Journal of Engineering Science *Vol. XXIX, no. 1 (2022), pp. 142 - 150*
Fascicle *Food Engineering* *ISSN 2587-3474*
Topic *Food Technologies and Food Processes* *eISSN 2587-3482*

https://doi.org/10.52326/jes.utm.2022.29(1).13
CZU 637.146.34:667.275.5

COLOR STABILITY OF YOGURT WITH NATURAL YELLOW FOOD DYE FROM SAFFLOWER (*CARTHAMUS TINCTORIUS L*)

Liliana Popescu, ORCID ID: 0000-0003-3381-7511,
Aliona Ghendov-Moșanu, ORCID ID: 0000-0001-5214-3562,
Alexei Baerle, ORCID ID: 0000-0001-6392-9579,
Alexandra Savcenco*, ORCID ID: 0000-0002-1962-3959,
Pavel Tatarov, ORCID ID: 0000-0001-9923-8200

Technical University of Moldova, 168 Stefan cel Mare Blvd., Chisinau, Republic of Moldova
*Corresponding author: Alexandra Savcenco, *alexandra.savcenco@tpa.utm.md*

Received: 11. 28. 2021
Accepted: 01. 18. 2022

Abstract. The article elucidates the possibility of using the yellow dye, obtained from saffron petals (*Carthamus Tinctorius L.)* in the food industry. This powdered dye, added to yoghurt in concentrations of 0.3 - 0.4%, in addition to its yellow color, gives the yoghurt samples a light aroma of saffron flowers and demonstrates high stability during storage at 4° C. for 28 days. The values of the chromatic coordinates, L *, a *, b *, of the yogurt samples did not undergo considerable changes during the storage stage, the color difference ΔE being ⩽ 0.79, which indicates a high stability of the dye in yogurt. samples. The chromatic analysis data correlate with the results obtained by the reverse phase HPLC method, which identified the presence of four intact yellow chalcones in the yoghurt. The results of this study will encourage the cultivation of saffron, the extraction of natural dyes and their use in the food industry.

Keywords: *chromaticity coordinates, color analysis, food additive, natural food.*

Rezumat. Articolul elucidează posibilitatea de utilizare a colorantul galben, obținut din petale de șofrănel (*Carthamus Tinctorius* L.) in industria alimentara. Acest colorant, adăugat în formă de pudră la iaurt în concentrații de 0,3 - 0,4%, pe lângă culoarea galbenă, conferă probelor de iaurt o aromă ușoară de flori de șofrănel și demonstrează stabilitate ridicată în timpul păstrării la 4°C timp de 28 de zile. Valorile coordonatelor cromatice, L*, a*, b*, ale probelor de iaurt nu au suferit modificări considerabile în timpul etapei de păstrare, diferența de culoare ΔE fiind ⩽ 0,79, ceea ce indică o stabilitate ridicată a colorantului în iaurt. mostre. Datele analizei cromatice sunt corelate cu rezultatele obținute prin metoda HPLC în fază inversă, care a identificat prezența a patru calcone galbene intacte în iaurt. Rezultatele acestui studiu vor încuraja cultivarea șofrănelului, extragerea coloranților naturali și utilizarea lor în industria alimentară.

Cuvinte cheie: *coordonate cromatice, analiza culorii, aditiv alimentar, alimente naturale.*

Introduction

We are currently witnessing an increase in consumer demands for natural foods, as people aspire to a healthier lifestyle. Natural food colorings play a special role in this trend.

Quantum transitions (absorption) corresponding to the wavelengths of visible light are due to the presence of a system of conjugated double bonds (CDB) in molecules. However, the cause of dyes instability is that CDB systems are unstable to the action of active oxidizing agents and free-radicals. That is why, natural dyes have biological activity (and chemical instability): the ability to neutralize free radicals which causes oxidative stress and even mutations.

Modern science finds thousands of natural dyes with potential biological activity in flowers and fruits of higher plants. Mostly red dyes, anthocyanins, are very well known to the consumer, which cannot be said about chalcones – majorly yellow and less often – red compounds [1]. Chalcones are phenolic dyes with an unclosed benzopyran skeleton. These compounds are characteristic for the *Asteraceae* family. Safflower (*Carthamus Tinctorius, Asteraceae*) is often confused even in scientific literature with Saffron (*Crocus Sativus, Iridaceae*), because partial similarly names and visual aspect of dye-containing raw material. Safflower petals contain, according to various sources, 5 - 11 different yellow chalcones, the most famous of which are: Hydroxisafflower Yellow A (HSYA), Anhidrosafflower Yellow B (AHSYB) and Precarthamin [2]. Being the basic phenols of the Safflower petals, these compounds, in addition to its coloring properties, exhibits a wide range of biological functions, including anticoagulant, vasodilation and antioxidant activities [3].

Color is an important factor in the quality of fruit yogurt, influencing the acceptability of the product to the consumer [4]. It is one of the first characteristics perceived and is used by consumers to appreciate the quality of food [5, 6]. Unfortunately, the attractive color of fruit yogurt is not preserved during storage [6]. The food industry uses food coloring to improve the color and acceptance of fruit yogurts [7].

The goal of the study was to evaluate color stability of yogurt during storage by determining the chromaticity coordinates, measured using the CIE color space L* a* b*. Our results could be useful for the development of an effective strategy for the manufacturing of dairy products with high color stability.

Materials and methods

Plant material. Yellow florets of Safflower, grown in Republic of Moldova, were used. Petals were dried in the dark, at max 40°C in the dark to relative humidity less than 2%.

Yellow Food Dye from Safflower. YFDS was prepared by modifying of previously patented method [8]. Dried Safflower petals (40g) were extracted with 400g of a Na_2CO_3 2% (w) cold solution. The resulting dark-brown opaque extract was treated with 1g microcrystalline cellulose Flocel-102 (India) and 2g of activated carbon („Balkan Pharmaceuticals", Moldova) and centrifuged for 10 minutes at 6000 rpm, resulting brown (very concentrated yellow) solution. Citric acid crystals were added to this solution until pH=4.5. Resulted dark-brown solution was concentrated in a rotary evaporator. Then, three volumes of food grade Ethyl Alcohol, 96% (v), were added and mixed intensively, resulting formation of the brown viscous phase. This phase was dried at 70-75°C under vacuum of 80-100mbar and mechanically powdered. The yield of dry YFDS powder was of 13g.

Yogurt preparation. Yogurt was obtained using milk with a fat content of 2.6%, skimmed milk powder and natural yellow coloring from Safflower petals (0.1; 0.2; 0.3 and

0.4% (w/w)). The standardized milk was filtered, pasteurized at a temperature of 90-94°C for 2 - 8 min, cooled to a temperature of 40 - 42°C, seeded with yogurt starter cultures and packed in glass jars. The packaged yogurt samples were kept at a temperature of 39 -42°C until the formation of the curd with a pH of 4.50-4.55, then were cooled in the refrigerator to a temperature of 2-6°C and stored there for 28 days.

Sensory analysis of yogurt was done by scoring in accordance with SM ISO 22935-3:2015 [9].

Syneresis Index. The syneresis rates of the yoghurts were determined by a centrifugal acceleration test. 10 grams of yogurt sample were placed in a test tube and centrifuged at 1200×g for 10 minutes at room temperature. The amount of serum separated from the samples was measured to estimate the syneresis index, which was expressed in % (w/w) [10].

Viscosity was determined with Brookfield DV-III Ultra rotational viscometer at the storage temperature of yogurt samples according to the method [10] with some modifications. The yogurt samples were lightly mixed 30 times for homogenization. Viscosity was determined at the shear rate (75 min^{-1}).

The pH was measured with a digital pH-meter (Mettler Toledo, SUA) at 20°C.

Fat content was measured by the Gerber methods [11].

Dry matter content was measured by the SM ISO 6731:2014 [12].

HPLC analysis. Photodiode Array (PDA) integrated HPLC instrument "Provenience-i LC-2030C 3D-Plus (by "Shimadzu"), equipped with reversed-phase C_{18}-column "Phenomenex" (4.6×150mm×4μm) was used. A gradient elution technique was realized with two mobile phases: Water, containing Acetic Acid 1.0% (v) (Phase A) and Acetonitrile, containing Acetic Acid 1.0% (v) (Phase B). Default flow: Phase B 5% at the constant rate of 0.5 mL/min. Constant oven and detection cell temperatures were of 25°C. Elution gradient program: 0-2 min – default flow; 2-18 min – Phase B 5%-40%; 18-20 min – Phase B 40%-90%; 20-24 min – Phase B 90%; 24-25 min - Phase B 90%-5%; 25-40 min – default flow.

Color analysis. The CIE-Lab parameters, L* (brightness), a* (green versus red coordinate) and b* (blue versus yellow coordinate), were measured on a white calibration block using a Chroma Meter CR-400/410 colorimeter (Konica Minolta, Tokyo, Japan) according to the method of Loypimai P. et al. [13]. The instrument was calibrated with a standard white and black reference tiles. A sample of yogurt was homogenized and placed in a 2 cm thick glass cuvette. Each sample was analyzed at five distinct points.

Color analysis of the two types of yogurt were conducted based on the variations of L*, a*, b*, and the color differences (ΔE) were determined. The determination of ΔE was performed using the following Equation (1):

$$\Delta E = \sqrt{(L_i^* - L_o^*)^2 + (a_i^* - a_o^*)^2 + (b_i^* - b_o^*)^2}, \quad (1)$$

where: L_o^*, a_o^* and b_o^* are the values of the samples at zero time and : L_i^*, a_i^* and b_i^* are the values of each sample with time. ΔE comparisons were made based on two different factors: in relation to the color obtained at t = 0 days with respect to the control sample; and based on its own color at t = 0 days [14].

Statistical Analysis. Confidence interval was appreciated for 95% confidence level.

Results and Discussion

Sensory and physico-chemical property of yogurt

The YFDS was added to the yogurts during the production to form the concentrations of 0.1; 0.2; 0.3 and 0.4% (w/w). The sensory analysis of the yogurt was made on the basis of the 5-point scale by the commission of 10 trained evaluators.

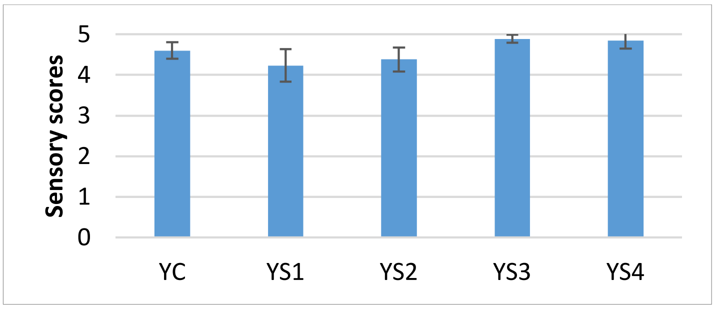

Figure 1. Sensory evaluation of yogurt with YFDS.
YC: classic yogurt (control sample); YS1-YS4: yogurts with YFDS, 0.1%-0.4% (w/w), respectively.

Following the sensory analysis, it was found that all yogurt samples had a firm curd, without removal of whey. As the amount of Safflower dye increases, so does the intensity of the yellow color of the yogurt samples. The introduction of Safflower dye in proportions of 0.3 and 0.4% in addition to the yellow color gives the samples of yogurt and a light aroma of Safflower flowers. Finally, it can be concluded that the addition of Safflower dye has an attractive yellow color and can simulate the color of apricots and other fruits in yogurt.

Similar results were obtained when using safflower petal extract in ice cream samples [15].

The physicochemical composition of the yogurts is shown in Table 1.

Table 1

Physico-chemical properties of yogurt samples

Yogurt Code*	YC	YS1	YS2	YS3	YS4
YFDS	**none**	**0.1%**	**0.2%**	**0.3%**	**0.4%**
Dry substance, %	11.7 ± 0.1	11.8 ± 0.1	11.9 ± 0.2	12.0 ± 0.2	12.1 ± 0.2
Fats, %	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

*YC: classic yogurt (control sample); YS1 – YS4: yogurts with YFDS, 0.1-0.4% (w/w), respectively.

The dry matter content was found to be slightly higher in the samples of Safflower dye yogurt than in the control. A similar trend was observed for the viscosity of yogurt. It was found that the fat content decreases with increasing dye concentration. Yogurt samples with the addition of Safflower dye have a pH value similar to the dye-free yogurt sample. Therefore, the addition of dye obtained from Safflower petals induces the yellow color of yogurt without influencing its physico-chemical properties.

The evolution of the yogurt color depending on the amount of dye

The color of fruit yogurt has a remarkable influence on consumer acceptance and is also an indicator of changes in the concentration of pigments that occur during storage [6].

The values of the chromaticity coordinates, L *, a *, b *, of the yoghurts with the addition of natural dye from Safflower petals are presented in Table 2. As a control sample, the classic yogurt without additives was used.

Table 2

CIE-Lab parameters of classic yogurt (control sample) and yogurts with YFDS

Chromaticity coordinates	Yogurt*				
	YC	YS1	YS2	YS3	YS4
L*	75.08	75.31	74.79	74.40	74.62
a*	-2.90	-4.18	-4.71	-5.20	-5.21
b*	9.06	13.29	15.53	17.96	18.41
ΔE (YS (dyed) vs YC (control)	-	4.43	6.72	9.22	9.64

*YC: classic yogurt (control sample); YS1 – YS4: yogurts with YFDS, 0.1-0.4% (w/w), respectively.

The color differences between classic yogurt and YFDS-added yogurt were determined by ΔE. When 0<ΔE<1, the assessors does not perceive the color difference, 1<ΔE<2 only the experienced assessors can observe the color difference, finally, if 2<ΔE<3.5, the color differences are observed even the inexperienced assessors [16].

According to the data presented in Table 2, ΔE increases in proportion to the increase in the concentration of Safflower dye in yogurt, from 4.43 to 9.64. ΔE correlates with the results of sensory analysis, through which with the naked eye a slight increase in the intensity of the shade of yellow is detected along with the increase in the concentration of safflower dye.

Color stability during storage of yogurt

Stability of yogurt color with the addition of natural dye Safflower YS3 and YS4 was determined during 28 days of storage (Table 3). As a control sample, the yogurt with the addition of synthetic dye E 102 of yellow color similar to that of Safflower dye was used, so that the color of the yogurt with E 102 is very close to that of the yogurt YS4, ΔE being 0.86. Dye E 102 is used in the manufacture of dairy products at the industrial level, demonstrating a high stability during storage of finished products.

Table 3

Behavior of chromaticity coordinates of the yogurts with the addition of natural dye Safflower as a function of storage time

Parameters	Storage time, days	Yogurt*		
		YS3	YS4	YT
L*	0	74.40	74.62	75.18
	3	74.42	74.51	75.17
	7	74.35	74.46	75.12
	14	74.54	74.29	75.23
	21	74.58	74.03	75.29
	28	74.62	73.97	75.48
a*	0	-5.20	-5.21	-5.85
	3	-5.15	-5.30	-5.84
	7	-5.00	-5.35	-5.84
	14	-5.08	-5.34	-5.85
	21	-5.10	-5.32	-5.89
	28	-5.12	-5.32	-5.90

Continuation Table 3

b*	0	17.96	18.41	18.29
	3	18.34	18.62	18.21
	7	18.70	18.91	18.11
	14	18.32	18.90	18.30
	21	18.25	18.89	18.46
	28	18.05	18.86	18.65
h°	0	106.15	105.80	107.74
	3	105.69	105.89	107.78
	7	104.97	105.80	107.87
	14	105.50	105.78	107.73
	21	105.61	105.73	107.70
	28	105.84	105.75	107.55
C*	0	18.70	19.13	19.20
	3	19.05	19.36	19.12
	7	19.36	19.65	19.03
	14	19.01	19.64	19.21
	21	18.95	19.62	19.38
	28	18.76	19.60	19.56

*YT: yogurt with synthetic dye E 102; YS3, YS4: yogurts with YFDS, 0.3 and 0.4% (w/w), respectively.

As can be seen in Table 3, the L* parameter showed 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 value t=0 days. Regarding the coordinate a*, the average values were between -5.90 and -5.00 units. The values of the chromatic coordinates a* indicate a negative value that represents the green area. In the case of the b* chromaticity coordinate, the average values were between 17.96 and 18.91 and all are located on the positive side of the axis, which represents the yellow area. The samples showed uniformity in the three chromatic coordinates L*, a*, b* throughout the storage period for both the control yogurt and the yogurt with natural coloring from Safflower petals.

The color shade (h°) remained almost constant during the storage of yogurts with coloring from Safflower petals, which indicates that the color of the yogurt does not change during storage. Most likely there is no degradation of chalconic dyes in the composition of YFDS and in the lipid-protein matrix of the yogurt.

During storage, neither significant differences in chromium (C*) were observed for all yogurt samples, indicating that the color of the yogurt remains as intense over time.

In general, the chromatic coordinates of the samples of yoghurt with coloring of Safflower petals did not undergo considerable changes during the storage stage.

Photodiode Array detection demonstrate presence of four yellow chalcones in Yogurt: Hydroxysafflower Yellow A, Precarthamin, Unidentified (but with UV-Vis pattern, characteristic for yellow chalcones of Safflower), and Anhydrosafflower Yellow B (Figure 2).

Chromatographical data, obtained by calculation of relative peak areas of chalcones in the samples of YFDS and YFDS-colored Yogurts, demonstrate no significant changes in the chalcones ratio during storage period (Table 4).

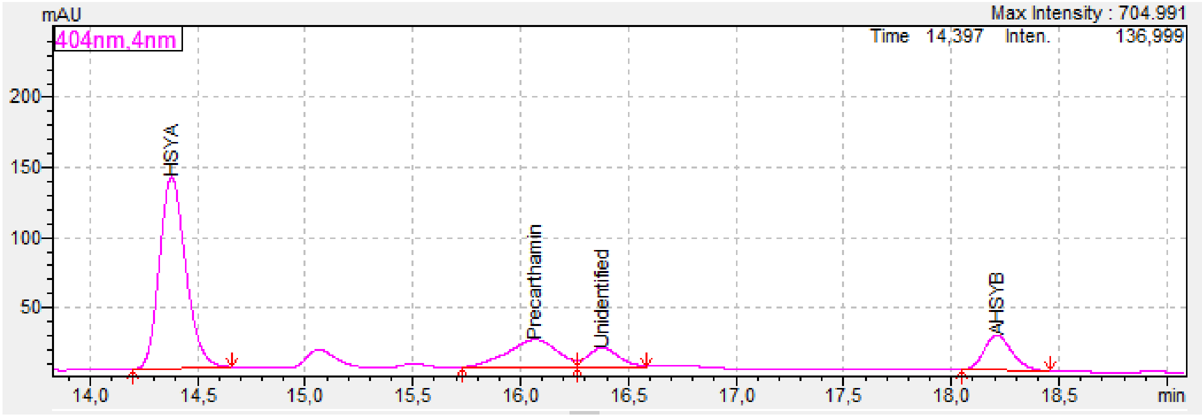

Figure 2. HPLC profile of chalcones in YFDS-colored yoghurts after 14 days of storage.

Table 4

Relative content of yellow chalcones in Yogurt after 14 days of storage

Yellow chalcones in Yogurt	R_T, min	λ_{max}, nm	Relative peak Area, %
Hydroxysafflower Yellow A	14.4	339, 402	62
Precarthamin	16.1	327 sh, 407	18
Unidentified	16.4	406	8
Anhydrosafflower Yellow B	18.2	335 sh, 409	12

Chemical degradation of chalconic components in YFDS could result in significant changes in brightness during yogurt storage, but HPLC data confirm that yellow substances remain chemically intact during yogurt storage.

Color differences during the storage period were determined by ΔE. Figure 3 shows the color differences (ΔE) obtained during the 28 day storage for synthetic dye and Safflower dye yogurts.

The color difference ΔE for samples with natural dye from Safflower petals throughout the storage period was ⩽ 0.79, which is an extremely satisfactory result, as ΔE ⩽ 3.0 cannot be detected with the naked eye [17].

The above facts indicate that the natural colorant of Safflower petals was stable throughout the storage period at refrigeration temperatures, with no significant changes in any of the three color coordinates.

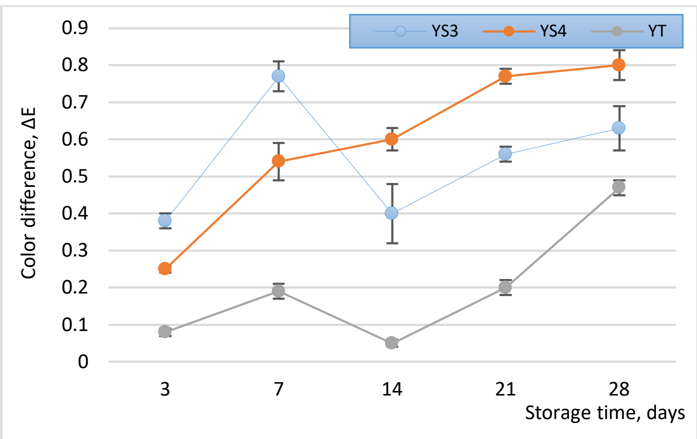

Figure 3. Color difference (ΔE) of the yogurt with YFDS of storage time. ΔE comparisons were made based on its own color at t=0 days.

YT: yogurt with synthetic dye Tartrazine (E 102); YS3, YS4: yogurts with YFDS, 0.3 and 0.4% (w/w), respectively.

The high stability of the natural coloring of Safflower petals in the protein-lipid matrix of yogurts taken in this study contrasts with [18], in which were pointed out that colors from natural sources tend to lose their coloring power or disappear over time in storage studies.

Conclusions

The possibility of using Yellow Food Dye from Safflower to form the natural yellow color in yogurts has been demonstrated. Principal yellow chalcones of Safflower (HSYA, Precarthamin and AHSYB) ratio, does not suffering significant changes in the composition of Yogurt during storage period. Yogurt production allows YFDS to be packaged in the lipid-protein matrix of the dairy product. The color parameters remain stable during the 28 days of storage. The results of this study contribute to encouraging the use of Safflower dyes in natural foods. This study confirms, that Safflower petals (usually wastes, resulting of the Safflower growing) are valuable source for the production of harmless yellow food dyes for the dairy industry of Moldova and Romania.

Acknowledgments. The research was funded by State Project no. 20.80009.5107.09 ”*Improving of food quality and safety through biotechnology and food engineering”*, running at Technical University of Moldova.

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