ULTRASOUND-ASSISTED EXTRACTION OF COMPOUNDS FROM FOODS

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ABSTRACT

The optimization process of an ultrasound assisted extraction method for compounds in solid food samples is shown. A fractional factorial experimental design allowed for the determination

of the effects of several extraction variables. Relationships between all variables were examined. By applying graphical analysis, the best extractions conditions were obtained. The most important variables were the extracting liquid and the extraction temperature. Later, a central composite design was applied for optimizing temperature and the composition of the extracting liquid.

The optimized method was applied to grapes and to winemaking by-products, as well as to soy beans.

Introduction

Ultrasound-assisted extraction (UAE) can be used for extraction methods with liquid solvents applied to analytes in solid matrices. This extraction process is fast in comparison with the traditional methods, because of the contact surface area between solid and liquid phase is much greater, due to particle disruption taking place.[1]

The application of UAE to plants has produced very interesting results [2], to the extent that industrial processing has been proposed for obtaining compounds with pharmacological properties.[3]

In this paper the UAE of different compounds presents in two solid food samples is shown. Tartaric and malic acids in grapes as well as isoflavones in soy beans were analyzed by HPLC after the extraction.

Experimental

Samples

Red grapes of the Napoleon variety and grape seeds from grapes of the white Palomino Fino variety, obtained from winemaking byproducts, were used.

Commercial soy beans were used as starting material for isoflavones analyses.

Around 1 g of solid grapes or 0.5 g of soy beans were used in the extractions. All samples were freeze-dried before the extraction in order to increase sensitivity of the analysis and because of different grape seeds could have different moisture.

Extraction

A high intensity probe ultrasound generation system of 200 W, 24 kHz was used. The instrument was a model UP 200S from dr.Hielscher GmbH (Teltow, Germany). Its amplitude controller allows the ultrasonic vibrations at the probe microtip to be set at any desired level in the 10-100% range of the nominal power. Also the cycle controller allows the duration of the application of the ultrasound to be set, to a fraction of a second in the 0.1-1.0 range.

Results and discussion

Extraction variables

The experimental design was applied only to grape seeds and the fine tuning of the extraction method was developed for both grape seeds and whole grapes separately.

A fractional factorial experimental design was carried out in order to determine the more significant variables for the extraction process. The experimental conditions and the concentrations of tartaric acid and malic acid found in the extracts are shown in Table 1. It also shows the concentrations of tartaric and malic acid found in the extracts obtained. All the concentrations are shown relative to the amount found using the most effective conditions (100%).

Analyzing the main effect plots, it can be concluded that the more significant variables for the extraction process are temperature and the solvent used as extracting liquid. It can be seen that the higher the temperature, the higher the recovery. This effect is much higher for malic acid than for tartaric acid.

Table 1. Extraction conditions in the fractional factorial experimental design.

experiment	temp	solvent	vol	time	probe	amplitude	cycle	Tartaric Acid (%)	Malic Acid (%)
1	20	methanol	25	5	2	30	0.2	3.5	28.7
2	50	water	25	5	2	30	0.8	75.6	57.0
3	20	water	100	5	2	70	0.2	53.6	9.8
4	50	methanol	100	5	2	70	0.8	1.0	38.4
5	20	water	25	15	2	70	0.8	84.5	51.5
6	50	methanol	25	15	2	70	0.2	5.0	40.3
7	20	methanol	100	15	2	30	0.8	0.0	16.0
8	50	water	100	15	2	30	0.2	100.0	43.2
9	20	methanol	25	5	7	70	0.8	6.0	30.7
10	50	water	25	5	7	70	0.2	44.7	26.5
11	20	water	100	5	7	30	0.8	44.7	3.4
12	50	methanol	100	5	7	30	0.2	17.8	100.0
13	20	water	25	15	7	30	0.2	40.3	24.5
14	50	methanol	25	15	7	30	0.8	1.0	7.4
15	20	methanol	100	15	7	70	0.2	2.2	1.6
16	50	water	100	15	7	70	0.8	46.4	1.7

Temp: temperature (°C), vol: volume of extracting liquid (mL), time: time (min), probe: diameter of probe used (mm), solvent: extracting solvent, amplitude: amplitude of ultrasounds (% of maximum ultrasonic power), cycle: pulse of ultrasound in fractions of second.

From the graphical analysis, it can be concluded that the best conditions for extracting the two acids are: 100 mL of extracting liquid, rather than 25 mL; a thin probe (2 mm) rather than a thick probe (7 mm); 30% of amplitude and 0.2 seconds of cycle time, rather than 70% and 0.8 seconds, respectively.

Optimization of extraction time

The extraction time must be adjusted to obtain quantitative recoveries of both acids. To determine the time needed, different extractions were done using increasing extraction times to establish the kinetic of the extraction.

Both grape seed and whole grapes were used to determine separately the best extraction time. The kinetic obtained was compared with the kinetic of the extraction method of maceration and continuous magnetic stirring (at 1000 rpm), to determine the influence of ultrasound on the recoveries. The resulting graph for malic acid is shown in Figure 1.

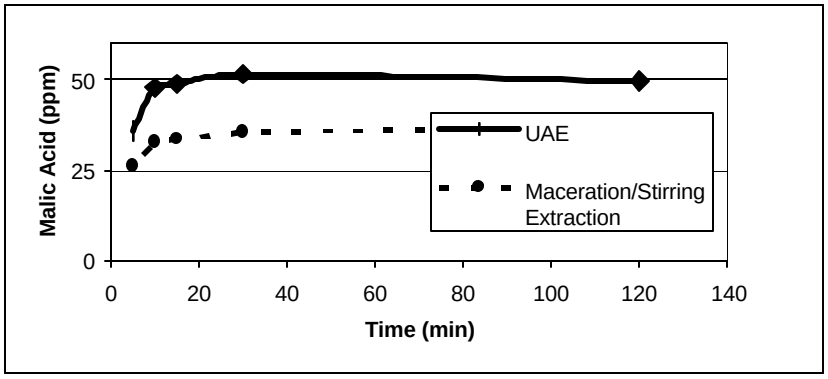

Figure 1. Kinetics of extraction obtained for malic acid from grape seeds.

Application of UAE to soy isoflavones

Several extracting solvents were used for extracting isoflavones from soy beans. They were selected based on the usual solvents for the analyses of isoflavones with classical extraction methods, i.e., pure ethanol, 50% ethanol in water, pure methanol and 50% methanol in water.

The same conditions were applied for all the extractions. The relative recoveries obtained for the extractions are shown in figures 2 for daidzin.

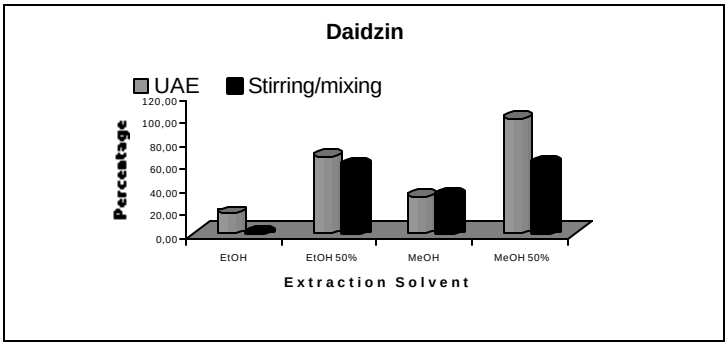

Figure 2. The relative recoveries obtained for the extractions of daidzin

The results obtained by UAE were compared to the corresponding recoveries obtained applying a soaking/stirring extraction method at the same temperature. With the only exception of 100%

methanol for a couple of compounds, the UAE produced at least a 10% higher recovery that the soaking/stirring extraction method. The differences are more significant for the solvent which produced the best recoveries, i.e. 50% methanol in water. It was obtained between 30% and 200% higher recoveries using UAE.

Conclusions

Under the optimized extraction conditions, quantitative recovery is obtained for tartaric and malic acids after 30 minutes of extraction and the method has high repeatability.

For grape seeds, UAE offers considerable advantages over the conventional maceration/stirring extraction method, but for grapes the differences are less marked.

For isoflavones, significant higher recoveries were obtained by UAE vs the soaking/stirring extraction for all the extracting solvents assayed.

Acknowledgements

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