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EVALUATION OF THE COLORIMETRIC STABILITY OF PIGMENTS FROM *Sphagneticola trilobata* EXTRACTED WITH OIL FROM *Glycine max* (SOJA)

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Abstract: Soybean oil is the most consumed vegetable oil in Brazil. As it is an oil extracted with a fossil solvent, a refining step is necessary for food safety reasons. However, in refining most of the bioactive compounds in the oil are removed. *Sphagneticola trilobata* (L) Pruski, known as Wedélia, is an herb native to Brazil, whose compounds have already been linked to herbal treatments. Luteolin isolated from its flowers is a flavonoid that, in addition to having biological activity, is a natural dye responsible for giving the flower its yellow color. Currently, the use of natural dyes has assumed a relevant position in food processing, due to the benefits associated with a more natural diet and also the correlation of some synthetic dyes with adverse effects on human health. Thus, in this work, the effect of the Drying at method of Wedelia flowers on the colorimetric stability of commercial soybean oil containing fat-soluble compounds from the flower was evaluated, in the CIE L*a*b* system. The process was carried out by immersion, for 60 days, of the pinnules in soybean oil in the proportion 1:9 (m/m), respectively. Flowers dehydrated by four Drying at techniques were used. For comparative purposes, a sample containing pure soybean oil was used as a standard. Analysis by repeated measures ANOVA and Tuckey test indicated a significant effect of Drying at flower methods at all points in the color spectrum of the samples. However, cold dehydration was the only one that showed a significant difference between treatments in terms of distance from the standard. This result indicated the potential of using the Wedelia pigment as a natural food coloring and as a source of bioactive compounds from sustainable processing, expanding the use of soybean oil as a renewable solvent in the formulation of new products.

Keywords: *Asteraceae*, Colorimetry, Bioactive compounds, Lyophilization, microwave.

INTRODUCTION

Brazil is the largest producer and exporter of soy in the world. In 2021, according to a survey by ABIOVE, Brazilian production of the grain was around 138 million tons. In relation to the oil obtained from this legume, in that same year, production approached 9.4 million tons, 81% of which was directed to the domestic market (ABIOVE, 2021).

Because it is sold at a more competitive price, soy oil is present in the basic food basket of the majority of the Brazilian population. However, before reaching the consumer's table, the oil extracted from the bran of the grain needs to go through a refining process.

The refining of vegetable oils, in addition to reducing degradation compounds, aims to make them more pleasant, in terms of taste, odor and appearance. However, this process involves steps that end up removing compounds with biological functions from the oils, reducing their nutraceutical value (LANFER-MARQUEZ, 2003).

Since the end of the 20th century, there has been a continuous behavioral change in consumers, making them more demanding about the quality of food and its effect on human health (CRUZ et al., 2007; RIBEIRO, 2019). This new market reinforces the interest in the study of biological activities promoted by flavonoids present in plants, as well as strategies to enable their application on an industrial scale.

A *Sphagneticola trilobata* (L) Pruski is a native herb belonging to the Asteraceae family. In the scientific literature, several studies address its use for herbal purposes. In recent years, compounds from Wedelia have been linked to hypoglycemic effects; anti-inflammatory, antiseptic and antioxidant (CZEPULA, 2006). No scientific studies were found reporting side effects of its use for human consumption.

Luteolin, a flavonoid present in flower

pinnules, is one of the major secondary compounds, which, in addition to having anti-inflammatory, antioxidant and antinociceptive functions; manifests coloring property (yellow natural dye) (MANZOOR et al., 2017; VANKAR; SHUKLA, 2018). Thus, a study on the application as a dye of this plant is relevant for the food sector.

Among the most commonly used synthetic yellow dyes in the food industry is tartrazine yellow. This compound, however, represents a great risk to the environment and human health and its use is related to numerous cases of allergy, and its ban is even discussed in several countries (BRASIL, 2011; FREITAS, 2012).

Given the above, natural dyes have been gaining relevance within the food sector. Considering that the Wedelia flower has not yet been explored as a source of pigments for the sector, this work sought to evaluate the influence of Drying methods of Wedelia flowers on the colorimetric properties of commercial soybean oil, used as extracting solvent.

MATERIAL AND METHODS

The materials used in this study were Wedelia flowers, collected from the flower beds on the UFRJ-Fundão campus, and organic soybean oil (Korin Agropecuária Ltda., Rio de Janeiro, RJ., Brazil) (Figure 1).

DRYING OF THE PINNULES

Wedelia flowers were harvested, in the flowering team, and then used to study the influence of Drying at methods of the flower on its coloring properties when incubated by immersion in soybean oil. For this purpose, the following Drying at techniques were selected: Lyophilization; microwave; Drying in an incubator chamber (10 °C) and by natural convection (25 °C). The parameters of each process are described in Table 1.

INCUBATION IN SOYBEAN OIL

After Drying at, the flowers were incubated at a rate of 10% (m/m) in soybean oil in 4 amber glasses, in order to avoid the influence of light on the color of the samples, each with about 10 g of oil. The 4 samples had their time zero (initial time) of incubation started simultaneously and the flowers were kept in contact with the oil for a period of 60 days, based on results reported by Freitas, Souza and Carvalho (2019). After this time interval, the samples were filtered in a Büchner funnel to separate the pinnules from the liquid phase. In addition to the samples containing extract of Wedelia flowers, pure organic soybean oil was also used as a control, packed in amber glass.

COLORIMETRIC ANALYSIS

For colorimetric analysis, the color space L* a* b* was used, defined by the CIE (Commission Internationale de l'Eclairage), where the variables "a" and "b" have different color meanings depending on the signal that the precedes and the combination of these variables can represent different colors. The L* factor ranges from zero to 100 and indicates the luminosity of the sample. As a function of these coordinates, the color difference between the samples and the pattern was correlated through a mathematical equation (ΔE*ab), to stipulate the distance between their points within this color space (LOPES, 2009). The calculations used to differentiate the stains of the samples from the standard are shown below.

Amostra = sample

Padrão = standard

$$\Delta L^* = L^*_{Amostra} - L^*_{Padrão}$$
$$\Delta a^* = a^*_{Amostra} - a^*_{Padrão}$$
$$\Delta b^* = b^*_{Amostra} - b^*_{Padrão}$$

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

Figure 1 - Materials used: Wedelia flower and organic soybean oil

Source: Authors, 2019.

Process	Parameters	Equipment
Lyophilization	Time of Lyophilization: 1 h	Lyophilization Machine L101 LIOTOP
Traditional Micro-waves	Potency of micro-waves: 400 W.kg^{-1} Time of Drying at: 5 minutes	Micro-waves cônsul facilite
Incubator	Temperature: 10 °C Time of Drying at: 24 h	Incubator B.O.D. NI1704
Natural convection	Temperature: 25 °C Time of Drying at: 24 h	Kraft paper bags

Table 1 - Parameters of the processes of Drying

Source: Authors, 2019.

where:

a^*_{sample} = value obtained from the analysis of the sample.

$a^*_{standard}$ = value obtained from the analysis of the standard used.

Δa^* = difference in red/green axis.

b^*_{sample} = value obtained from the analysis of the sample. b*standard = value obtained from the analysis of the standard used.

Δb^* = difference in yellow/blue axis.

L^*_{sample} = value obtained from the analysis of the sample. L*standard = value obtained from the analysis of the standard used.

ΔL^* = difference in brightness/darkness axis.

ΔE_{ab}^* = total amount of color difference.

Analyzes were performed using a 15 mL aliquot of each sample and standard. To reduce experimental error, the same optical path was used for all samples and the influence of ambient lighting on the analysis was tested. The experiment was conducted with a Chroma meter CR-400 colorimeter and the results were analyzed right after filtration (t = 0) and after one year (t = 365 days). The reading of the variables was done in quintuplicate.

STATISTICAL ANALYSIS OF DATA

The significance of Drying at techniques, storage time and interaction of these factors on oil color was evaluated by repeated measures analysis of variance (ANOVA). Subsequently, the data were analyzed by the Tuckey post hock test with 95% significance ($p < 0.05$), using the Statistica program (v.10) to evaluate the results obtained.

RESULTS AND DISCUSSION

During the incubation period of Wedelia flower in soybean oil, it was possible to visualize a clear change in the color of pure soybean oil, which changed from light yellow to orange, as illustrated in Figure 2.

The statistical analysis of the data, ANOVA of repeated measures and the Tuckey test, found that the interaction Drying method at*time of storage had no significant effect on the responses obtained for the dependent variables L* and a*. However, their separate effects showed significance in the confidence interval used. In this case, the impacts of these variables on the color of the samples were evaluated individually, as shown in Tables 2 and 3.

The samples dried at 25 °C, in microwaves and by Lyophilization, are statistically different from Pure oil, but not statistically different from each other. The sample dried at 10 °C showed lower luminosity compared to the microwave and lyophilized treatment, but it did not differ from the sample dried at 25 °C. Regarding the a* value, which represents the green-red axis, it can be seen that, regardless of the treatment, the oil acquires a more reddish color after incubation with the flowers (Table 2). This result was already expected since the pigment of interest gives an orange color.

For all samples, there was a clear difference ($p < 0.05$), for the intensity values of the a* variable, which was significantly higher (1.91 to 2.92) compared to Pure oil (-1.77). In this case, the samples showed greater intensity of red color in relation to Pure oil. It can also be observed a significant difference in the value of a* from the first to the last day of the storage team. It is also observed that after 365 days there was a small variation of intensity, of approximately 15%, of the reddish color. This decay can be explained by the degradation of the pigment throughout the study team (Table 3).

From Table 3, it can be concluded that the storage time significantly affected the luminosity of all samples, regardless of the Drying at method. In all experiments, there was an increase in opacity (reduction in the L* value) of the sample compared to Pure oil,

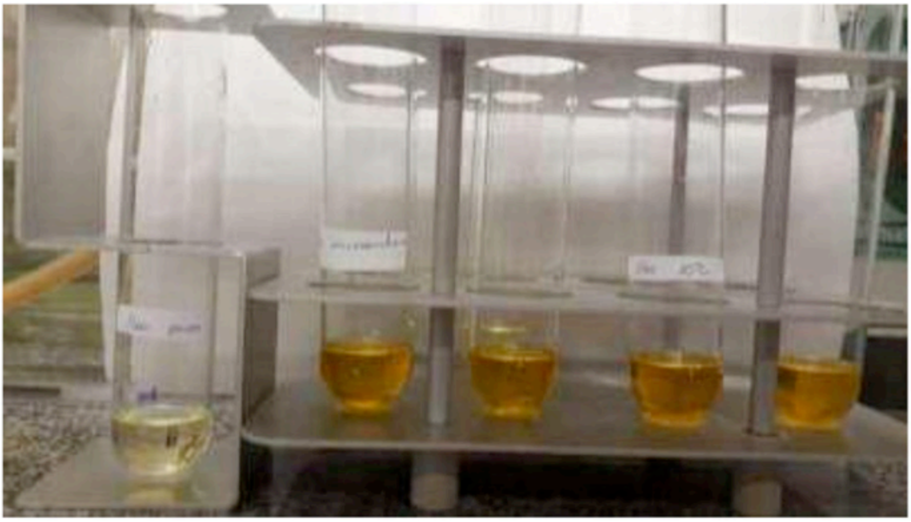

Figure 2 - Color difference between in natura soybean oil and oil incubated at 10 °C with dried flowers in microwaves

Source: Authors, 2019.

Samples	Variables	Average ± DP
Pure oil	L*	24,58±3,07[c]
	a*	-1,77±0,29[D]
Drying at 25°C	L*	21,85±3,11[a,b]
	a*	2,29 ±0,23[A,B]
Drying at 10°C	L*	20,38±3,36[b]
	a*	2,92±0,51[C]
Drying at microwaves	L*	22,12±3,90[a]
	a*	1,91±0,22[A]
Lyophilization	L*	22,47±3,54[a]
	a*	2,58±0,33[B,C]

Caption: Equal letters indicate that the results do not differ statistically. The uppercase letters refer to the variable a* and the lowercase letters to the variable L.

Table 2 - Results obtained for the color parameters, L* and a*, of the soybean oil according to the different techniques of Drying:

Time	Average ± DP
L* 0	19,23 ± 1,60[a]
L* 365	25,33 ± 1,50[b]
a* 0	1,71 ± 1,88[B]
a* 365	1,46 ± 1,95[A]

Caption: Equal letters indicate that the results do not differ statistically. The uppercase letters refer to the variable a* and the lowercase letters to the variable L.

Table 3 - Effect of storage time on color parameters, L* and a*, of soybean oil

indicating partial transport of the pigment into the oil on the first day of the test.

The results expressed in Table 3 indicate that, when compared to each other, the incubation of flowers, regardless of the Drying at methodology, resulted in an increase in luminosity after 12 months, compared to time 0.

The more orange color and the more floral aroma are organoleptic changes observed in the oil and that can have a positive impact from the sensory point of view of the product.

In the case of the variable b*, the interaction time versus treatment was significant ($p < 0.05$). In this case, the results referring to the interaction were also evaluated. Table 4 shows the effects of Drying at and storage time techniques on the b* value.

From the data presented in Table 4, it was possible to verify that, except for the treatment at 25 °C at time 0, all samples differ significantly ($p < 0.05$) from the in natura oil. The sample incubated with the dried flowers at 10 °C, when compared to the others, was the one with the highest intensity of yellow color during storage. Microwave-dried and freeze-dried samples do not differ in this color variable (b*).

The sample incubated with the dried flowers at 10 °C showed a color closer to yellow, which may give a more attractive sensory characteristic to the oil. Also according to Table 4, the pure oil preserved the light yellow hue during storage. This same behavior was observed in samples incubated with dried flowers at 10 °C, freeze-dried and microwaved. Only the sample incubated with the dried flowers at 25 °C showed a significant increase in the yellow hue over the 12 months.

The results regarding the global parameter, delta E, can be seen in Table 5.

From the results presented in Table 5, it appears that the sample incubated with the dried flowers at 10 °C was the only one that differed from the other treatments and showed a greater color difference when compared to the oil *in natura*, which confirms the discussion of individual parameters. These results indicate that the cold drying process favored the color stability of the Wedelia flower. This fact may be explained by a lower degree of luteolin degradation when drying was carried out at milder temperatures.

No data on extraction of natural pigments using vegetable oils as solvent were found in the literature. However, luteolin extracted with methanol presented a color spectrum with a green hue (VANKAR; SHUKLA, 2018) while in the present work the predominant hue was orange. This was possibly due to the difference in polarity between the solvents used.

CONCLUSIONS

In this study, it can be observed that the technique developed for extracting pigments from Wedelia flowers by incubating them directly in the oily matrix, allowed the efficient transport of pigments and other fat-soluble compounds without the use of fossil solvents.

Depending on the drying process of the flowers and the incubation time, the oil obtained presented colors with different intensities. However, when the process was carried out with the flowers dried at 10 °C, the oil showed better parameters in the color space, particularly in the product brightness. Therefore, the potential of using the Wedelia pigment as a stable natural food coloring and as a source of bioactive compounds was verified, expanding the use of soybean oil in the formulation of new products, in particular margarines.

Studies involving the characterization, quantification of luteolin, sensory tests and nutraceutical analysis of the oil obtained, as well as techniques for isolation and application of the dye for other purposes are suggestions for future work.

Treatment	Time	Average ± DP
Drying at a 25 °C	B0	4,84 ± 0,30[c]
Pure oil	B365	5,29 ± 0,12[c]
Pure oil	B0	5,58 ± 0,10[c]
Drying at a 25 °C	B365	7,31 ± 0,35[a]
Lyophilization	B0	7,63 ± 0,16[a]
Drying at microwaves	B0	7,74 ± 0,05[a,b]
Microwave drying	B365	8,21 ± 0,40[a,b]
Lyophilization	B365	8,31 ± 0,77[a,b]
Drying at 10 °C	B0	9,33 ± 0,41[b,d]
Drying at 10 °C	B365	10,14 ± 1,40[d]

Caption: Equal letters indicate that the results do not differ statistically.

Table 4 - Effect of the storage time on the value of parameter b*

Source: Authors, 2019.

Treatment	Average ± DP
Drying at 25 °C	5,13 ± 0,63[a]
Microwave drying	5,19 ± 0,26[a]
Lyophilization	5,52 ± 0,43[a]
Drying at 10 °C	7,66 ± 1,34[b]

Caption: Equal letters indicate that the results do not differ statistically.

Table 5 - Effect of the drying treatment on the delta parameter value: E_{ab}

Source: Authors, 2019.

REFERENCES

ABIOVE. **Brasil - Complexo Soja**. 2021 Disponível em: https://abiove.org.br/estatisticas/. Acesso em: 25 de janeiro de 2022.

BRASIL. Câmara dos Deputados. **Projeto de lei n.º 1.271, de 5 de maio de 2011**. Proíbe a utilização do corante Amarelo Tartrazina na indústriafarmacêutica, cosmética e alimentícia e dá outras providências. Brasília: Câmarados Deputados, 2011.

CRUZ, G. F. R. *et al.* O comportamento do consumidor de alimentos funcionais*In:* SIMPÓSIO INTERNACIONAL DE GESTÃO DE PROJETOS, INOVAÇÃO E SUSTENTABILIDADE, 6., 2007, São Paulo. **Anais[...]**, São Paulo: 2007. p. 1-16.

CZEPULA, A. I**. Desenvolvimento de preparações semi-sólidas contendo extratos de *Sphagneticola trilobata (L.) Pruski* (*Acmela brasiliensis, Wedelia paludosa*) (*Asteraceae*) e avaliação da atividade antiinflamatória tópica in vivo**. 2006. 121 f. Dissertação (Mestrado em Ciências farmacêuticas) – Universidade Vale do Itajaí, Itajaí, 2006.

FREITAS, A. S. Corante artificial amarelo tartrazina: uma revisão das propriedades e análises de quantificação. **Revista Acta Tecnológica.** v. 7, n. 2,p. 65-72, 2013.

FREITAS, S. P.; SOUZA, T. R.; CARVALHO, M. C. S. Effect of the drying processon mass transport of S*phagneticola trilobata* pigments using soybean oil as solvent. *In:* EUROPEAN DRYING CONFERENCE, 7., 2019, Torino. **Book of Abstracts [...]**, Torino: 2019. p. 165-179.

LANFER-MARQUEZ, U. M. O papel da clorofila na alimentação humana: uma revisão. **Revista Brasileira de Ciências Farmacêuticas**, São Paulo, v. 39, n. 3, p. 227-242, set. 2003.

LOPES, L. C. **Controle metrológico da cor aplicado à estamparia digital de materiais têxteis**. 2009. 142f. Dissertação (Mestrado em Metrologia) – PontifíciaUniversidade Católica, Rio de Janeiro, 2009.

MANZOOR, M. F. *et al.* Food based phytochemical luteolin their derivatives, sources and medicinal benefits. **International Journal of Agriculture and Life Sciences**, v. 3, n. 11, p.195-207, 2017.

RIBEIRO, P. C. E. **Motivações do consumidor em relação à escolha de alimentos biológicos.** 2019. 112f. Dissertação (Mestrado em Publicidade e Marketing) – Escola Superior de Comunicação Social, Lisboa, 2019.

VANKAR, P. S.; SHUKLA, D. Spectrum of colors from reseda luteola and other natural yellow dyes. **Journal of Textile Engineering & Fashion Technology**, v.4, n. 2, p. 107-120, 2018.