Stabilization of luteolins from weld (*Reseda luteola* L.) and sawwort (*Serratula tinctoria* L.) by microencapsulation for natural dyeing

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Abstract

Weld and sawwort, are herbaceous plants that have been used, since ancient times, for natural dyeing, concerning to the presence of luteolins and derivatives. However, natural dyes have been limited due to lower stability and higher production prices when compared with synthetic dyes. The weld plant was acquired in France and sawwort was produced in June 2020 in Portugal, with a low success rate. The leaves having been harvested at flowering stage (July 2021) for extraction of the coloring matter. The aim of this work was to study the effect of luteolins microencapsulation by freeze-drying and spray-drying on color stability, using maltodextrin and Arabic gum as wall material. Weld extracts and microencapsulates showed higher total phenolic compounds, and encapsulation efficiency (EE) for freeze-drying process, than extracts and microencapsulates of sawwort, but lower (EE) for spray-drying process. The analysis of the results shows that the solubility of the dye increases with microencapsulation and the stability of encapsulated pigments and the color of the plant extracts, in buffer solutions, was higher at pH 3 for weld, and at pH 4 for sawwort. LC-HRMS/MS analysis have shown that the main yellow chromophore are luteolin-O-glucoside. Weld extract contains luteolin-3',7-O-glucoside isomers, luteolin-7-O-glucoside and luteolin-4´-O-glucoside, apigenin-O-glucoside, chrysoeriol-O-glucoside, luteolin, apigenin and chrysoeriol whereas glucoside and glucuronide isomers of luteolin and quercetin-3-O-methyl ether are the color compounds in the sawwort extract. Microencapsulation by spray-drying and by freeze-drying produces microcapsules with different morphologies and sizes, allowing to maintain their original color, contrary to what happens with the extracts. Microencapsulation is a promising process to improve the stability of natural dyes because it allows them to be obtained in powder form, enabling better preservation and easy transport. Weld and sawwtort plants have the potential to be applied as natural dyes, in different technological processes: handmade, semi-industrial and industrial, by companies with good environmental practices.

Keywords: luteolin, dyes, pH, freeze-drying, spray-drying, sustainability

INTRODUCTION

In Europe dyeing substances were extracted from more than 30 plants (Menzi, 1956), many of them resulting from the influence of the Romans and the commercial exchanges imported from the East, whose processes were developed in large Italian cities, such as Venice, Genova and Florence, once established as the great trading post of the Mediterranean. However, it was in Europe that the dyeing plants cultivation and their processing, for the dyeing of textiles, became modernised and where, for example, the woad, in Thüringen, Germany, and mader, in the Netherlands and in areas of southern France,

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gained economic, social, political and cultural importance.

In the 19th century, with the development of chemical synthesis and the understanding of the principles of dyeing, new synthetic dyes, more effective than natural appeared. There was a break in the use of natural dyes and the large-scale production of coloring plants gave rise to huge factories producing synthetic dyes. We thus witnessed the decline of the large-scale plantations of "dyes" that Europeans had been promoting in their colonies since the 16th century.

However, due to the great environmental damage caused by the dyeing process, namely in what concerns the discharge of synthetic dyes in effluents, without the proper treatment, since the 90s the demand for natural dyes has been increasing, mainly in the last years, due to a greater consumer awareness about the highly pollutant procedures, which involve the dyeing of textiles or leather with synthetic dyes. Natural dyes present as positive factors their biodegradable character, being anti-allergenic, non-carcinogenic and of low toxicity (Chequer et al., 2013).

The weld (Goffer, 1980), *Reseda luteola* L., a plant of the *Resedaceae* family, introduced in Ancient Rome to yellow dye, was one of the most widely used plants in dyeing in Western Europe. In Portugal (Serrano et al., 2007, 2008), since the 17th century, it was grown mainly in the North in the Douro and Alentejo regions for use in dyeing. After flowering the plant was harvested, appearing on the market as dried bundles of plants.

Sawwort, *Serratula tinctoria* L. belongs to the *Asteraceae* family and was used as a yellow dye in Europe since the Middle Ages until the 19th century, especially in areas where weld was not cultivated (Cardon, 2007).

A coloring agent belonging to the flavonoid group, called flavones, is extracted from the seeds and upper branches of weld and from the leaves of sawwort, which major compound luteolin-7-O-glucoside. The weld extract contains other compounds that include the isomers of luteolin-3',7-O-glucoside, luteolin-4'-O-glucoside, apigenin-O-glucoside; chrysoeriol-O-glucoside, luteolin, apigenin and chrysoeriol. Two isomers of quercetin-3-O-glucuronide and luteolin were found in the sawwort extract. Flavones, have a yellow color and are present in plants combined with glucoside units, in the form of very water-soluble heterosides, which are normally hydrolysed during dyeing, and progressive heating of the bath releases the dyeing aglycones. However, there are some limitations to its commercial application due to the high cost of the raw material and poor stability in sunlight because of the high sensitivity to oxidation.

The development of appropriate technologies that facilitate an increase the luteolins stability will improve the application of these plants and promote the growth of aromatic and medicinal plant producers. In this context, although luteolins represent a potential application for textiles, the use has been limited due to instability, which is affected by several factors during processing, such as temperature, pH and light leading to the quinones formation (Cavalcanti et al., 2011).

Many authors (Feller, 1994; Egerton and Morgan, 1970; Beek, and Heertjes, 1966; Cumming et al., 1956) have studied the temperature influence on the stability of flavone aglycones from various sources at different pH values, proving that heating has a detrimental effect on the color and content of flavone aglycones, apigenin, luteolin, and chrysoeriol were stable at pH 3, but degraded steadily at pH 5 or 7. The most rapid degradation occurred with luteolin, at pH 7 (Sadilova et al., 2007; Woodward et al., 2009). For this, it is essential the natural dyes stabilization by encapsulation that allows dyes to be involved by a polymeric membrane, in order to protect them from external conditions such as light, humidity, acidity/alkalinity, oxidation, etc (Ersus and Yurdagel, 2007; Akhavan Mahdavi et al., 2016). The coloring matter is dispersed in the polymeric material, leading after solidification of the drops, by freeze-drying or spray-drying to production the microcapsules. These processes allow to obtained dyes in powder form, facilitating transport and storage by converting liquid materials into solid capsules, powders or granules. On the other hand, it also allows the use of shorter processing techniques, with fewer cleaning operations and better dyes solubility, without need new incorporation phases, etc.

The aim of this work was to study the potential dye plants for the encapsulation of

luteolins, using maltodextrin and Arabic gum as wall material.

MATERIAL AND METHODS

Chemicals and reagents

Ethanol p.a, hydrochloric acid (370 g L^{-1}), iron (II) sulfate heptahydrate, iron (III) chloride hexahydrate, sodium acetate trihydrate, were purchased from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent, and potassium hexacyanoferrate (III), gallic acid (990 g L^{-1}) were purchased from Sigma (Sternheim, Germany). Sodium carbonate anhydrous was obtained from BDH (Poole, UK), while 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ, 990 g L^{-1}) and ferric chloride were acquired from Fluka (Buchs, Germany), and anhydrous sodium sulfate from Panreac (Barcelona, Spain). Ethanol absolute anhydrous was purchase from Carlo Erba (Marseille, France). Luteolin-7-O-glucoside was acquired from Extrasynthése (Genay, France). Maltodextrin (DE 16.5-19.5) and Arabic gum was purchased from Sigma-Aldrich (Darmstadt, Germany). All other unlabeled chemicals and reagents were analytical or HPLC-MS Optima grade.

Plant material

Weld dried was purchased in PMA28 (Varize, France) enterprise, and Sawwort was provided dried by a local producer (São João da Madeira, Portugal). The plants were powered in a mill (IKA Micro Fine Mill Culatti) using a 1.0 mm thick sieve, stored under vacuum in a packaging film polymer (LDPE 60 µm / PA 30 µm) (Amcor Flexibles, Portugal) and placed in desiccators until further analysis.

Flavonoids laboratory-scale extraction

The flavonoids dye compounds, in weld and sawwort plants, were extracted according Moiteiro et al. (2008) by taking powered plant with ethanol:water (80:20 v/v), at a ratio of 1:20, containing $CaCO_3$ (0.17 M) at pH=3.0, into erlenmeyer flask (500 mL). The solution was placed on a hot plate at 55°C with stirring at 4500 rpm (Are, Velp Scientifica), during 45 min. The extracts were centrifuged (Sigma and Laborzentrifugen, 1k15) at 3100 rpm for 10 min at 5°C. Finally, supernatant was collected and evaporated under vacuum (40°C, 178 mbar) by a rotary evaporator (Buchi R-114 Rotavapor Vap System). The residue was weighed and stored at -20°C until further use. Each weighed dry sample was then reconstituted in 400 mL of water and stored in the dark at a low temperature (4°C) until testing. The assays were performed in duplicate.

Preparation of microcapsules flavonoids extracts by spray-drying (SD) and freeze-drying (FD)

To produce the water-soluble shell-coated matrix capsules maltodextrin or Arabic gum were used as carrier agents. A stock solution of 40% (w/w) maltodextrin, or 35% (w/w) Arabic gum (wall materials) were placed in Erlenmeyer flask and distilled water was added. Each solution was placed on a hot plate at 60°C with stirring at 200 rpm until complete dissolution. These solutions were added to 20% (w/w) sorghum extract and the pH was adjusted to 3.5. Each solution was placed on a hot plate at 45°C with stirring at 4500 rpm for 1 h, and was spray dried and frozen. The dye extracts without carrier agents were frozen at -80°C and dried by freeze-drying. The solution was dehydrated in a laboratory scale spray dryer (Buchi B-290, Labortechnik AG, Flawil, Switzerland), with a 0.7 mm diameter nozzle, a main spray chamber of 500×215 mm, and a standard cyclone. The gas flow (nitrogen) of the drying air was about 35 m3 h^{-1}. The following parameters were fixed: pump (10%), aspirator (100%), inlet temperature (150°C) and outlet temperature (80°C).

The samples were dehydrated in a laboratory freeze dryer (Scanvac Cool Safe, Labogene Scadinavian by Design). The vacuum pressure of the freeze drier was set at 0.2 hPa, the plate temperature was 20°C, and the condenser was at -50°C for 24 h. The powders obtained by spray and freeze drying were stored in glass flasks (Schott 250 mL) in a desiccator containing silica gel in the dark at room temperature.

Liquid chromatography and tandem mass spectrometry

Triplicates of the extract samples were analyzed by HPLC-DAD-MS on a Dionex Ultimate 3000SD system with a diode array detector coupled online to a LCQ Fleet ion trap mass spectrometer equipped with an ESI source, operating in the negative mode (Thermo Scientific TM, Waltham, MA, USA). High resolution tandem mass spectra were obtained on an UHPLC Elute interfaced with a QqTOF Impact II mass spectrometer equipped with an ESI source, operating in the negative mode (Bruker Daltonics, Bremen, Germany). Chromatographic separation were carried out with a Kinetex C18 column 100 Å (150×2.1 mm, 2.6 µm particle size, Phenomenex), using a gradient of elution of 0.1% of acid formic in water and acetonitrile. For details see Sharif et al. (2022).

Microcapsules characterization

1. Encapsulation yield (EY), encapsulation efficiency (EE), and solubility (S), and total phenolic compounds (TPC).

The encapsulation yield was calculated according methodology given by Jimenez-Gonzalez et al. (2018), using the follow equation: EY (%) = QE *100/ER + E, where QE was the amount (g) of flavonoids encapsulated, ER is the extraction residue and E is carrier agent (g).

The encapsulation efficiency, the solubility and the total phenolic compounds was calculated according to methodology described by Serrano et al. 2020. The encapsulation efficiency was calculated using the following equation: EE (%) = (TP – SP)/TP × 100, where TP is the total phenolic compounds the content from a known amount of powder particles after rupture, and SP the total phenol compounds content contained in the surface of the same amount of powder particles.

2. Morphology and particle size distribution by scanning electron microscopy (SEM).

The morphology of the weld particles was observed by scanning electronic microscopy (SEM). Each sample was covered with a fine layer of gold through Sputer Coating Attachment of Quorum Q150R ES in vacuumed evaporators. The equipment used for observations was a scanning electron microscope (Hitachi, S-3400N, Tokyo, Japan) working with a voltage of 20 kV. The sawwort microcapsules the powder samples were previously fixed on metal pins with carbon tape and coated with gold in a Jeol JFC-1200 metallizer and observed in a Jeol JSM-5200 LM scanning electron microscope.

The microphotographs were carried out witha camera coupled to the microscopic. The samples were systematically observed with 1000× and 3000× magnification. The particle size was determined by examination of SEM micrographs.

3. Colorimetry assays.

Color parameters (L*, a*, b*) of flavonoid solutions were measured using a colorimeter Chroma meter CR-400 (Konica Minolta, Japan), using an illuminant D 65 and a 2° observation angle. The color difference degree between of flavonoid solutions and standard white, ΔE* values was calculated according the formula: $\Delta E^* = \sqrt{(\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})}$ where L* represent lightness, a* redness, b* yellowness and ΔL*, Δa* Δb* the difference between each parameter for flavonoid solutions and white samples. The flavonoid compounds encapsulated stability was evaluated by the loss of color which is the ratio (%) between the ΔE * values of the sample after and before thermal evaluation.

Stability tests of non-encapsulated and encapsulated released flavonoid compounds

1. pH effect.

Encapsulated and non-encapsulated dye extracts solutions were prepared by weighing 0.03 g, of each sample, into volumetric flasks (10 mL), and completed the volume with sodium citrate buffer solutions at four pH values (3, 4, 5 and 6). The buffer solution at pH 3, was prepared by weighed 0.27 g of sodium citrate dehydrate (294.0 g mol^{-1}), 1.74 g of citric acid (192.12 g mol^{-1}), and distilled water was added until complete a volume (flask 100 mL).

The other pH values (4, 5 and 6) were achieved with NaOH solution (1 M). Then 5 mL of the previously solutions were measured into 10 mL tubes hermetically sealed, and placed in a thermostat bath (Unitronic-OR, Selecta, Barcelona). The solutions scanning spectra were plotted at 350 nm using a UV–visible spectrophotometer (double-beam; Hitachi U-2010). The blank solutions were constituted by the citrate buffer solutions. The assays were performed in mass duplicate.

2. Temperature effect.

Approximately 0.03 g of encapsulated and non-encapsulated dye extracts were weighed into volumetric flasks (25 mL) and completed with buffer solution at pH 4. Then 5 mL of the previously prepared solutions were measured into 10mL tubes hermetically sealed. The tubes were placed in a thermostat bath (Unitronic-OR, Selecta, Barcelona) at 80°C (maximum temperature to apply in the wool dyeing bath) for 1.5, 3, 6, 12, 15 and 18 h. After the heat treatment the tubes were immediately cooled in an ice bath to stop thermal degradation. The absorbance was determined at 350 nm using a UV–visible spectrophotometer (double-beam; Hitachi U-2010).

3. Kinetic parameters.

The kinetic parameters of flavonoids degradation were determinate according to Petrucci (2007). The first order reaction rate constants (Kd), half-lives (t1/2), i.e. the time that is necessary for degradation of 50% of flavonoids were calculated by the fowling equations:

$$\ln(C_t/C_0) = -k \times t$$

$$t_{1/2} = -\ln(0.5) \times k^{-1}$$

where C_0 is the initial 3-deoxyanthocyanidins content and Ct the 3-deoxyanthocyanidins content after t minutes of heating at given temperature.

Statistical analysis

Experimental design for thermal stability study and all statistical analysis were performed using StatisticaTM v8.0 software (Statsoft, Inc., Tulsa, OK, USA). Differences between extracts were tested with analysis of variance (ANOVA). To satisfy ANOVA assumptions data were transformed, followed by multiple comparisons tests (Tukey HSD) to identify differences between groups. Statistical analyses were tested at a 0.05 level of probability.

RESULTS AND DISCUSSION

The extracted dye quality was the first step in the development of the encapsulation process, since it has to take into account the dye yield, the color parameters and the flavonoid compounds extracted, which affects the ratio of the active agent/carrier and its stability at temperature and pH.

Phenolic profile by liquid chromatography – tandem mass spectrometry

Table 1 summarizes the chromatographic profile of the yellow chromophores identified in the extracts of weld and sawwort, obtained by HPLC-DAD and LC-HRMS/MS. Compounds were identified based on their UV-VIS data and accurate m/z values released as deprotonated molecules $[M-H]^-$. Each molecular formula was validated by extracting the ion chromatograms from the raw data, and the accurate mass, isotopic, and fragmentation pattern were evaluated. The typical UV-VIS spectra obtained for major yellow components revealed a band I with a maximum of absorption between 304 and 350 nm, pointing to a flavonol structure. The compounds were confirmed by comparison with analytical standards or published data (Marques et al., 2009). The chromatographic profile of yellow dyes from the non-encapsulated weld extracts showed 12 chromophore compounds and in sawwort were identified nine.

Table 1. HPLC-DAD and LC-ESI(-)-HRMS/MS characterization of the main yellow chromophores present in the weld and sawwort extracts.

Rt (min)	λ max (nm)	MF	[M-H]⁻ [(*m/z*) (±Δ ppm)]	MS/MS [(*m/z*) (Δ ppm) (attribution)]	Proposed compound	Weld	Sawwort
5.1	270; 330	$C_{27}H_{30}O_{15}$	593.1526 (-2.3)	503.1245 (-6.0) ($^{0,3}X_{H1}$⁻)[$C_{24}H_{23}O_{12}$] ⁻; 473.1119 (-6.2) ($^{0,2}X_{H1}$⁻)[$C_{23}H_{21}O_{11}$] ⁻; 383.0797 (-6.4) ($^{0,2}X_{H1}{}^{0,3}X_{H2}$⁻)[$C_{20}H_{15}O_8$] ⁻; 353.0687 (-5.7) ($^{0,2}X_{H1}{}^{0,2}X_{H2}$⁻)[$C_{19}H_{13}O_7$] ⁻	Apigenin-6,8-di-*C*-Glc	x	–
5.3	268; 334	$C_{27}H_{30}O_{16}$	609.1470 (-1,2)	447.0949 (-2.7) [Y_1⁻] [$C_{21}H_{19}O_{11}$]⁻; 285.0411 (-2.2) [Y_0⁻] [$C_{15}H_9O_6$]⁻	Luteolin-di-*O*-Glc	x	–
5.7	268; 340	$C_{27}H_{30}O_{16}$	609.1460 (+0,2)	447.0939 (-1.5) [Y_1⁻] [$C_{21}H_{19}O_{11}$]⁻; 285.0409 (-1.4) [Y_0⁻] [$C_{15}H_9O_6$]⁻	Luteolin-3',7-di-*O*-Glc	x	–
6.3	254; 348	$C_{21}H_{20}O_{11}$	447.0934 (-0.2)	285.0409 (-2.1) [Y_0⁻] [$C_{15}H_9O_6$] ⁻;284.0338 (+1.3) [Y_0-H]•⁻ [$C_{15}H_8O_6$]•⁻; 133.0304(-6.4) ($^{1,3}B$⁻) [$C_8H_5O_2$]⁻	Luteolin-7-*O*-Glc[a]	x	x
6.4	-	$C_{21}H_{18}O_{12}$	461.0739 (-2.1)	285.0412 (-2.6) [Y_0⁻][$C_{15}H_9O_6$]⁻	Luteolin-*O*-Glr[a]	-	x
6.7	338	$C_{26}H_{28}O_{15}$	579.1360 (-0.8)	447.0921(+2.7) [Y_1⁻] [$C_{21}H_{19}O_{11}$]⁻; 301.0346 (2.6) [Y_0⁻][$C_{15}H_9O_7$]⁻; 300.0278 (-0.6)[Y_0-H]•⁻ [$C_{15}H_8O_7$]•⁻	Quercetin-3-*O*-deoxyhex- -pen	x	–
6.8	266; 336	$C_{21}H_{20}O_{10}$	431.0985 (-0.3)	269.0441 (+5.4) [Y_0⁻] [$C_{15}H_9O_5$]⁻; 268.0400(-5.6) [Y_0-H]•⁻ [$C_{15}H_8O_6$]•⁻	Apigenin-7-*O*-Glc	x	–
6.9	266; 342	$C_{21}H_{20}O_{11}$	447.0934 (-0.2)	285.0409 (-2.1) [Y_0⁻] [$C_{15}H_9O_5$]⁻; 284.0338 (+1.3) [Y_0-H]•⁻ [$C_{15}H_8O_6$]•⁻; 133.0304 (-6.4) ($^{1,3}B$⁻) [$C_8H_5O_2$]⁻	Luteolin-3'-*O*-Glc	–	x
7.0	268; 336	$C_{21}H_{18}O_{12}$	461.0739 (-2.1)	285.0413 (-2.8) [Y_0⁻] [$C_{15}H_9O_6$]⁻	Luteolin-*O*-Glr isomer	–	x
7.0	268; 344	$C_{22}H_{22}O_{11}$	461.1089 (+0.1)	446.0864 (-2.1) [M-H-•CH_3]•⁻; 299.0543 (+6.1) [Y_0⁻] [$C_{16}H_{11}O_6$]⁻; 298.0483(-0.0) [Y_0-H]•⁻ [$C_{16}H_{10}O_6$]•⁻	Chrysoeriol-7-*O*-Glc	x	–
7.1	268; 338	$C_{21}H_{20}O_{11}$	447.0946 (-2.7)	285.0412 (-2.4) [Y_0⁻] [$C_{15}H_9O_6$]⁻	Luteolin-4'-*O*-Glc	x	–
7.1	266; 340	$C_{22}H_{22}O_{12}$	477.1037 (-1.7)	315.0515 (-1.4) [Y_0⁻] [$C_{16}H_{11}O_7$]⁻; 300.0280 (-1.5) [Y_0-•CH_3]•⁻[$C_{15}H_8O_7$]•⁻; 271.0249 (-0.3) [$C_{14}H_7O_6$]⁻; 255.0301 (-0.4) [$C_{14}H_7O_5$]⁻	Quercetin-3-methyl ether - -7-*O*-Glc	–	x
7.2	366: 346	$C_{22}H_{20}O_{13}$	491.0832 (-4.1)	315.0515 (-1.4) [Y_0⁻] [$C_{16}H_{11}O_7$]⁻; 300.0281 (-1.7) [Y_0-•CH_3]•⁻ [$C_{15}H_8O_7$] •⁻; 271.0259 (-2.7) [$C_{14}H_7O_6$]⁻; 255.0308 (-3.4) [$C_{14}H_7O_5$]⁻	Quercetin-3-methyl ether- -7-*O*-Glr	–	x
7.4	266; 338	$C_{22}H_{22}O_{12}$	477.1032 (-1.2)	315.0512 (-1.2) [Y_0⁻] [$C_{16}H_{11}O_7$]⁻; 300.0281 (-1.7) [Y_0-•CH_3]•⁻[$C_{15}H_8O_7$]•⁻; 271.0269 (-7.1) [$C_{14}H_7O_6$]⁻	Quercetin-3-methyl ether-*O*-Glc isomer	–	x
8.3	266; 346	$C_{15}H_{10}O_6$	285.0412 (-2.4)	151.0034 (-0.7) [$^{1,3}A$⁻] [$C_7H_3O_4$]⁻; 133.0283 (9.0) [$^{1,3}B$⁻] [$C_8H_5O_2$]⁻	Luteolin[a]	x	x
8.5	264;354	$C_{16}H_{12}O_7$	315.0517 (-2.0)	300.0278 (-0.8) [M-H-•CH_3]•⁻ [$C_{15}H_8O_7$]•⁻;271.0268 (-0.7) [$C_{14}H_7O_6$]⁻; 255.0291 (+3.1) [$C_{14}H_7O_5$]⁻; 243.0305 (-2.7) [$C_{13}H_7O_5$]⁻; 227.0358 (-3.4) [$C_{13}H_7O_4$]⁻	Quercetin-3-methyl ether	–	x
9.2	268; 336	$C_{15}H_{10}O_5$	269.0449 (-2.3)	151.0034 (-0.7) [$^{1,3}A$⁻] [$C_7H_3O_4$]⁻; 117.0283 (9.0) [$^{1,3}B$⁻] [C_8H_5O]⁻	Apigenin[a]	x	–.
9.4	268; 342	$C_{16}H_{12}O_6$	299.0551 (+3.6)	284.0310 (+5.8) [M-H-•CH_3]•⁻ [$C_{15}H_8O_6$]•⁻; 256.0363 (+5.3) ([M-H]-CO-•CH_3]•⁻ [$C_{14}H_8O_6$]•⁻; 151.0031 (+4.0) [$^{1,3}A$⁻] [$C_7H_3O_4$]⁻	Chrysoeriol	x	–

[a]Identified with an analytical standard.

Glc, glucoside; Glr, glucuronide; Deoxyhex, deoxyhexoside; Pen, pentoside.

Solubility, encapsulation yeld (EY), encapsulation efficiency (EE), and total phenolic compounds (TPC)

In Table 2, it can be seen that the two encapsulation processes, for both encapsulating agents, caused a significant increase ($p>0.05$) in the solubility of the hydroalcoholic extracts of weld and sawwort.

Table 2. Physicochemical parameters (mean±SD) weld (W) and sawwort (S) extracts non-encapsulated (NE) and encapsulated with maltodextrin (M-FD) and Arabic gum (AG-SD) by freeze-drying (FD) and spray-drying processes (SD).

		Freeze-drying			Spray-drying	
		M-FD	AG-FD	NE	M-SD	AG-SD
Solubility (%)	W	49.92^{a}±3.58	45.16^{a}±1.40	16.39^{b}±1.08	46.92^{a}±0.16	44.37^{a}±1.07
	S	43.26^{a}±0.02	41.78^{a}±0.41	16.67^{b}±0.20	45.37^{a}±1.11	42.05^{a}±0.36
EY (%)	W	79.88^{b}±7.52	89.19^{a,b}±8.92	15.41^{b}±1.41	24.96^{a,b}±2.32	24.55^{a}±2.48
	S	54.04^{b}±6.22	59.18^{a,b}±5.14	10.38^{b}±3.46	48.76^{a,b}±1.01	55.36^{a}±1.02
EE (%)	W	84.34^{b}±1.56	83.18^{a,b}±1.07	-	81.42^{a,b}±1.42	77.71^{a}±0.96
	S	86.48^{b}±1.07	92.97^{a,b}±1.31	-	75.65^{a,b}±5.80	81.23^{a}±3.87
TPC (mg g^{-1})	W	4.40^{a}±0.03	5.03^{a}±0.33	4.02^{a}±0.36	4.06^{a}±0.73	3.96^{a}±0.13
	S	3.49^{a}±0.06	5.86^{a}±0.07	3.71^{a}±0.88	1.55^{b}±0.15	2.31^{b}±0.23

Mean ± standard deviation of the analyses in duplicate. Values with different letters per row indicate significant differences ($p<0.05$).

Regarding the flavonoid compounds encapsulation yield and encapsulation efficiency, significant differences were also observed for the spray-drying process ($p>0.05$), and it was showed lower values than the freeze-drying process for both plants, regardless of the encapsulation agents. This fact may be due to the adhesion and deposition of the microcapsules on the wall of the atomizer chamber, which leads to lower particle collection efficiency, as previously reported by other authors (Jimenez-Gonzalez et al., 2018, Serrano et al., 2020). Regarding TPC, it was found that there were no significant differences for the non-encapsulated and encapsulated sawdust extracts for the two drying processes. However, the sawdust extract, showed differences in the spray drying process for both encapsulating agents, indicating that these compounds are more thermolabile.

Morphology and particle size distribution by scanning electron microscopy (SEM)

The weld powder particles produced with maltodextrin and Arabic gum exhibited a wrinkled rounded outer surface but no cracks in the microstructure. Relatively the morphology of the particles obtained with the sawwort extracts, for both encapsulants, they showed microcapsules with a more irregular and wrinkled outer surface than the microcapsules obtained with the weld extracts. The size distribution of maltodextrin microcapsules exhibited a size range beteween 1.0-12.0 µm and those of Arabic gum a wider size range 0.8-20.8 µm (Figure 1). On the sawwort microcapsules maltodextrin morphology exhibited a size range between 0.9 and 10 µm, and those of Arabic gum ranging between 0.7 and 18.8 µm (Figure 2).

Color measurements of the encapsulated flavonoid compounds released at different pHs

The results obtained for the total color differences (ΔE) of the encapsulated flavonoid compounds released at different pHs (Table 3), showed no significant differences ($p<0.05$), for weld (W) extracts, and the smallest differences were obtained at pH=3 and for sawwort (S) at pH=4, for both encapsulating agents and drying processes.

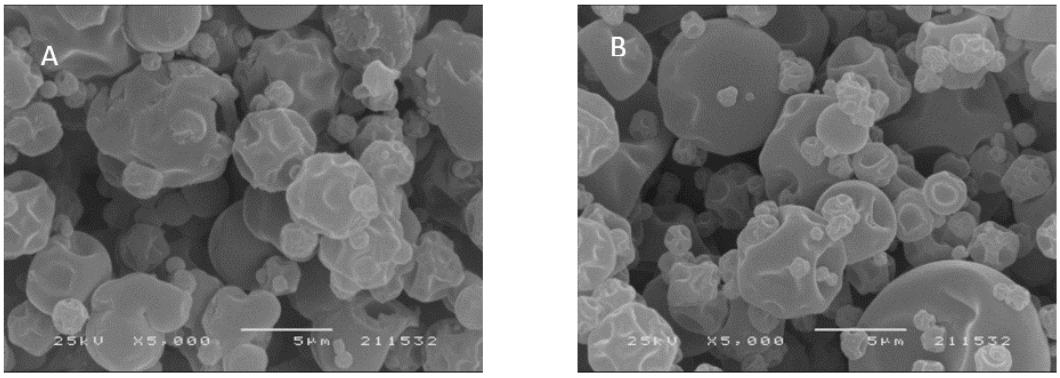

Figure 1. SEM images of weld microcapsules obtained by spray-drying by maltodextrin (A) and Arabic gum (B) (magnification 5000×).

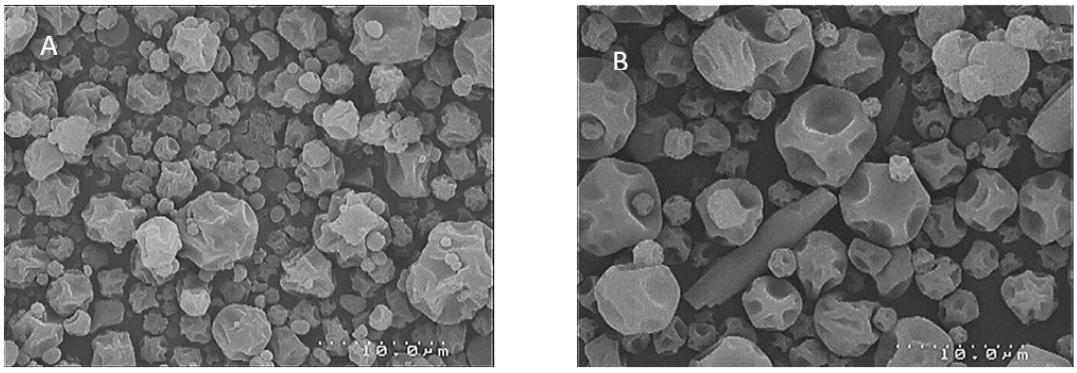

Figure 2. SEM images of sawwort microcapsules obtained by spray-drying by maltodextrin (A) and Arabic gum (B) (magnification 3000×).

Table 3. Total color differences (ΔE) of the microcapsules solubilized at different pH in the CIE coordinates L* a* b*, from weld (W) and sawwort (S).

		$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$			
		pH 3	**pH 4**	**pH 5**	**pH 6**
M-FD	W	43.84^{a}±4.25	56.46^{a,b}±0.57	61.96^{a,b}±0.32	63.22^{a,b}±1.89
	S	44.52^{a}±0.54	41.19^{a,b}±1.45	51.69^{a,b}±5.51	58.59^{a,b}±0.69
AG-FD	W	40.65^{a}±3.59	53.32^{b}±2.75	57.11^{a,c}±1.63	60.05^{a,b}±1.45
	S	55.02^{a}±0.72	41.35^{b}±6.48	53.56^{a,c}±2.56	60.10^{a,b}±4.95
M-SD	W	40.62^{a}±0.94	60.37^{c}±0.75	62.52^{a}±0.94	64.19^{a,b}±1.86
	S	61.20^{a}±3.68	58.49^{c}±2.46	64.51^{a}±2.23	71.98^{a,b}±0.82
AG-SD	W	38.19^{a}±2.53	50.16^{c} ±0.67	56.03^{c}±0.69	57.57^{c}±2.54
	S	38.68^{a}±0.50	36.00^{c} ±2.61	47.02^{c}±6.50	54.73^{c}±1.44

Mean±standard deviation of the analyses in duplicate.

Values with different letters per row indicate significant differences ($p<0.05$).

Color measurements and degradation kinetics

In the Figure 3 it can be observed the CIE chromaticity diagrams for the values of the parameters, a* and b* for the extracts of the weld samples, non-encapsulated and encapsulated. The points, for the same sample, show different temperature exposure times. In general, for all the samples analyzed for the two drying processes and two encapsulants,

there is a shift to the right (yellow zone) as the temperature exposure time increases. Thus, for most points sets, the rightmost point corresponds to the zero temperature exposure time and the leftmost point represents the result after 18 h of temperature exposure. The sample without encapsulant shows a yellowish color that, as the degradation time increases, moves toward the green area. The samples encapsulated with maltodextrin and obtained by freeze-drying are those which offer the best color stabilization for application in dyeing.

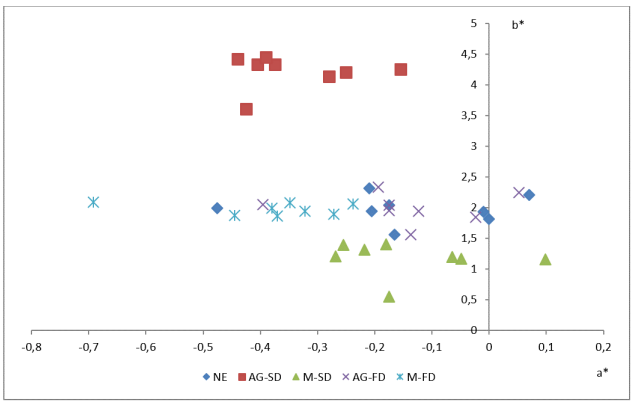

Figure 3. CIE chromaticity diagram for weld extract (W) non-encapsulated (NE) and encapsulated with maltodextrin (M), and Arabic gum (AG) obtained by freeze-drying (FD) and spray-drying (SD).

The degradation kinetics of flavonoid compounds, non-encapsulated and encapsulated with two encapsulating agents and two drying processes, was carried out at 80°C and pH=3, since these conditions would be used in the dyeing bath. The experimental data obtained were adjusted to a 1st order model using the least squares method through Excel v.2010 software.

Figure 4 presents the influence of temperature on the dye solutions over time, and the slopes of the straight lines presented provides the values of the degradation constant (Kd) and the half-life times ($t_{1/2}$) at the temperature of 80°C. The degradation speed of flavonoid compounds present in non-encapsulated and encapsulated weld shows the longer time of exposure to temperature, the greater the degradation constant and, consequently, the shorter half-life of flavonoids.

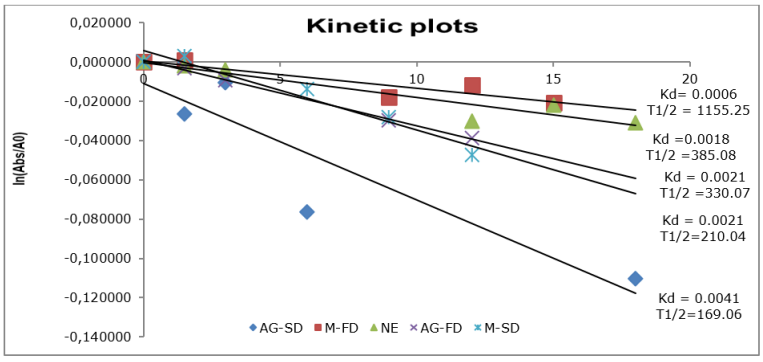

Figure 4. Degradation kinetics of non-encapsulated and encapsulated flavonoids in weld extract as funtion of temperature (T=80°C).

The Kd values confirm a greater degradation of the dye obtained from weld encapsulated with Arabic gum obtained by spray-drying, presented an estimated half-life time of 169.06 h, and the less degradation was obtained for weld extracts encapsulated with

maltodextrin obtained by freeze-drying with a estimated half-time of 1155.25 h. The results also show that the major degradation occurs in the spray-drying process with both encapsulates and Arabic gum is also the carrier agent that least protects flavonoids.

For the sawwort the quatity of plant supplied did not allow the fiasibility of the kinetics study.

Profile of encapsulated yellow dye extracts from weld (W) and sawwort (S) in released tests

The variation in the relative peak areas of each yellow dye presents in weld and sawwort microcapsulas as a funtion of the encapsulation with maltodextrin and Arabic gum obtained by freeze-drying and spray-drying processes is shown in 3D graphs.

Figures 5 and 6 show that the encapsulation of the extracts of the two plants present the same number of flavonoid compounds with high relative abundances that were identified in the release assays of the compounds from the microcapsules, obtained from the two drying processes. Regarding the two encapsulants the results suggest that the microcapsules prepared with Arabic gum show higher relative abundances, than those obtained with maltodextrin, indicating that the yellow flavonoid chromophores are more efficiently retained by the former encapsulant. This can be explained by the larger size of the microcapsules obtained with Arabic gum, allowing greater retention of the compounds, compared to those with maltodextrin.

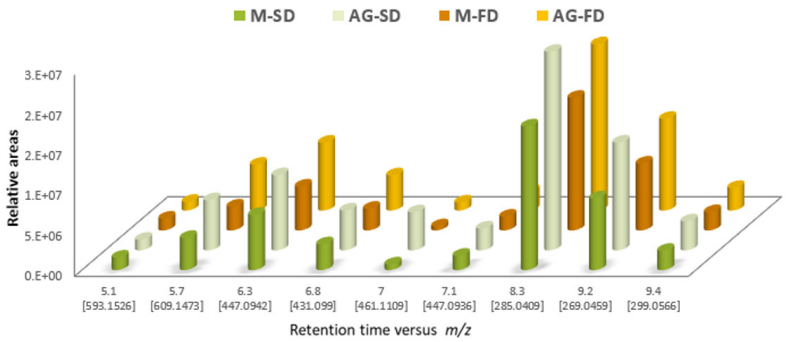

Figure 5. Comparative diagram of the relative abundances of flavonoid compounds present in freeze-drying of weld extracts encapsulated with maltodextrin (M-FD) and Arabic gum (AG-FD), and spray-drying extracts encapsulated with maltodextrin (M-SD) and Arabic gum (AG-SD), respectively.

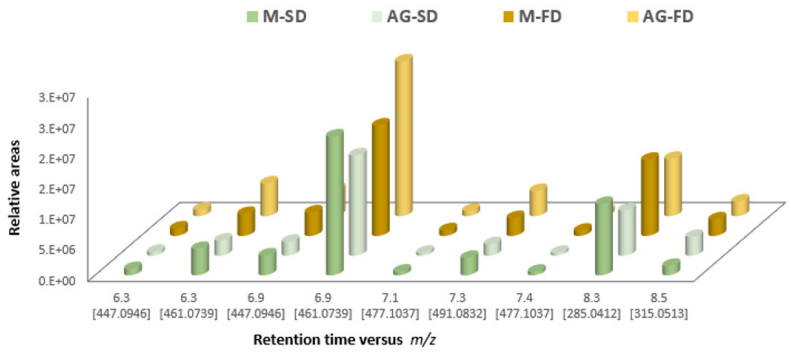

Figure 6. Comparative diagram of the relative abundances for the main yellow chromophores present in freeze-drying of sawwort extracts encapsulated with maltodextrin (M-FD) and Arabic gum (AG-FD), and spray-drying extracts encapsulated with maltodextrin (M-SD) and Arabic gum (AG-SD), respectively.

CONCLUSIONS

For weld and sawwort, the results obtained demosntrated that it is possible to extract high proportions of luteolins using a mixture of water and ethanol at pH=3, as solvent, at 55°C, under stirring for 45 min. The non-encapsulated freeze-dried weld extracts, as well as the microencapsules in maltodextrin and Arabic gum by freeze-drying and spray-drying did not present significant differences between the contents of the total phenolic compounds. In the case of sawwort the TPC showed significant differences for the spray-drying process. The dispersion of plants extracts encapsulated with maltodextrin and Arabic gum showed to be more soluble in water than the non-encapsulated extracts. Microencapsulation by spray-drying produces microcapsules with a spherical shape with a smooth surface and no cracks, while microencapsulation by freeze-drying forms microcapsules with an amorphous shape.

The profiles of the encapsulated dye extracts, with the two encapsulating agents and for both encapsulation processes, allowed the identification of a high number of flavonoid compounds, indicating that the encapsulation is a process that allows the preservation of the dyes.

Microencapsulation by freeze-drying conferred, for the weld, color protection and temperature stability, with no variations in color parameters over 1155.25 h, making this process promising for wool dyeing.

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