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OBTAINING AND USING NATURAL DYES FROM SAFFLOWER PETALS (*Carthamus tinctorius* L.) IN FOOD TECHNOLOGY

253. 06. BIOLOGICAL AND CHEMICAL TECHNOLOGIES IN FOOD INDUSTRY

Abstract of the PhD in engineering sciences dissertation

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CONTENTS

INTRODUCTION

Actuality and importance of the topic addressed. From the point of view of the continuous modernization of industrial food manufacturing technologies, simultaneously with the improvement of their nutritional value, there is the problem of manufacturing food of superior sensory quality. Dyes have a significant impact on the formation of the sensory quality of food. Currently, the use of food dyes internationally is regulated by the Codex Alimentarius Commission of the UN on Food and Agriculture and the World Health Organization, WHO. At the national level, the use of food colorings is coordinated by the Codex Alimentarius National Committee of the Republic of Moldova.

In recent years, special attention has been paid to the problem of improving the sensory quality and harmlessness of foods containing synthetic dyes. Therefore, one of the important directions of the countries of the European Union, including the Republic of Moldova, is intended for investigations regarding the identification of new raw materials - sources of natural dyes. The problem of combating the abuse of synthetic dyes in food, being very important, was also studied in previous years. In the years 2000 – 2010, based on the State Programs of the Academy of Sciences of Moldova, AȘM, coordinated by the academician Gheorghe Duca, the problem of obtaining dyes from grape waste was studied. At the Technical University of Moldova, Faculty of Food Technology, scientific research was carried out (led by associate professor Boris Carabulea), aimed at obtaining dyes from beetroot [1]. In the years 2016 - 2020 following scientific research, carried out by dr. hab. Aliona Ghendov-Moșanu and dr. hab., Prof. Rodica Sturza, the possibility of obtaining dyes from fruit and vegetable waste and their use in the technology of obtaining functional foods was demonstrated [2, 3].

Currently, investigations related to the identification of raw materials of plant origin for obtaining dyes continue. One of the main problems presents the high consumption of energy resources in the technologies of obtaining dyes from traditional raw materials and their waste. Therefore, further investigations are needed to identify non-traditional plant sources, high in pigments with improved color stability, which will ensure reasonable energy consumption in economically feasible production technology.

The petals of the safflower plant (*Carthamus tinctorius* L.) are rich in yellow and red pigments of a chalcone nature. Therefore, this plant is of particular interest as an alternative source, which would ensure the obtaining of food dyes. The development of the technology for obtaining pigments requires the study of the physico-chemical and technological properties, as well as the field of use of these pigments in the food industry.

For consumers, the appearance of food, especially color, presents an important indicator of quality, influencing the acceptability of food by the consumer, which characterizes the sensory quality and harmlessness of food products. In order to obtain the color, synthetic and natural dyes are used. Usually, natural dyes are less stable than synthetic ones.

However, in recent years, the concern of the European Union regarding the use of synthetic dyes in food products has grown considerably. Recent studies have shown that there is a problem with the use of synthetic dyes, the consumption of which negatively affects the health of the population. The use of a wide range of synthetic dyes raises food safety issues. The disadvantage of using synthetic dyes refers to their toxicological properties, because, being produced by synthesis, many dyes contain residues of toxic elements (Pb, As, Cu, Cd, Se, U, Hg) or organic compounds (solvents, aromatic hydrocarbons, etc.). Compounds with "azo" type chromophore groups (amaranth, tartrazine) according to some data are carcinogenic, which is why in European countries these dyes are prohibited for use in food compositions.

Investigations related to the development and implementation of the technology for manufacturing dyes from the petals of the safflower plant, currently harvested in the Republic of Moldova only on experimental plots, are current, because they will contribute to solving the problem of harmless coloring of food products and reducing the negative effects of synthetic dyes, respectively , to improve the quality of food and the health of the population.

Based on the above, the theme of the thesis corresponds to the strategic priority for the development of science in the Republic of Moldova, namely: "Sustainable agriculture, food security and food safety". A large part of this thesis was carried out within the State Program, project 20.80009.5107.09: "Improving the quality and safety of food through biotechnology and food engineering" (2020 – 2023), director Rodica Sturza, PhD, prof.

The purpose of the work: it consists of theoretical and experimental investigations, aimed at assessing the physico-chemical and technological properties of the dyes from safflower petals (*Carthamus tinctorius* L.), the development of the technology for obtaining and using new food dyes, which will be able to replace synthetic dyes in technology to obtain food products. The work is part of the priority direction of EU investigations, aimed at improving the quality of food products by increasing the weight of the use of natural dyes in the food industry.

Research objectives:

Analysis of the chemical structure and physico-chemical properties of pigments from saffron petals, cultivated in the Republic of Moldova;

Establishing the correlation between the chemical structure of pigments and the coloring ability of model systems and food products;

Theoretical and experimental elucidation of the mechanism of the process of extracting pigments from safflower petals;

Determination of the optimal duration of the extraction process of pigments from safflower petals by kinetic modeling of the process;

Development of the technological system for the production of stable yellow and red dyes from safflower petals;

The use of yellow dye from safflower petals in the manufacture of some food products.

In order to achieve the goal of the research, the following scientific hypotheses were formulated:

For the extraction of pigments from safflower petals, it is necessary to ensure the mobility of pigment molecules inside the petal phase and to apply the hydrodynamic regime of pigment diffusion according to Fick's first law.

The use of yellow and red pigments, obtained from safflower petals, as food colorings is possible due to the fact that the pigment molecules display the ability to hydrate and diffuse in a liquid medium, while preserving the stability of the chromophore groups of the pigments and the ability to color food compositions.

THESIS CONTENT

1. OBTAINING AND USING NATURAL DYES IN FOOD COMPOSITIONS

The first chapter represents the analysis of information related to the problem of obtaining and using natural dyes in food compositions, the classification of dyes, the general principles of using dyes in the food industry, the impact of changing chemical compounds on the appearance of food products, the general characteristics of the safflower plant and the characteristics of the pigments in the petals of the plant. The problem of obtaining and using natural dyes for the food industry remains current from the theoretical and applied points of view. The structure and properties of the colorants used influence food quality assurance. Therefore, solving the problems related to obtaining and using natural dyes from new vegetable sources in the food industry is current for the Republic of Moldova. A large number of bibliographic sources indicate that the safflower plant presents a promising source of yellow and red dyes, necessary for use in the food industry, but few publications, especially local ones, include research in this field. At the same time, not enough information was found about the technological properties of safflower petals, used for the extraction of dyes. There is also a lack of information on the scientific principles of the extraction methods of yellow and red pigments from safflower petals. The chapter ends with conclusions and the formulation of objectives, based on the analysis of bibliographic sources,

which reflect the problem in the field of food technology, namely, in the field of the use of synthetic dyes in the manufacture of food products, which have a negative effect on human health.

2. MATERIALS AND RESEARCH METHODS

Chapter 2 reflects the information about the studied raw material, chemical, physico-chemical and technological research methods. The chemical reagents, the laboratory materials used and the statistical processing methods of the obtained results are presented. The study was carried out in the scientific laboratories of the Faculty of Food Technology, Technical University of Moldova.

The main objects of study are the yellow and red dyes obtained under laboratory conditions through three steps: the extraction of the dyes from the petals, the elimination of the native cartamine from the liquid medium, and the obtaining of the yellow dye in the solid state.

When conducting experimental research, standard methods, approved for use in the food industry, were used, such as spectroscopy in the UV-Vis field (DR-5000, "Hach-Lange", USA-Germany), which allowed the determination of the degree of separation of yellow and red dyes from the extract obtained from the petals. Determination of cartamine stability in pure state and in complex with cellulose, by color evolution at different pH values, using RGB code analysis method. From modern industrial methods, for the separation and identification of dyes in a purified state; high-performance liquid chromatography (Shimadzu LC 2030-C 3D-Plus) was used to identify the components of the yellow dye and to determine the stability of the cartamine-cellulose complex at high temperatures in the dry state. The analysis of the formation of new bonds and the transformations of the cartamine molecule in the cellulose phase were recorded by the infrared spectroscopy method ("Shimadzu IR Prestige-21").

The analysis of the process of extracting the pigments from the petals was carried out by the kinetic modeling of the change in the pigment concentration over time. The confidence limits of the mean of the examined parameter, Xm, and the dispersion of the numerical values, σ, were determined. The confidence probability, P, is 0,95.

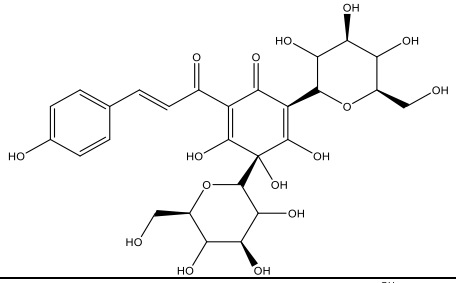
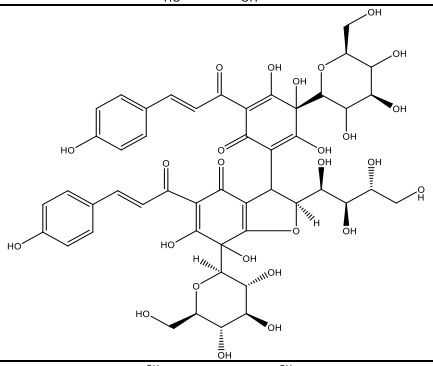
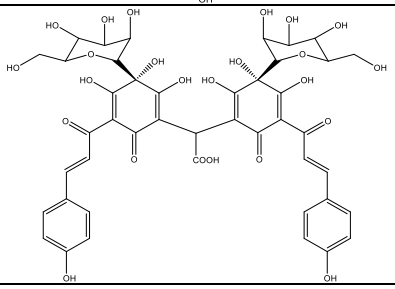
3. PHYSICO-CHEMICAL PROPERTIES OF DYES FROM SAFFLOWER PETALS

3.1. Biosynthesis of chalcones in the raw material

In order to initiate and carry out the study of the physico-chemical properties of the dyes, extracted from safflower, which would be suitable for the food industry, it was necessary to know their history in the plant raw material (petals), i.e., their changes during biosynthesis.

The fact that cartamine biosynthesis is a complex process is confirmed by the data obtained in the present thesis by using the HPLC method with PDA detector [4]. According to the data, safflower petals, regardless of the degree of maturity, contain at least five yellow dyes (table 1), which have been identified [4]. Through the HPLC method, it was determined that the highest amount of yellow color in YFDS is represented by HSYA (R_T = 21,1), precarthamine (R_T = 24,39) and AHSYB (R_T = 22,3) in descending order.

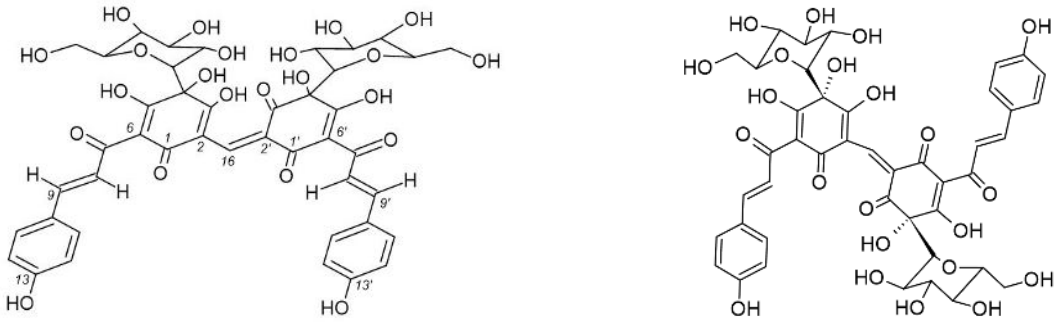
Table 1. Retention times of yellow pigments in safflower petals, which predominate in YFDS

The name of the chalcone	The raw formula	Chemical structure of chalcone	R_T, minutes	
			LC-1A	LC-1B
1	2	3	4	5
Hidroxisaffllor yellow A	$C_{27}H_{32}O_{16}$		21,1 ± 0,6	14,9 ± 0,4
Anhidrosaffllor yellow B	$C_{48}H_{52}O_{26}$		22,3 ± 0,6	16,7 ± 0,4
Precarthamine	$C_{44}H_{44}O_{24}$		24,4 ± 0,6	18,8 ± 0,5

The last step in the biosynthesis of the most valuable chalcones, carthamine and red isocarthamine, is the simultaneous decarboxylation/dehydrogenation of precarthamine – an enzymatic reaction, catalyzed by the enzyme decarboxylase, which takes place both in the petals and can be performed in vitro [5].

Carthamine and isocarthamine molecules retain two fragments with the chalcone structure in their structure. In these molecules, the conjugation of seventeen pairs of electrons is achieved (figure 1), this rather large chromophore ensures the appearance of the absorption maximum at

520 nm, which corresponds to the red color of carthamine and its solutions in water and in some organic solvents.



a) **b)**

Fig. 1. Chemical structure of carthamine (a) and isocarthamine (b) molecule

3.2. Influence of pH on the spectra and color of chalcones in solutions

UV-Vis spectra of extracts of model solutions with concentrations from 10^{-5} to 10^{-3} mol/L at different pH values were recorded (figures 2, 3).

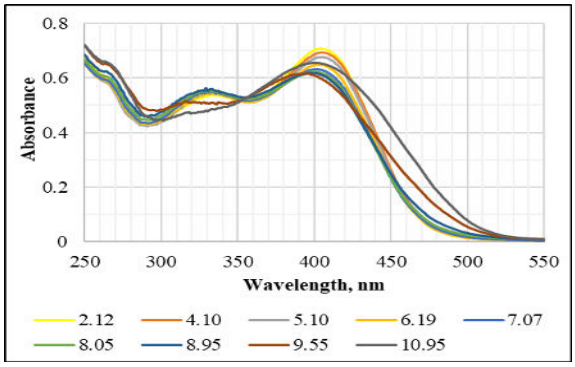


Fig. 2. Changes in the UV-Vis spectrum of the yellow dye extract 10^{-4} mol/L depending on pH

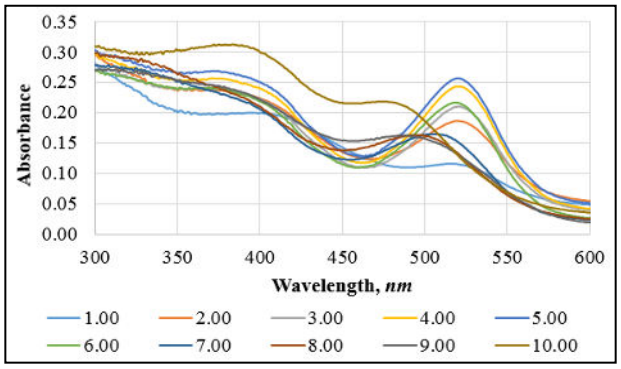


Fig. 3. Spectra of 10^{-5} mol/L solutions of carthamine as a function of pH

The spectrophotometric method demonstrates the moderate influence of the pH value on the color of the yellow chalcone extracts. The spectra of cartamine solutions differ significantly with pH, indicating structural changes in the chromophore.

The position of the absorption maximum of the yellow dye extract remains constant at the wavelength 404 nm in the pH range from 2 to 8. At pH values from 8 to 11 the changes in the UV – Vis spectra become significant (figure 2). For the spectra of carthamine, the absorption maximum corresponds to 520 nm in the pH range from 2 to 5. If the pH increases, the UV – Vis spectra show a shift to shorter wavelengths (figure 3), which denotes the fact of the change in structure of the chromophore and, most likely, disrupting the stability of the molecule.

3.3. The instability of carthamine in solutions

It is interesting to assess the rate constants of the carthamine decomposition process in solutions, which quantitatively characterized the stability of this chalcone.

For the determination of the current concentration Cτ, the cartamine solution was injected immediately after obtaining and filtering through the microfilter. Then one part of this solution was kept at +5°C (II), and the second part at +20°C (III). The next day, solution (II) and solution (III) were injected, obtaining peak area values of A (II) = 2369816 and A (III) = 479349. Carthamine decomposition rate constants at pH = 4,5 were calculated, which were $K_{I,278} = 0{,}00357\ h^{-1}$ and $K_{I,293} = 0{,}0835\ h^{-1}$.

The calculated rate constants allowed to calculate the activation energy E_A [6] of the decomposition process. The high value of the activation energy, which is 142,32 kJ, corresponds to the processes that take place in the liquid phase, that is, it does not involve the interface phenomena, for which the activity energy is < 40 kJ/mol.

Thus, the lifetime of cartamine in the dissolved state in water is only a few hours even at temperatures below 30°C.

3.4. Particularities of RGB profile of carthamine solution and wet carthamine – cellulose complex

The comparative study of the stability and RGB profile demonstrated that carthamine behaves differently in solution and in the phase of microcrystalline cellulose at the same pH values (table 2).

Table 2. RGB values (digitized) and visual appearance of carthamine solutions and wet carthamine-cellulose complex

pH	10	9	8	7	6	5	4	3	2	1
Cartamina în soluții apoase foarte diluate, C_M egală cu 10^{-4} mol/L										
<R>	232	233	229	234	241	240	232	230	228	224
<G>	176	165	167	161	143	133	130	137	147	176
<B>	84	102	118	123	141	141	136	139	135	128
	Oranj	**Oranj-roz**			**Roz**					**Bej**
Complexul umed cartamina-celuloză										
<R>	144	97	86	82	89	136	132	123	135	128
<G>	90	41	20	19	21	14	6	8	7	11
<B>	139	79	59	62	65	37	31	29	36	39
		Purpuriu				**Magenta**				

In the pH range of 1 to 5, freshly filtered samples of the complex in the wet state have a magenta color. At pH 6 to 9, wet CCC acquires a purple color, which is totally absent in the color

range of carthamine solutions. We assumed that such an "anomaly" can be explained by the significant influence of cellulose on the state of the chromophore groups and on the structure of the carthamine molecule as a whole [7].

3.5. The mechanism of carthamine stabilization in the cellulose phase

Unlike anhydrous cartamine (M = 910) [8] the structure of the hydrated carthamine molecule (M = 928) [8] provides possibilities for free rotation of quinochalcone groups in aqueous environment. The high stability of CCC allowed us to assume that the complexation of carthamine on cellulose prevents the decomposition of hydrocarthamine (figure 4).

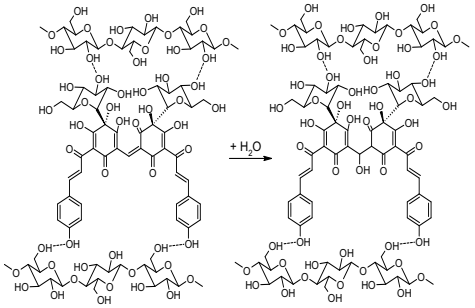


Fig. 4. Blocking the rotation of hydrocarthamine upon complexation with cellulose

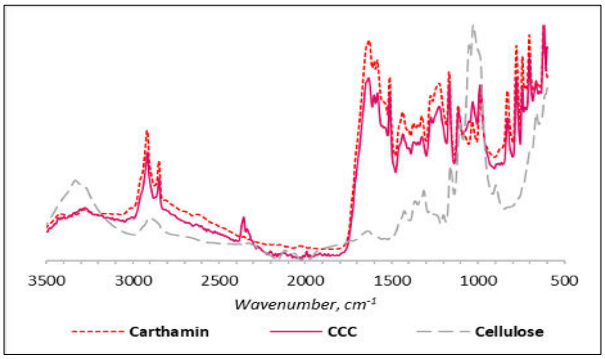


Fig. 5. "Normalized" FTIR ATR spectra of dry powders

The hypothesis about the strong effect of cellulose on the chromophore state of carthamine also finds confirmation by the analysis of FTIR spectra (figure 5). In the FTIR spectrum of CCC, new bands appear at 2340 cm^{-1} and 2360 cm^{-1}, which are not observed in either the spectra of cellulose or pure carthamine powder [7]. Although at present these two unusual bands have not been linked by us to any specific functional group, they indicate a strong interaction between cellulose and carthamine and therefore can be interpreted as an argument in favor of the formation of the carthamine–cellulose complex (CCC).

3.6. Influence of temperature on the yellow dye and carthamine – cellulose complex in powder form

It is of interest, from the point of view of protecting food products from inoculation with microorganisms, subjecting dyes to the sterilization process. The stability result of powder dyes at different temperatures is shown in table 3.

Table 3. The influence of temperature and time on the ratio of cartamine isomers in the cartamine – cellulose complex

Name of the isomer	Temperature, °C	Duration of heat treatment, minutes	Wavelength, λ, nm	Retention time, Rt, minutes	Area of the peak	Isomer content,%
Izocarthamine	100	5	520	5,91	39480	1,642
Carthamine			519	8,407	2365001	98,358
Izocarthamine	100	15	516	5,91	47778	1,948
Carthamine			519	8,408	2404771	98,052

The research results demonstrated that the degradation of carthamine from the CCC composition under the influence of different temperatures is insignificant. Thus, the non-thermally treated sample is characterized by the carthamine/isocarthamine ratio 98,1/1,9, and the sample treated at 100°C for 15 minutes is characterized by the carthamine/isocarthamine ratio by the same ratio. At the same time, when comparing the areas, a difference of only two 2% is observed between these samples, which does not exceed the admissible value of the experiment.

3.7. The influence of ultraviolet radiation on the stability of dyes

In order to study the stability of dyes in the form of powder under the action of UV-Vis radiation, an experimental stand was developed and set up to simulate the long-term action of UV rays on the samples (table 4).

Table 4. Influence of ultraviolet radiation on the yellow dye, YFDS

Sample analyzed	Name of the identified pigment	Wavelength, λ, nm	Retention time, Rt, minutes	Peak area	Pigment content, %
YFDS	HSYA	403	18,248	623985	35,342
	Unidentified	409	18,83	104648	5,927
	Precarthamine	411	19,98	575297	32,584
	AHSYB	411	21,999	461657	26,147
YFDS-UV	HSYA	403	18,238	648992	36,557
	Unidentified	409	18,825	97142	5,472
	Precarthamine	409	19,973	546886	30,806
	AHSYB	410	21,994	482250	27,165

Note: YFDS – yellow dye; YFDS-UV – UV irradiated yellow dye

Analyzing the areas of the chromatographic peaks of the yellow chalcones (table 4), it was demonstrated that the yellow dye YFDS in solid state does not undergo significant changes under the action of ultraviolet radiation. After treating YFDS samples with ultraviolet rays, the compounds of chalconic nature had the same values of retention times, which confirms that the structure of chalcones in the composition of the solid dye remains stable under UV irradiation.

The results, which characterize the stability of red chalcones, are presented in table 5.

Table 5. The influence of ultraviolet radiation on carthamine – cellulose complex

Sample analyzed	Name of the substance	Wavelength, λ(max), nm	Retention time, Rt, minutes	Area	Area, %
CCC	Izocarthamine	**513**/415/260/669/637	6,128	8894	2,659
	Carthamine	**519**/458/197/309/226	8,835	325578	97,341
CCC-UV	Izocarthamine	194/**522**/222/248/674	6,13	15068	5,203
	Carthamine	**520**/458/195/308/227	8,836	274557	94,797

Note: CCC – cartamine-cellulose complex; CCC – UV – cartamine-cellulose complex irradiated

An identical situation was followed in table 5. Carthamine and isocarthamine peak areas after exposure to light do not differ significantly from carthamine and isocarthamine peak area values obtained before exposure to UV rays. This fact once again confirms that the complexation of carthamine on cellulose blocks the rotation of the functional groups of the chromophore and does not allow the rearrangement of the structure of the carthamine molecule.

4. TECHNOLOGY FOR OBTAINING DYES FROM SAFFLOWER PETALS (*Carthamus tinctorius* L.)

The development of manufacturing technology of new food dyes presents a complex problem from a technological and technical point of view. In particular, the theoretical and experimental studies were intended to appreciate the mechanism of the pigment extraction process from the petals, simultaneously with the development of a mathematical model of the pigment diffusion process.

4.1. The mechanism of the process of extracting pigments from petals in liquid medium

Pigments move out of the petal mass by both molecular diffusion and turbulent motion as a result of mechanical agitation. Diffusion of pigments is expressed by Fick's first law. The principle of the process is that the mass of the extracted substance depends on the concentration gradient and the diffusion coefficient. In general, the differential equation of diffusion is presented in the form [9]:

$$\mathrm{dm} = -\mathrm{D}\frac{\mathrm{dc}}{\mathrm{dx}}, \quad (1)$$

For the analysis of the pigment diffusion process, the generalized model of the structure of the petals, reduced to an easy to calculate geometric body, was proposed in the thesis. The petals were presented in the form of a plate with the shape coefficient value $E_f = 1,0$ [9].

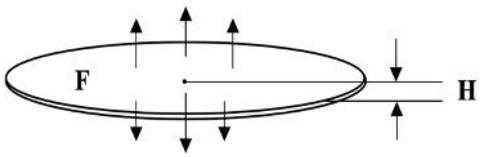


Fig. 6. Presentation of the safflower petal in the form of a plate

According to Fick's equation, the mass diffusion of the pigments on the surface of the petals in a liquid medium is expressed in differential form as follows:

$$dm = -D\frac{dc}{dF}Md\tau, \tag{2}$$

In the process of extracting the mass of pigments, dm, it migrates by diffusion from the center of the petals to the surface. The mass of displaced pigments, dm, is determined using the relationship:

$$dm = \beta \cdot M \cdot C \cdot F \cdot d\tau, \tag{3}$$

At the same time, there is a balance between the mass of pigments displaced from the center of the petals on their surface and the mass of pigments that diffuse from the surface of the petals to the outside environment. The mathematical relationship of this balance is presented in the form of a differentiale quation:

$$-D\frac{dC}{dF}Md\tau = \beta \cdot M \cdot C \cdot F \cdot d\tau, \tag{4}$$

After solving equation (4), the mathematical relationship of the process of extracting pigments from the petals was obtained, which looks as follows:

$$C = C_0 \exp\ (-0{,}5\ k\ F^2) \tag{5}$$

According to relation (5) the concentration of extracted pigments, C, depends on the concentration of pigments in the petals, C_0, the surface area of the petals to the power of two and the pigment extraction coefficient, k, which is the ratio of the transfer and diffusion coefficients, D and β.

The impact of the numerical values of the coefficients, D and β, on the yield of pigments in the petals was analyzed. For this purpose, three possible relationships between the pigment yield determined experimentally and calculated by applying the mathematical relationship (5) were examined.

It was found that the ratio of coefficients D/β ≈1 corresponds to the actual process of extracting pigments from the petals and ensures the maximum yield of 85% [10].

Table 6. The influence of the ratio of the coefficients, D and β, in the developed mathematical relationship and the application of the relationship in the analysis of the pigment extraction process

Ratio D/β	Number of extraction cycles	Pigments yield, grams	Pigments yield, %	Correlation of theoretical and experimental pigments yield
D > β (D = 3β)	4	7,0	63,0	Absent
D < β (3D = β)	2	10,7	97,0	Absent
D = β (D/β = 1)	4	11,0	85,0	Correspond 85,0% = 85,0%

The data of the pigment extraction process were analyzed from the point of view of the development of the process over time (figure 7). To simplify the determination of the pigment extraction coefficient, the experimental data of the reduction of pigment content over time are presented in semi-logarithmic coordinates (figure 8) [11].

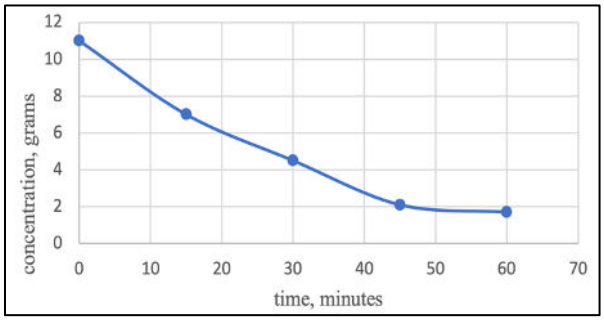


Fig. 7. Reduction of pigment content in the petal structure during the extraction process

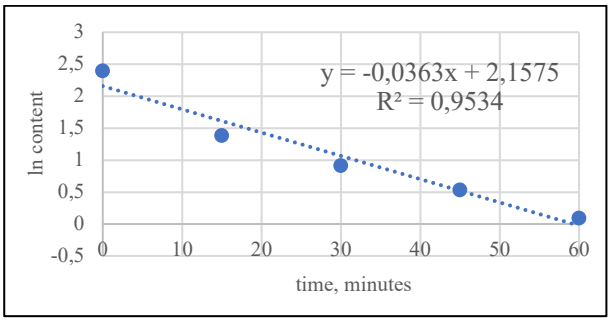


Fig. 8. Linear correlation between the logarithm of pigment concentration in petals, ln C, and the duration of the extraction process, minutes

The data, presented on figure 8, are arranged linearly, and the approximation credibility value $R^2 > 0,95$ is another good argument, which confirms its linearity.

$$\ln C = -0,0363\tau + 2,1575 \quad (6)$$

In the conditions, when the coefficient, k, is transferred from the semi-logarithmic equation to the linear equation, the exponent exp (-k) is determined; in this case $k = e^{-0,0363} = 0,97 \approx 1,0$.

Therefore, it was proved that the coefficient of the extraction process, k, determined experimentally, correlates with the numerical value, k, determined theoretically, the value of which is equal to $k = \beta/D \approx 1,0$.

4.2. Kinetic modeling of the pigments extraction process

Kinetic modeling is an experimental method based on the analysis of chemical process rates [12]. In general, the speed of the pigment extraction process is as follows:

$$-\frac{dC}{d\tau} = k_1 [C]^n \quad (7)$$

Tabelul 7. Changing the concentration and extraction speed of pigments

Duration of the pigment extraction process, τ, minutes	Modification in pigment concentration, C, kg	Logarithm of the change in concentration, ln C	Rate of change of concentration, C/τ, kg/min	Logarithm of the change in concentration, ln (C/τ)
0	11,0	2,4	-	-
15	7,0	1,95	0,47	- 0,76
30	4,5	1,5	0,15	-1,90
45	2,1	0,70	0,50	-1,61
60	1,7	0,53	0,03	-2,30

For the practical use of the differential equation (7), it was necessary to determine the numerical values of the reaction order, n, and the numerical value of the process duration coefficient, k_1.

To determine the order of the process, n, the differential equation (7) is presented in logarithmic form, thus transforming into the equation:

$$\ln\left(-\frac{dC}{d\tau}\right) = \ln k_1 + n \ln C \quad (8)$$

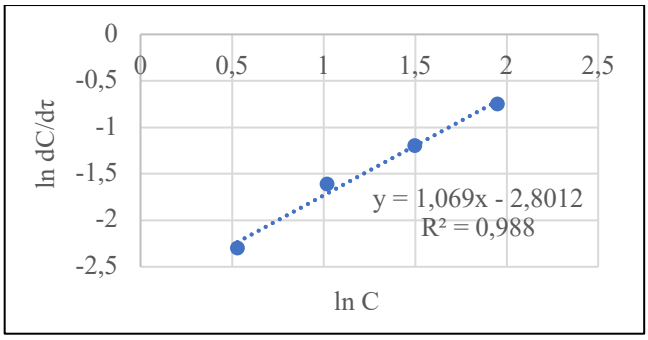


Fig. 9. Determination of process order

The slope of the straight line reflects the order of the process, n (figure 9), which is equal to 1,069, which reflects the fact that the order of the process n ≈1,0.

Therefore, the duration of the pigment extraction process is determined using the equation:

$$\tau = \frac{1}{k} \ln \frac{C_0}{C_0 - C} \quad (9)$$

The verification of the obtained data was carried out in order to appreciate the degree of correlation between the data obtained experimentally and the data calculated with the help of relations (8, 9). Also, the numerical values of the pigment extraction duration coefficient were analyzed according to the mass of processed petals from 1,0 to 50,0 kilograms (table 8).

Table 8. Numerical values of the pigment extraction duration coefficient

Mass of petals, kg	Mass of pigments, kg	Equation ln(C) = f(τ)	Extraction duration coefficient, k, h^{-1}	Duration of the extraction process, h
4,5	1,12 ± 0,02	ln C = - 0,036 τ + 0,12 R^2 = 0,9542	- 0,036	1,0
6,0	1,50 ± 0,05	ln C = - 0,032 τ + 0,40 R^2 = 0,8834	- 0,032	1,2
50,0	12,50 ± 0,05	ln C = - 0,031 τ + 2,52 R^2 = 0,9989	- 0,031	2,5

4.3. Dyes production system from safflower petals

Currently, one of the most effective methods of investigations intended for solving complex tasks is the methodology of the systemic approach in engineering [13, 14]. According to this methodology, the production technology of a food coloring presents a complex system, made up of several structural elements (figure 10).

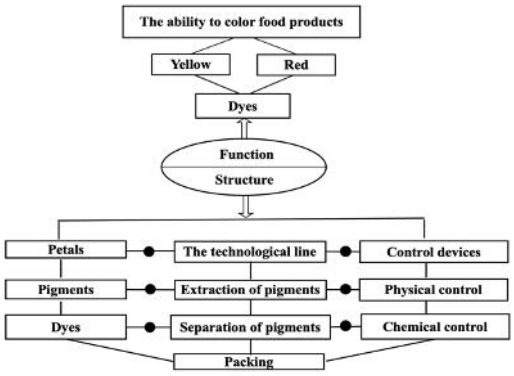


Fig. 10. Scheme of the production system of dyes from safflower petals with three hierarchical levels and links between system elements (-●-)

4.3.1. The technological line for obtaining dyes from safflower petals

For the materialization of the dye manufacturing technology, the technological line for processing safflower petals was developed (figure 11).

Position 1. Keeping the petals in perforated polyethylene bags at a temperature of 20±2°C, relative air humidity 70 – 75%. Shelf life up to 15-20 days.

Position 2. Inspection of petals, from which impurities are removed. Inspected petals are loaded into containers and weighed – position 3.

Position 4. The petals are subjected to hydration by stirring with potable water, ratio petals: water 1:10. At this stage, the water interacts with the pigment granules in the petals, forming the granule-water bond.

Positions 5 – 9. According to the results of experimental and theoretical research, discussed in chapters 2 and 3, it was demonstrated that the extraction is carried out by four consecutive cycles

of extraction of pigments from the petals. The yield of pigments reaches 80 – 85% of the total mass of pigments.

In the reactor, position 5, takes place the first cycle of the process of extracting the pigments from the petals with drinking water and the addition of sodium carbonate, Na_2CO_3, to obtain the environment with a pH value equal to 8,0. The extraction is carried out at a temperature of $20 \pm 2°C$, for 15 minutes, with continuous stirring until the constant color of the extract appears [15]. The extraction of pigments in the next three cycles is done with the addition of water, obtained by condensing the vapors resulting from the pigment concentration process, position 10.

The mixture of extract and petals accumulates in the container in position 6. The separation of the petals is carried out with the help of the decanter (position 7). The separated petals are transported using the vessel (position 9) to the reactor, position 5, for the next extraction cycle. This procedure is repeated through four extraction cycles, until the petals fade.

Position 8. The concentration of pigments in the total volume of the extract is small and varies between 1,5 and 2,8%.

Position 10. Industrial rotary evaporator. It is used for the first concentration of pigment extract from 1,5 – 2,8% to 24 – 25%. Evaporation of the water is carried out in a rotating device under vacuum: vaporization temperature 50°C, at pressure P = < 10 kPa.

Position 11 and 12. The concentrated extract accumulates in the intermediate container 11 and is further pumped into the container 12, where the separation and sedimentation of the solid red dye [16] from the liquid medium of the mixture of yellow and red dyes takes place. The yellow dye remains in the extract.

Position 14. Centrifuge. The red dye in the form of carthamine – cellulose complex accumulates in the intermediate container 13. To remove the rest of the yellow dye and treat the sediment with drinking water, the carthamine – cellulose complex passes through centrifugation, where the water is removed 14. After removing the water, the red dye, is subjected to dehydration by drying with IR rays, position 20, at a temperature of 60 – 70°C.

Position 16. From container 12, the yellow pigment extract is introduced into the rotary evaporator, position 15, for the second concentration of the yellow pigment extract from 24 – 25%, until obtaining the yellow dye in the form of a paste with a concentration of 30 – 35 %. Concentration conditions: vaporization temperature 50°C, pressure Pa = <10 kPa. The finished product – yellow dye in the form of a paste is transported for dosing and packaging, position 18. Also, from the container 16, a part of the paste is sent to dry with IR rays, plant 20, temperature 60 – 70°C, to obtain the yellow dye in powder form.

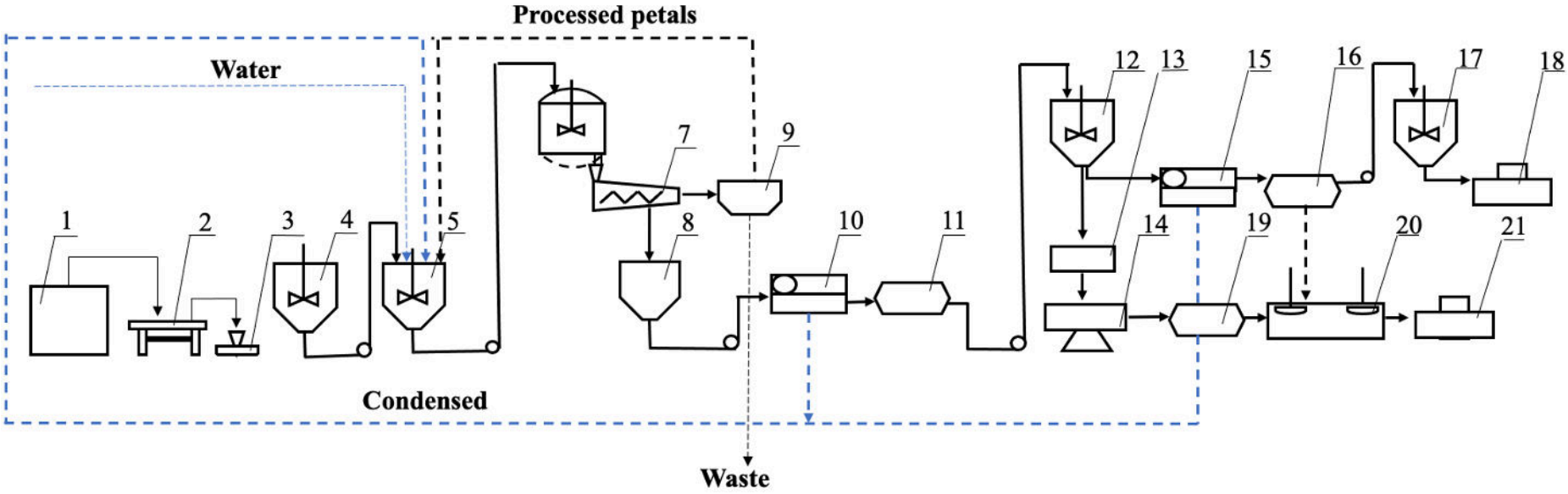


Fig. 11. Technological line for obtaining dyes from safflower petals

1 – petals storage, **control point 1**; 2 – petals inspection table; 3 – weigher; 4 – reactor for the formation of the system petal:water; 5 – reactor for extraction pigments from petals, **control point 2**; 6 – container for collecting pigment extract together with petals; 7 – decanter for separating the petals from the liquid phase; 8 – pigment extract collector; 9 – container for partially and totally discolored petals; 10 – rotary evaporator; 11 – reactor for the concentrated extract; 12 – reactor for red dye sedimentation, **control point 3**; 13 – container for wet red dye; 14 – centrifuge; 15– rotary evaporator; 16 – container for collecting the yellow dye in paste form; 17 – reactor for the yellow paste dye with the programmed concentration, **control point 4**; 18 – aparat for dosing the yellow dye in paste form; 19 – container for collecting the wet red dye; 20 – IR instalation for red dye and part of yellow dye; 21– aparat for dosing yellow and red powders, **control point 5**.

5. POSSIBLE DIRECTIONS OF USE OF YELLOW DYE FROM SAFFLOWER PETALS IN FOOD TECHNOLOGY

For the manufacture of a food product with the use of chalcone dyes, obtained within the thesis, there is a need to develop a modernized technology for the manufacture of the respective food.

5.1. Principles of using natural dyes from safflower petals in food compositions

The choice of the optimal dye concentration is opportune to achieve based on the following considerations. The optical density (absorbance) of sufficiently colored systems with a thickness of 1 cm, falls within the limits of 0,2 to 2,0. Solutions with optical density values less than 0,2 will have gri, undeterminable hues. The color of systems with an optical density above 2,0 will be so intense that the addition of the dye will not produce significant changes.

It was experimentally demonstrated that the value of the molar extinction coefficient of the yellow dye, calculated with reference to precarthamine, is 16500 $L \cdot mol^{-1} \cdot cm^{-1}$ for pH equal to 5,0. For the yellow dye, the optimal concentration values are estimated by using the Beer-Lambert law (eq. 5.1).

$$m = \frac{A}{\varepsilon \cdot l} \cdot M(\text{precarthamine}) = \frac{A * 957 g \cdot mol^{-1}}{0{,}05 cm \cdot 16500\ L\ cm^{-1} mol^{-1}} = 1{,}16 \cdot A, \qquad (5.1)$$

Thus, the mass of the yellow dye, which corresponds to optical densities, A, of 0,2 to 2,0, will fall within the mass values of 0,116 to 1,16 g/L. The estimated range of yellow dye concentrations for various solid food products will be within the range of 0,24 to 2,4 g/L.

Starting from the molar extinction coefficient value of 3800 $L \cdot mol^{-1} \cdot cm^{-1}$ for carthamine, the concentration of CCC falls within the limits of 4 to 16 g/kg solid product.

5.2. The use of yellow dye from safflower petals in the technology of manufacturing caramel masses

To study the possibility of using the yellow dye from safflower petals in the manufacture of caramel masses, the technologies based on the use of three anticrystallizers were used: glucose syrup, molasses and isomalt.

5.2.1. Determining the quality indices of caramel masses

It was determined that with the increase in the concentration of yellow dye from safflower petals, the value of the acidity index increases in all the manufactured series. This increase has a

slow character and can be explained by the fact that the precarthamine in the composition of the yellow dye contains the acid carboxylic group. At the same time, these values fall within the permitted limits of 7,1 – 16,0 degrees of acidity for all samples. The largest deviations from the control sample and the tartrazine sample are obtained for the caramel samples with 1,0% YFDS.

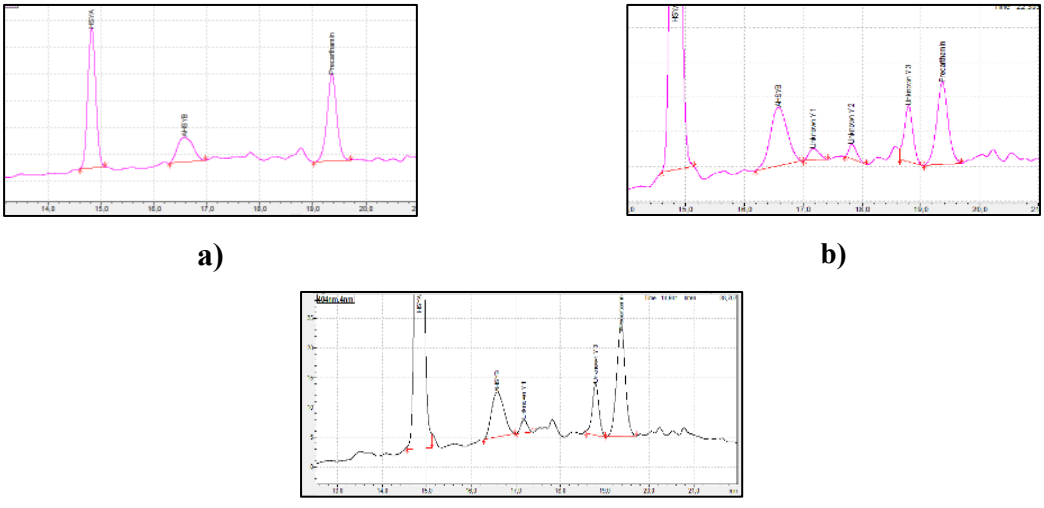
The moisture content of the samples of the caramel masses decreases considerably with the increase in the content of natural yellow coloring added. This effect can occur as a result of the increase in the acidity of the samples, which, in turn, influences the ease of removal of free water at the final stages of the formation of the caramel masses.

The ash content in the samples with 0,1 – 0,3% natural dye does not exceed the permissible value of 0,2% ash, for all types of caramel, and in the samples with the concentration of 0,4% and 1,0% of YFDS the content is not correspondingly as in samples with tartrazine.

The content of reducing sugars in the analyzed samples demonstrates that the addition of natural dye, YFDS, does not significantly influence their content. In all samples of caramel masses, the values of reducing sugars fell within the limits established, 23±2%, by Government Decision number 204 regarding the approval of the Technical Regulation "Confectionery Products" [17].

5.2.2. Color indices and stability of yellow dye in caramel masses

The caramel samples were dissolved in distilled water, the obtained solutions were filtered through a PES filter and immediately chromatographed according to the HPLC method (figure 12).



a) **b)**

c)

Fig. 12. Chromatograms profils of caramel masses based on glucose syrup (a), isomalt (b) and molasses (c)

According to the chromatograms, three weeks after manufacture, all caramel samples contain three chalcones: hydroxysafflor yellow A, anhydrosafflor yellow B and precarthamine,

which predominates in the mixture. The data obtained demonstrate that the parameters of the technological manufacturing processes, the type of anticrystallizer and the storage time do not cause significant degradation of the chalcones from YFDS in the caramel masses.

5.2.3. The influence of the anticrystallizer on the chromatic parameters of caramel masses

The color parameters of the caramel masses were recorded with the Chroma Meter CR-400 device by photographing the samples of the caramel masses.

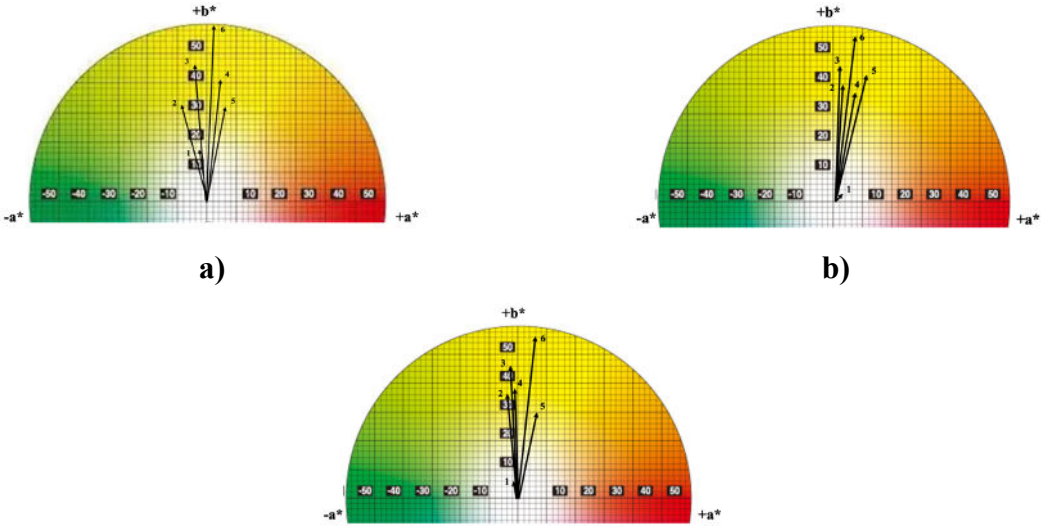


Fig. 13. Chromatic profiles of caramel masses based on: a) glucose syrup; b) isomalt; c) molasses in CIELab coordinates

From figure 13 it can be seen that all the control samples of the caramel masses are positioned closest to the intersection of the axes (points 1) and refer to the "grey" color shades. Caramel masses with natural dye content and based on synthetic dye tartrazine, are found in the yellow color coordinates of the CIELab system, depending on the amount of natural dye added. The samples of the caramel masses with a content of 0,4 and 1,0% natural dye, YFDS, are positioned in the part of the profile with the shade of orange color, which once again confirms that the optimal concentration for the use of the dye in the caramel masses must be no more than 0,3% (points 3).

The analysis of the results demonstrates that the optimal concentration of yellow dye from safflower petals is 0,3%, but as an anticrystallizer the most suitable for the manufacture of caramel masses is isomalt.

5.3. Use of yellow dye in yogurt manufacturing technology

In order to study the possibility of incorporating the yellow dye into some dairy products, the study of the stability of the dye in the yogurt composition during the storage period was carried out.

5.3.1. Determination of quality indices of yogurt samples

It is important to make sure that the added doses of yellow dye do not substantially modify the physico-chemical properties of the yogurt samples. The results of the determination of the physico-chemical parameters are presented in table 9 [18].

Table 9. Physico-chemical properties of yogurt samples

Indicators	YC	YS1	YS2	YS3	YS4
Total dry matter, %	11,7 ± 0,1	11,8 ± 0,1	11,9 ± 0,2	12,0 ± 0,2	12,1 ± 0,2
Fat, %	3,00 ± 0,11	2,97 ± 0,10	2,96 ± 0,09	2,96 ± 0,09	2,96 ± 0,09
pH	4,40 ± 0,03	4,41 ± 0,03	4,46 ± 0,03	4,48 ± 0,03	4,44 ± 0,03
Viscosity, Pa·s	3,31 ± 0,17	3,90 ± 0,20	3,84 ± 0,19	3,93 ± 0,20	3,97 ± 0,18
Syneresis index, %	70,86 ± 0,71	66,31 ± 0,67	62,84 ± 0,63	60,78 ± 0,61	61,65 ± 0,62

Note: YC: classic yogurt (control sample); YS1-YS4: yogurt samples with added YFDS at concentrations from 0,1% to 0,4% (w/w)

From table 9 it can be seen that the yogurt viscosity was found to be slightly higher in the yogurt samples with dye from safflower petals than in the control sample. The fat content was found to decrease slightly with increasing dye concentration and the dry matter content increased moderately as well, which is natural since YFDS dye represents the non-lipid soluble dry matter. The dependence on the dye dose of the syneresis index values is not linear and does not essentially influence the yogurt quality. Yogurt samples with the addition of dye from safflower petals show a pH value similar to the yogurt sample without dye.

5.3.2. Dye stability during 28 days of storage

Yogurt samples with 0,3 and 0,4% (w/w) addition of yellow dye, YFDS, were monitored to determine the stability of the dye during storage.

The obtained data demonstrate that the L* parameter presents average values between 73,97 and 75,48 units during the 28 days of storage, indicating that the samples had a high brightness, similar to the initial value obtained on the day yogurt manufacturing. Regarding the a* coordinate, the mean values were between -5,90 and -5,00 units. The values of the chromatic coordinates a* are negative, which corresponds to the green zone of the CIELab space. In the case of the chromaticity coordinate b*, the average values were between 17,96 and 18,91 and all are located on the positive side of the axis, which corresponds to the yellow area. The samples showed

uniformity in the three color coordinates L*, a*, b* throughout the storage period for both the control yogurt and the yogurt with natural yellow safflower petal dye. The degree of hue angle ho remained almost constant during the storage of yogurts with natural yellow dye. This result indicates that the color shade of the yogurt does not change during storage, which confirms that the chalcone compounds in the composition of the yellow dye in the yogurt environment do not undergo essential structural degradation. During storage, no significant differences in the parameters, C*, were observed for all yogurt samples, indicating that the color of the yogurt remains as intense throughout the monitoring period [18].

The yogurt sample prepared for chromatography was analyzed by the HPLC method - 1 (chapter 2 of the thesis). The samples of 5 ml of yogurt were centrifuged at 6000 revolutions/minute, for 15 minutes, being subjected to stratification in three phases (protein, aqueous and lipid). The colored aqueous phase was taken up with the syringe, then filtered through 0.45 micron PTFL and injected. The results of the identification of the chalcones are confirmed by the HPLC chromatogram (figure 14).

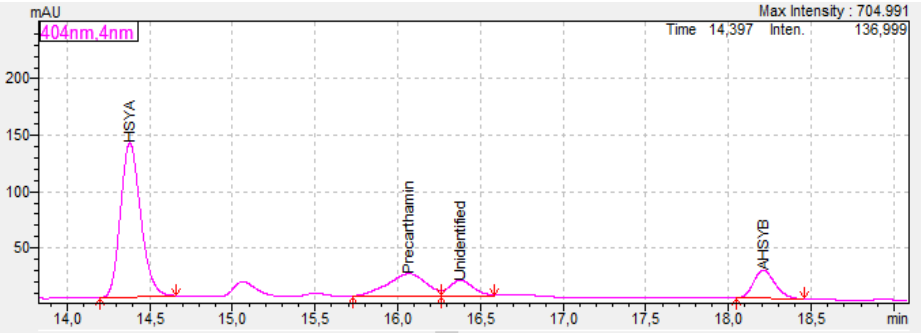


Fig. 14. HPLC profile of chalcones in yogurt sample with addition of natural yellow dye from safflower petals

Chromatographic data, obtained by calculating the relative peak areas of chalcones (hydroxysafflor yellow A, anhydrosafflor yellow B, precarthamine) did not demonstrate any significant change in the ratio of chalcones during the storage period.

Color differences during the storage period were determined by ΔE. The color differences, ΔE, recorded for samples with natural dye from safflower petals throughout the storage period was ≤ 0,79, which is an extremely satisfactory result, since ΔE ≤ 3,0 cannot be detected with the free eye [19].

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Following the realization of theoretical and practical research and according to the analysis of the results obtained in the doctoral thesis with the title: "Obtaining and using natural dyes from safflower petals (*Carthamus tinctorius* L.) in food technology", the following conclusions were formulated:

1. For the first time, the scientific principles of the operation of the technological system for the simultaneous production of yellow and red natural dyes from the non-traditional raw material of safflower petals (*Carthamus tinctorius* L.) were developed, the chemical composition, physico – chemical and technological properties of the dyes were determined, their ability to color and improve the appearance of food compositions of vegetable and animal origin has been appreciated.

2. A production system of yellow and red dyes from safflower petals with programmed physico-chemical properties was developed, which includes the following basic elements: raw material; the general technology of obtaining dyes, the manufacturing technology line and the technological flow control system.

3. As the raw material for obtaining YFDS and CCC dyes, safflower petals with a content of 24,0 – 26,0% of red and yellow pigments from the mass of dry petals must be used. The yellow pigment is water soluble, constitutes 18,0 – 20,0% of the mass of the petals and contains chalconic compounds, of which 5 have been identified [4]. The red pigment contains carthamine and isocarthamine – practically insoluble compounds in water, and constitutes 4,5 – 5,5 % from the mass of the petals.

4. The physico-chemical properties of the pigments were determined: the yellow pigments show high thermal stability, being resistant to heating up to 120°C, they possess stability to the action of UV rays and stability in solutions in a wide range of pH, from 1,0 to 9,0 [4]. The red pigment, carthamine, exhibits stability in acidic environments, at pH 1,0 to 5,0 [7].

5. The physico-chemical method of stabilizing the red dye, carthamine, by forming the complex with cellulose in a ratio of 1:5 – 1:10 [16], which consists in preventing the rotation of the hydrocarthamine molecule, was elaborated and explained. The structure of the complex was confirmed by the methods of IR spectroscopy, the analysis of the color of the complex in the wet state, the analysis of the extraction kinetics of carthamine from its complex with cellulose, which confirms that the carthamine-cellulose complex can be proposed as a food colorant, stable at pH up to 5,0 and at temperatures up to 70°C for 15 – 30 minutes [7].

6. It was demonstrated that the mechanism of the pigment diffusion process corresponds to the exponential function and proceeds according to Fick's first law. The appropriate mathematical model was developed, which describes the extraction process of pigments [10].

7. Through the kinetic modeling of the pigment extraction process, the mathematical relationship for determining the duration of the pigment extraction process from the petals at the constant temperature 20,0 ± 2,0°C was developed. The deviation between the calculated data and the experimental data of the process duration varies within 3,0 – 5,0%.

8. It was found that the yield of yellow and red dyes constitutes 80,0 – 85,0% of the initial concentration of the dyes in the petals [10]. The advantage of the system from an economic point of view, consists in the fact that the manufacture of dyes is carried out on the basis of circular technology with low consumption of thermal energy and potable water (there is practically no need to dry the petals).

9. The theoretical principle of the food coloring methodology was elaborated, which allows the expression of color by numerical values, based on the standard scales of yellow and red dyes from safflower petals:

- for the yellow dye, this value is from 0,116 to 1,16 g/L;
- for the carthamine-cellulose complex, CCC, from 0,24 to 2,4 g/L.

10. It was found that the C-glycosylic chalcones in the YFDS dye remain intact in the process of making caramel masses and it can be used as a food dye without affecting the physico-chemical properties of the finished product. The optimal concentrations of yellow dye in the caramel masses are 0,3%, which ensures a color very similar to the color of samples containing tartrazine.

11. It was confirmed that the YFDS dye can be incorporated into the lipid-protein matrix of the lactic acid product, due to the high stability of C-glycosyl chalcones in yogurt manufacturing technology. The optimum concentration of natural yellow dye, YFDS, required for a sufficient color of the yogurt, is from 0,3 to 0,4%. The color parameters remain stable during the 28 days of storage [18].

Recommendations:

1. It is recommended to continue researching the chemical composition of the parts of the safflower plant, as a rich source of biologically active compounds: natural dyes from the petals and the polyunsaturated fatty acids of the seed oil.

2. It is recommended to develop a State project, which would include multilateral research, aimed at harvesting and processing the safflower plant (petals, seeds, etc.) and selecting food products, in which safflower dyes can be added, replacing synthetic dyes.

SELECTIVE BIBLIOGRAPHY

1. CARABULEA, B., MOTRIUC, S. Extragerea coloranților naturali. In: *Conferința tehnico – științifică a colaboratorilor, doctoranzilor și studenților,* 15 – 23 noiembrie, 2013, Chișinău, Republica Moldova, pp. 69-72. ISBN 978-9975-45-310-3.

2. GHENDOV-MOȘANU, A., CRISTEA, E., STURZA, R., NICULAUA, M., PATRAȘ, A. Synthetic dyes substitution with chokeberry extract in jelly candies. In: *Food Science & Technology.* 2020, 57(12), pp. 4383-4394. ISSN 2076-3417.

3. GHENDOV-MOȘANU, A. The use of dog-rose hips (rosa canina) fruits in the production of marshmallow-type candy. In: *Foof and Environment Safety Journal*. 2018, nr. 17(1), pp. 59-65. ISSN 2068 - 6609.

4. **SAVCENCO, A**. Spectral and chromatographic characterisation of the yellow food dye from safflower. In: *Journal Engineering Science.* 2022, vol. 29, nr. 3, pp. 189-195. ISSN 2587-3474.

5. MAN-HO, C., TAE-RYONG, H. Purification and characterization of precarthamin decarboxylase from the yellow petals of *Carthamus tinctorius* L. In: *Biochemistry and Biophysics.* 2000, nr. 382(2), pp. 238-244. ISSN: 0003-9861.

6. TATAROV, P. Principii conceptuale ale calității alimentelor și capabilității proceselor tehnologice. Chișinău: Editura „Tehnica-UTM", 2019. 160 p. ISBN 978-9975-45-572-5.

7. BAERLE, A., **SAVCENCO, A.,** TATAROV, P., FETEA, F., IVANOVA, R., RADU, O. Stability limits of a red carthamin-celulose complex as potential food colourant. In: *Food & Function*. 2021, nr.12, pp. 8037-8043. ISSN 2042-6496.

8. LAURSEN, R., MOURI, C. Decomposition and analysis of carthamin in safflower-dyed textiles. In: *e-Preservation Science*. 2013, nr.10, pp. 35-37. ISSN 1581-9280.

9. BĂISAN, I. *Operații și tehnologii în industria alimentară* [online]. Iași, 2011 [citat 15.02.2023]. Disponibil: https://mec.tuiasi.ro/diverse/otiam.pdf.

10. **SAVCENCO, A.** Particularitățile procesului de extracție a pigmenților din petale de șofrănel (*Carthamus tinctorius* L.). In: *Conferința tehnico – științifică a studenților, masteranzilor și doctoranzilor*, 5 – 7 aprilie, 2023. Chișinău, Republica Moldova, vol. 2, pp. 234-237. ISBN 978-9975-45-956-3.

11. ATKINS, P., PAULA, J. *Physical Chemistry.* New York: W. H. Freeman and Company, 2006. ISBN 0-7167-8759-8.

12. BIRDI, K. *Surface and Colloid Chemistry.* 3rd ed. USA: CRC Press Taylor & Francis Group, 2009. 756 p. ISBN 978-0-8493-7327-5.

13. FOSSNES, T., FORSBERG, K., FISHER, G., INCOSE, C. *Systems ingineering handbook* [online]. USA: International Council on Systems Ingineering, 2004 [13.05.2023]. Disponibil: http://www.las.inpe.br/~perondi/21.06.2010/SEHandbookv3.pdf

14. GAINDRIC, C. *Abordări sistemice în luarea deciziilor* (suport de curs). Universitatea Academiei de Științe a Moldovei, 1017. 156 p.

15. **SAVCENCO, A.,** BAERLE, A., TATAROV, P., IVANOVA, R. Procedeu de obținere a coloranților din petale de șofrănel (*Carthamus tinctorius* L.) Brevet de invenție 1453 (13) Z, C09B 61/00. Universitatea Tehnică a Moldovei. Nr. depozit s2019 0133. Data depozit 23.12.2019. Publicat 31.08.2020. In BOPI. 2020, nr. 8, pp. 59-60.

16. **SAVCENCO, A.,** BAERLE, A., TATAROV, P., IVANOVA, R. Procedeu de obținere a colorantului roșu în formă de pulbere din petale de șofrănel. Brevet de invenție 1609 (13) Z, C09B 61/100. Universitatea Tehnică a Moldovei. Nr. depozit s2020 0159. Data depozit 24.12.2024. Publicat 31.03.2022. In: BOPI. 2022, nr. 3, pp. 59.

17. Hotărârea Guvernului Republicii Moldova pentru aprobarea Reglementării tehnice "Produse de cofetărie", № 204 din 20.03.2009. În: *Monitorul Oficial al Republicii Moldova*, nr. 57-58.

18. 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.). In: *Journal Engineering Science.* 2022, nr. 29 (1), pp. 142-150. ISSN 2587-3474.

19. HONG, S., HAN, J., KROCHTA, J. Optical and Surface Properties of Whey Protein Isolate Coatings on Plastic Films as Influenced by Substrate, Protein Concentration, and Plasticizer Type. In: *Journal Applied Polymer Science.* 2004, nr.92(1), pp. 335-343. ISSN 0021-8995.

LIST OF THE AUTHOR'S PUBLICATIONS ON THE SUBJECT OF THE THESIS

1. Specialty books

1.2. Specialized collective books

1.2.1. SANDU, I., **SAVCENCO, A.,** BAERLE, A., TATAROV, P., MACARI, A. Capitolul 2. Stabilizarea proprietăților senzoriale și activității biologice a substanțelor biologic active din compoziții alimentare. In: *Ameliorarea calității alimentelor prin biotehnologie și inginerie alimentară.* Chișinău: Ed. Tehnica-UTM, 2023, pp. 34-58

1.2.2. BULGARU, V., CUȘMENCO, T., POPESCU, L., CEȘCO, T., **SAVCENCO, A.,** BAERLE, A., ȚĂRNĂ, R., MACARI, A., STURZA, R., GHENDOV-MOȘANU, A., SANDULACHI, E., GUREV, A., TATAROV, P. Capitolul 6. Tehnologii de fabricare a produselor

lactate fermentate cu adaosuri vegetale. In: *Ameliorarea calității alimentelor prin biotehnologie și inginerie alimentară.* Chișinău: Ed. Tehnica-UTM, 2023, pp. 136-165

2. Articles in scientific journals

2.1. in journals from the Web of Science and SCOPUS -1 databases -1.

2.1.1. 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. In: *Food and Function*, 2021, nr. 12, pp. 8037-8043. Disponibil: https://doi.org/10.1039/D1FO01376A **(I.F. > 6)**

2.2. in journals from other databases accepted by ANACEC (AGRIS, DOAJ, IDN) - 3

2.2.1. 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.). In: *Journal of Engineering Science*, 2022, 29 (1), pp. 142-150. Disponibil: https://doi.org/10.52326/jes.utm.2022.29(1).13

2.2.2. **SAVCENCO, A.** Spectral and chromatographic characterisation of the yellow food dye from safflower. In: *Journal of Engineering Science,* 2022, 29 (3), pp. 189-195. Disponibil: https://doi.org/10.52326/jes.utm.2022.29(3).16

2.2.3. **SAVCENCO, A.** The use of food dyes: problem, solution and source of perspective. In: *Journal of Social Sciences*, 2023, 30 (3), pp. 173-187. Disponibil: https://doi.org/10.52326/jes.utm.2023.30(3).13

3. Articles in conference proceedings and other scientific events

3.3. in the works of scientific events included in the Register of materials published on the basis of scientific events organized in the Republic of Moldova -1

3.1.1. **SAVCENCO, A.** Particularitățile procesului de extracție a pigmenților din petale de șofrănel (*Carthamus tinctorius* L.). In: *Conferința Tehnico-Științifică a Studenților, Masteranzilor și Doctoranzilor,* 2023, vol. 2, 234-237. ISBN 978-9975-45-956-3 **(2nd degree diploma).**

4. Patents and other intellectual property

4.2. issued by the State Agency for Intellectual Property -3

4.2.1. **SAVCENCO, A.,** BAERLE, A., TATAROV, P., IVANOVA, R. Procedeu de obținere a coloranților din petale de șofrănel (*Carthamus tinctorius* L.). Brevet de invenție MD 1453 (13) Z. Publicat In: BOPI nr. 8/2020, pp. 59-60. Disponibil**:** https://www.agepi.gov.md/sites/default/files/bopi/BOPI_08_2020.pdf#page=7

4.2.2. **SAVCENCO A.,** BAERLE A., TATAROV P., IVANOVA R. Procedeu de obținere a colorantului roșu cartamină sub formă de pulbere. Brevet de invenție MD 1609 (13) Z. Publicat In: BOPI nr. 3/2022, pp. 59-60. Disponibil: https://www.agepi.gov.md/sites/default/files/bopi/BOPI_03_2022.pdf#page=7

4.2.3. POPESCU, L., **SAVCENCO, A.,** BAERLE, A., TATAROV, P., GHENDOV - MOȘANU, A., STURZA, R., PATRAȘ, A. Procedeu de fabricare a iaurtului. Brevet de invenție MD 1625 (13) Z. Publicat In: BOPI nr. 6/2022, pp. 56. Disponibil: https://www.agepi.gov.md/sites/default/files/bopi/BOPI_06_2022.pdf#page=7

5. Other works and achievements specific to different scientific fields

5.1. theses in the proceedings of international scientific conferences (abroad) -4

5.1.1. **SAVCENCO, A.,** NETREBA, N., BANTEA-ZAGAREANU, V. Perspective of using ziziphus jujuba mill in the production of marmelade-pastile products. In: *11th International Symposium Euro-Aliment,* 2023, Galați, România. pp. 42-43. ISSN 1843-5114**.** Disponibil: http://www.euroaliment.ugal.ro/index.php/en/

5.1.2. **SAVCENCO, A.,** BAERLE, A. State of carthamin in water and steam extracts of safflower (*Carthamus tinctorius* L.) petals. *In: 4th International Scientific Conference Agrobiodiversity Nutrition, Health and Quality of Human and Bees Life*, 2019, Nitra, Slovacia, pp. 186. ISBN 978-80-552-2037-6. Disponibil: http://www.slpk.sk/eldo/2019/dl/9788055220703/9788055220703.pdf

5.1.3. **SAVCENCO, A.**, BAERLE, A., TATAROV, P., MACARI, A., RUGINA, D., RANGA, F. Chalconic profile of yellow food powder-form pigment, obtained from safflower petals. In: *Conference Euro-Aliment, the 10th International Symposium*, 2021, Galați, România, pp. 54. ISBN 1843-5114. Disponibil: https://ibn.idsi.md/ro/author_articles/64513?coll_str=alt

5.1.4. BAERLE, A., **SAVCENCO, A.,** POPESCU, L., TATAROV, P. Smart technology for obtaining of natural yellow food colorants from safflower. In: *The 16th International Conference of Constructive Design and Technological Optimization in Machine Building Field,* 2021, Bacău, România, pp. 73. ISSN 2457 – 3388. Disponibil: https://oproteh.ub.ro/assets/abstracts.pdf

5.2. theses in the works of international scientific conferences (Republic of Moldova) - 3

5.2.1. **SAVCENCO, A.,** BANTEA-ZAGAREANU, V., BAERLE, A., TATAROV, P. Use of yellow food dye from safflower petales in producing of caramel. In: *International Conference MODERN TECHNOLOGIES IN THE FOOD INDUSTRY,* 2022, Chișinău, Republica Moldova, pp. 105. ISBN 978-9975-45-851-1. Disponibil: https://mtfi.utm.md/files/Materialele_Conferintei_MTFI-2022.pdf

5.2.2. **SAVCENCO, A.,** BAERLE, A., IVANOVA, R., TATAROV, P. Regression analysis of carthamin extraction from safflower (*Carthamus tinctorius* L.). In: *International Conference MODERN TECHNOLOGIES IN THE FOOD INDUSTRY,* 2018, Chișinău, Republica Moldova, pp. 243-244. ISBN 978-9975-87-428-1. Disponibil: http://repository.utm.md/bitstream/handle/5014/3727/Conf_Tehnol_2018_pg243_244.pdf?sequence=1&isAllowed=y

5.2.3. POPESCU, L., GHENDOV-MOȘANU, A., **SAVCENCO, A.,** BAERLE, A., TATAROV, P. Color stability of yogurt with natural dye obtained from safflower (*Carthamus tinctorius* L.). In: *Conferința Intelligent Valorisation of Agro-Food Industrial Wastes*, 2021, Chișinău, Republica Moldova, pp. 49. ISBN 978-9975-3464-2-9. Disponibil: https://ibn.idsi.md/ro/vizualizare_articol/140465

5.3. theses in the works of national scientific conferences -3

5.3.1. **SAVCENCO, A.,** LAICO, V. Coloranții chalconici din șofrănel pentru corecția culorii produselor alimentare. In: *Conferința tehnico – științifică a studenților, masteranzilor și doctoranzilor*, 2021, Chișinău, Republica Moldova, pp. 444-445. Disponibil: https://ibn.idsi.md/ro/vizualizare_articol/134045 **(Diploma de gradul III)**

5.3.2. **SAVCENCO, A.,** BAERLE, N., SANDU, I., BAERLE, V. Unusual pH – behavior of safflower extract. In: *Conferința tehnico – științifică a studenților, masteranzilor și doctoranzilor*, 2019, Chișinău, Republica Moldova, pp. 514. ISBN 978-9975-45-588-6. Disponibil: https://ibn.idsi.md/ro/vizualizare_articol/98847 **(Diploma de gradul II)**

5.3.3. **САВЧЕНКО,** А., БАЕРЛЕ, А., ТАТАРОВ, П., ИВАНОВА, Р. Экстракция биологически активных веществ из лепестков сафлора красильного In: *Conferința tehnico – științifică a studenților, masteranzilor și doctoranzilor,* Ed. Volumul II, 2017, Chișinău: Universitatea Tehnică a Moldovei, 2017, pp. 40-41. ISBN 978-9975-45-545-9 Disponibil:https://ibn.idsi.md/ro/vizualizare_articol/169276

- POPESCU, L., **SAVCENCO, A.,** BAERLE, A., TATAROV, P., GHENDOV-MOȘANU, A., STURZA, R., PATRAȘ, A. Procedeu de fabricare a iaurtului. The 7th Innovation and Creative Education Fair for Youth ICE-USV, 2023. **Medalia de Bronz**.
- **SAVCENCO, A.,** BAERLE, A., TATAROV, P., IVANOVA, R. Process for obtaining red dye carthamin in powder form. The 25th International exhibition of inventions, INVENTICA, Iasi, Romania, 2021, pp. 249. ISSN 1844-7880. **Medalia de Argint.** Disponibil: https://ini.tuiasi.ro/exhibition/wpcontent/uploads/sites/5/2021/06/Volum%20INVENTICA%202021.pdf
- **SAVCENCO, A.,** BAERLE, A., TATAROV, P., IVANOVA R. Process for producing dyes from Safflower petals (*Carthamus tinctorius* L.). European Exhibition of Creativity and Innovation: proceed. of the 13th edition EUROINVENT, Iasi, Romania, 2021, pp. 170. ISSN 2601-4564. **Medalia de Aur.** Disponibil: http://repository.utm.md/bitstream/handle/5014/20356/Exb_EUROINVENT_2021_p170_Md18.pdf?sequence=1&isAllowed=y
- **SAVCENCO, A.,** BAERLE, A., TATAROV, P., IVANOVA, R. Process for producing dyes from Safflower petals (*Carthamus tinctorius* L.). Expoziţia Internaţională Specializată INFOINVENT, Chișinău, Republica Moldova, 2021, pp. 63. **Medalia de bronz.** Disponibil: https://infoinvent.md/assets/files/catalog/catalog-2021.pdf
- **SAVCENCO, A.** Elaborarea tehnologiei de obținere a colorantului galben alimentae din petale de șofrănel. International fair of innovation and creative education for youth, ICE - USV, 5th Edition, Suceava, România, 2021, pp. 14-15. ISBN 978-606-8992-18-1. **Medalia de Bronz.** Disponibil: https://utm.md/wp-content/uploads/2021/05/ICE-USV2021-volum.pdf
- **SAVCENCO A.,** BAERLE, A., TATAROV, P., IVANOVA, R. Method for isolating of red and yellow dyes from safflower. Salonul Internațional al Cercetării Științifice, Inovării și Inventicii PRO INVENT, ediția a XVIII-a, Cluj-Napoca, România, 2020. ISBN 978-606-737-480-3. **Diploma de Excelență, Medalia de Aur și premiul ”Augustin Maior„**. Disponibil: https://proinvent.utcluj.ro/img/catalogs/2020.pdf
- POPESCU, L., **SAVCENCO, A.,** BAERLE, A., TATAROV, P., GHENDOV-MOȘANU, A., STURZA, R., PATRAȘ, A. Process for produsing yogurt. Salonul Internațional al Cercetării Științifice, Inovării și Inventicii PRO INVENT, ediția a XX-a, Cluj-Napoca, România, 2022. ISSN 2810 – 2789. **Medalia de Aur și Diploma de Apreciere de la Ministerul Sănătății al Republicii Moldova, Agenția Națională pentru Sănătate Publică.** Disponibil: https://proinvent.utcluj.ro/img/catalogs/2022.pdf

ADNOTARE

Savcenco Alexandra: "Obținerea și utilizarea coloranților naturali din petale de șofrănel (*Carthamus tinctorius* L.)" în tehnologia produselor alimentare, teza de doctor în științe inginerești, Chișinău, 2024.

Structura tezei: teza constă din introducere, cinci capitole, concluzii generale și recomandări, bibliografie (157 titluri), 8 anexe. Textul de bază a tezei conține 134 pagini, inclusiv 64 figuri, 30 tabele. Rezultatele obținute au fost publicate în două capitole în monografie colectivă, 10 lucrări științifice, dintre care un articol în revista cu IF 6, 317, trei în revista B^+ și un articol în culegeri științifice a conferinței internaționale. Au fost publicate 3 brevete de invenție.

Cuvinte cheie: petale de șofrănel, extracția, sistem tehnologic de producție a coloranților, colorant galben (YFDS), colorant roșu (cartamina), complex cartamina-celuloză (CCC).

Scopul lucrării constă în conceptualizarea și fundamentarea științifică a tehnologiei de extracție și stabilizare a coloranților alimentari calconece de culoare galbenă și colorantului nou de culoare roșie, obținute din petale de șofrănel și aplicarea lor în fabricarea produselor alimentare.

Obiectivele cercetării: analiza structurii și compoziției chimice ale petalelor de șofrănel, realizarea cercetărilor teoretice și experimentale a mecanismului procesului de extracție și stabilizare a pigmenților; elaborarea sistemului tehnologic de producție a coloranților galben și roșu; determinarea proprietăților funcționale și tehnologice ale coloranților galben și roșu; aplicarea coloranților alimentari obținuți în fabricarea produselor alimentare.

Noutatea și originalitatea științifică: pentru prima dată a fost elaborat și realizat procedeul de obținere a coloranților naturali calconece din petale de șofrănel, rentabil din punct de vedere economic. În premieră a fost elaborată tehnologia de fabricație a coloranților de culoare galbenă și roșie din același lot de petale. Au fost determinate metodele de stabilizare și proprietățile tehnologice ale coloranților, care permit încorporarea lor în diferite compoziții alimentare.

Rezultatele obținute în soluționarea problemei științifice: modelele matematice elaborate ale modificării concentrației pigmenților în procesul de extracție și a determinării duratei optime de extracție rezolvă problema obținerii coloranților calconece pe scară industrială. Stabilirea mecanismului de formare a complexului CCC contribuie la determinarea condițiilor de utilizare a colorantului roșu natural în industria alimentară.

Semnificația teoretică: constă în identificarea pigmenților calconece din petale de șofrănel, determinarea structurii chimice și a proprietăților fizico-chimice ale moleculelor pigmenților de culoare galbenă și roșie, elucidarea mecanismului de stabilizare a cartaminei prin formarea complexului cartamina-celuloză.

Valoarea aplicativă: s-a elaborat sistemul tehnologic de producție a coloranților calconece galben și roșu din petale de șofrănel. Randamentul liniei tehnologice de fabricație a coloranților constituie peste 80%. Valoarea aplicativă a fost confirmată prin obținerea a 3 brevete de invenție a Republicii Moldova.

Implementarea rezultatelor științifice: s-a demonstrat eficacitatea aplicării coloranților calconece alimentari galben (YFDS) și roșu (CCC) în obținerea produselor zaharoase și iaurtului cu stabilitate înaltă a culorii pe durata depozitării.

АННОТАЦИЯ

Савченко Александра: "Получение и использование натуральных красителей из лепестков сафлора красильного (*Carthamus tinctorius* L.) в технологии пищевых продуктов", диссертация на соискание ученой степени доктора технических наук, Кишинэу, 2024.

Структура диссертации: диссертация состоит из введения, пяти глав, общих выводов и рекомендаций, библиографии (из 157 наименований), 8 приложений. Основной текст диссертации содержит 134 страницы, в том числе 64 рисунка, 30 таблиц. Полученные результаты опубликованы в двух главах коллективной монографии, 10 научных статьях, из них одна статья в журнале „Food and Functions" с ISI IF 6,317, три в журнале B+ и одна статья в научном сборнике международной конференции. Опубликовано 3 патента на изобретения.

Ключевые слова: лепестки сафлора красильного, экстракция, технологическая система производства красителей, желтый краситель (YFDS), красный краситель (картамин), картамино-целлюлозный комплекс (CCC).

Цель работы: разработка научной концепции и детальное обоснование этапов технологии экстракции и стабилизации пищевых халконовых красителей желтого и красного цвета из лепестков сафлора красильного (*Carthamus tinctorius* L.) и их применения при производстве пищевых продуктов.

Задачи исследования: анализ структуры и химического состава лепестков сафлора, проведение теоретических и экспериментальных исследований механизма экстракции и стабилизации полученных пигментов; разработка технологической системы производства желтого и красного красителей; определение функционально-технологических свойств желтых и красных красителей; использование полученных красителей при производстве пищевых продуктов.

Научная новизна и оригинальность: впервые разработана технология получения желтого и красного красителей из одной партии лепестков. Установлены способы стабилизации и технологические свойства красителей, позволяющие использовать их формы и оптимальные концентрации в различных пищевых композициях.

Результаты, полученные при решении научной задачи: разработанные математические уравнения изменения концентрации пигментов в процессе экстракции и определения оптимального времени проведения процесса экстракции, решают задачу энергоэффективного получения халконовых красителей в промышленных масштабах.

Теоретическая значимость: заключается в идентификации халконовых пигментов из лепестков сафлора красильного, определении химического строения и физико – химических свойств молекул желтых и красных пигментов, выяснении механизма стабилизации картамина в фазе биополимера.

Практическая значимость работы: разработана технологическая система производства халконовых красителей желтого и красного цвета из лепестков сафлора красильного. Выход технологической линии по производству красителей составляет более 80%. Прикладная ценность подтверждена выдачей 3-х патентов на изобретения Республики Молдова.

Внедрение научных результатов: показана эффективность применения халконовых пищевых красителей желтого (YFDS) и красного (CCC) при получении карамели и йогурта с высокой стабильностью цвета при хранении.

ABSTRACT

Savcenco Alexandra: "Obtaining and using natural dyes from safflower petals (*Carthamus tinctorius* L.) in food technology", PhD Thesis in engineering sciences, Chișinău, 2024.

Structure of the thesis: introduction, five chapters, general conclusions and recommendations, bibliography (157 titles), 8 appendices. The main text of the dissertation contains 134 pages, including 64 figures, 30 tables. The results obtained were published in two chapters of a collective monograph, 10 scientific articles, one article was published in a journal „Food and Functions" with an ISI IF of 6,317, three articles were published in the B+ journal and one article in a scientific collection of republican conferences. Three patents for inventions have been published.

Keywords: safflower petals, extraction, technological dye production system, yellow food dye (YFDS), red dye (carthamine), carthamine-cellulose complex (CCC).

Purpose: consist in to develop the concept and scientific substantiation of the technology for extraction and stabilization of yellow and red food chalcone dyes from safflower petals (*Carthamus tinctorius* L.) and their use in food production.

Objectives: structure and chemical composition analysis of the safflower petals, theoretical and experimental studies of the extraction and stabilization mechanism for the resulting pigments; development of a technological system for the production of yellow and red dyes; determination of functional and technological properties of yellow and red dyes; use of the obtained dyes in food production.

Scientific novelty and originality: for the first time, a technology for producing yellow and red dyes from one batch of petals has been developed. Methods of stabilization and technological properties of dyes have been established, allowing their use in various food compositions.

The results obtained when solving a scientific problem: the developed mathematical equations for changing the concentration of pigments during the extraction process and determining the optimal time for the extraction process contribute to solve the problem of producing chalcone dyes on an industrial scale.

Theoretical significance: consist in the identification of chalcone pigments from safflower petals, determination of the chemical structure and physicochemical properties of the molecules of yellow and red pigments, elucidation of the mechanism of carthamine stabilization due to the formation of a carthamine-cellulose complex.

Applicative value: a technological system has been developed for the production of yellow and red chalcone dyes from safflower petals. The yield of the dye production line is more than 80%. The applied value is confirmed by the receipt of 3 patents for inventions of the Republic of Moldova.

Implementation of scientific results: demonstrated the effectiveness of using chalcone food dyes: yellow (YFDS) and new red (CCC) in producing of caramel and yogurt, both with high color stability during storage, was demonstrated.

SAVCENCO ALEXANDRA

OBTAINING AND USING NATURAL DYES FROM SAFFLOWER PETALS (*Carthamus tinctorius* L.) IN FOOD TECHNOLOGY

253. 06. Biological and chemical technologies in the food industry

Abstract of the PhD in engineering sciences dissertation

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