EXTRACTION OF BIOACTIVE COMPOUNDS OF SAFFRON (*CROCUS SATIVUS L.*) BY ULTRASOUND ASSISTED EXTRACTION (UAE) AND BY RAPID SOLID-LIQUID DYNAMIC EXTRACTION (RSLDE)

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**Abstract**  
*Crocus sativus L.* (Iridaceae), commonly known as saffron is not only used as a spice in food, but also for its medicinal properties. For these reasons, in this study the comparison between a conventional extraction technique using ultrasounds (UAE) and a cyclically pressurized solid-liquid extraction with the Naviglio extractor (NE) or Rapid Solid-Liquid Dynamic Extraction (RSLDE) was performed, in order to obtain qualitative and quantitative data related to bioactive compounds of saffron. The extracts obtained were analyzed for their antioxidant activity using ABTS, DPPH and FRAP assays, for their total polyphenol content (Folin-Ciocalteu) and for the metabolic profile using liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). The efficiency of extraction of active principles obtained with the Naviglio extractor was about four times higher than that resulting from the sonication extraction. The results obtained showed that the extracts of the Naviglio extractor had significant advantages in terms of extraction efficiency and quality of extract; moreover savings of time and therefore represented an important data in anticipation of the use of such extracts for the preparation of functional food and also phytodrugs.

**Keywords:** Saffron, ultrasound, Naviglio extractor, antioxidant activity, polyphenols
Introduction

*Crocus sativus* L. (*Iridaceae*), commonly known as saffron, is a perennial herbaceous plant widely cultivated in Iran and other countries such as India, Greece and Italy. The flowers are very valuable because the saffron is obtained from stigmas, whose cost is high because all harvesting operations are done by hand, from the plant setting of bulbs, to the gathering of flowers, to the separation of the stigmas from the flower, to packaging. The most delicate operations mainly include two phases:

- the wilting, i.e. the separation of the stigmas from the flower, red filaments that once dried constitute the saffron. This phase requires special attention, because the stigmas are red only in the part that protrudes from the calyx, while the remaining part is white and his presence in the final product decreases the purity and value of the spice;

- the roasting or drying during which the filaments are brought to the temperature of 50-55°C for a period of time determined from time to time depending on the moisture present in the initial product.

Only experience allows to evaluate the right point of drying to ensure the best yield and the best shelf life. The interest in this plant dates back to the nineteenth century during which many researchers were been interested in the yellow color extraction and established the glycosidic nature. (*Vogel, H.C.A.1991; Meyer, J.E.,1984*)

Carbohydrates are naturally present in the saffron in a concentration of 15% (w/w) consisting mainly of glucose and gentiobiose. Among other substances are present active dyes: crocetin, a carotenoid characterized by two carboxyl groups at the end of a chain of twenty carbon atoms with seven double bonds and four methyl groups and its glycosylated esters, the crocins, responsible for the coloring power of saffron, of bright yellow, soluble in water; these last compounds are rapidly hydrolyzed to crocetin, insoluble in water and having red colour (Fig.1). In saffron were also found bitering and odorous substances which the most important is the picrocrocin (Fig.2), that is hydrolyzed in water into glucose and safranal responsible for the aroma of saffron (*Liakopoulou-Kyriakides,M,2002: Alonso, G.L.1998*). The good quality of the saffron is determined by the concentration of crocin, at around 30% (w/w). The yellow color imparted to foods and beverages is due to the presence of carotenoids and in particular to crocetin and its esters. Moreover, saffron has antioxidant activity (*Verma, S.K.1998; Martinez Tome, M.2001*) anti-bacterial and anti-viral as well as diuretic, hypoglycemic and hypcholesterolemic (*Wintherhalter, P.2000*). From the Eastern peoples (China) stigmas were used in the form of infusion and decoction to treat pathologies, such as asthma, arthritis, cough, acne, skin diseases and even infertility. At high doses it acts on the CNS (Central Nervous System) as a
narcotic, but it is also nephrotoxic, causes allergies and in some cases can even be lethal (Feo, F.1997; Lucas, C.D.2001; Wuthrich, B.1997).

![Fig. 1](image1.png)

**Fig. 1**

![Fig. 2](image2.png)

**Fig. 2** R=R= gentiobiose

In the food industry saffron was of great importance both as a preservative and as a dye for food and beverages. Saffron is not only a spice, but also a very popular medicinal plant used in traditional medicine against cramps, asthma, and bronchospasm, menstrual disorders, liver disease and pain, with soothing and invigorating effect on the gastrointestinal tract. The application as a stimulant is very important, in fact it is aphrodisiac and antidepressant and it is even used in Indian Ayurvedic medicine (Wintherhalter, P.2000). Saffron is today mainly used in the food industry as a dye and as a spice, particular uses are in the confectionery and liqueurs. Its limited use is caused by the high cost determined by the complexity of the production process and for this reason is often adulterated with cheaper substitutes such as *Calendula officinalis*, *Carthamus tinctorius* and *Arnica montana* or with colored fibers by safranin or nitrophenols. The increased attention paid to the protection of human health has resulted in the return to natural products although more expensive and a renewed scientific interest in the study of the active ingredients they contain.

Modern pharmacological and clinical research in the field have confirmed many of the traditional knowledge related to the effects of saffron. Extracts of saffron containing crocin and crocetin may be useful in the treatment of neurodegenerative diseases and to improve the ability of learning and memory (Abe, K.2000); also improves the blood flow to the eye level facilitating the recovery of retinal function for which it could be used to
treat the ischemic retinopathy and/or macular degeneration associated with aging (Xuan,B.1999)

About ten years ago, antitumor effects have been highlighted on different types of cells: the viability of healthy cells has remained unchanged, while the saffron has demonstrated selective cytotoxic effects on malignant cells with effective doses in the micro molar (Abdullaev, F.I.2004; Abdullaev, F.I.2002). In literature very discordant investigation of the safety and toxicity of saffron are reported. While the toxic effects have been highlighted with a dose of 5 g and lethal effects with a dose of 20 g, in some cases, adverse effects such as vomiting, diarrhea, bleeding have been reported already with doses of 1.2 g, and abortive effect already at dose of 4 g (Lucas, C.D.2001; Wuthrich, B.1997).

Several extraction techniques and solvents are used for obtaining antioxidant extracts from plant sources (Ferrara, L.2002; Gallo, M.2010). Among these, the cyclically pressurized solid-liquid extraction with a Naviglio extractor (NE) is a relatively new method used for the extraction of natural products (Naviglio, D.2013). Such extraction is a process in which the pressure applied to the extracting liquid in which samples are immersed for hydration periodically varies during an established range of time. The process was performed at room temperature, and the energy cost was primarily due to liquid pressurization. The Naviglio extractor is formed by two extraction chambers, each consisting of a steel cylinder with a piston. Two porous septa that allow only the liquid phase to pass through are on the bottom of the chambers. The two extraction chambers are connected by a pipe with an electric valve that is closed during the extraction process and opened to evacuate the liquid from the system at the end of the cycle. When the maximum value of the programmed pressure is reached, it is maintained for a predetermined time (generally two minutes); in this manner, equilibrium between the solid and liquid matrices (static phase) is established. After this time (static phase), the pressurized air that acts on the pistons is quickly evacuated with a consequent pressure reduction, resulting in a negative pressure gradient between the inside and outside of the solid matrix (dynamic phase). During this phase, compounds that are not chemically bound to the solid matrix are physically extracted and transferred to the liquid phase, and the active ingredients are thus extracted (Naviglio, D.2003).

Summarizing, for the reasons stated above, the well-known beneficial effects of saffron, but the high costs of production, that led to several attempts at adulteration of this spice, can be interesting to have an exhaustive extraction method, but also fast and cheap. For this purpose, in this work two methods were compared, in order to evaluate the most efficient extraction method: an extraction technique utilizing conventional ultrasound and a
technique of solid-liquid extraction using the Naviglio extractor. Furthermore, to obtain data on bioactive compounds of saffron, the extracts were analyzed for their antioxidant activity.

Materials and Methods

Chemicals. All reagents and solvents HPLC grade were purchased from Merck (Darmstadt, Germany). Crocus sativus were bought in a local market from a specialized retailer of spices for human consumption. Each sample was contained in a small glass bottle sealed with a plastic screw cap. (S)-(−)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2’-azino-bis(3-ethyl-benzothiazoline-6’-sulfonic acid) diammonium salt (ABTS), gallic acid, potassium persulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride, Folin Ciocalteau, anhydrous sodium acetate, reagent and 2,4,6-tri(2-pyridyl-s-triazine) (TPTZ), were purchased from Sigma (Milan, Italy).

Ultrasound assisted extraction (UAE). Sample (1.00 g) was extracted with ethanol/water (50:50 v/v, 100 mL), the extractions were performed in a ultrasonic bath (Astrason the Heat System( Germany) with a working frequency of 33 KHz. The samples after sonication at room temperature for 30 minutes, were centrifuged to 4000 rpm, at 4 °C, filtered with filter paper and analyzed by mass spectrometry. The ultrasonic plant material extraction procedure was repeated three times.

Naviglio extractor (NE). Naviglio Extractor 500 mL model (Atlas Filtri Engineering, Padua, Italy), was used for the extractive process. The ethanol/water (50:50 v/v) extracts were obtained with the following conditions:

Sample weight: 5.00 grams of saffron threads were extracted with 500 mL ethanol/water (50:50v/v) in the Naviglio extractor according to a method previously reported in the literature (.Ferrara, L.2002). Briefly static phase: 2 min.; dynamic phase: 5 cycles with 12 sec. of stop piston (2 min.); total cycles: 30 (2 hours). After evaporation of the solvent was obtained 2480 mg of a residue, which was dissolved in methanol and analyzed.

LC/MS/MS analysis of bioactive compounds. Chromatographic separation was performed using an HPLC apparatus equipped with two Micropumps Series 200 (Perkin Elmer, Shelton, CT, USA), an UV/VIS series 200 (Perkin Elmer, Shelton, CT, USA) detector set at 280 nm and a Prodigy ODS3 100 A column (250 x 4.6 mm, particle size 5 Km) (Phenomenex, CA, USA). The eluents were: A water 0.2% formic acid; B acetonitrile/methanol (60:40 v/v). The gradient program was as follows: 20-30% B (six min), 30-40% B (10 min), 40-50% B (8 min), 50-90% B (eight min), 90-90% B (three min), 90-20% B (three min) at a constant flow of 0.8
mL/min. The LC flow was split and 0.2 mL/min was sent to the mass spectrometry. Injection volume was 20 mL.

MS and MS/MS analyses of extracts were performed on an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonSpray negative or positive ion mode depending on metabolites chemical structure, as shown in Table 1.

The analyses were performed using the following settings: drying gas (air) was heated to 400°C, capillary voltage (IS) was set to -4000 V in negative mode and +5500 V in positive mode, nebulizer gas (air) 12 (arbitrary units), curtain gas (N₂) 14 (arbitrary units), collision gas (N₂) 4 (arbitrary units). The declustering potential (DP), focus potential (FP) and the collision energy (CE) for standards were optimized for each compound infusing directly into the mass spectrometer standard solutions (10 µg/mL) at a constant flow rate of 8 µL/min using a model 11 syringe pump (Harvard Apparatus, Holliston, MA, USA).

To identify the metabolites, information dependent acquisition (IDA) was carried out using the range m/z 50-1100 with a cycle time of 0.5 sec and a step size of m/z 0.2. Identified compounds were than analyzed in MRM (multiple reaction monitoring).

**ABTS assay.** Antioxidant capacity assay was performed using an UV-VIS recording spectrophotometer (Shimadzu, Japan) by the improved ABTS. + method as described by Re et al (1999).

ABTS.⁺ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate after incubation at room temperature (23 °C) in dark for 16 h. The ABTS. solution was diluted with ethanol to an absorbance of 0.700 ± 0.050 at 734 nm. The filtered sample was diluted with 70% methanol so as to give 20–80% inhibition of the blank absorbance with 0.1 mL of sample.

ABTS.⁺ solution (1 mL, with absorbance of 0.700 ± 0.050) was added to the tested samples (0.1mL) and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 2.5 min and the absorbance was immediately recorded at 734 nm. Trolox standard solution (final concentration 0-15 µM) in methanol was prepared and assayed at the same conditions. The absorbance of the resulting oxidized solution was compared to that of the calibrated Trolox standard. Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, mmol Trolox equivalents per 100 g dry weight of plant (dw)). All determinations were performed in triplicate (n = 3).

**DPPH assay.** The DPPH radical-scavenging activity was determined using the method proposed by Yen and Chen (1995). DPPH (100 µM) was dissolved in pure ethanol (96%). The radical stock solution was prepared fresh daily. The DPPH solution (1 mL) was added to the polyphenol extract
(1 mL) with ethanol (3 mL). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm at 10 min. The results were corrected for dilution and expressed in μM Trolox per 100 g dry weight (dw). All determinations were performed in triplicate (n = 3).

**FRAP assay.** This assay was based on the reducing power of antioxidant compounds toward ferrie salt (Benzie, I.F.F. 1996). Antioxidants reduce the ferric ion (Fe$^{3+}$) to the ferrous ion (Fe$^{2+}$) and the latter forms a blue complex (Fe$^{2+}$/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 μM, pH 3.6), a solution of 10 μM TPTZ in 40 μM HCl, and 20 μM FeCl3 at 10:1:1 (v/v/v). The reagent (300 μL) and sample solutions (10 μL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of Trolox. All solutions were used on the day of preparation. The results were corrected for dilution and expressed in μM Trolox per 100 g dry weight (dw). All determinations were performed in triplicate (n = 3).

**Determination of total phenolics (Folin-Ciocalteu).** Total polyphenol content was measured using the Folin-Ciocalteu colorimetric method described previously by Gao et al (2000). Extracts (100 μL) were mixed with Folin-Ciocalteu reagent (0.2 mL) and H$_2$O (2 mL), and incubated at room temperature for 3 min. Following the addition of 20% sodium carbonate (1 mL) to the mixture, total polyphenols were determined after 1 h of incubation at room temperature. The absorbance of the resulting blue colour was measured at 765 nm with a UV-VIS spectrophotometer. Quantification was done with respect to the standard curve of gallic acid. The results were equivalents (GAE), milligrams per 100 g of dry weight (dw). All determinations were performed in triplicate (n = 3).

**Results and Discussion**

**LC/MS/MS analysis of Crocus sativus.** Bioactive compounds were identified by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). Antioxidants extracts were identified by IDA analysis that generate a survey scan, single MS spectra with molecular mass information, product ion spectra and extracted ion fragmentograms (XICs). Phenolic compounds were then identified comparing their molecular weight, the pattern of fragmentation and their retention time (RT) with standards or with data reported in literature. Successively, the extracts were analysed in MRM (Multiple Reaction Monitoring) in which are monitored the selected transition for each precursor ion selected. LC/MS/MS analysis were performed on ultrasonic and NE extracts (chromatograms not shown), but
significative differences in the metabolic profile of the two different extracts were not outlined.

In Table 1 are reported the LC/MS/MS characteristics of polyphenolic compounds identified in the hydroalcoholic extract of *Crocus sativus*. This analysis performed on conventional and NE not showed significant differences in the qualitative profile on two different extracts.

Tandem mass spectrometry analysis of *Crocus sativus* extracts showed the presence of metabolites belonging to the group of crocins, in particular, *trans* or *cis* crocetin di-(s-D gentibiosyl)ester, *trans* or *cis* crocetin (s-D-gluco(syl)-β-D-gentibiosyl) ester and *trans* crocetin di-(s-D glucosyl)ester. The isomers *trans* and *cis* were identified comparing the chromatographic profile and retention time with literature data (Caballero-Ortega, H.2007; Carmona,M.2007). According to Tarantilis et al. (1995), kaempferol diglucoside was identified in the extract at retention time of 14.72 min.

The peak at retention time of 12.71 min showed in IDA a precursor ion at *m/z* 515 [M+Na]+ and a fragment *m/z* 339. This peak was identified, according with Carmona et al.(2007), as 4-hydroxy-2,6,6-trimethyl-cyclohexen-1-carbaldehyde 4-O-β-D-gentibioside that is an aroma precursor and this hypothesis was confirmed by an increased UV absorption at 280 nm (UV chromatogram).

In *Crocus sativus* the bioactive compound is mainly represented by an unusual apocarotenoid, the crocetin, which is esterified with one or two sugar moieties of glucose, gentibiose or neapolitanose (crocins) (Fig. 2). The crocetins are water soluble, in contrast to most families of carotenoids. The bibliography on crocetin is abundant and their determination is reported by HPLC, by liquid chromatography coupled to mass spectrometry with electro-spray ionization LC-ESI-MS. In the literature, there are few studies reporting the use of mass spectrometry for the identification of the flavonoid fraction in saffron spice. Carmona et al.(2007) reported, for the first time, five kaempferol derivatives identified by LC-DAD-MS/MS-ESI, but a complete LC/MS/MS investigations of saffron bioactive metabolites was not described yet.

It is hoped that in the next few years the use of NE in analysis laboratories may increase, especially for the reasonable cost of the equipment. In addition, the results obtained may be useful for the efficient development of large-scale extractions of bioactive compounds from other vegetable matrices of food and pharmaceutical interest.

In this study, extraction procedures for total phenols with ultrasound assisted extraction (UAE) and cyclically pressurized solid-liquid extraction with the Naviglio extractor (NE) were investigated in order to exhaustively extract bioactive metabolites from saffron (*Crocus sativus*). Three
extractions were carried out with each method. After extraction, the sample was measured in a graduated cylinder and the volume recovered for each sample was recorded. The extracts were stored in the freezer under dark conditions before antioxidant analysis. The analysis of total polyphenols (Folin) of two extracts obtained suggested that the NE method was more effective compared to the ultrasound extraction method and it could be used as an effective method to extract antioxidant components considering factors such as the extraction time and the solvent wastage. Moreover, the extraction to NE showed a recovery of antioxidants of about 4 times higher for *Crocus sativus* (Table 2).

In particular, in hydroalcoholic ultrasonic extracts of *Crocus sativus* total antioxidant concentration was of 518 mg/100g on dry weight, this result was according with literature data which reported amount of polyphenolic compounds of 526 mg/100g on dry weight (Proestos, C.2005). The experiments were performed employing as extracting solvents 50% (v/v) aqueous EtOH, considering a recent paper present in literature (Martino, E.2006), which reports that the best extraction of phytocomponents from flowering tops of *Melilotus officinalis* was obtained employing 50% aqueous ethanol with ultrasound assisted extraction.

*Crocus sativus* was rich in phenolic constituents and demonstrated good antioxidant activity measured by different methods. The antioxidant activity was carried out using ABTS (Re, R.1999), DPPH (Yen, G.1995) and FRAP assay (Benzie, I.F.1996), which determined the disappearance of free radical solutions using a spectrophotometer. In particular, results from ABTS, DPPH and FRAP activity highlighted that the extracts obtained using NE were richer in antioxidant metabolites than those obtained by ultrasonic extraction, being a good source of antioxidants. The antioxidant activity evaluated with the ABTS test allowed to obtain the results reported in Table 3.

In particular, NE extract of saffron, showed an antioxidant effectiveness 20 times higher than the extract obtained with ultrasound assisted extraction considering the ABTS assay. In the case of DPPH, the values of antioxidant activity of *Crocus sativus* extracts obtained from the two extractive techniques are comparable (Table 3). The data obtained with FRAP assay are represented in Table 3.

The extracts analyzed showed an antioxidant activity highest for the NE extraction (260 mmoles trolox/100 g).

Results allow to claim that the NE extraction is a particularly advantageous technique for saffron hydroalcoholic extraction, which showed an antioxidant activity about 30 times higher than ultrasound assisted extract. In literature it was reported that saffron extract showed a total antioxidant activity calculated as sum of hydrophilic and lipophilic antioxidant activity
of about 73.94 mmoles of Fe+2/100 g dry weight (Pellegrini, N.2006). The results obtained for ultrasound extracts showed an antioxidant activity expressed as mmol of trolox/100g of dry matter of 8.55; this result allow to assert that saffron was the matrix better extracted using NE probably for its typical structure that enables an advantageus recovery of bioactive metabolites.

The control more and more careful on additives used in foods and in particular of artificial colorings has often highlighted the harmful effects that synthetic dyes have on our health. Accordingly, recently, it is promoting a return to the use of natural substances, voted more healthy, although if

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Retention time (min)</th>
<th>Precursor ion [M-H] m/z</th>
<th>Product ions m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaemperol-diglycoside</td>
<td>14.72</td>
<td>633</td>
<td>449 287</td>
</tr>
<tr>
<td>Trans crocetin di-(β-D gentibiosyl) ester (Crocin 4)</td>
<td>21.27</td>
<td>999</td>
<td>675 329 511</td>
</tr>
<tr>
<td>Trans crocetin (β-D-glucosyl)-(β-D-gentibiosyl) ester (Crocin 3)</td>
<td>23.95</td>
<td>837</td>
<td>675 329</td>
</tr>
<tr>
<td>Trans crocetin di (β-D glucosyl) ester (Crocin 2)</td>
<td>26.40</td>
<td>675</td>
<td>513</td>
</tr>
<tr>
<td>cis crocetin di-(β-D gentibiosyl) ester (Crocin 4)</td>
<td>29.19</td>
<td>999</td>
<td>675 329 511</td>
</tr>
<tr>
<td>cis crocetin (β-D glucosyl)-(β-D-gentibiosyl) estere (Crocin 3)</td>
<td>31.11</td>
<td>837</td>
<td>675 329</td>
</tr>
</tbody>
</table>

**Table 1:** LC/MS/MS characteristics of phenolic compounds identified in *Crocus sativus* extracts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant activity (ABTS) mmol Trolox/100 g</th>
<th>Antioxidant activity (DPPH) % inhibition</th>
<th>Antioxidant activity (FRAP) mmol Trolox/100 g</th>
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<tbody>
<tr>
<td></td>
<td>UAE</td>
<td>RSLDE</td>
<td>UAE</td>
</tr>
<tr>
<td>Saffron</td>
<td>0.042</td>
<td>0.858</td>
<td>14.692</td>
</tr>
</tbody>
</table>

**Table 2.** Total phenolic contents of saffron extracts obtained with ultrasound and RSLDE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant activity (ABTS) mmol Trolox/100 g</th>
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</tr>
</tbody>
</table>

**Table 3.** Antioxidant activity of saffron extracts obtained with UAE and RSLDE

**Conclusion**

The discovery of interesting pharmacological activities has given new impetus to the search for the coloring matter, although he revived some quantitative issues related to the extraction of the main components and their
subsequent determination. In this study two different methods for the bioactive metabolites extraction (ultrasound and solid-liquid extraction) were compared. The analysis of total polyphenols (Folin) of two extracts obtained suggested that the solid-liquid extraction was more convenient compared to the ultrasound extraction. The antioxidant activity measured with ABTS, DPPH and FRAP assays activity highlighted that the extracts obtained using Naviglio extractor were richer in antioxidant metabolites than those obtained by ultrasonic extraction, being a good source of antioxidants. Therefore, this extraction method allowed to obtain many advantages, especially related to the good reproducibility of the results with the ability to perform a standardization of the extraction method. In addition, compared with other methods, both cold and hot, it allowed a comprehensive extraction within a much reduced showing the best features of the extract. The active ingredients are not degraded and no leaks are volatile molecules as a result of the extraction technique in container tightly closed. Finally, an industrial application of this method would be particularly advantageous allowing to carry out extraction in a short time, with considerable reduction of production costs. Moreover, for their beneficial effects, the extracts of saffron may be used for the formulation of functional foods or phytodrugs to improve health.

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