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SPECTRAL AND CHROMATOGRAPHIC CHARACTERISATION OF THE YELLOW FOOD DYE FROM SAFFLOWER

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Abstract. Recently, an increasingly trend of public concern for the food safety is observed. Use of additives in food industry growing steadily. Present study deals with separation and identification of compounds from Yellow Food Dye from Safflower (YFDS). Spectral and chromatographic characteristics of YFDS were obtained and discussed. Dry powders and solutions of YFDS were examined using Thin Layer Chromatography (TLC), UV-Vis spectroscopy and reversed-phase HPLC. TLC was carried out in three systems I (HCl 0.1 M), II (aqua 50%, ethanol 45%, citric acid 5%) and III (water, butan-1-ol, acetic acid). Spectral determinations in the range of 200 to 600 nm were carried out at various pH values. HPLC method was carried out by the gradient elution technique. Chromatographic method showed that it is impossible to separate and to identify the components of the YFDS by paper chromatography. UV-Vis Spectra demonstrated that the most successful interval for the practical use of YFDS is in the pH range of pH = 4...5, since in this range the coloration activity of dyes is maximal. HPLC method demonstrated that YFDS-compounds corresponds to the composition of dry Safflower petals, which confirms its high biological activity. Powdered yellow pigment from Safflower petals is containing natural chalcones and can be successfully used in the dairy producing.

Keywords: *Anhydrosafflower Yellow B, Chalcones, Hydroxisafflower Yellow A, Izosafflomin C, Precarthamine, Safflomin C.*

Rezumat. Actualmente se observă o creștere din ce în ce mai mare a îngrijorării consumatorilor față de siguranța alimentelor consumate. Utilizarea aditivilor în industria alimentară este în continuă creștere. Studiile în acest domeniu demonstrează, că apar noi aspecte ale toxicității coloranților sintetici. În studiul de față, s-a efectuat separarea și identificarea compușilor din amestecul de compuși a colorantului galben alimentar din șofrănel (YFDS). Au fost testate caracteristicile spectrale și cromatografice ale YFDS. Pulberile uscate și soluțiile de YFDS au fost studiate, utilizând cromatografia în strat subțire (TLC), spectroscopia UV-Vis și HPLC cu fază inversă. TLC a fost efectuat în trei sisteme I (HCl 0,1 M), II (aqua 50%, etanol 45%, acid citric 5%) și III (apă, butan-1-ol, acid acetic). Determinările spectrale s-au efectuat în intervalul de la 200 la 600 nm la diferite valori a pH-ului. Metoda HPLC a fost efectuată prin tehnica de eluare cu gradient. Metoda cromatografică

a arătat că este imposibil de separat și de identificat componentele YFDS prin cromatografie pe hârtie. Spectroscopia UV-Vis a demonstrat că intervalul cel mai de succes pentru utilizarea practică a YFDS este în intervalul de pH de la 4 la 5, deoarece în acest interval activitatea de colorare a coloranților este maximă. Metoda HPLC a demonstrat că compușii YFDS corespund compoziției petalelor uscate de șofrănel, ceea ce confirmă activitatea sa biologică ridicată. Pigmentul galben sub formă de pulbere din petalele de șofrănel conține chalconi naturali și poate fi utilizat cu succes în fabricarea produselor lactate.

Cuvinte cheie: *Anhidrosafflora Galben B, Chalcone, Hydroxisaflomină Galben A, Izosaflomină C, Precartamină, Safflomină C.*

1. Introduction

Consumers striving for naturalness choose a food product not only in appearance, but also in composition, rightly considering this factor to be extremely important. The existing demand for partial or complete abandonment of synthetic dyes in food products is expressed by a decrease in the growth rate of the production of synthetic dyes, and an increase in the production of natural ones [1]. At the same time, existing natural sources of food colors are already actively exploited, and they are clearly insufficient. Therefore, in order to effectively replace of the synthetic dyes with natural ones, it is necessary to found new and suitable sources of the latter.

The perspective plant which can be used as a source of food colours is Safflower (*Carthamus tinctorius* L.) [2]. Safflower seeds are an important sourse for extracting oil in Asia, North and Central America [3]. Safflower florets are widely used in cosmetics, modern and popular medicine [4]. By Safflower petals extraction it can be obtained dyes of two colors: yellow and red [5]. According to the chimical structure, the dyes represent different chalcones [6].

There are many reasons, which causes confusions in the taxonomy of the sources of raw materials and/or of the compounds, obtained from these sources. Safflower (*Carthamus tinctorius L., Asteraceae*) is often confused in Internet, also in traditional printed scientific sources, with consonant Saffron (*Crocus sativus L., Iridaceae*) [7]. Due to this confusion, Carthamin is considered a dye, extracted from Saffron. But Saffron does not include red dimeric chalcone Carthamin in composition: red compounds of Saffron are anthocyanins [8]. Instead of correct “Safflower Yellow”, a mistakeous name “Carthamin Yellow” is used in some research papers [9], but in commerce and Internet especially. So, “Carthamin Yellow” is not a compound, instead of the correct name, which is not Carthamin (red) but this a yellow dye derived from Safflower. The Yellow Precarthamin is the biochemical precursor of Red Carthamin. Precartamin is enzymatically converted to Red Carthamin both in vivo and in vitro [10]. In the strong acid environment, red Carthamin isomerizes to yellow Izocarthamin [11]. Main goal of this paper is to stable the physico-chemical properties of powered yellow colour from Safflower petals, growned in Republic of Moldova, in order to its utilisation in food industry.

2. Materials and Methods

Safflower petals. Safflower was grown in the experimental fields of the Institute of Genetics and Plant Protection, Chisinau, Moldova. The petals were collected manually. The green sepals and light gray (almost white) seeds were carefully separated from the petals.

Purified petals were dryed in the dark to an absolute humidity of no more than 5% and were stored in an airtight container.

Solvents. Food grade ethanol 96% (v/v) ("Kvint" distillery, Moldova), freshly prepared bidistillate, acetic acid purum ("Severodonetsk Azot Association", Ukraine) and HPLC-grade acetonitrile, n-butanol and methanol ("Merck KGaA", Germany) were used for spectrophotometric and chromatographic analysis.

Separation of the Yellow Food Dye from Safflower (YFDS). Dried safflower petals were treated with a sodium carbonate solution with a hydromodule of 1:10 at a temperature of 18…20°C. The resulting mixture was pressed in four steps to give a yellow extract. This solution was centrifuged for 10 minutes at 6000 rpm. Yellow liquid solution, which remained after the carthamine was removed [12], was purified with activated carbon and cellulose 1 gram per liter for 10 minutes. After filtration, solution was evaporated in a rotary evaporator at 60-75°C, with speed of 150-210 rpm, under pressure 60-100 mbar. In obtained dark-brown viscous solution were added 3 volumes of ethyl alcohol and intensely mixed to obtain very viscous mass, which was dried in vacuum at 65-80 °C and 80-100 mbar. The dry mass of YFDS constituting 40 % of dry petal mass.

Thin Layer Chromatography (TLC). Chromatograms were obtained in vertical camera on 24×3cm sheets of Whatman Chromatographic Paper, by ascension technique. Three chromatographic systems with different polarity were used as liquid mobile phases: System I – acidic medium (HCl 0.10 mol/L); System II – (50% distilled water, 45% ethanol, 5% citric acid); System III – (4 parts distilled water, 5 parts butan-1-ol, 1 part acetic acid).

UV-Vis spectra. Standard solution of YFDS was prepared by dissolving of 0.2 g of powder in 200mL distilled water (Solution P1). For farther directly spectroscopic measurements, Solution P1 was diluted 5.0 times and adjusted to different pH value by adding crystals of sodium carbonate and citric acid. Distilled water was used as a reference sample. Spectra of Safflower extracts were recorded at Hach-Lange "DR 5000" spectrophotometer in the range of 200...600 nm, step of 1 nm, using quartz cell with l = 10mm.

Photodiode Array (PDA) coupled HPLC. Shimadzu "Provinience-i LC-2030C 3D-Plus", with integrated Photodiode Array Detector (PDA), on reversed-phase C_{18} column "Phenomenex" (4.6x150mm, particle size 4µm, pores 80nm), gradient elution technique by two mobile phases: Water, containing 0.1% (v) Acetic Acid (Phase A) and Acetonitrile containing 0.1% (v) Acetic Acid (Phase B) were used. Default flow: Phase B 5% at the constant rate of 0.8 mL/min. Constant oven and detection cell temperatures of 30 °C. Elution gradient program: 0...2 min – Phase B 5% (default flow); 2...18 min – Phase B from 5% to 40%; 18...20 min – Phase B from 40% to 90%; 20...24 min – Phase B 90%; 24...25 min - Phase B from 90% to 5%; 25-40 min – Phase B 5% (default flow). Data acquisitions and interpretations were performed using Shimadzu LabSolutions software.

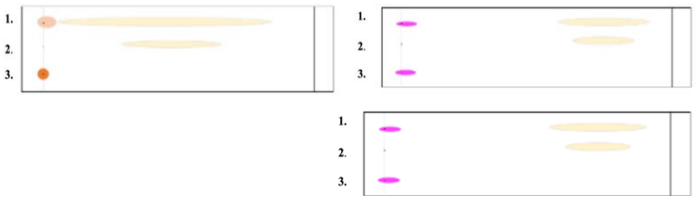
3. Results and Discussion

3.1. TLC of red and yellow Safflower dyes

Investigations of the chromatography conditions of the Yellow Food Dye from Safflower (YFDS) and Cartamine dyes were performed using three chromatography systems, Table 1. Most acid System I (HCl 0.1 mol/L) confirms the instability of the Carthamin in the environment with pH = 1.00 [11]. Chromatographic systems II and III demonstrated the effective separation of Carthamine from yellow dyes. At the same time, we failed to separate the components of yellow dyes by paper chromatography.

Table 1

Thin Layer Chromatography of the Safflower Pigments

System	I	II	III, superior layer
Composition	HCl 0.1 mol·L^{-1}	Aqua, 50% (v) Ethanol, 45% (v) Citric Acid, 5% (m)	Water, 4 parts (v) Butan-1-ol, 5 parts (v) Acetic Acid, 1 part (v)
View			
Rf (Yellow)	0.51 ± 0.05	0.71 ± 0.05	0.35 ± 0.05
Rf (Crt)	0.00	0.03 ± 0.02	0.03± 0.02

*Spotted consecutively: 1 - Safflower extract; 2 - YFDS; 3 - Carthamin

Thus, to obtain a bright yellow (and not orange) YFDS, it is necessary to treat the extract with a suspension of microcrystalline cellulose.

3.2. UV-Vis Spectroscopy and pH-sensitivity of YFDS

Prepared samples of solutions with different pH values were spectrophotometrically, and two diagrams were built from the obtained spectra. The spectra were processed using the Excel program.

The UV-Vis spectra of the yellow dye in Safflower were analysed as a function of pH (Figure 1). Almost all curves have the same shape with maximum wavelengths between 392 nm and 412 nm and the presence of the left shoulder. The spectra, obtained at acidic (5.10) and basic (10.95) pH values show an obvious deviation. The maximum absorption of the yellow pigment solutions takes place in an acid medium (2.12) with a peak wavelength of 405 nm. From the analysis of the spectra of the liquid yellow dye at different pH, it follows that a change in pH causes a shift in the absorption maxima of the dyes. This is clearly seen in the graphs λmax = f (pH). The most successful interval for the practical use of dyes is the pH range = 4...5, since in this range the absorption of dyes is maximum and color of the solutions is bright yellow.

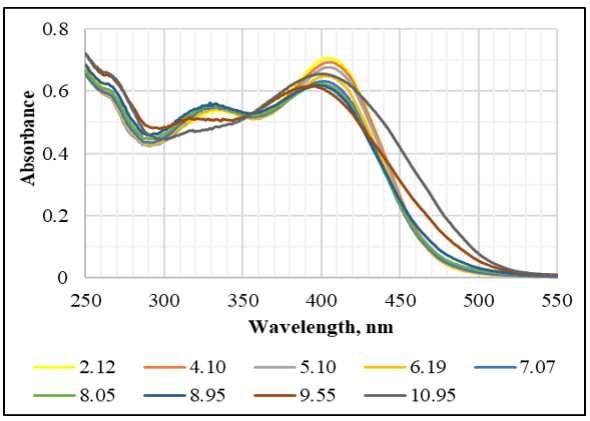


Figure 1. Spectra of YFDS, pH function.

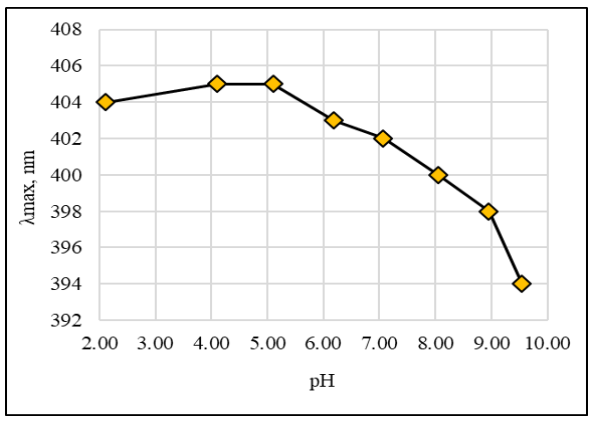


Figure 2. YFDS absorbtion maximum, pH function.

Thus, despite the removal of the Cartamine, highly sensitive to pH, YFDS does not completely lose sensitivity to the pH value. At the same time, minor changes in the position of the absorption maximum, 394-405 nm (Figure 2), cannot significantly affect color perception, which means that YFDS can be successfully used over a wide pH range.

3.3. HPLC profile of Safflower petals extract and YFDS

HPLC profile of Safflower petals and YFDS were analysed using the same method. The HPLC results (Figure 3) show the five separate peaks were at the retention times of 18.861, 19.589, 20.844, 22.273, 24.398 in YFDS extract, what corresponded to the peaks and retention time in petals extract. The peaks show the clear separation of yellow compounds and identification of Safflomin C, Izosafflomin C, HSYA, AHSYB and Precarthamin. This fact that the composition of the YFDS, obtained from Safflower petals correspond the composition of the petals, known for its biological activity [13], suggests that the YFDS can be used as a natural food yellow colorant, which can be successfully in the production of dairy products [14-16].

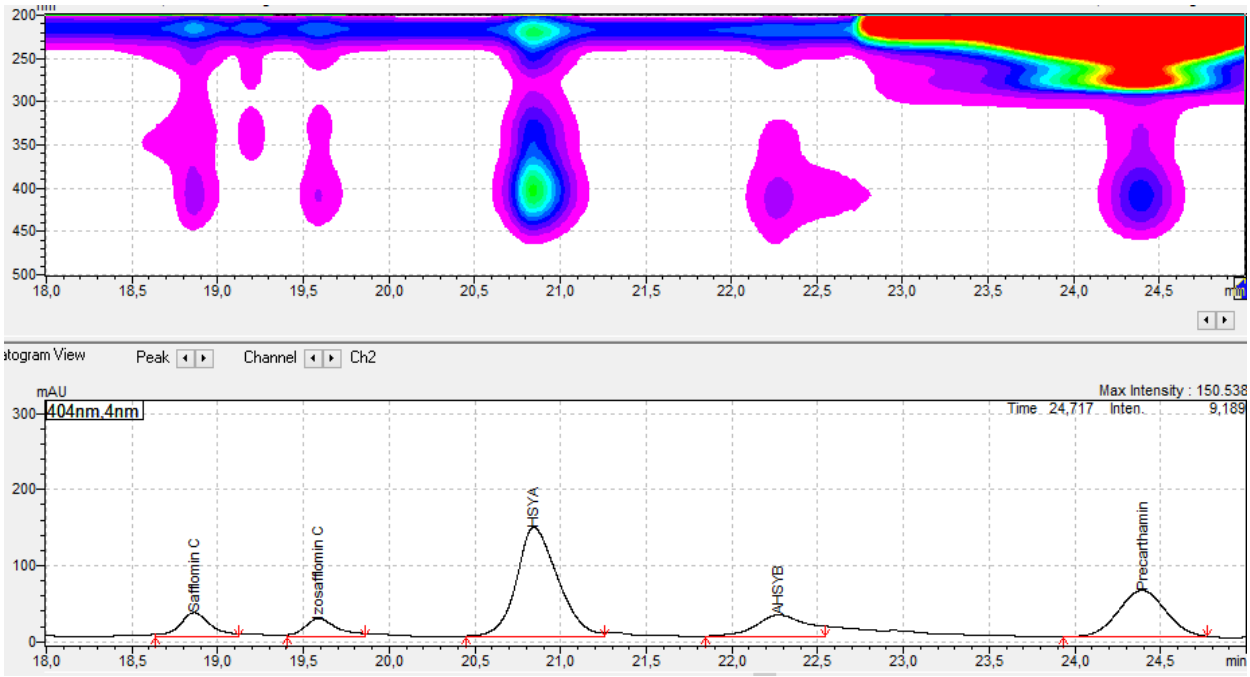


Figure 3. Multichromatogram UV-Vis = f (Time), and ordinary chromatogram at 404nm.

Table 2

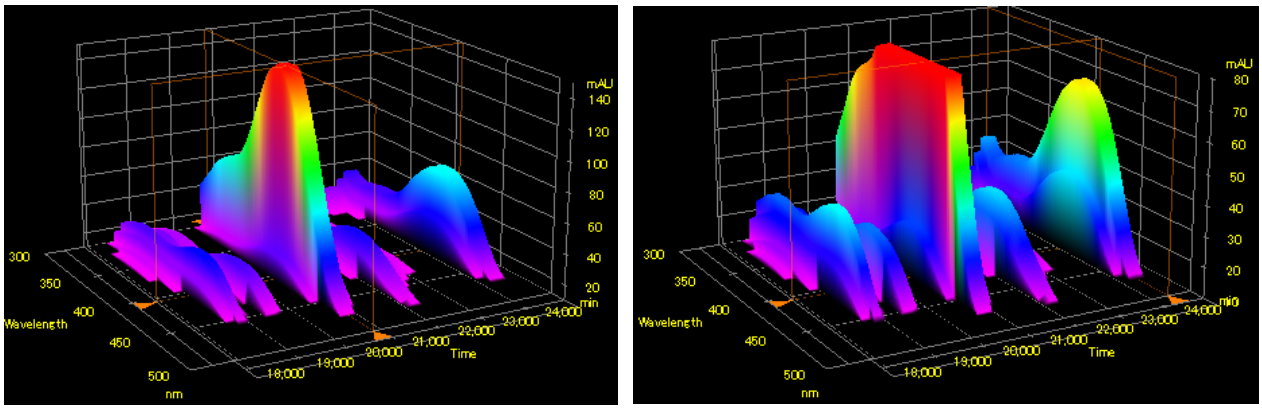
Chromatographic data for yellow compounds from Safflower petals and YFDS

Peak No.	R_T (min)	Area	Area (%)	λ_{max} (nm)	Identification
		Safflower Petals Extract			
1	18.62	263361	2.3	218, 332, 406	Safflomin C
2	19.40	241525	2.2	216, 315, 404	Izosafflomin C
3	20.63	8997460	80.1	223, 335sh, 402	HSYA
4	22.32	628858	5.6	219, 335sh, 410	AHSYB
5	24.67	1098554	9.8	335sh, 407	Precarthamin

Continuation Table 2

Yellow Food Dye from Safflower					
1	18.86	394512	8.1	215, 332, 408	Safflomin C
2	19.59	313120	6.4	216, 327, 408	Izosafflomin C
3	20.84	2410258	49.5	220, 335sh, 402	HSYA
4	22.27	594323	12.2	218, 335sh, 412	AHSYB
5	24.39	1159907	23.8	335sh, 408	Precarthamin

The UV-Vis spectra clearly seen that the mixture of dyes is yellow in colour (Figure 1), however, the presence of which yellow components in the mixture are most present, is impossible to identify using spectroscopy method. Thanks to 3D chromatogram, it was possible to establish that the largest amount of yellow colour in YFDS is accounted for by HSYA (R_T = 20.84, Figure 4a). Precarthamin (R_T = 24.39), AHSYB, Safflomin C and Isosafflomin C also give yellow colour, in correspondingly decreasing order (Figure 4b).



a) b)

Figure 4. YFDS 3D-chromatogram: a) at 140 mAU resolution; b) at 80 mAU resolution.

4. Conclusions

Five yellow dyes of the chalcone class with a similar chromophore structure were confirmed in the Safflower petals extract and in the powdered pigment which was obtained at the concentration of this extract.

Essential condition to obtain bright yelloe colour, is the absence of traces of red carthamine, which is will achive by treatment of Safflower extract with cellulose.

The separation of yellow substances into individual components is not advisable, since they have similar UV-Vis absorption spectra, and hence the colour. Therefore, there is no need to separate the yellow powder into individual components for its further use in the food industry**.**

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Conflicts of Interest. The authors declare no conflict of interest.

References

1. Global Food Coloring Market: Intensive Growth in the Natural Segment. [online]. [accesat 10.05.2022]. Available:

https://www.giord.ru/news/novosti_otrasli/mirovoy_rynok_pishchevykh_krasiteley_intensivnyy_rost_naturalnogo_segmenta/ - [in Russian].
2. Jadhav, B.; Joshi, A. Extraction and quantitative estimation of bio active component (yellow and red carthamin) from dried safflower petals. *Indian Journal of Science and Technology*, 2015, 8, pp. 1-5. http://dx.doi.org/10.17485/ijst/2015/v8i16/42380
3. Mateaș, I. M.; Tabără, A.; Albai, A.; Niță S. The degree of branching in safflower (Carthamus tinctorius L.) a few lines under the influence of sowing. *Journal of Agricultural Science*, 2014, 46, pp. 26-28.
4. Fatahi, N.; Carapetian, J. Comparing stability of carthamin and safflower yellow pigments at pH, temperature and light, from Safflower (Carthamus tinctorius L.) florets. *Journal of Biological Sciences*, 2009, 4, pp. 250-253.
5. Fatahi, N.; Carapetian J.; Heidari R. Spectrophotometric measurement of valuable pigments from petals of safflower (Carthamus tinctorius L.) and their identification by TLC method. *Journal of Biological Scinces*, 2008, 3, pp. 761-763.
6. Hye – Ryeon, Y.; Young – Sook, P. Isolation of two quinochalcones from carthamus tictorius. *Applied Biological Chemistry*, 2008, 51, pp. 169 – 171.
7. Koshi, Saito; Masatoshi, Katsukura. On manganese-induced reddening of florets from dyer's saffron capitula. *Food Chemistry*, 1992, 44, pp. 349-355. https://doi.org/10.1016/0308-8146(92)90267-6
8. Khazaei, K.M.; Jafari, S.M.; Ghorbani, M.; A. Hemmati Kakhki, A.H.; Sarfarazi, M. Optimization of Anthocyanin Extraction from Saffron Petals with Response Surface Methodology. *Food Analytical Methods*, 2016, 9, pp. DOI: 10.1007/s12161-015-0375-4
9. Wan, L.; Chen, J.; Li, L.; Xiong, W.; Zhou, L. Protective effects of Carthamus tinctorius injection on isoprenaline-induced myocardial injury in rats. *Pharmaceutical Biology*, 2011, 49, pp. 1205. https://doi.org/10.3109/13880209.2011.576348
10. Cho, M.H.; Paik, Y.S.; Hahn, T.R. Enzymatic Conversion of Precarthamin to Carthamin by a Purified Enzyme from the Yellow Petals of Safflower. *Journal Of Agriculture And Food Chemistry*, 2000, 48, pp. 3917. https://doi.org/10.1021/jf9911038
11. Baerle, A.; Savcenco, A.; Tatarov, P.; Fetea, F.; Ivanova, R.; Radu, O. Stability limits of a red carthamin – cellulose complex as a potential food colourant. *Food & Function*, 2021, 12, pp. 8037 – 8043. DOI: 10.1039/d1fo01376a.
12. Savcenco, A.; Baerle, A.; Tatarov, P.; Ivanova, R. Process for producing dyes from Safflower petals. *Patent of Moldova*, MD-1453, issued 31.03.2021.
13. Xinbing, W.; Huiqing, L.; Xia, S.; Fengua, F.; Xiumei, Z.; Jin, W.; Jie, A.; Hua, D. Hydroxysafflor yellow A protects rat brains against ischemia-reperfusion injury by antioxidant action. *Neuroscience Letters*, 2005, 386, pp. 58-62. https://doi.org/10.1016/j.neulet.2005.05.069
14. Machewad, G.; Ghatge, P.; Chappalwar, V.; Jadhav, B.; Chappalwar, A. Studies on extraction of safflower pigments and utilization in ice cream. *Journal Of Food Proccesing & Technology*, 2012, 3, 3p. DOI: 10.4172/2157-7110.1000172
15. Popescu, L.; Ghendov-Moșanu, A.; Baerle, A.; Savcenco, A.; Tatarov, P. Color stability of yogurt with natural yellow food dye from safflower (Carthamus tinctorius L.). *Journal Of Engineering Science*, 2022, 29, pp. 142 - 150. https://doi.org/10.52326/jes.utm.2022.29(1).13
16. Popescu, L.; Savcenco, A.; Baerle, A.; Tatarov, P.; Ghendov-Moșanu, A.; Sturza, R.; Patraș, A. Process for producing yogurt. *Patent of Moldova*, MD-1625, issued 30.06.2022

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