreb, Croatia). Phospholipon 90G (P90G) was supplied by Lipoid GmbH (Ludwigshafen, Germany). All chemicals used for experimental procedures were of analytical or HPLC grade.

Methods

Preparation of banana peel samples

The maturity stage of bananas was determined following the standard colour chart of Tapre and Jain (2012), by which they exhibited a value of 6 (full yellow).

Different preparation techniques of banana peel were explored, drying in the oven (D) (Instrumentaria ST-06, Zagreb, Croatia) and freeze-drying (Christ, Alpha 1-2 LD plus, Germany) (F) with (B) or without (NB) previous blanching in water (100 °C, 7 min) to inactivate endogenous enzymes. Dried banana peels were pulverized in a domestic coffee mill to obtain a representative sample. Water extracts were prepared by mixing powdered banana peel with water in ratio 1:20 (w/v), followed by heating to 80 °C for 30 min and the separation of the extract by centrifugation (Thermo Scientific SL8R, Fisher Scientific, Göteborg, Sweden). The extracts were prepared in duplicate. The extract selected to prepare liposomes was further concentrated in a vacuum evaporator (IKA RV8, Staufen, Germany) and freeze-dried (Christ, Alpha 1-2 LD plus, Germany). The obtained extracts were characterized spectrophotometrically by the determination of total phenolic content, antioxidant capacity by DPPH and ABTS assays and dopamine content by HPLC-DAD, as described in the following sections.

Determination of total phenolic content (TPC) and antioxidant capacity

TPC was determined spectrophotometrically (Genesys 10S UV-VIS Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA), according to the method of Singleton and Rossi (1965). The calibration curve was constructed on gallic acid standard and the results were expressed as gallic acid equivalents on dry matter basis (mg GAE/g dmb). Antioxidant capacity was determined using DPPH and ABTS radical cation decolourization assays by Brand-Williams et al. (1965) and Re et al. (1999), respectively. Trolox was used for the external standard calibration curve, and the results were expressed as Trolox equivalent per dry matter basis of the sample (mg Trolox/g dmb). All measurements were performed in triplicate.

HPLC-PAD determination of dopamine

HPLC analysis was performed on Agilent Series 1200 chromatographic system (Agilent Technologies, Santa Clara, CA, USA) coupled with a Photodiode Array Detector (PAD, Agilent Technologies, Santa Clara, CA, USA) and Zorbax Extend C18 (4.6 × 250 mm, 5 µm i.d.) chromatographic column (Agilent Technologies, Santa Clara, CA, USA). The elution was performed in a gradient with three-component mobile phase consisting of (A) 100 % acetonitrile, (B) 2 % (v/v) formic acid solution in methanol and (C) 2 % (v/v) formic acid solution in water, as follows: 0 min – 0 % A, 3 % B, 97 % C; 5 min – 0 % A, 3 % B, 97 % C; 10 min – 0 % A, 5 % B, 95 % C; 30 min – 30 % A, 30 % B, 40 % C; 35 min – 30 % A, 30 % B, 40 % C; 45 min – 0 % A, 3 % B, 97 % C; 60 min – 0 % A, 3 % B, 97 % C. The flow was 1 mL/min, the injection volume 20 µL and the column temperature 25 °C. The chromatograms were recorded at 280 nm. Dopamine identification was performed by comparing the retention time of pure external standard and its characteristic absorption spectrum (190–400 nm). Quantification was enabled by establishing calibration curve for dopamine (20-100 µg/mL). All samples were filtered through a 0.45 µm syringe filter (Nylon Membranes, Supelco, Bellefonte, PA, USA) prior to the analysis.

Encapsulation of banana peel powdered extract into liposomes

Liposomes were prepared following the proliposome method as described by Perrett et al. (1991). Briefly, 1 g of P90G was dissolved in 1.25 mL of ethanol (96 %) and heated to 60 °C for 2 min in water bath with constant stirring. Freeze-dried banana peel extract was dissolved in 2 mL water in different concentrations: 25 mg/mL, 50 mg/ml and 100 mg/mL, and each mixed with the prepared P90G. The mixture was again heated at 60 °C for 2 min and left to cool down to room temperature. Finally, 48 mL of distilled water was added drop-wise (Perfusor Compact Plus, B. Braun, Germany) through a metal needle (G 23) into P90G-extract mixture while stirring to allow the formation of liposomes (L20, L10 and L5 liposome samples for the concentrations of the extract 25, 50 and 100 mg/mL, respectively).

Physico-chemical characterisation of liposomes

Size, polydispersity index (PDI) and zeta potential of liposomes were measured using Malvern Nano-ZS Zetasizer (Malvern, UK).

Determination of encapsulation efficiency

The suspensions of liposomes were centrifuged (6000 rpm, 1 h) and the supernatants separated, while the liposomes concentrated in the residue were washed once with water, centrifuged (6000 rpm, 30 min, supernatant discarded) and then suspended in 1 mL of distilled water and dissolved in chloroform-methanol (1:1, v/v) mixture. The mixture was vortexed for 1 min and left until the phases separated. The upper water-methanol phase was collected and subjected to analyses. In order to measure the encapsulation efficiency, determination of TPC, antioxidant capacity and dopamine content, as described in previous chapters, were performed both in collected supernatants and water-methanol phases.

Thiobarbituric acid reacting substances assay (TBARS)

The peroxidation of the liposomes was determined spectrophotometrically (Genesys 10S UV-VIS Spectrophotometer, Thermo Fisher Scientific, US) using the TBARS assay. Plain liposomes and liposomes with encapsulated powdered extract were placed into UV-pulsed device with radiant energy per pulse 1.27 J/cm^2 (Polytec, Germany). Small aliquots of samples were taken after 0, 10, 20, 30, 40, 50, 70, 90, 110 and 130 UV pulses for the TBARS assay. Briefly, 0.2 mL of liposome sample was mixed with 3 mL of 20 % (w/v) trichloroacetic acid and 1 mL of solution containing 2 % (w/v) thiobarbituric acid and 20 % (v/v) perchloric acid in ratio (1:1, v/v). The mixture was well mixed and placed into water bath (100 °C) for 25 min. The reaction was stopped by placing the tubes into cold water for 5 min. To remove the resulting precipitate, the tubes were centrifuged (9500 rpm, 10 min). The absorbance of the supernatant was measured at 532 nm.

Results and Discussion

Preparation of banana peel samples

In order to efficiently preserve polyphenolic compounds in banana peel, otherwise susceptible to enzymatic degradation by endogenous polyphenol oxidase, banana peel was prepared in 4 different ways by combining oven- and freeze-drying with or without blanching. The results of the TPC, antioxidant capacity and dopamine content in water extracts of differently prepared banana peels are shown in Table 1.

Table 1. Bioactive evaluation parameters of water extracts of differently prepared banana peel extracts

Sample	TPC (mg GAE/g dmb*)	Antioxidant capacity		Dopamine (mg/g dmb)
		DPPH (mmol Trolox/g dmb)	ABTS (mmol Trolox/g dmb)	
B&F banana peel	25.59 ± 0.38	0.156 ± 0.000	0.160 ± 0.000	9.94 ± 0.08
NB&F banana peel	15.55 ± 0.19	0.116 ± 0.010	0.110 ± 0.000	7.05 ± 0.15
B&D banana peel	17.73 ± 0.88	0.113 ± 0.010	0.134 ± 0.000	8.87 ± 0.09
NB&D banana peel	3.44 ± 0.04	0.025 ± 0.010	0.018 ± 0.000	0.23 ± 0.04

*B&L= blanched and freeze-dried; NB&L=non-blanched and freeze dried; B&D= blanched and oven-dried; NB&D=non-blanched and oven-dried; *dmb = dry matter basis of the sample*

The highest TPC (25.59 mg GAE/g dmb), antioxidant capacity (DPPH: 0.156 mmol Trolox/g dmb; ABTS: 0.160 mmol Trolox/g dmb) and dopamine content (9.94 mg/g dmb) were determined for banana peel prepared by combining blanching and freeze-drying, while the lowest values of the measured bioactive evaluation parameters were determined for oven-dried banana peel without previous blanching (TPC: 3.44 mg GAE/g dmb; DPPH: 0.025 mmol Trolox/g dmb; ABTS: 0.018 mmol Trolox/g dmb; 0.23 mg dopamine/g dmb). The results indicate that the blanching process (100 °C; 7 min) was sufficient to inactivate the enzyme polyphenol oxidase, while freeze-drying proved to be a better option than oven-drying. Similar results were obtained by Hsu et al. (2003) who reported that freeze-drying preserved more antioxidant capacity of the yam flours than hot air-drying and drum-drying. Blanched and freeze-dried banana peels were used further in the experimental work.

Physico-chemical characterization of liposomes

Size, zeta potential and PDI of formulated liposomes, prepared by varying the concentration of banana peel extract, are presented in Table 2. The size of plain liposomes was 316.1 nm, while the size of dopamine-loaded liposomes ranged between 317.6 nm (liposomes L5) to 350.8 nm (liposomes L20). Lin et al. (2018) stated that when the zeta potential is <-30 mV or >30 mV, as in the case of plain liposomes, then it can provide sufficient repulsive force to maintain a stable system. However, from the presented results, it is obvious that the addition of the banana peel extract into liposomes negatively affected their stability. Regarding the particle size distribution and system homogeneity, presented as PDI values, all formulated

liposomes were found to be monodisperse and homogenous since PDI values were below 0.5 (Balanč et al., 2016).

Table 2. Physico-chemical properties of liposomes

Sample	Size (nm)	Zeta potential (mV)	PDI
Plain liposomes	316.1 ± 6.4	-31.2 ± 0.5	0.369 ± 0.043
Liposomes L5	317.6 ± 5.8	-29.4 ± 0.5	0.338 ± 0.007
Liposomes L10	319.1 ± 1.7	-27.3 ± 1.6	0.333 ± 0.045
Liposomes L20	350.8 ± 7.2	-22.3 ± 1.7	0.303 ± 0.010

L20-liposomes prepared with 1 g of P90G and 50 mg of extract; L10-liposomes prepared with 1 g of P90G and 100 mg of extract; L5-liposomes prepared with 1 g of P90G and 200 mg of extract

Encapsulation efficiency (EE) of bioactive parameters of formulated liposomes

EE in terms of TPC, antioxidant capacity determined by DPPH and ABTS assays and dopamine content is presented in Table 3.

Table 3. Encapsulation efficiency evaluated by total phenolic content (TPC), antioxidant capacity (DPPH and ABTS) and dopamine content

Sample	Encapsulation efficiency (%)			
	TPC	Antioxidant capacity		Dopamine
		DPPH	ABTS	
Liposomes L5	33.71	36.54	35.24	35.10
Liposomes L10	45.93	36.19	33.58	38.42
Liposomes L20	35.39	37.23	33.67	41.12

L20-liposomes prepared with 1 g of P90G and 50 mg of extract; L10-liposomes prepared with 1 g of P90G and 100 mg of extract; L5-liposomes prepared with 1 g of P90G and 200 mg of extract

It is evident that liposomes L20, prepared with the lowest ratio of P90G to extract dry matter, resulted in the highest EE with respect to dopamine content (41.12 %) and antioxidant capacity determined by DPPH assay (37.23 %), while liposomes L10 were characterised with the highest EE with respect to TPC (45.93 %). In the available literature, liposomes proved to be highly efficient in the encapsulation of different bioactive compounds, such as curcumin (Li et al., 2017) and resveratrol (Feng et al., 2019). Relatively low EE for dopamine can be attributed to the extremely hydrophilic character of dopamine. Further research on the development of dopamine-loaded liposomes is required.

Thiobarbituric acid reacting substances (TBARS) assay

TBARS assay was used for the determination of lipid peroxidation induced by UV light exposure. The principle of the assay is the reaction between thiobarbituric acid and malondialdehyde, a carbonyl product of lipid hydroperoxide decomposition (Janero, 1990), that results in pink-coloured reaction mixtures. The results of TBARS assay are presented in Fig. 1.

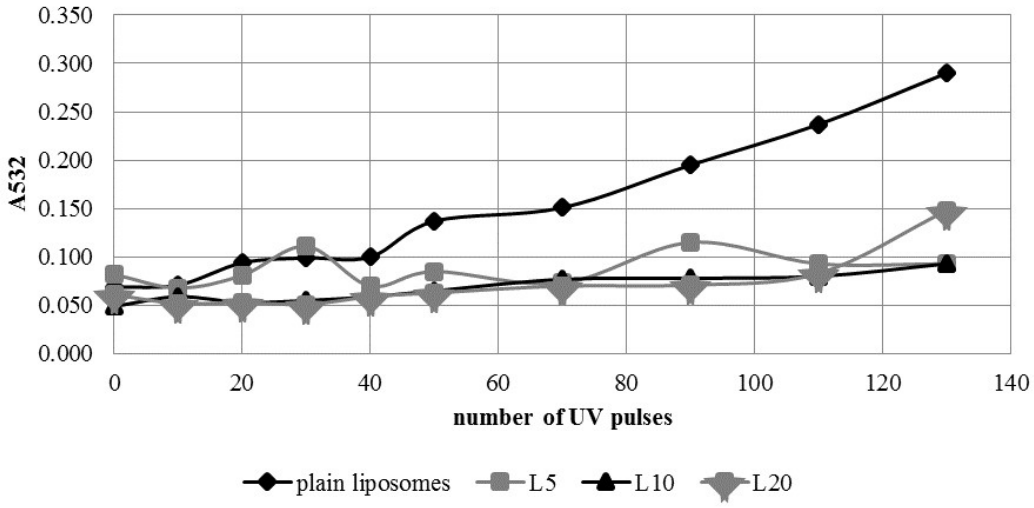

Figure 1. Results of the TBARS assays for plain and dopamine-loaded liposomes (*L20-liposomes prepared with 1 g of P90G and 50 mg of extract; L10 liposomes prepared with 1 g of P90G and 100 mg of extract; L5-liposomes prepared with 1 g of P90G and 200 mg of extract*)

It is obvious that plain liposomes showed much higher absorbance at the same amount of UV radiation than the loaded liposomes. The results indicate on the protective effect of encapsulated extracts on the overall peroxidation stability of liposomes, coming presumably from polyphenols and dopamine which both exert antioxidant activity (Scalbert et al., 2005; Kanazawa and Sakakibara, 2000). Plain liposomes were probably more susceptible to peroxidation reactions because they did not contain encapsulated polyphenols. Encapsulated banana peel extracts were efficient in preventing lipid peroxidation in all evaluated extract concentrations and for all examined UV exposure density.

Conclusions

Combination of blanching and freeze-drying enabled sufficient inactivation of endogenous polyphenol oxidase which resulted in the highest content of banana peel's bioactive compounds preserved. Banana peel presents a valuable source of dopamine (9.94 mg/g dmb). Liposomes prepared with the lowest ratio of banana peel extract to P90G were characterised with the highest encapsulation efficiency in terms of dopamine content (41.12 %) and the smallest PDI value (0.303), indicating a high homogeneity of the prepared liposome suspension. Liposomes prepared with the highest ratio of banana peel extract to P90G were the smallest in size (317.6 nm) and most stable according to determined zeta potential (-29.4 mV). Banana peel extract in the prepared liposomes inhibited malondialdehyde formation and thus showed lipid peroxidation-protecting efficiency acting as a high-level scavenger of radicals.

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