ULTRASOUND-ASSISTED EXTRACTION OF PHOTOSYNTHETIC PIGMENTS FROM DRIED DILL (ANETHUM GRAVEOLENS)

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Abstract: *Extraction is an important step in studies involving the isolation of active compounds from plant materials. Ultrasound assisted extraction (UAE) is a simple, inexpensive and efficient technique due to shortened extraction time and reduced organic solvent consumption compared with other extraction method.*
Chlorophyll extracts are used extensively as dye in coloring inks, resins, soaps and waxes, edible fats, cosmetics, lotions perfume. Some experimental data suggest also that chlorophyll may ameliorate drug side effects, may have some anti-mutagenic and anti-carcinogenic potential and it may help protect against some toxins.
The aim of this study was to determine the influence of ultrasound extraction parameters, like acetone volume (80%, 90%, 100%), ultrasounds exposure time and ratio solvent/material on the extraction of photosynthetic pigments from dill (Anethum graveolens).
Ultraviolet spectroscopy was used to evaluate the efficiency of this extraction method and it was also used to quantitatively analyse the extracted chlorophylls and carotenoids.
Results show that the extraction efficiency of photosynthetic pigments from dried dill was higher when using acetone 90% comparative to the other two concentrations, because the highest level of total chlorophyll obtained was of 4,53mg/g dried dill in acetone 90% after 15 min. exposure to ultrasounds.
The results suggest that UAE method is an efficient technique for extraction of photosynthetic pigments.

Keywords: *chlorophyll, carotenoids, spectrophotometric quantification*

1. Introduction

Dill is a highly versatile seasoning herb. The dill is classed among species reducing the risk of cancer [1]. Its consumption also lowers the level of cholesterolaemia [2] while its components show antioxidative properties [3, 4]. Besides, seasoning herbs, including the dill, enrich the main dishes with complementary compounds, such as vitamins, mineral salts, and also compounds affecting the sensory traits of food. One of the most important sensory traits is the colour. The basic pigments of seasoning leafy herbs are chlorophylls, always accompanied by carotenoids. Acids, temperature, light, oxygen, and enzymes easily destroy the chlorophylls [5, 6], while carotenoids are fairly resistant to technological procedures [7].

Extraction is an important step in studies involving the isolation of active compounds from plant materials.

The extraction methods applied and compared in this study were: conventional extraction and ultrasound assisted extraction. Ultrasound assisted extraction has been employed as an efficient technique for plant pigments, phenolics and antioxidants extraction in the last years [8, 9].

Chlorophyll is one of the valuable bioactive compounds that can be extracted from plants.

Chlorophylls and carotenoids concentration correlate to the photosynthetic potential of plants giving some indication of the physiological status of the plant [10].

However, the content of pigments in plants is important, not only due to the coloration and physiological function, but also due to their acknowledged roles in health [11].

The interest in new data on carotenoids in edible plants is increasing due to a more extensive use of natural compounds in the food, following the directives of European Community in favor of natural rather than synthetic compounds [12].

Chlorophyll provides a chelating activity which can be used in ointment treatment for pharmaceutical benefits.

Chlorophyll has also been investigated as source of pigments in cosmetics. Because of its strong green colour and consumers demand for natural foods, chlorophyll gained importance as a food additive [13]

Traditional methods for analysis of photosynthetic pigments were employed based on spectroscopy and extinction coefficients that had been calculated for a range of solvents.

For whole-leaf extracts these methods allowed the accurate calculation of chlorophyll *a* and *b* concentration, but were limited to a pooling of the carotene pigments to give total carotenoids content [14].

Chlorophylls are highly susceptible to degradation during processing and storage [15, 16, 17].

Daood, Czinkotai, Hoschke, and Biacs [18] reported that chlorophyll a is more sensitive to degradation induced by thermal processing. The degradation process of chlorophyll a occurred faster according to Canjura and Schwartz [19] that studied the relative degradation of chlorophyll a with respect to b and showed too that the first degrades faster depending on temperature. The fact that chlorophyll a is more sensitive to thermal treatment was proved by the reduced chlorophyll a/b ratio.

The present study was undertaken with an aim to evaluate the pigments content in dried dill.

2. Experimental

Dill was purcheased from the local venders. The plants used in the research work were as fresh as possible and grown without using conventional pesticides and fertilizers. Also the raw material was healthy without traces of yellowing. The time from purchasing to the beginning of the analyses and technological processing of the raw material did not exceed 2 h. The plant material was dried in a drier at 40^0C till the brittleness of the leaves.

All solvent were purchased from Chemical Company and were of spectrophotometric grade.

Different concentrations of acetone (100%, 90%, 80%) were applied for choice of suitable solvent for the extraction of photosynthetic pigments from dried dill.

The experiments of one step extraction of chlorophylls and carotenoids from dried dill were performed by contacting the plant material with acetone at different concentrations for 5, 10 and 15 minutes. The ultrasound assisted extraction was realized in an ultrasonic bath at fixed ultrasonic power of 200W.

Ultraviolet-visible spectroscopy was used to evaluate the efficiency of the extraction and also to quantitatively analyse the photosynthetic pigments.

The equations used for the determination of chlorophyll a and b and total carotene concentrations are presented in table 1 [20-24].

Table 1
Simultaneous equations for the determination of chlorophyll a (C_a), chlorophyll b (C_b) and total carotene ($C_{(x+c)}$)

Solvent	Equation for chlorophylls and total carotene concentrations (µg/ml)
Acetone 100%	Ca=11.24A661.6+2.04A644.8 Cb=20.13A645+4.19A662 C(x+c)=(1000A470-1.9Ca-3.14Cb)/214
Acetone 90%	Ca=11.93A664+1.93A647 Cb=20.36A647+5.5A664 C(x+c)=(1000A470-1.9Ca-3.14Cb)/214
Acetone 80%	Ca=12.25A664+2.55A647 Cb=20.31A647+4.91A664 C(x+c)=(1000A470-1.82Ca-85.02Cb)/198

3. Results and Discussion

In the present study the ultrasound assised extraction technique was studied comparively with the conventional extraction. Different concentrations for acetone were used to determine the suitable solvent. Also the extraction tehniques were applied for 5. 10 and 15 minutes. Figs. 1(a). 1(b) and 1(c) present the influence of mixtures acetone/water and extraction time on the concentration of chlorophyll a. chlorophyll b and total carotene by conventional extraction. The highest content of chlorophyll a 2.55mg/g dried dill was obtained through conventional extraction with acetone 90%.

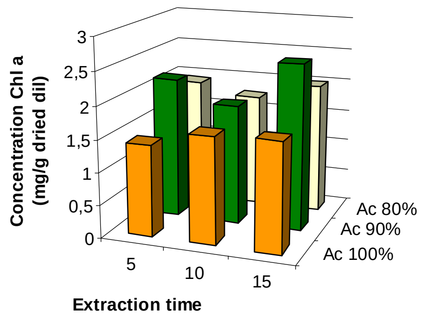

Figure 1(a). Influence of mixtures of acetone/water and extraction time on the concentration of chlorophyll a using conventional extraction

The highest content of chlorophyll b. 1.44mg/g dried dill was also obtained using acetone 90%. The extraction of total carotene through conventional method was optimum using acetone 100%.

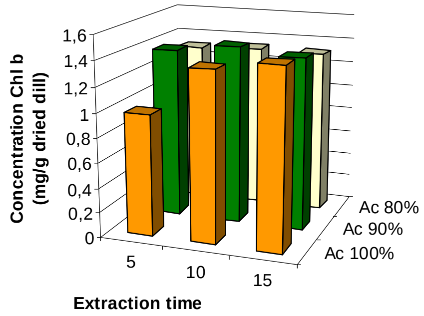

Figure 1(b). Influence of mixtures of acetone/water and extraction time on the concentration of chlorophyll b using conventional extraction

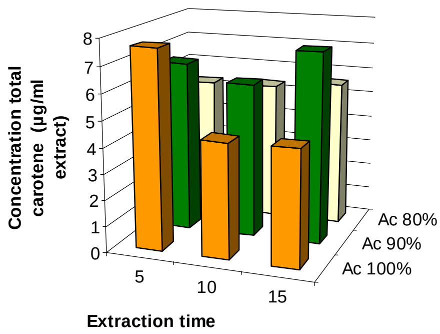

Figure 1(c). Influence of mixtures of acetone/water and extraction time on the concentration of total carotene using conventional extraction

Regarding the ratio of chlorophyll a versus chlorophyll b concentration in extracts the highest ratio of 1.83 was obtained using acetone 90%. The results are presented in Fig. 2.

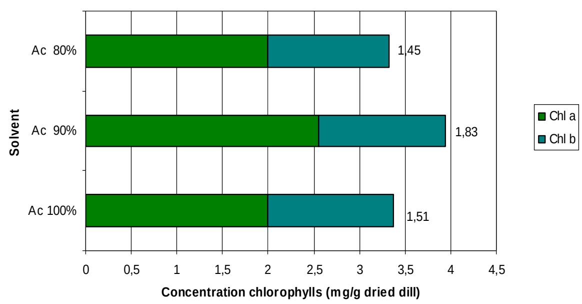

Figure 2. Concentration of individual chlorophyll and ratio of chlorophyll a/b obtained by conventional extraction

The extraction efficiency of the ultrasounds assisted method was higher then of the conventional method. For chlorophyll a the extraction using ultrasounds yielded 2.8 mg/g dried dill and for chlorophyll b 1.73 mg/g dried dill. Acetone 90% was also the most efficient solvent in the case of ultrasound assisted extraction. The influence of the same extraction parameters on the concentration of photosynthetic pigments are presented in Figs. 3(a). 3(b). 3(c).

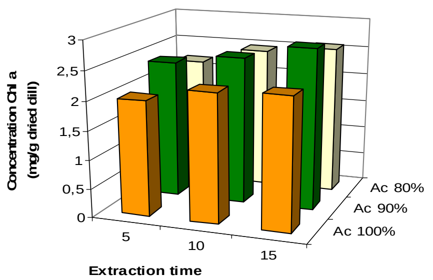

Figure 3(a). Influence of mixtures of acetone/water and extraction time on the concentration of chlorophyll a using ultrasounds assisted extraction

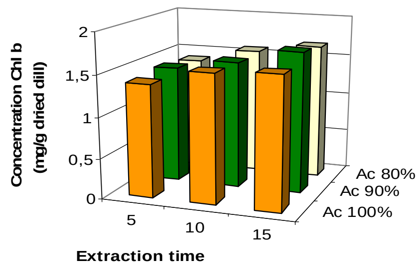

Figure 3(b). Influence of mixtures of acetone/water and extraction time on the concentration of chlorophyll b using ultrasounds assisted extraction

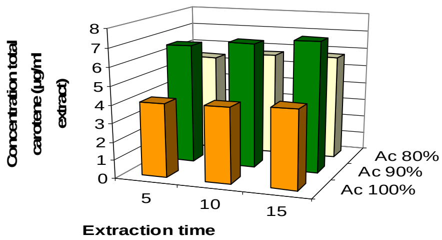

Figure 3(c). Influence of mixtures of acetone/water and extraction time on the concentration of total carotene using ultrasounds assisted extraction

The highest ratio of chlorophyll a versus chlorophyll b obtained was 1.61. Results are presented in Fig. 4.

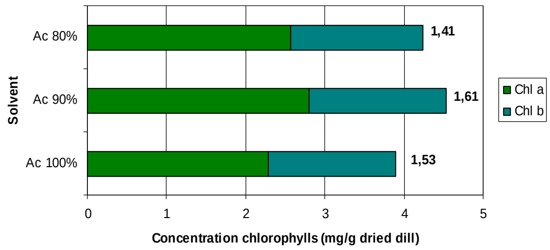

Figure 4. Concentration of individual chlorophyll and ratio of chlorophyll a/b obtained by ultrasound assisted extraction

4. Conclusion

In terms of concentration of chlorophylls the results showed that ultrasound assisted extraction is a promising extraction technique giving the highest efficiency extraction. As conventional extraction and Soxhlet extraction are not always acceptable for industrial applications due to long extraction time. large consumption of solvents and other disadvantages. ultrasound assisted extraction could be an alternative. as giving the best isolation efficiency of chlorophylls in advantageously much shorter extraction time because of the disruption of the plants cells.

Regardind the different concentrations of acetone used for the extraction of photosynthetic pigments. acetone 90% proved to be the most efficient both in conventional extraction and ultrasouds assisted extraction.

5. References

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