Journal of Engineering Science *Vol. XXIX, no. 4 (2022), pp. 138 - 149*
Fascicle *Food Engineering* *ISSN 2587-3474*
Topic *Food Technologies and Food Processes* *eISSN 2587-3482*

https://doi.org/10.52326/jes.utm.2022.29(4).11
UDC 547.973:582.788.1

MICROENCAPSULATION OF ANTHOCYANINS FROM CORNELIAN CHERRY FRUITS IN WHEY PROTEIN ISOLATE AND PECTIN

Iuliana-Maria Enache [1], ORCID: 0000-0002-4691-7311,
Liliana Lucescu Ciurlă [1], ORCID: 0000-0003-2874-9825,
Nicoleta Stănciuc [2], ORCID: 0000-0002-4763-8656,
Liviu Mihai Irimia [1], ORCID: 0000-0003-1356-1774,
Antoanela Patraș [1]*, ORCID: 0000-0002-4054-4884,
Camelia Vizireanu [2], ORCID: 0000-0002-8704-2662

[1] *"Ion Ionescu de la Brad" Iasi University of Life Sciences", Department of Sciences, Iasi, Romania*
[2] *"Dunărea de Jos" University of Galați, Department of Food Science, Food Engineering, Applied Biotechnology, Galati, Romania*
*Corresponding author: Antoanela Patraș, *apatras@uaiasi.ro*

Received: 10. 25. 2022
Accepted: 12. 03. 2022

Abstract. Cornelian cherry (*Cornus mas* L.) is one of the most important forest fruits, considered as a valuable horticultural resource of bioactives, such as anthocyanins - cyanidin-3-glucoside, flavonoids, vitamins (e.g. vitamin C), carotenoids (e.g. β-carotene). The aim of this study was to obtain designed delivery systems of bioactive from cornelian cherry, as microencapsulated powders in order to assure their controlled release and to develop stable and natural additives for different application. Anthocyanin's (concentrated extract) from cornelian cherry fruits were microencapsulated in a complex, biopolymeric matrice, formed by whey protein isolate (WPI) and pectin (PT). Two experimental variants were obtained by varying the ratio between WPI and PT, such as 1:1 (PT1) and 1:2 (PT2). The powders were tested for encapsulation efficiency of the anthocyanins, phytochemical profile of the extract and freeze-dried powders, as well as colorimetric analysis. Encapsulation efficiency of the anthocyanins varied between 80.04 and 82.11% with an important level of biologically active compounds (total polyphenols, total flavonoids) and remarkable antioxidant activity. Colorimetric analysis reveals a red colour of the powders, associated with their anthocyanin content. Both experimental variants proposed in this study protected the anthocyanins from cornelian cherry fruits. Moreover, microencapsulated powders can be used as natural food additives due to their red colour and phytochemical profile.

Keywords: *complex biopolymeric matrice, Cornus mas L., encapsulation efficiency, colour.*

Rezumat. Cornul de pădure (*Cornus mas* L.) este unul dintre cele mai importante fructe de pădure, considerat o resursă horticolă valoroasă de compuși bioactivi, cum ar fi antociani - cianidin-3-glucozidă, flavonoide, vitamine (de exemplu, vitamina C), carotenoide (de exemplu, β-caroten). Scopul acestui studiu a fost de a obține sisteme de eliberare a substanțelor bioactive din fructele de corn, sub formă de pulberi microîncapsulate, pentru a

asigura eliberarea lor controlată și pentru a dezvolta aditivi naturali și stabili pentru diferite aplicații. Antocianii (extract concentrat) din fructele de corn au fost microîncapsulați într-o matrice biopolimerică complexă, formată din izolat de proteic din zer (IPZ) și pectină (PT). Două variante experimentale au fost obținute prin variația raportului dintre IPZ și PT, cum ar fi 1:1 (PT1) și 1:2 (PT2). Au fost testate pudrele pentru eficiența de încapsulare a antocianilor, profilul fitochimic al extractului și al pulberilor liofilizate, precum și din punct de vedere colorimetric. Eficiența de încapsulare a antocianilor a variat între 80.04 și 82.11%, cu un nivel important de compuși biologic activi (polifenoli totali, flavonoide totale) și o activitate antioxidantă remarcabilă. Analiza colorimetrică relevă o culoare roșie a pudrelor, asociată cu conținutul de antociani. Ambele variante experimentale propuse în acest studiu au protejat antocianii din fructele de corn. În plus, pulberile microîncapsulate pot fi utilizate ca aditivi alimentari naturali datorită culorii roșii și profilului lor fitochimic.

Cuvinte cheie: *matrice biopolimerică complexă, Cornus mas L., eficiența încapsulării, culoare*.

1. Introduction

Cornelian cherry (*Cornus mas* L) is one of the forest fruits found in 38 spontaneous crop in Romania, considered as a rich resource of biologically active compounds (polyphenols: anthocyanins, flavonoids, vitamins and carotenoids etc) [1]. According to a study conducted by Yilmaz, (2009) [2], cornelian cherry, known also as *dogwood*, only 4 of the 65 species are edible.

Anthocyanins represent an important subgroup of flavonoids, perhaps the most important of them. Over time studies have revealed a number of essential characteristics of flavonoids, of which the best known can be considered: antioxidant, antimicrobial, antibacterial, antifungal and antiviral activity [3]. A study conducted by Manganaris [4] concluded that in the *Plantae* kingdom the most popular compounds with antioxidant properties are: ascorbic acid, carotenoids, tocopherol and phenolic compounds.

To the best of our knowledge, one of the most important categories of biologically active compounds found naturally in fresh fruits and vegetables are anthocyanins. The 700 anthocyanins identified since now are associated with some specific colour of leaves, flowers and fruits, such as orange, red, blue and purple colours and the shades between them. The compounds above-mentioned are derived from aglycons, being known as *anthocyanidins*. Each of six more commonly anthocyanidins found in nature are correlated with a maximum absorbance, as well as with a specific colour, respectivelly: pelargonidin (494 nm, identified by orange colour), cyanidin and peonidin (506 nm, correlated with orange-red colour), delphinidin and petunidin (red colour, 508 nm) and malvinidin (510 nm, bluish-red colour components) [5, 6].

A remarkable property of anthocyanins is their antioxidant capacity. A study conducted by Einbond (2004) [7] suggests that the most common anthocyanin is thought to be cyanidin. According to the same study, cyanidin-3-glucoside is considered to be the most active anthocyanin associated with a high level of antioxidants. Therefore, based on the above-mentioned information, cyanidin-3-glucoside is a natural compound considered one of the most popular anthocyanins correlated with an important antioxidant capacity.

Moreover, the literature highlights [8] that anthocyanin extraction is strongly influenced by the solvent or solvents used, including the ratio between them, table 1 shown the most commonly of them.

Table 1

The most commonly solvents used for anthocyanins extraction

Anthocyanins	Method of extraction/ Solvent(s)
Cyanidin-3-glucoside	Maceration/Methanol [7]
Delphinidin-3-glucoside	Maceration/Methanol [7]
Cyanidin-3-glucoside	Conventional extraction/Ethanol 60-100% [1]
Cyanidin-3-rutinoside	Combined method (hydroalcoholic solvent and ultrasound extraction)/ Ethanol 70% [9]

On the another hand, the anthocyanins extraction yield can be influenced by several factors. The influencing factors of the anthocyanins extraction yield and recommended variant can be considered: type of solvent (e.g. organic/inorganic solvent), quantity of solvent (expressed as µL/mL), type of extraction (e.g. maceration/ conventional extraction/ ultrasound extraction), temperature extraction (e.g. 30 – 50 °C), duration of the extraction (e.g. 15-45 min) [1, 9].

In order to obtain a functional extract of phenolic compounds from black rice, as well as for anthocyanins extraction, previous studies used random parameters (extraction time and temperature, concentration of solvent), as well as a mathematical method (Response Surface Methodology) for the optimization phenolic compounds extraction yield [10 -15]. The most widely used method for determining anthocyanins is the differential pH method [16].

As for the health benefits of anthocyanins, the most common examples can be considered the relief or treatment of conditions such as cancer, allergies, viral infections, low immunity, inflammation, diabetes [17].

In terms of determination and quantification methods, antioxidant activity can be achieved after microwave-assisted extraction, ultrasound-assisted extraction, maceration or conventional extraction using various methods, shown in table 2:

Table 2

Analytical methods used for the quantification of antioxidant activity [17, 18].

Method	Reagent/ Method explication
DPPH	2,2-diphenyl-1-picrylhydrazyl
ORAC	Oxygen Radical Absorbance Capacity
TEAC	Trolox Equivalent Antioxidant Capacity
FRAP	Ferric Radical Absorbance Capacity
CUPRAC	Cupric Radical Absorbance Capacity
ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid

It can be considered that anthocyanins are the most common biologically active compounds in nature, but they degrade very quickly.

In order to protect anthocyanins against external factors (pH, light, storage temperature, storage time, etc.) it is necessary to find viable, efficient and cost-effective alternatives. The objectives listed above can be achieved by applying microencapsulation, and a number of technologies can be used, including spray drying, ionic gelation, freeze drying, etc. [17-19].

Freeze-drying involves removing water from the sample by freezing. This technique consists of two stages: (1) sublimation in the primary drying stage *and* (2) desorption in the secondary drying stage. Among the advantages of using freeze-drying are: applicability in

various fields (e.g. pharmaceuticals, biological products, flowers, cultured micro-organisms, bulk pharmaceuticals, medical and cosmetic devices, chemicals, pigments, enzymes etc.), thermolabile products (e.g. heat-sensitive foods, products unstable in water, high storage stability over time. The extraction of water from food by freeze-drying involves the following steps: freezing the food → sublimation of the ice formed directly into water vapor → removal of the water vapor. Freeze-drying is considered to be complete when the sublimation process is completed. The quality of the final product obtained by freeze-drying is dependent on three essential parameters that must be set very correctly: the pressure of the equipment (e.g., 10 Pa), the working time (e.g., 48 hours) and the operating temperature of the equipment (e.g., -42 ^{o}C) [9, 20, 21]. An important role in the freeze-drying process is played by the excipients used (bulking agents, antimicrobial agents, surfactants, cosolvents) [22].

Several studies showed the importance of the practical applications of microencapsulation in the food industry and biotechnology. In terms of specific applications for the food industry, a wide range of lactic acid bacteria, biologically active compounds extracted from fruits and vegetables, can be subjected to the microencapsulation process, including *Bifidobacterium lactis* [23], pineapple, acerola cherries, guava, papaya, mango [24], pomegranate [25], cherries [26], bananas [27], black rice [28], grape peel [29], strawberry [30], tomato peel [31], eggplant peel [32], yellow onion peel [33], black beans [34], blackcurrants [35], cornelian cherry fruit [9, 36].

Nowadays, a negative impact on human health is given by the consumption of food synthetic additives, associated with many diseases, such as: obesity, cancer, diabetes, hypertension, dementia or eye diseases [37].

Wall materials (including those used in this work) can be correlated with many health benefits. Whey protein isolate is associated with antimicrobial and antiviral effect, anticancer effect, cardiovascular health, immunological activity, improves cognitive performance, physical performance, and weight management [38]. The pectin's effects for human health include maintenance of low level of cholesterol, prevention of colon and prostate cancer; remove heavy metals from body [39].

The main aim of this study was to obtain and characterise two complex biopolymeric matrices of cornelian cherry extract (performed with whey protein isolate and pectin) by the variation of their ratio (1:1, 1:2). The final goal was to demonstrate their benefits for future applications as natural food additives due to their important level of anthocyanins, polyphenols and flavonoids content, as well as antioxidant capacity.

2. Materials and Methods

Reagents used in this study (extraction: ethanol; total antioxidant activity: methanol, 2,2-Diphenyl-1-picrylhydrazyl (DDPH), 6-Hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid (Trolox); total polyphenols content: Folin–Ciocalteu reagent, sodium carbonate, gallic acid; total flavonoids content: sodium nitrate, aluminium chloride, sodium hydroxide, catechin; total anthocyanins content: cyanidin-3-glucoside, sodium acetate, potassium chloride; encapsulation efficiency: acetic acid) were purchased from Sigma-Aldrich (Steinheim, Germany).

The cornelian cherry fruits (CCF) were purchased from a local market (Galati, Romania) in 2018. Fruit washed with distilled water and dried with paper towels had the stones removed.

CCF extract was obtained by hydroalcoholic extraction (70% ethanol) combined with ultrasound extraction (30 min, 40^{o}C, 100 W and 40 kHz - MRC Scientific Instrument), followed

by filtration. Finally, the liquid extract was centrifuged at 5000 rpm for 30 min at 4°C (Universal 320R, Tuttlingen, Germany). Microencapsulation of anthocyanins from CCF was achieved by mixing the concentrated extract (2.5 g) with wall materials: whey protein isolate (WPI) and pectin (PT). Encapsulation of the bioactive compounds from *Cornus mas* fruits was achieved by mixing the concentrated extracts fruits with whey protein isolate and pectin as follows: variant 1: 2.5 g concentrate extract of cornelian cherry fruits, 1 g whey protein isolate, 1 g pectin were mixed and brought to the mark with ultrapure water using a 100 mL volumetric flask. For the development of the second experimental variant, the same protocol was used, except that 2 g of pectin was added. Two microencapsulated powders were obtained, respectively: WPI:PT=1:1 (PT1) and WPI:PT=1:2 (PT2). Both experimental variants were left for 4 hours on a magnetic stirrer (650 rpm) in order to completely homogenize the ingredients of the mixture. Subsequently, the samples were kept for 24 hours at -4 °C in order to hydrate the mixture, and then freeze-dried (-42°C, for 48 hours at a pressure of 10 Pa - CHRIST Alpha 1-4 LD plus, Osterode am Harz, Germany).

Phytochemical profile and antioxidant capacity of the extract and microencapsulated powders was performed by: total anthocyanin content (TAC) – pH differential method (results expressed as mg cyanidine-3-glucoside per gram of dry weight (C3G/g DW), total polyphenols content (TPC) – Folin-Ciocalteu method (results expressed as mg gallic acid equivalent (GAE)/g DW) total flavonoid content (TFC) – colorimetric method (results expressed as mg catechin equivalent (CE)/g DW), while total antioxidant activity (T-AA) was studied by DDPH radical scavenging method (mMol Trolox Equivalent (TE)/g DW) [9, 35, 36]. The spectrophotometer UV-Vis Jenway was used to read to absorbance for each sample (OSA, UK).

Encapsulation efficiency is the percentage of encapsulated anthocyanins in the wall materials used, by measuring the surface anthocyanin content (SAC), by extracting the anthocyanins in a mixture of methanol and ethanol in a ratio of 1:1, (v/v) and the total anthocyanin content (TAC), by extracting in a mixture of methanol: acetic acid: ultrapure water (25:4:21, v/v/v), according to equation 1:

$$EE(\%) = \frac{TAC-SAC}{TAC} x\ 100 \quad (1)$$

The colorimetric analysis was performed by determining the colour parameters (L*, a*, b*) using the colorimeter. According to the CIEL*a*b* system protocol, the L* parameter indicates the brightness of the sample (0 - for black, 100 - for white), the a* parameter provides information about the green/red spectrum of the sample (positive values are associated with red shades, while negative values are associated with green shades), and the b* blue/yellow parameter indicates the tendency of the analysed sample to have yellow (positive values) or blue (negative values) colour [40].

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

$$h* = arctang\left(\frac{b*}{a*}\right) \quad (3)$$

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (4)$$

The overall colour difference or total colour difference (ΔE*) was obtained as follows: for the experimental variants of microencapsulated powders, the difference between the initial L*, a*, b* values obtained for the concentrated extract of cornelian cherry fruit and the obtained powders (PT1, PT2) was taken into account, while the ΔE* for the concentrated

extract was calculated as the difference between the L*, a*, and respectively, b* values of the cornelian cherry fruit and the values of the concentrated extract.

All the analysis of this study were performed in triplicate and the results were expressed as mean ± standard deviation. Microsoft Excel Package Tools and IBM SPSS Statistics 21 were used to perform statistical analysis. Tukey test with 95% confidence interval was applied.

3. Results and Discussion

The anthocyanins content, polyphenolic content and flavonoids content, as well as the antioxidant capacity of the cornelian cherry extract and microencapsulated powder are showed in table 3.

Table 3

Bioactive compounds, antioxidant activity and encapsulation efficiency of concentrated extract and microencapsulated powders

Tested parameter	Value
Concentrated extract	
Total antioxidant activity (mMol TE/g DW)	185.15 ± 1.15
Total polyphenolic content (mg GAE/g DW)	17.41 ± 0.67
Total anthocyanins content (mg C3G/g DW)	12.86 ± 0.80
Total flavonoid content (mg CE/g DW)	5.08 ± 0.13
Microencapsulated powders	
PT1	
Encapsulation efficiency (%)	80.04 ± 0.81[a]
Total antioxidant activity (mMol TE/g DW)	53.54 ± 0.27[a]
Total polyphenolic content (mg GAE/g DW)	10.45 ± 0.06[a]
Total anthocyanins content (mg C3G/g DW)	5.15 ± 0.12[a]
Total flavonoid content (mg CE/g DW)	3.76 ± 0.13[a]
PT2	
Encapsulation efficiency (%)	82.11 ± 0.14[b]
Total antioxidant activity (mMol TE/g DW)	53.59 ± 0.11[a]
Total polyphenolic content (mg GAE/g DW)	10.79 ± 0.67[a]
Total anthocyanins content (mg C3G/g DW)	5.71 ± 0.06[b]
Total flavonoid content (mg CE/g DW)	3.69 ± 0.18[a]

Note. For each powders parameter tested, values that are for the same parameter (e.g. total antioxidant activity) that do not have the same lowercase letters ((a) or (b)) are statistically different at $p < 0.05$ based on the Tukey method with 95% confidence interval

In table 3 can be observed that the characteristics of the concentrated extract were well preserved in the two microencapsulated powders with the exception of the total antioxidant activity, which decreased with 71%. Generally, the results of the PT2 variant were slightly better compared to PT1 (excepting the total flavonoid content), but the differences

are extremely small. The encapsulation efficiency of the anthocyanins was higher than 80%, highlighting the efficiency of the biopolymeric combination used in this study to retain the bioactive compounds. The obtained results are in accordance with previous researches [36], which reported an encapsulation efficiency of 95.46±1.30 % for anthocyanins extracted from black currant fruits microencapsulated in WPI, chitosan and inulin. In another study [9], were microencapsulated cornelian cherry fruits extracts in WPI and casein or inulin. In this case, encapsulation efficiency varied between 77.97±0.57 % (variant with casein) and 79.03 ± 0.72% (variant with inulin).

Table 4

Comparative analysis of the cornelian cherry parameters previously published

Determined parameter	Solvent used	Method of determination (λ, nm)	Value	Unit of measurement	References
Antioxidant activity	Ethanol 80%	DPPH (515 nm)	0.43-1.32	$(1/IC_{50})\cdot 100$	[41]
	Methanol	DPPH (515 nm)	38.98-82.37	%	[42]
Anthocyanins content	Ethanol 80%	pH differential method (510 nm, 700 nm)	0.06-3.03	mg C3G/ g	[41]
	Methanol; Hydrochloric acid (1%, v/v)	pH differential method (510 nm, 700 nm)	106.89-442.11	mg C3G/ 100 g	[42]
Flavonoids content	Methanol; Hydrochloric acid (1%, v/v)	Colorimetric method (NaOH) (510 nm)	321.71-669.00	mg CE/100 g fresh fruit	[42]
	Methanol Water Ethyl acetate Acetone Petroleum ether	Colorimetric method–$AlCl_3$ (415 nm)	7.18 ± 0.10 3.53 ± 0.39 41.49 ± 0.57 8.05 ± 0.76 6.91 ± 0.09	mg RE /g extract	[43]
Polyphenols content	Hydrochloric acid: methanol: water (2:80:18)	Folin Ciocâlteu method (765 nm)	2.61±0.21 8.11±0.40	g GAE/ kg fresh fruit	[44]
	Methanol Water Ethyl acetate Acetone Petroleum ether	Folin Ciocâlteu method (765 nm)	31.36±0.34 12.77±0.81 179.05±0.53 55.38±0.86 27.14±0.30	mg GAE/g extract	[43]

Comparing data with the literature is difficult due to the different extraction techniques used and the results expression, but the results of present study are close to the previously published values, if converted to similar units.

Another concern of the food industry is the colour of food. One of the major objectives of this study was to demonstrate that the phytochemical composition of our samples change in a similar manner to the change of their red colour (a* parameter), and the obtained results prove it.

It is obvious that the phenolic compounds, and especially the anthocyanin pigments due to their red colour in acidic media, influence the chromatic parameter a*. Table 5 presents the chromatic parameters of the two microencapsulated powders, compared to the concentrated extract. The values obtained for a* parameter prove that all the analysed samples are red coloured, as the value of a* is positive (table 5). The red colour is able to create a positive psychological impact on the consumer, who is very attracted to brightly coloured foods [45].

Table 5

Chromatic analysis of the concentrated extract and microencapsulated powders

Chromatic parameter tested	Value
Concentrated extract	
Luminosity (L*)	53.72±0.83
Green/red colour component (a*)	20.22±0.32
Blue/yellow colour component (b*)	7.04±0.03
Tone (hue angle, h*)	21.41±0.15
Chroma (colour intensity, C*)	0.33±0.01
Total colour difference (ΔE*)	57.82±0.63
Microencapsulated powders	
PT1	
Luminosity (L*)	36.79±0.59[a]
Green/red colour component (a*)	10.95±0.74[a]
Blue/yellow colour component (b*)	5.85±0.45[a]
Tone (hue angle, h*)	12.42±0.79[a]
Chroma (colour intensity, C*)	0.49±0.03[a]
Total colour difference (ΔE*)	38.83±0.79[a]
PT2	
Luminosity (L*)	35.04±0.68[b]
Green/red colour component (a*)	11.65±0.42[b]
Blue/yellow colour component (b*)	5.52±0.14[a]
Tone (hue angle, h*)	12.89±0.43[a]
Chroma (colour intensity, C*)	0.44±0.01[a]
Total colour difference (ΔE*)	37.34±0.51[b]

Note. For each powders parameter tested, values that are for the same parameter (e.g. luminosity) that do not have the same lowercase letters ((a) and (b)) are statistically different at $p < 0.05$ based on the Tukey method and the 95% confidence interval.

The colour parameters (table 5), as well as the previously measured parameters (table 3), were well preserved in the microencapsulated powders, with very closed values between the 2 variants (PT1 and PT2). The total colour difference of powders (compared to the concentrated extract) proved that PT2 was closer to the initial extract, compared to PT1

(smaller ΔE* value) and this confirms, also from the chromatic point of view, that PT2 is a better encapsulating solution than PT1 (but the difference is small). Double amount of pectin than the amount of whey protein isolate has been shown to slightly improve the properties of the microencapsulated powder, but an economic analysis would be required to verify whether this small improvement is worth the cost difference.

4. Conclusions

In order to protect the anthocyanins from cornelian cherry fruits against environmental factors (light, storage, temperature etc.), two walls materials were used (whey protein isolate and pectin). The scientific novelty of present research comes from the development, integration and implementation of two variants of microencapsulations with complex biopolymeric matrices that have not been previously applied for the protection of the bioactive compounds from cornelian cherry fruits, according to our knowledge. The biopolymeric wall materials used in this study for the microencapsulation of the cornelian cherry fruit anthocyanins allowed obtaining two powders with high encapsulation efficiency and satisfactory phytochemical profile. Colorimetric analysis highlighted a light red colour with yellow shades in both experimental variants. Further studies are needed to test the *in vitro* digestion of these anthocyanins and also, to test cornelian cherry fruits as source of natural food additives, researches which are currently on-going in our laboratories.

Acknowledgments: The authors are grateful for the technical support provided by the Integrated Center for Research, Expertise and Technological Transfer in Food Industry (*http://www.bioaliment.ugal.ro/index_en.html*) - „Dunărea de Jos" University of Galați and also, to the Horticultural Research Center (*http://www.uaiasi.ro/horticultura/centru_cercet.php*) - "Ion Ionescu de la Brad" Iași University of Life Sciences. The results of this study were presented at the International Conference, Modern Technology in the Food Industry, MTFI-2022, 20-22 October 2022, Chisinau, Republic of Moldova.

Conflicts of Interest: The authors declare no conflict of interest

References

1. Enache, I.M.; Coman, G.; Rosca, S.; Vizireanu, C.; Mihalcea, L. Optimization conventional extraction of bioactive compounds from *Cornus mas* by RSM and determination of favorability factors by GIS technique. *Not. Bot. HortiAgrobot. Cluj-Napoca.* 2021, 49 (2), 12307.DOI: https://doi.org/10.15835/nbha49212307 (a)
2. Yilmaz, K.U.; Ercisli, S.; Zengin, Y.; Sengul, M.; Kafkas, E.Y. Preliminary characterisation of cornelian cherry *(Cornus mas* L.) genotypes for their physico-chemical properties. *Food Chem.* 2009, 114 (2), pp. 408–412.
3. Tapas, A.R.; Sakarkar, D.M.; Kakde, R.B. Flavonoids as nutraceuticals: a review. *Trop. J. Pharm. Res.* 2008, 7 (3), pp. 1089–1099. https://doi.org/10.4314/tjpr.v7i3.14693.
4. Manganaris, G.A.; Goulas, V.; Vicente, A.R.; Terry, L.A. Berry antioxidants: Small fruits providing large benefits. *J. Sci. Food Agric.* 2013, 94 (5), pp. 825–833. https://doi.org/10.1002/jsfa.6432
5. de Pascual-Teresa, S.; Sanchez-Ballesta, M.T. Anthocyanins: from plant to health. *Phytochem. Rev* 2008, 7, pp. 281–299. https://doi.org/10.1007/s11101-007-9074-0
6. Wallace, T.C.; Giusti, M.M. Anthocyanins. *Adv. Nutr.* 2015, 6 (5), pp. 620–622. doi:10.3945/an.115.009233
7. Einbond, L.S.; Reynertson, K.A.; Luo, X.D.; Basile, M.J.; Kennelly, E.J. Anthocyanin antioxidants from edible fruits. Food Chem. 2014, 84 (1), pp. 23–28. https://doi.org/10.1016/S0308-8146(03)00162-6
8. Escribano-Bailón, M.T.; Santos-Buelga, C.; Rivas-Gonzalo, J.C. Anthocyanins in cereals. *J. Chromatogr. A.* 2004, 1054 (1–2), pp. 129–141. https://doi.org/10.1016/j.chroma.2004.08.152
9. Enache, I.M.; Vasile, M.A.; Crăciunescu, O.; Prelipcean, A.M.; Oancea, A.; Enachi, E.; Barbu, V.V.; Stănciuc, N.; Vizireanu C. Co-microencapsulation of anthocyanins from cornelian cherry (*Cornus mas* l.) fruits and lactic acid bacteria into antioxidant and anti-proliferative derivative powders. *Nutrients* 2022. 14, 3458. https://doi.org/10.3390/nu14173458

10. Phetpornpaisan, P.; Tippayawat, P.; Jay, M., Sutthanut, K. A local Thai cultivar glutinous black rice bran: A source of functional compounds in immunomodulation, cell viability and collagen synthesis, and matrix metalloproteinase-2 and -9 inhibition. *J. Funct. Foods*. 2014, 7, pp. 650–661. https://doi.org/10.1016/j.jff.2013.12.020
11. Shao, Y.; Xu, F.; Sun, X.; Bao, J.; Beta, T. Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (*Oryza sativa* L.). *J. Cereal Sci*. 2014, 59 (2), pp. 211–218. https://doi.org/10.1016/j.jcs.2014.01.004
12. Bassani, D.C.; Nunes, D.S.; Granato, D. Optimization of phenolics and flavonoids extraction conditions and antioxidant activity of roasted yerba-mate leaves (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) using response surface methodology. *An. Acad. Bras. Cienc*. 2014, 86 (2), pp. 923–934. https://doi.org/10.1590/0001-3765201420130019
13. Granato, D.; de Araújo Calado; V.Ô. M.; Jarvis, B. Observations on the use of statistical methods in Food Science and Technology. *Food Res. Int*. 2014, 55, pp. 137–149.
14. Granato, D.; Grevink, R.; Zielinski, A.A.F.; Nunes, D.S.; Van Ruth, S. M. Analytical strategy coupled with response surface methodology to maximize the extraction of antioxidants from ternary mixtures of green, yellow, and red teas (*Camellia sinensis* var. Sinensis). *J. Agric. Food Chem*. 2014, 62 (42), pp. 10283–10296.
15. Pedro, A.C.; Granato, D.; Rosso, N.D. Extraction of anthocyanins and polyphenols from black rice (*Oryza sativa* L.) by modeling and assessing their reversibility and stability. *Food Chem*. 2016, 191, pp. 12–20. https://doi.org/10.1016/j.foodchem.2015.02.045
16. Turturică, M.; Stănciuc, N.; Bahrim, G.; Râpeanu, G. Effect of thermal treatment on phenolic compounds from plum (*Prunus domestica*) extracts – A Kinetic study. *J. Food Eng*. 2016, 171, pp. 200–207. https://doi.org/10.1016/j.jfoodeng.2015.10.024.
17. Veberic, R.; Jakopic, J.; Stampar, F.; Schmitzer, V. European elderberry (*Sambucus nigra* L.) rich in sugars, organic acids, anthocyanins and selected polyphenols. *Food Chem*. 2009, 114 (2), pp. 511–515. https://doi.org/10.1016/j.foodchem.2008.09.080
18. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot, D.A. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants* 2017, 6 (4), 23. https://doi.org/10.3390/plants6040042
19. Dinda, B.; Kyriakopoulos, A.M.; Dinda, S.; Zoumpourlis, V.; Thomaidis, N.S.; Velegraki, A.; Markopoulos, C.; Dinda, M. *Cornus mas* L. (cornelian cherry), an important European and Asian traditional food and medicine: Ethnomedicine, phytochemistry and pharmacology for its commercial utilization in drug industry. *J. Ethnopharmacol*. 2016, 193, pp. 670–690. https://doi.org/10.1016/j.jep.2016.09.042
20. Nireesha, Gr. L.; Divya, C.; Sowmya, N.; Venkateshan, M.; Niranjan Babu, V.L. Lyophilization/freeze drying - an review. *Int. J. Novel Trends Pharm. Sci*. 2013, 3 (4), pp. 87-98.
21. Ciurzyńska, A.; Lenart, A. Freeze-drying - application in food processing and biotechnology - a review. *Polish J. Food Nutr. Sci*. 2011, 61 (3), pp. 165–171, https://doi.org/10.2478/v10222-011-0017-5
22. Baheti, A.; Kumar, L.; Bansal, A.K. Excipients used in lyophilization of small molecules. *J. Excip. Food Chem*. 2010, 1 (1), pp. 41–54.
23. Saarela, M.; Virkajärvi, I.; Alakomi, H.L.; Sigvart-Mattila, P.; Mättö, J. Stability and functionality of freeze-dried probiotic *Bifidobacterium* cells during storage in juice and milk. *Int. Dairy J*. 2006, 16 (12), pp. 1477–1482. https://doi.org/10.1016/j.idairyj.2005.12.007
24. Marques, L.G.; Silveira, A.M.; Freire, J.T. Freeze-drying characteristics of tropical fruits. *Dry. Technol*. 2007, 24 (4), pp. 457–463. https://doi.org/10.1080/07373930600611919
25. de Ciriano, M.G.I.; Rehecho, S.; Calvo, M.I.; Cavero, R.Y.; Navarro, Í.; Astiasarán, I.; Ansorena, D. Effect of lyophilized water extracts of *Melissa officinalis* on the stability of algae and linseed oil-in-water emulsion to be used as a functional ingredient in meat products. *Meat Sci*. 2010, 85 (2), pp. 373–377. https://doi.org/10.1016/j.meatsci.2010.01.007
26. Zorić, Z.; Dragović-Uzelac, V.; Pedisić, S.; Kurtanjek, Ž.; Garofulić, I.E. Kinetics of the degradation of anthocyanins, phenolic acids and flavonols during heat treatments of freeze-dried sour cherry *Marasca paste*. *Food Technol. Biotechnol*. 2014, 52 (1), pp. 101–108.
27. Jiang, H.; Zhang, M.; Mujumdar, A.S.; Lim, R.X. Drying uniformity analysis of pulse-spouted microwave–freeze drying of banana cubes. *Dry. Technol*. 2015, 34 (5), pp. 539–546. https://doi.org/10.1080/07373937.2015.1061000

28. Bulatao, R.M.; Samin, J.P.A.; Salazar, J.R.; Monserate, J.J. Encapsulation of anthocyanins from black rice (*Oryza sativa* L.) bran extract using chitosan-alginate nanoparticles. *J. Food Res.* 2017, 6 (3), pp. 40–47. https://doi.org/10.5539/jfr.v6n3p40
29. Stănciuc, N.; Turturică, M.; Oancea, A.M.; Barbu, V.; Ioniţă, E.; Aprodu, I.; Râpeanu, G. Microencapsulation of anthocyanins from grape skins by whey protein isolates and different polymers. *Food Bioproc. Tech.* 2017, 10 (9), pp. 1715–1726. https://doi.org/10.1007/s11947-017-1938-8
30. Ursache, F.M.; Andronoiu, D.G.; Ghinea, I.O.; Barbu, V.; Ioniţă, E.; Cotârleţ, M.; Dumitraşcu, L.; Botez, E.; Râpeanu, G.; Stănciuc, N. Valorizations of carotenoids from sea buckthorn extract by microencapsulation and formulation of value-added food products. *J. Food Eng.* 2018, 219, pp. 16–24, https://doi.org/10.1016/j.jfoodeng.2017.09.015
31. Gheonea (Dima), I.; Aprodu, I.; Cîrciumaru, A.; Râpeanu, G.; Bahrim, G.E.; Stănciuc, N. Microencapsulation of lycopene from tomatoes peels by complex coacervation and freeze-drying: Evidences on phytochemical profile, stability and food applications, *J. Food Eng.* 2021, 288, 110166, pp. 1-8, https://doi.org/10.1016/j.jfoodeng.2020.110166
32. Condurache, N.N., Aprodu, I., Crăciunescu, O.; Tatia, R.; Horincar, G.; Barbu, V.; Enachi, E.; Râpeanu, G.; Bahrim, G.E.; Oancea, A.; Stănciuc, N. Probing the functionality of bioactives from eggplant peel extracts through extraction and microencapsulation in different polymers and whey protein hydrolysates. *Food Bioproc. Tech.* 2019, 12, pp. 1316–1329. https://doi.org/10.1007/s11947-019-02302-1
33. Milea, Ș.A.; Aprodu, I.; Vasile, A.M.; Barbu, V.; Râpeanu, G.; Bahrim, G.E.; Stănciuc, N. Widen the functionality of flavonoids from yellow onion skins through extraction and microencapsulation in whey proteins hydrolysates and different polymers. *J. Food Eng.* 2019, 251, pp. 29-35. https://doi.org/10.1016/j.jfoodeng.2019.02.003
34. Vasile, M.A.; Milea, Ș.A.; Enachi, E.; Barbu, V.; Cîrciumaru, A.; Bahrim, G.E.; Râpeanu, G.; Stănciuc, N. Functional enhancement of bioactives from black beans and lactic acid bacteria into an innovative food ingredient by comicroencapsulation. *Food Bioproc. Tech.* 2020, 13, pp. 978–987. https://doi.org/10.1007/s11947-020-02451-8
35. Enache, I.M.; Vasile, A.M.; Enachi, E.; Barbu, V.; Stănciuc, N.; Vizireanu, C. Co-microencapsulation of anthocyanins from black currant extract and lactic acid bacteria in biopolymeric matrices. *Molecules* 2020, 25, 1700, pp. 1-12. doi: 10.3390/molecules25071700
36. Enache, I.M.; Vasile, A.M.; Enachi, E.; Barbu, V.; Stănciuc, N.; Vizireanu, C. Co-microencapsulation of anthocyanins from cornelian cherry fruits and lactic acid bacteria in biopolymeric matrices by freeze-drying: evidences on functional properties and applications in food. *Polymers* 2020, 12 (906), pp. 1–12. https://doi.org/10.3390/polym12040906.
37. Boeing, H.; Bechthold, A.; Bub, A.; Ellinger, S.; Haller, D.; Kroke, A.; Leschik-Bonnet, E.; Müller, M. J.; Oberritter, H.; Schulze, M.; Stehle, P.; Watzl, B. Critical review: Vegetables and fruit in the prevention of chronic diseases. *Eur. J. Nutr.* 2012, 51, pp. 637–663. https://doi.org/10.1007/s00394-012-0380-y
38. Solak, B.B.; Akin, N. Health benefits of whey protein: a review. *J. Food Sci. Eng.* 2012, 2, pp. 129-137.
39. Ghai, K.; Gupta, A.K.; Gupta, P.K. Pectin: A versatile biopolymer with numerous health benefits and medical uses. *J. Biol. Act. Prod. Nat.* 2012, 2 (4), pp. 250–255. https://doi.org/10.1080/22311866.2012.10719132
40. Enache, I.M.; Benito-Román, Ó.; Coman, G.; Vizireanu, C.; Stănciuc, N.; Andronoiu, D.G.; Mihalcea, L.; Sanz, M.T. Extraction optimization and valorization of the cornelian cherry fruits extracts: evidence on antioxidant activity and food applications. *Appl. Sci.* 2021, 11, p. 0729. https://doi.org/10.3390/app112210729
41. Popović, B.M.; Štajner, D.; Slavko, K.; Sandra, B. Antioxidant capacity of cornelian cherry (*Cornus mas* L.) – Comparison between permanganate reducing antioxidant capacity and other antioxidant methods. *Food Chem.* 2012, 134 (2), pp. 734–741. https://doi.org/DOI: 10.1016/j.foodchem.2012.02.170
42. Hassanpour, H., Yousef, H., Jafar, H., Mohammad, A. Antioxidant capacity and phytochemical properties of cornelian cherry (*Cornus mas* L.) genotypes in Iran. *Sci. Hortic.* 2011, 129 (3), pp. 459–463. https://doi.org/10.1016/j.scienta.2011.04.017
43. Stankovic, M.S.; Zia-Ul-Haq, M.; Bojovic, B.M.; Topuzovic, M.D. Total phenolics, flavonoid content and antioxidant power of leaf, flower and fruits from cornelian cherry (*Cornus mas* L.). *Bulg. J. Agric. Sci.* 2014, 20, pp. 358–363.
44. Rop, O.; Mlcek, J.; Kramarova, D.; Jurikova, T. Selected cultivars of cornelian cherry (*Cornus mas* L.) as a new food source for human nutrition. *Afr. J. Biotechnol.* 2010, 9 (8), pp. 1205–1210. https://doi.org/DOI: 10.5897/AJB09.1722

45. Spence, C. On the psychological impact of food colour. *Flavour* 2015, 4 (21), pp. 1-16. https://doi.org/10.1186/s13411-015-0031-3

Citation: Enache, I.M.; Lucescu Ciurlă, L.; Stănciuc, N.; Irimia, L.M.; Patraș, A.; Vizireanu, C. Microencapsulation of anthocyanins from cornelian cherry fruits in whey protein isolate and pectin. *Journal of Engineering Science* 2020, 29 (4), pp. 138-149. https://doi.org/10.52326/jes.utm.2022.29(4).11.

Submission of manuscripts: jes@meridian.utm.md