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REMOVAL OF TOXIC COMPOUNDS FROM NATURAL EXTRACTS USING GREEN TECHNIQUES

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Abstract: The aim of the present study was compare two natural bio adsorbent materials with different commercial adsorbents, such as Granular Activated Carbon (CAG) and Zeolite, for the elimination of pesticides in extracts of interest. Two natural bio adsorbents were developed from rice husk and almond skin. Artichoke and lemon extracts were obtained from artichoke by-product and citrus by-product respectively. The trend is supported by the potential of these food wastes, which could be used for the production of valued products. In fact, peels, seeds, stems, or vegetable pulps are considered raw materials to obtain bioactive ingredients with multiple applications, mainly for the production of food ingredients, cosmetics, or nutraceuticals. Furthermore, bioactive compounds of vegetable wastes have demonstrated antioxidant and antimicrobial actions. The results obtained show the effectiveness of the different adsorbents in the adsorption of pesticides that artichoke and lemon extracts contain. Adsorbents from almond and rice by-product were effective, reducing in case of almond skin adsorbent by half the concentration of Metalaxyl and reducing a large amount of Azoxystrobin and Difenoconazole. Rice bio-adsorbent was less effective than the other adsorbates even though it was more effective than zeolite. The use of agri-food waste allows solving not only an environmental problem of pollution of high importance, but also a problem of management of large amounts of waste generated in the agri-food industry contributing to the promotion of circular economy. Two adsorbents have been developed from almond and rice by-products which are effective for the elimination of different toxic compounds through adsorption processes such as pesticides.

Keywords: *Pesticides, green tehcniques, adsorbents, natural extracts, organic compound.*

1. Introduction

Nowadays, consumption of fruit and vegetable is very widespread as they provide health benefits. These benefits are partly due to the organic compounds that products contain. These bioactive molecules have antioxidant, antithrombotic, anti-inflammatory, and antidiabetic properties, among others [1–4]. Artichoke (Cynara scolymus L.) is an herbaceous perennial plant belonging to Cynara genus and Asteraceae family. Commonly known as globe artichoke, it is traditionally consumed in the Mediterranean diet. Their cultivation is considered an important activity of the agro-economy of the semediterranean countries [5,6]. The Mediterranean region has an annual production of about 770,000 tons. The edible portion of this plant includes the receptacle of immature flowers and the inner bracts, named “capitula” or heads. During the artichoke processing, the

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residues, principally external leaves or stems, represent approximately 60–80% of the total harvested plant material, which is translated in more than 460,000 tons of wastes generated annually. Nevertheless, these by-products possess a great content of bioactive phenolic compounds, inulin, fibers, and minerals [7–11]. This information highlighted the significance of the evaluation of artichoke wastes for the extraction of bioactive compounds.

Despite its content in these interesting compounds, it is the presence of bioactive compounds that has aroused greater interest, especially phenolic compounds [12, 13]. The interest in their phytochemicals has been linked to various pharmacological activities exerted on humans. Therefore, the artichoke has been used for medicinal purposes since antiquity, being considered a functional food [14, 15, 16, and 17]. Consequently, the re-valorization of food by-products as a bioactive source material has experienced a great growth due to the economic and environmental benefits that it produces. In the case of artichoke, recent studies based their research on external bracts, leaves and floral stems by-products, which are considered the principal discarding parts of the artichoke processing because they are not suitable for human consumption [18].

The processing of citrus fruits in the agro-industry has different phases from the collection of raw materials to obtaining the desired products. During this process, a significant amount of waste is generated.

The nature of the mentioned waste is low-quality fruits and, especially, parts of the fruits without commercial value (crust) removed during the transformation process. The huge volume of citrus waste is not been managed and eliminated using advanced scientific and technical approaches, but it is mainly done in an inadequate way implying negative environmental effects.

Discarded parts of citrus fruits have a very high content of cell wall polysaccharides that are source of dietary fiber and constitute up to now the only raw material for the production of pectin used by the food industry as stabilizers or gelling agents.

This trend is supported by the potential of these food wastes, which could be used for the production of valued products. In fact, peels, seeds, stems, or vegetable pulps are considered raw materials to obtain bioactive ingredients with multiple applications, mainly for the production of food ingredients, cosmetics, or nutraceuticals. Furthermore, bioactive compounds of vegetable wastes have demonstrated antioxidant and antimicrobial actions in developed food additives used for conservative purposes

The current need of a sustainable food chain demands an implementation of a circular economy approach in the processing industries. The major focus of this approach is to revalorize the discarding parts of vegetables due to their great contents in bioactive compounds [19, 20].

The pesticide-based treatments that are applied to fruit and vegetables are present in the by-products generated after industrial processing. These by-products that are currently used for their revaluation through the extraction of compounds of interest give rise to the dragging of these pesticides and other toxic substances such as heavy metals and mycotoxins, concentrated in the extracts, making their use as ingredients in the food, nutraceutical and cosmetic industry unfeasible because these substances are potentially toxic to humans being related to various diseases including cancer and decreased fertility, among others [21-22].

The use and extraction of compounds of interest from fruit and vegetable waste reduces the risk of accumulation of waste in the environment, preventing the negative effect on the environment of waste treatment such as incineration or landfilling, as well as the cost and investment of labor in the process.

The adsorption of toxic compounds on the surface of adsorbent materials is presented as a promising, effective, sustainable and low-cost measure, compared to other types of treatments, looking for low-cost and environmentally friendly adsorbents obtained from biomass. [23-25].

The possibility of using agri-food waste as potential precursors for the preparation of adsorbents provides the opportunity to have a wide range of adsorbents, because they are generated on a large scale and have a low cost compared to commercial adsorbents.

The use of bio-adsorbents allows solving not only an environmental problem of pollution of high importance, but also a problem of management of large amounts of waste generated in the agri-food industry contributing to the promotion of circular economy. Therefore, in this study, bio-adsorbents have been developed from agri-food residues for the elimination of pesticides in extracts containing compounds of interest and the result of adsorption has been compared with commercial adsorbates such as Granular Activated Carbon (GAC) and zeolite.

The selection of appropriate substrates in adsorption systems for the decontamination of pesticides from aqueous streams is of utmost importance. Drobeket al. studied a natural adsorber from rice by-product showing that it has a significantly high potential to adsorb the pesticides atrazine and imidacloprid. Al-Qodah et al. carried out the adsorption of the pesticides Deltamethrin and Lambda-Cyhalothrin using ash from shale oil as adsorbent, proving to be very effective and very low cost, compared to other metallic adsorbers and activated carbon.

Currently, the scientific community focuses on adsorption as a promising, effective, sustainable and low-cost measure, compared to other types of treatment, seeking low-cost and environmentally friendly adsorbents obtained from biomass [28-30]. Gupta et al publish a study that reveals an alternative approach to the management of fruit residues with the production of value-added products and their use of residues as a bio adsorbent.

The aim of the present study was comparenatural bio adsorbent materials with different commercial adsorbents, such as Granular Activated Carbon (CAG) and Zeolite, for the elimination of pesticides in extracts of interest.

2. Materials and methods

Adsorbent materials

Adsorption experiments were performed with granular activated carbon adsorbent, with granular size range 0,5-3,13 mm, and zeolite adsorbent with granular size range 0,5-3,13 mm,were supplied by Panreacapplichem.

Production of different bio-adsorbent materials

Two bio-adsorbent were performed in adsorption experiments. The natural adsorbent of almond by-product and rice by-product were activated by treating almond skin and rice husk through a process of washing, and activation with solution of 2% of H_2O_2thus obtaining two natural adsorbents.

Extracts of interest

Extracts of interest were prepared as follows: artichoke or lemon by product were mixed with wáter 1:1 proportion. The mixture was heated at 100ºC and have been kept in constant agitation for 1 hours. The different mixture have been filtered, the treated aqueous extract has been frozen and dehydrated through a lyophilization process.

Use of adsorbent in the food laboratory scale system

200 grams of the different adsorbates (CAG, Zeolite, almond and rice adsorbent) have been mixed with 1 liter of the aqueous extract of artichoke orlemon extract and have been kept in constant agitation for 3 hours.

After 3 hours, the different adsorbates have been filtered, the treated aqueous extract has been frozen and dehydrated through a lyophilization process.

HPLC Analysis

The determination of pesticides were carried out by the liquid chromatography technique coupled to mass spectrometry with a triple quadrupole LC-MS / MS QQQ analyzer (QuEChERS method). The application range is established between the limit of quantification (0.01 mg / Kg) and the maximum limit (2 mg / Kg). For pesticides with MRLs greater than 2 and less than or equal to 6 mg / Kg, the maximum limit will be 7 mg / Kg.

This procedure is based on the SANCO Document "Guidance document on analytical quality control and validation procedures for pesticides residues analysis in food and feed", standard UNE-EN 15662, UNE-EN 12393-1 / 3 (61,62) and Directive 96 / 23 / EC (63). Two stock solutions of each of the specified pesticides will be prepared from commercial certified active ingredients coded by the laboratory, using acetonitrile as solvent.

An internal standard (ISTD), Triphenyl phosphate, will be prepared from its commercial certified active material, also coded by the laboratory, using acetonitrile as a solvent in the same way. From this stock standard, a 1 ppm concentration is prepared, which will be the one added to each of the dilutions of the calibration line, to have a constant ISTD concentration of 0.05 ppm in each of the points on the line.

From said stock solutions, two multipatterns will be prepared in acetonitrile, the analytes of which will be found at a concentration of 1 ppm.

With one of the multipatterns, dilutions with white matrix extracted in acetonitrile will be prepared, from which we will obtain the calibration line.

The response factor (FR) of each point of the function will be calculated, according to the following expression:

$$FR=(A\_pesticide/AISTD)/(C\_pesticide/CISTD)$$

Next, the mean and coefficient of variation of the response factors obtained will be calculated, which must be ≤ 20%.

Both the calibration line and the correlation coefficient, r, the FR, mean and coefficient of variation of said response factors are calculated by the Agilent Mass Hunter Workstation software.

The minimum quantity of sample necessary to carry out the test will be 250g.

The concentrations of the pesticides in the test sample will be calculated by introducing the ratio of areas between the active matter and the ISTD detected in the sample, in the calibration line. This process is carried out automatically by the equipment software, Agilent Mass Hunter Workstation.

Statistical analysis

All determinations and experiments were performed in triplicate andthe presented results are the average values of three determinations and standard deviation

3. Results and Discussion

Content of pesticides and organic compounds of interest in artichoke extract and lemon extract

Table 1 presents thevalues of pesticides and organic compounds obtained in artichoke extracts.

Table 1.

Content of pesticides and organic compounds of interest in artichoke. Results are expressed as mean values of triplicates ± S.D.

Compounds of interest (mg/kg)	
Chlorogenic acid	25359 ± 378
Cynarine	19232 ± 138
Pesticides (mg/kg)	
Azoxystrobin	0.42 ± 0.01
Difenoconazole	12.2 ± 0.1
Dimethoate	2.8 ± 0.1
Metalaxyl	59.5 ± 1.6

Table 1 shows that the artichoke extract has compounds of interest such as Caffeic Acid, Chlorogenic Acid and Cynarin.With respect to its pesticide content, the presence of azoxystrobin, difenoconazole, metalaxyl and dimethoate were detected.

Table 2 presents the values of pesticides and organic compounds obtained in lemon extracts. Table 2 shows that the lemon extract has compounds of interest such as Hesperidin and Limonin.With respect toits pesticide content, the presence of Imazalil and Pyrimetanilwere detected.

Table 2.

Content of pesticides and organic compounds of interest in lemon extract. Results are expressed as mean values of triplicates ± S.D.

Compounds of interest (mg/kg)	
Hesperidin	16478 ± 178
Lemonin	2110 ± 44
Pesticides (mg/kg)	
Imazalil	11.2 ± 0.5
Pyrimetanil	1.3 ± 0.1

Adsorption process

In order to comparethe effectiveness of the different adsorbents, artichoke and lemon extract were treated with bio adsorbents, zeolite andactivated carbon.

After performing the adsorption process for the reduction of pesticides with the different adsorbates using the procedure described above, different pesticides and compounds of interest were analized.

Table 3 shows the values of pesticides and organic compounds of interest in artichoke extract after adsorption process with bio-adsorbents, activated carbon and zeolite adsorbents.

Table 3.

Content of pesticides and organic compounds of interest in artichoke Results are expressed as mean values of triplicates ± S.D.

	No treatment	Zeolite	GAC	Adsorbent Rice	Adsorbent Almond
Determination of Compounds Interest (mg/kg)					
Chlorogenic acid	25359 ± 378	22947 ± 316	18566 ± 122	24186 ± 178	21512 ± 164
Cynarine	19232 ± 138	18394 ± 101	15081± 92	16654 ± 133	14914 ± 145
Pesticide determination (mg/kg)					
Azoxystrobin	0.42 ± 0.01	0.32 ± 0.04	0.029 ± 0.01	0.53 ± 0.09	0.12 ± 0.03
Difenoconazole	12.2 ± 0.1	10.5 ± 0.8	0.44 ± 0.11	6.7 ± 0.2	1.5 ± 0.1
Dimethoate	2.8 ± 0.1	2.8 ± 0.1	1.1 ± 0.2	2.4 ± 0.3	2.2 ± 0.1
Metalaxyl	59.5 ± 1.6	46.1 ± 1.3	2.5 ± 0.3	50.0 ± 2.5	25.4 ± 2.1

Granular activated carbon was the most efficient adsorbate.Adsorbents from almond and rice by-product were effective. reducing in case of almond skin adsorbent by half the concentration of Metalaxyl and reducing a large amount of Azoxystrobin and Difenoconazole. Rice bio-adsorbent was less effective than the other adsorbates even though it was more effective than zeolite. partly reducing Difenoconazole and Metalaxyl. The artichoke extracts obtained preserved the compounds of interest in similar concentrations and pesticides were greatly reduced.

Table 4 shows the values of pesticides and organic compounds of interest in lemon extract after adsorption process with bio-adsorbents. activated carbon and zeolite adsorbents.

Table 4.

Content of pesticides and organic compounds of interest in lemon extract. Results are expressed as mean values of triplicates ± S.D.

	No treatment	Zeolite	GAC	Adsorbent Rice	Adsorbent Almond
Determination of Compounds Interest (mg/kg)					
Hesperidin	16478 ± 178	15020 ± 125	16909 ± 82	15156 ± 96	16703± 120
Limonin	2110 ± 44	1505 ± 38	958 ± 28	1486 ± 31	461 ± 11
Pesticide determination (mg/kg)					
Imazalil	11.2 ± 0.5	5.9 ± 0.3	8.9 ± 0.2	6.8 ± 0.2	-
Pyrimetanil	1.3 ± 0.1	1.5 ± 0.2	0.48 ± 0.03	1.0 ± 0.1	0.24 ± 0.06

Almond bio-adsorbent was the most effective in completely eliminating Imazalil and greatly reduced Pyrimetanil. With respect to the other adsorbents used greatly reduced pesticides. The lemon extracts obtained preserved the compounds of interest in similar concentrations.

4. Conclusion

The use of agri-food waste allows solving not only an environmental problem of pollution of high importance. but also a problem of management of large amounts of waste generated in the agri-food industry contributing to the promotion of circular economy. In addition. the elimination of toxic compounds in extracts with large concentrations of compounds of interest is one of the key points for the use of these extracts in the food. nutraceutical and cosmetic industry due to the toxicity of these compounds to health. Two adsorbents have been developed from almond and rice by-products which are effective for the elimination of different toxic compounds through adsorption processes such as pesticides. recovering extracts containing compounds of interest by reducing the concentration of these toxic compounds.

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