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High Efficiency and New Potential of RSLDE: A Green Technique for the Extraction of Bioactive Molecules from Not Completely Exhausted Plant Biomass and Organic Industrial Processing Waste

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Abstract: A product is characterized by low environmental impact if, during the whole process (from extraction of raw materials from solid natural matter to disposal), its negative contribution to environment modification is significantly reduced or eliminated. According to circular economy, it is important to take into consideration other aspects, such as the possibility to improve the efficiency of extraction process by modifying the principle on which it is based and allowing the recovery of not completely exhausted waste, obtaining other active ingredients, and favoring the recycling of normally eliminated materials. The purpose of this work was to propose more efficient and greener alternatives to conventional solid–liquid extraction processes. Major features are the rapidity of the process, extraction at room temperature and high yields. Rapid Solid–Liquid Dynamic Extraction (RSLDE) represents an innovative solid–liquid extraction technology that allows the solid matrices containing extractable substances in an organic or inorganic solvent and their mixtures to be exhausted in shorter time than current techniques. The principle at the basis of this novel process consists of the generation of a negative pressure gradient between the inside and the outside of the solid matrix, which induces the extraction of compounds not chemically linked to the solid matter, being insoluble in the extractant liquid. Therefore, this work focuses on how RSLDE can potentially bring several improvements in the field of solid–liquid extraction, especially for industrial applications.

Keywords: solid–liquid extraction; RSLDE; bioactive compounds; waste; green techniques; antioxidant activity

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1. Introduction

In order to find the best operating parameters, solid–liquid extraction procedure is generally based on a trial and error technique. Nowadays, there are not universal models of this phenomenology that can anticipate the results and therefore the best a posteriori extraction conditions must be studied for each individual solid–liquid application. The most employed trial and error approach consists in the variation of one single operating parameter per time, such as the kind of natural matter, previous machination pretreatment, humidity of the material, extracting solvent, temperature, pressure and composition of the system. In order to create an operative map, each parameter needs to be varied by maintaining the other constant. In the literature, in fact, there are no cases of an extraction process that anticipates the results [1]. Rapid Solid–Liquid Dynamic Extraction (RSLDE) carries out an innovative solid–liquid extraction process, as it makes the phenomenon active.

Exploiting the pressure–depression effect, this process forces the release of substances that are not chemically linked to the main insoluble structure of solid matrix and accelerates the process [2].

Conventional techniques such as maceration and percolation or solid–liquid extraction occurs essentially for two effects: diffusion and osmosis [3,4]. For this reason, they are defined as passive processes, as it is necessary to wait for the slow diffusion times to complete the process. The increase in temperature and the occasional agitation of the extraction system are the only parameters that can speed up the process. Unfortunately, even if the increase of temperature helps to speed up diffusion (Fick's Law), on the other hand it degrades the molecules extracted from the plant matrices, which in most cases are thermolabile [5–7]. On the other hand, RSLDE works by alternating a static phase with a dynamic one [8,9]; during the static phase, the extracting solvent is brought to a pressure of about 9 bar and kept in this state for a time sufficient to reach an equilibrium between the pressure of the liquid outside the solid matrix and the liquid inside it (generally 1 to 3 min): the static phase is followed by the dynamic one, produced by the displacement of the pistons, which causes the pressure reduction [10]. At this point, the extraction of the solid matrix takes place, made possible by a pressure difference between the inside and outside of the sample. The extractable substances, at each extraction cycle, are dragged for a sucking effect and that is why this extraction process is considered "active". Furthermore, the dynamic phase (following static phase) allows the rapid and complete mixing of the solid matrix and an instant diffusion of the extracted substances throughout the mass of the liquid, avoiding oversaturation phenomena that can take place around solid matrix: a high concentration of extracted compounds brings a premature equilibrium that stops the extractive process.

This extraction technology represents a valid alternative to other currently existing solid–liquid extraction techniques and brings considerable innovations and advantages to obtain high-quality extracts. The first feature that makes RLSDE technique different from other techniques is the elimination of the necessity of heating the extraction system, as the action exerted is of a mechanical type. In comparison, current extraction techniques (percolation, Soxhlet, steam distillation, ultrasound) tend to increase temperature to improve extraction efficiency in order to enhance extraction yield, according to the well-known Fick's law [11]. Extraction by diffusion and osmosis are temperature-dependent phenomena. However, in case of thermolabile compounds, the increase in temperature may result in their degradation; therefore, the best operating conditions will be chosen by controlling the maximum temperature at which the bioactive compounds of the plant matrix will not be degraded.

The other advantage of RSLDE is linked to the scalability of the process; indeed, a lab-scale plant has first been developed, with an internal volume between 0.5 L to 2 L. In order to verify the replicability on a large scale, a pilot plant has been also built, whose mean volume is 40 L. Finally, the successful use of these two previous versions brought the possibility of providing an industrial scale layout, with a volume of 200 L.

Since the extraction action in RSLDE is of a mechanical type, a few extraction cycles (about twenty) of 2 h each could be enough to obtain high yields of extraction with any solid matrix that contains extractable materials. Therefore, this is the same assumption on which all the conventional extraction processes are based. Even if maceration is considered the official extraction method according to Pharmacopoeia guidelines for active principles from officinal plants [12], RSLDE is fast and exhaustive at the same time [13]. Moreover, RSLDE gives the possibility to carry out the aqueous extraction of vegetables, thanks to the reduced times, which is impossible to achieve by maceration because of the fermentation process that takes place more rapidly than the extraction of actives. As a very common example for this general assumption, we could consider the observation that common vegetables cannot be extracted only with water at room temperature and pressure. It is well-known that in these cases, it is necessary to add preservatives to the water in order to avoid fermentation or microbiological growth.

Moreover, RSLDE is an inexpensive technique and requires minimal energy expenditure when compared to other innovative extraction techniques, such as Supercritical Fluid Extraction (SFE) [14] or Accelerated Solvent Extraction (ASE) [15]. Therefore, RSLDE is a valid alternative to existing extraction techniques and finds applications in the extraction of active ingredients of different origins [16]. The fields of application of the RSLDE are diverse, such as the pharmaceutical, cosmetic, herbal, but also the food and beverage fields [17]. These applications can be realized on both laboratory and industrial scale due to the scalability of machineries. At an industrial level, the extraction is generally carried out using percolators, as an alternative to the slow process of maceration, that allows one to obtain a large amount of extract in a short time, using a mild heating (between 30 °C and 50 °C) of the system. Despite the use of percolation process and a higher temperature, the overall yield of the process is not optimal (<60%) [18,19], and the solid matrix is not completely exhausted. Therefore, the matrix can be considered as waste material that can be used as a by-product and extracted completely using RSLDE. In addition, the importance of obtaining extracts with non-degraded active ingredients lies in the fact that in this way, the pharmacological activity of the extracts themselves increases.

From this point of view, the main advantages of RSLDE are: (1) rapid exhaustion of solid matrices containing extractable substances in reduced times (hours *versus* days or week); (2) low operating temperatures (ambient or sub-ambient); (3) the possibility to concentrate the extractant liquid toward saturation by extracting among two and three times the amount of new aliquots of fresh vegetable with the same enriched liquid; (4) high reproducibility of the extraction, keeping the operating conditions unchanged. Because of this, the extracts are standardized for the content of active ingredients and these characteristics guarantee the production of quality extracts in reduced operating times (from 4 to 24 h).

In this work, a series of applications of RSLDE on various plant matrices are reported to evaluate the potential of this innovative technique through the reduction of extraction times, the increase in extraction yields, and the lower formation of waste products associated with a reduction of energy and economic expenditure. Therefore, RSLDE represents a powerful environmentally friendly technique since its principle of extraction is not based on osmosis and diffusion phenomena, but on the gradient of pressure created during static and dynamic phases of the process. Therefore, many processes of extraction that require organic solvents can be substituted by RSLDE for its lower environmental impact. Because of very short time of extraction, RSLDE can be used to treat new solid matrices, while rapidly concentrating the liquid extractant towards saturation, with a significantly reduced energy consumption.

2. Materials and Methods

2.1. Vegetable Matrices, Solvents and Reagents

All plant matrices were supplied by a local company (Polcaro Fitopreparazioni, Nola, Napoli). The reagents and solvents used were of analytical grade. Ethanol 96% (*v*/*v*) and Ethanol absolute were purchased from (Sigma Aldrich, Saint Louis, MO, USA). Folin–Ciocâlteu's reagent and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) were purchased from Sigma-Aldrich (Darmstadt, Germany). Sodium carbonate, citric acid and potassium sorbate were purchased from Carlo Erba (Milan, Italy) (Sigma Aldrich, Saint Louis, MO, USA). A synthetic dye named E124 was purchased by Sigma Aldrich (Saint Louis, MO, USA).

2.2. RSLDE versus Maceration

In order to evaluate its potential, RSLDE was compared with maceration, a conventional extraction technique still used both at laboratory and industrial scales. Maceration was performed at room pressure and temperature, in absence of light for a time included between 1 and 4 weeks, without a continuous stirring, with no particular limit in volume treatments.

Concerning RSLDE process, in detail, 130 g of sweet orange peel was extracted with 500 mL of hydroalcoholic solution (62%, v/v). In particular, the amount of material obtained over time using both extraction systems was evaluated in terms of polyphenol content and their antioxidant activity. Furthermore, again to compare the two methods, an extraction from the vanilla beans (120 g), a material very resistant to extraction, was carried out in a hydroalcoholic environment solution (65%, v/v) and subjected to the same tests carried out for the sweet orange peel. The functioning of the process is depicted in Figure 1.

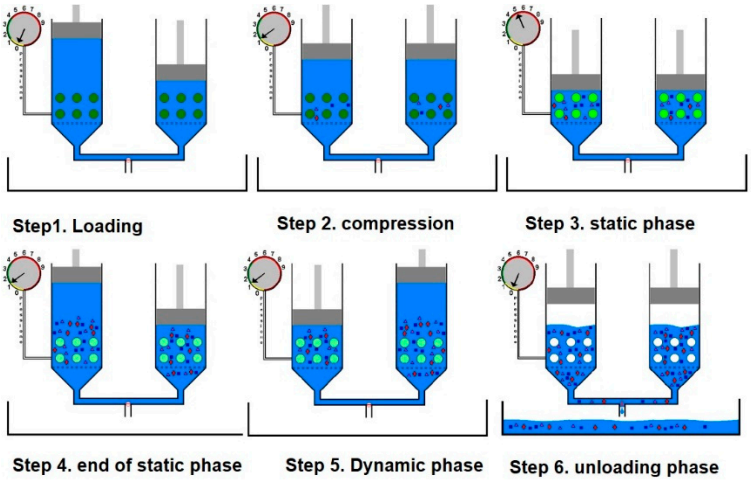


Figure 1. Description of the RSLDE process.

This process consists of 6 subsequent steps; the first one is the loading of the solid matrix into the extractive chambers. Then, after closing the vessel, the compression starts up to an average of 5 bar. Then, the static phase begins, consisting of leaving the system under pressure for 1–2 min. At the end of the static phase, the decompression starts, consisting of the dynamic phase, that allows the active principles extracted to diffuse across all the aqueous system. After repeating this static/dynamic cycles for several times, the unloading phase begins, discharging the liquid concentrated in the leached substances.

2.3. Determination of the Dry Residue

During the extraction phase, 10 mL of extract was withdrawn, at 2, 4, and 24 h respectively; subsequently, they were placed in an oven at 105 °C for 24 h, in order to evaporate the water liquid and determine the weight (on dry residue basis). This determination was repeated 3 times.

2.4. Folin-Ciocalteu Assay

The determination of the polyphenol content present in the extracts was determined by the Folin–Ciocalteu assay [20]. Briefly, 100 µL of sample was introduced into a 10 mL flask, after the addition of 500 µL of Folin–Ciocalteu reagent (Sigma Aldrich, Saint Louis, MO, USA) and 1.5 mL of sodium carbonate solution 20% (w/v). After an incubation period of 30 min in the dark at room temperature, the absorbance was measured at the wavelength of 760 nm using a spectrophotometer (SmartSpec 3000, Bio-Rad Laboratories, Inc. Hercules, CA, USA) and, to check the repeatability, with another spectrophotometer (V750, Jasko Manufacturing, Oklahoma City, OK, USA).

2.5. DPPH Assay

The evaluation of the antioxidant potential of the extracts against free radicals was determined by the 2,2-diphenyl-1-picrylidrazyl (DPPH) assay, using a stable colorimetric probe for the detection of free radical scavengers [21]. The procedure was performed using 100 µL of sample, reacted with 900 µL of 0.05% DPPH methanolic solution. After incubation in the dark for 30 min, measurements were carried out at a wavelength of 517 nm and the

results have been expressed as the percentage of antioxidant capacity. The color of the solution is inversely proportional to the antiradical power.

2.6. Extraction Kinetics for Efficiency and Yield of Extraction

To determine the total amount of extractable molecules in aqueous environment, an experimental protocol was carried out on a mix of officinal plants, such as chamomile (*Matricaria chamomilla* L.), valerian (*Valeriana officinalis*), rosehip (*Rosa canina* L.) and passion flower (*Passiflora incarnata*). This natural matter has been purchased from Acef (Piacenza, Italy) in dried and shredded form.

The procedure used is described as follows: 50 g of natural matter (solid mixture of 1:1:1:1 on mass basis) was subjected to a pre-maceration process for 10 min with 600 mL of stabilized water in a porous bag with the aim to hydrate dry material and to allow the liquid to completely fill the porous bag. Subsequently, the matrix and the solvent used for the pre-maceration were introduced into the RSLDE; the extraction, as described in Section 2.2, had a total processing time of 2 h. After this period, the solvent rich in active ingredients was recovered and an equal volume of new solvent was added. This sequence was repeated 5 times.

2.7. Reuse of Waste Material after Extraction of Peppermint Leaves

The use of peppermint (*Mentha piperita*) leaf extracts as a basis for the constitution of an *amaro* liquor is widely recognized in tradition [22]. However, after the traditional maceration extraction, the plant matrix still contains a large amount of active ingredients that can be extracted through a change of solvents and by using RSLDE. For this reason, a first extraction of 4 h to obtain the alcoholic extract of peppermint leaves was performed. Subsequently, the exhausted material was introduced into the RSLDE for 4 h with stabilized water, obtaining an aqueous extract.

2.8. Pre-Treatment of the Plant Matrix with 99.9° Ethanol

The experimentation campaign was carried out using Phlegraean mandarin (*Citrus reticulata*) peels for the production of Mandarinetto, a typical Italian liquor [23]. The botanical authority has been guaranteed by local producers that are traditionally owners of mandarin trees in the specific Phlegrean area (near Naples, Italy). Generally, in the production of liqueurs, an extraction of the plant matrix in 96% (v/v) alcohol is carried out. This allows one to extract essential oils, but also unwanted substances that can affect the quality and stability of the food product. To limit this problem, a purification pre-treatment of the plant matrix was carried out. In particular, 130 g of mandarin peels were treated using ethanol absolute 99.9° for 20 min using RSLDE, and the kinetics of dehydration of the peels were obtained through an alcoholic density meter (Density Meter, Notimin, China). This time was sufficient for pre-treating the plant matrix, limiting the loss of essential oils. After the first 20 min of pre-treatment, the extraction process began with 96° ethanol for the production of the traditional liqueur.

2.9. Extraction of Coloring Molecules from Vegetable Waste

During the production of herbal tea based on hibiscus flowers (*Hibiscus sabdariffa*), 50 g of dried flowers is generally used and left to soak for 30 min in 500 mL boiling water. This traditional process is effective, but it is characterized by several disadvantages such as the degradation of active molecules and the "cooking" of solid matrix that produces artefacts due to the effect of temperature [24]. By RSLDE, 50 g of dried flowers was extracted for 10 min in 500 mL of water at room temperature, allowing less degradation of the active molecules. After the aqueous extraction, the exhausted matrix was extracted again with ethanol 96% (v/v), for 2 h. This process allowed us to obtain only the coloring molecules creating an intense red extract. This liquid was concentrated by rotavapor (Buchi RII, Italy) at 40 °C under vacuum in about 1 h. The product could be used as a food coloring.

2.10. Gas-Chromatographic Analysis

GC analyses were performed on HP 5890 (Hewlett Packard-Agilent, Palo Alto, CA, USA) gas-chromatograph equipped by FID detector and a split/splitless inlet; the column was an Agilent J&W HP-5 (5%-phenyl methylpolysiloxane) nonpolar column, l = 30 m; i.d. 0.25 mm; f.t. 0.2 µm. Temperature program was 50 °C, hold for 3 min; increase 8 °C/min to 250 °C, hold for 5 min. Carrier gas: helium at flow 2 mL/min. FID temperature was 250 °C. Injected volume was 2 µL.

2.11. Statistical Analysis

The data were analyzed using multifactor Analysis of Variance (ANOVA). The experiments were repeated in triplicates and the results obtained were practically overlapped.

3. Results

3.1. Extraction of Sweet Orange Peel and Vanilla Pods

Figure 2 (black line) shows the extraction kinetics of sweet orange peels using RSLDE. As can be seen, after 24 h from the beginning of the RSLDE extraction process, a sample concentration equivalent to that extracted for 21 days by maceration is reached (Table 1). Similarly, the extraction in hydroalcoholic solution of the vanilla pods shows the same amount of material extracted in a time of 24 h by RSLDE compared to the 21 days of maceration (Figure 2). As it is possible to see, the points obtained for the two extractions (sweet orange in black and vanilla pods in red) are practically overlapped. This overlapping result is just a case, but defines clearly that the two natural matters are extracted with the same kinetics using RSLDE at defined operating conditions.

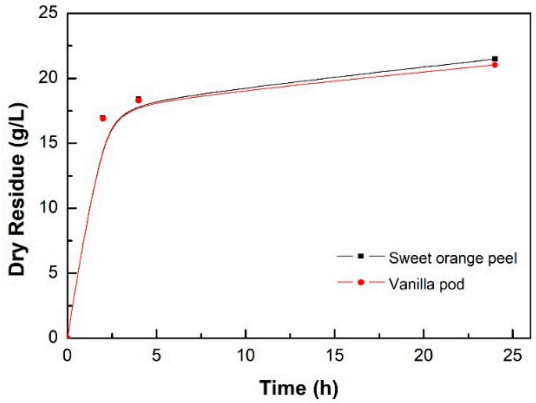


Figure 2. Extraction kinetics of sweet orange peels using RSLDE in 24 h (black) and extraction kinetics of vanilla pods using RSLDE in 24 h (red).

Table 1. Comparison of sweet orange peel and vanilla pod extraction by maceration and RSLDE.

Matter	Extraction	Dry Residue (g/L)	Polyphenol Content (g/L)	Antioxidant Capacity (%)
Sweet orange peel	Maceration (21 days)	27.9 ± 0.2	4.73 ± 0.51	75 ± 1
	RSLDE (24 h)	21.5 ± 0.3	3.99 ± 0.35	83 ± 1
Vanilla pod	Maceration (21 days)	26.8 ± 0.2	5.97 ± 0.65	62 ± 1
	RSLDE (24 h)	21.4 ± 0.3	4.97 ± 0.28	65± 1

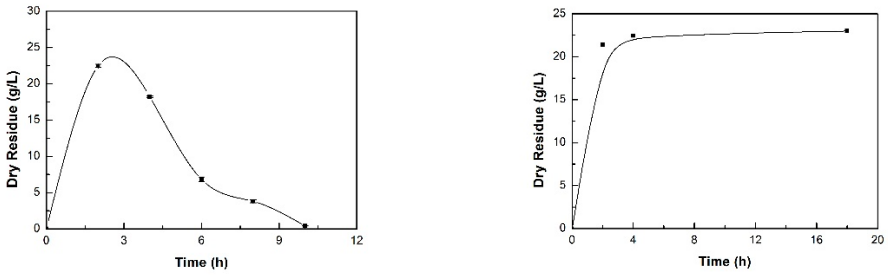
Table 1 shows the comparison of sweet orange peel extraction by maceration for 21 days and RSLDE. After 24 h, the extract obtained by using RSLDE is slightly defective (5.7%) compared to maceration but could be improved by squeezing the soaked plant material or by extending the extraction time. Furthermore, in Table 1, the values of the polyphenol content and the antioxidant capacity (DPPH) are reported. As it is possible to note, although it is higher in polyphenol content, the sample produced by maceration

shows a greater dispersion of the measure, which suggests a non-homogeneous distribution of polyphenols. Finally, the antioxidant capacity of the sample extracted by RSLDE is eight points higher than the sample extracted by maceration. This fact can be explained by reduced times of contact between solid and liquid in RSLDE, with respect to the longer time of maceration that can result in the degradation of solid matrix; this phenomenon can produce artefact compounds and colloidal solution.

As can be seen from Table 1, after 24 h, the extract obtained by using the RSLDE is slightly defective (5.82%). However, this amount could be increased by squeezing the soaked plant material, or by extending the extraction time to 30 h. Furthermore, in Table 1, the values of the polyphenol content and the antioxidant capacity (DPPH) are reported. As it is possible to notice, although it is higher in the polyphenol content, the sample produced by maceration shows a greater dispersion of the measure, which suggests a non-homogeneous distribution of the polyphenols [25]. Finally, the antioxidant capacity of the sample extracted by RSLDE is three points higher than the sample extracted by maceration; therefore, there is no great difference for this parameter.

3.2. Exhaustive Extraction of Plant Matrices

Based on the results of the extraction using RSLDE, it can be deduced that the quantity of extractable active ingredients for the mixture of officinal plants in an aqueous environment is 22.45 g/L. Comparing the graphs shown in Figure 3a,b it can be seen that the maximum concentration of solid matter extracted is obtained in the interval between 4 and 19 h with a maximum value of 22.95 g/L; moreover, the maximum theoretical value of the dry residue equal to 28.16 g/L provides a recovery rate of 81.5%. This result underlines how RSLDE is a very effective extraction method in recovering the total extractable substances in aqueous solution.



(**a**) (**b**)

Figure 3. Extraction kinetics of mixed plants using RSLDE in 5 process steps of 2 h each for a maximum of 10 h (**a**) and using RSLDE in 1 step of 20 h (**b**).

Figure 3a shows the kinetics of extraction performed after the first step of extraction using RSLDE, leaving an amount equal to 18.51% of active substances that were extractable with the following steps. The previous solvent was eliminated and substituted with the same volume of fresh one. This highlights that part of the exhausted material mixed with a percentage of fresh material could be reused for subsequent production, thus allowing a saving of raw material.

In order to study the obtaining of an exhaustive extraction of the bioactive compounds, the mixed plant matrix was subjected to five subsequent extractions by using water as extractant liquid. By sampling the extracts at regular time intervals and subjecting them to analysis, the full extraction kinetics at 10 h were obtained (see Figure 3a).

In order to demonstrate that the maximum peak is obtained in not more than 3 h of extraction (one to two steps), RSLDE was employed up to 20 h of operation, obtaining a maximum concentration almost similar to the one obtained at 3 h with the previous system. For this reason, it was demonstrated that it is practically useless to extract for more than 3 h, also from the energetic consumption.

The reported kinetics show both the total content of extractable molecules in aqueous environment and the content of polyphenols in the various extraction sequences. Table 2 summarizes the results obtained after 5 steps of extraction; as it is possible to see, there is still a polyphenol content equal to 1.25 (g/L) or 32.89% of the dry material extracted (3.8 g/L).

Table 2. Kinetics of exhaustive extraction from mix plant.

Stage of Extraction	Dry Residue (g/L)	Polyphenol Content (g/L)	Polyphenols on Dry Residue (%)
1st	22.5 ± 0.2	2.32 ± 0.51	10.3
2nd	18.2 ± 0.1	2.14 ± 0.33	11.8
3rd	6.8 ± 0.3	1.78 ± 0.15	26.1
4th	3.8 ± 0.2	1.25 ± 0.12	32.9
5th	0.4 ± 0.0	—	—

Furthermore, the exhausted mixed plant matrix has been subjected to a new extraction process by using ethanol as solvent to obtain a natural red dye, as reported later.

3.3. Reuse of Waste Material after Peppermint Extraction

Peppermint is widely recognized for its beneficial effects on the health of the body. These effects are mainly attributable to the presence of flavonoids and other antioxidants, in addition to the active ingredients contained in its essential oil, such as menthol and menton [26]. Peppermint has benefits both on the respiratory system and in counteracting the development of cancer [27].

Peppermint is widely used in the production of alcoholic extracts both for the production of menthol-rich candies and for the production of the mint liqueur [28,29].

Extraction of peppermint leaves via RSLDE performed in 4 h was compared to extraction performed in 30 days by maceration. From the comparison of the mass balances of the two extraction techniques shown Figure 4a,b, it can be seen that by means of RSLDE, about 27% greater amount of final extract is obtained than using the traditional maceration technique, prolonging also the operating times of extraction. RSLDE manages to improve the extraction, since it works dynamically with respect to the static operative environment of the maceration. This results in a reduced solid/liquid contact, favoring diffusion.

(**a**) (**b**)

Figure 4. Peppermint extract obtained using RSLDE in 4 h (**a**) and using the maceration method in 30 days (**b**).

After 4 h of extraction in an alcoholic environment, the exhausted material was extracted again by RSLDE using stabilized water with 0.15% w/v citric acid and 0.20% w/v potassium sorbate as solvent. Also in this case, the extraction lasted 4 h. The Folin–Ciolcateu test and the DPPH assay were carried out on the final extract. The results obtained with RSLDE showed a polyphenol content of 1.91 GAE/L and an antioxidant activity against free radicals of 95.5%. Despite the lower efficacy of the other traditional processes, it is necessary to consider that those methods do not completely exhaust the natural matrices deriving from several industries. Therefore, the recovery of these waste matrices can be

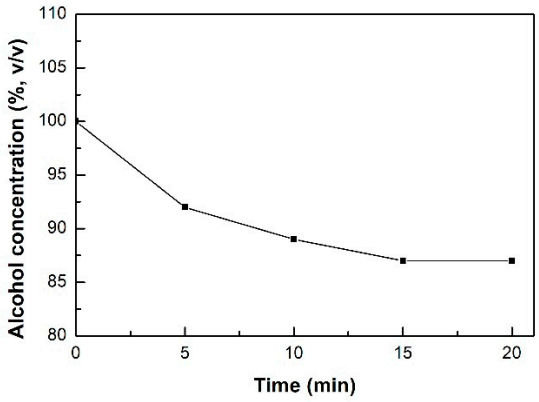
very advantageous as precursors for the production of new extracts, in accordance with the current principle of the circular economy of materials.

3.4. Pre-Purification Process of the Plant Matrix with 99.9° Ethanol

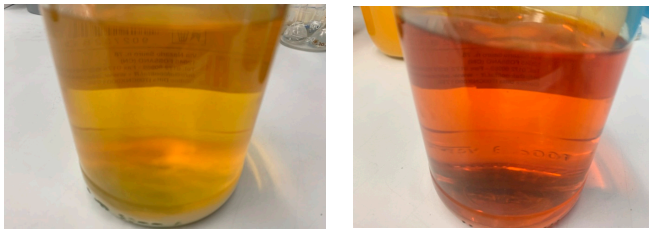
The pre-purification treatment was carried out by treating the mandarin peels in absolute ethyl alcohol 99.9° using RSLDE to improve the preparation of the Italian liqueur obtained from mandarin peels and named *Mandarinetto*. As known, the vegetable matrices are made up of a mixture of substances and have an average water content higher than 60%. This high water content is often an obstacle in the extraction phase process. Generally, according to the extraction methodology, materials are extracted from plants dried at temperatures above 50–60 °C. However, this causes degradation, especially of the thermolabile molecules. Therefore, to overcome this drawback, the vegetable matrices were dehydrated using absolute ethanol at room temperature.

The first step of extraction was performed using 130 g *mandarino* peels in 500 mL ethanol 99.9% *v*/*v* (as indicated in Methods). In particular, this process lasted 20 min; however, every 5 min, an aliquot of extract was withdrawn, distilled and measured from the alcohol content.

Figure 5a shows that alcohol content decreases due to the release of water molecules towards the extract until the alcohol content is lowered by about 13% in 20 min of RSLD treatment. The product has been withdrawn by an extractor and its visualization is depicted in Figure 5b, showing a white precipitate on the bottom. Then, the same *mandarino* peels have been subjected to a new step of extraction using 500 mL ethanol 96% *v*/*v* for 2 h. The visualization of the product is reported in Figure 5c. In particular, as it is possible to see, the appearance of the extract was clear in absence of any visible precipitates.



(**a**)



(**b**) (**c**)

Figure 5. Reduction in alcohol content (dehydration of solid matrix) due to the release of water molecules towards the extract (**a**); ethanolic extract of *mandarin* peels obtained by RSLDE (20 min) white precipitate visible at the bottom (**b**); absence of precipitate following pre-treatment with 99° ethyl alcohol (**c**).

A GC analysis was performed on the mandarin peels before treating them using RSLDE; the results reported in Figure 6 demonstrate that the pre-treatment did not affect

the content of essential oils significantly. Therefore, GC analysis assessed that the loss of essential oil compounds is negligible.

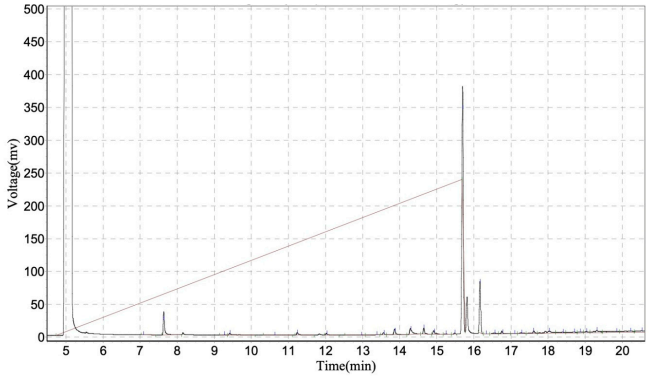


Figure 6. Gas-chromatogram of the extract of pre-treated mandarin peels.

3.5. Extraction of Coloring Substances from Vegetable Waste Matrix

Hibiscus flowers are commonly used for the production of Karkadè (as an experimental sample), an herbal tea rich in active ingredients, such as polyphenols and antioxidants, as well as a high content of vitamin C. The extraction process was conducted through the RSLDE at room temperature, thus not exposing the material to high temperatures (water boiling point) that occur using conventional techniques. The extraction time was set at 30 min. The extract has an intense red color and a high aromatic and fragrant power (Figure 7).

Figure 7. Extract of hibiscus flowers using RSLDE to obtain an experimental sample of Karkadè herbal tea.

After extraction, the exhausted matrix still has an intense red color that could be re-extracted with ethanol 99.9 absolute to obtain the pigmentation without extracting the aromatic molecules. The extraction was conducted for 2 h. The extract obtained was concentrated using a rotary evaporator to reduce the volume from 500 mL to 25 mL.

The dye obtained was compared with a commercial synthetic red dye (E124). In detail, 20 mL of distilled water was stained with two drops of synthetic dye and compared with 20 mL of distilled water stained with 500 µL of Karkade's concentrate. The colors are clearly visible in the solutions shown in Figure 8.

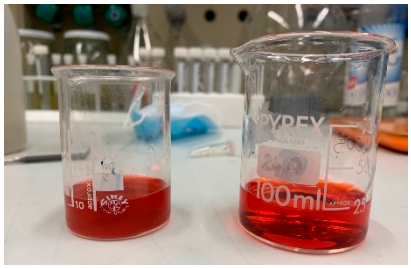


Figure 8. Comparison of the staining of a solution with artificial dye (**left**) and with Karkadè extract (**right**).

4. Discussion

In summary, the analysis of the results described above highlights some important aspects of RSLDE that improve the features of conventional solid–liquid extraction techniques:

1. The comparison of the extraction kinetics of different vegetable matrices by maceration and RSLDE showed that RSLDE allows obtaining, in just 72 h, more than 50% of product compared to what is obtained with maceration in two months.
2. The analysis of the kinetic curves shows that the maximum extraction yield occurs in just 12/24 h for the majority of vegetables compared to that obtained from maceration. It could be objected that the extract obtained using NE is slightly defective (5%), but this figure could be improved by squeezing the soaked plant material, or by lengthening the extraction time. Furthermore, the antioxidant capacity of the sample obtained by RSLDE is eight points higher than the sample extracted by maceration.
3. The study carried out on three types of extractive mixtures highlights the speed of RSLDE, as the maximum extractive yield of the products was reached in a maximum of 16 h. Due to its characteristics, RSLDE can be used in various sectors, such as pharmaceuticals, cosmetics, herbs, and food and beverages [17]. In the cosmetic sector, it is possible to produce extracts from vegetable matrices that contain pigments and odorous substances for the production and formulation of creams and perfumes [30,31]. In this context, a further resource is represented by the possibility of using the processing waste of vegetable matrices such as grape skins, rich in polyphenols, which can be easily reused in many different ways through RSLDE without adding any organic solvent or by applying heating or cooling processes that can cause loss of substances of interest [32,33].

In the herbal sector, RSLDE can be used to extract fluid bioactive molecules useful for herbal teas and/or infusions from plants and officinal herbs and for the production of liqueurs and elixirs. Running the process at room temperature keeps the active ingredients unaltered. Comparing the product obtained using RSLDE with that derived from the traditionally used method, namely maceration, the organoleptic tests performed show that the bitters produced with RSLDE were more appreciated than the bitters prepared by maceration [34].

The same comparison was made for the preparation of lemon liqueur (*limoncello*), a typical liqueur of Campania, famous all over the world, which is made with lemon peels macerated in ethyl alcohol. In fact, the use of RSLDE makes it possible to produce limoncello in just 2 h, avoiding the long traditional maceration that takes 7–14 days [35,36]. Furthermore, the efficiency of RSLDE was compared with another extraction technique that uses supercritical fluids (SFE), to obtain the extraction of acid compounds contained in hop flowers [37]. However, the sector where RSLDE is increasingly being applied is the food sector [17]. The previously cited work by Naviglio et al. [37] reports a cyclically pressurized soaking process for the rapid hydration of cannellini beans at room temperature. The hydration process using RSLDE is approximately ten times faster than the traditional soaking procedure and the microbial load developed at the end of the process is much

lower than that obtained using the traditional process [38]. Another sector where RSLDE finds application is in the recovery of processing waste, such as agri-food waste products, which can be important sources of bioactive compounds. Several studies have shown that tomato by-products contain significant amounts of bioactive phytochemicals, including lycopene, which when extracted from tomato peel could be used as natural antioxidants for the formulation of functional foods or as additives in food systems to extend their service life. RSLDE has been used to extract lycopene from tomato peel waste [39]. Another application of RSLDE in waste recovery concerns the extraction of bioactive compounds from the shells of sea urchins *Paracentrotus lividus* [40].

All plants, through their metabolic processes, elaborate and accumulate more or less complex chemical substances that are defined as "secondary metabolites" and have different functions for vegetables: essential oils, volatile compounds, and so on. Among these, those with a positive physiological action on the human body are defined as active ingredients, bioactives or phytocompounds. To obtain these phytocompounds, it is necessary to choose the most suitable extraction procedure and the best extractive conditions. As previously mentioned, currently, there are conventional and innovative extraction methods [41,42]. The first ones can be defined as more traditional methods with typical disadvantages such as the need for high amounts of solvent, low extraction selectivity, the need for long process times and thermal decomposition of thermolabile compounds (in case that high temperatures are required). As for the innovative extraction techniques, introduced relatively recently in the industrial field, these have numerous advantages, such as the reduction or elimination of the solvent, the reduction of energy consumption, the satisfaction of legal requirements in emissions, greater safety and control of the extraction technique, cost reduction and improvement of the quality and functionality of the entire process [43]. Among the innovative techniques, the RSLDE used in this work allows for extraction at room or lower temperatures, by exploiting the increase of the operating pressure due to the extracting liquid acting on the solid matrix. The principle on which the RSLDE is based, in addition to making the extraction process fast and efficient, makes the RSLDE a valid alternative to the innovative extraction processes currently existing [2].

In a global context in which the concept of productivity and income often conflicts with environmental sustainability, the use of new technologies more performant and environmentally friendly can resolve this dualism. According to our results, RSLDE could be considered as a valid alternative to conventional solid–liquid extractive processes such as maceration and percolation for the simplicity of its use, the low operative costs and for low environmental impact.

5. Conclusions

An extraction system is exhaustive when the right combination of three important elements is obtained: adequate preparation of the sample, suitable extraction solvent and efficient extraction method. Clearly, these criteria apply to both conventional and innovative solid–liquid extraction techniques. In addition, to date, great interest is directed towards innovative extraction techniques which aim to reduce extraction times, the consumption of organic solvents, improve extraction efficiency and reduce costs. Based on the results obtained in this work, RSLDE meets all these requirements.

In fact, this technique offers considerable advantages, such as short extraction times, higher production yield, low environmental impact and energy savings. Among other things, in addition to being efficient in different areas of application, RSLDE can be used to extract bioactive compounds also from agro-food waste and from not completely exhausted plants, favoring the recovery of these by-products in compliance with the current concept of circular economy. Furthermore, exhausted material obtained at the end of the extractive process can be disposed as manure, animal feed or as biomass.

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