J Biochem Tech (2018) Special Issue (2): 166-176
ISSN: 0974-2328

Extracting of Saponin from Acanthophyllum Roots Using Pulsed Electric Field (PEF) Pretreatment and Comparing it with Thermal Method and Studying of its Antioxidant and Foaming Properties

Hassan Abhari*, Amir Hosseinelhami Rad, Hojat Karajian, Abbas Abhari

Received: 25 February 2018 / Received in revised form: 25 May 2018, Accepted: 29 May 2018, Published online: 05 September 2018

Abstract

The thick root of *Acanthophyllum* is a rich source of Saponin compounds as it is considered the main compound in it, the existed saponin in the *acanthophyllum* extract has antioxidant, anti-microbial and emulsifier characteristics. The technology of pulsed electric fields is a non-thermal process that leads to maintain the food materials quality (no change in flavor and taste, nutritional value, and physical characteristics) in addition to inactivation of micro-organisms and high-efficiency extraction.The purpose of this research was to study the efficiency of PEF to study theextraction of saponin and total phenolic compounds from root of *acanthophyllum*. After removing and taking fat by Soxhlet method, extraction was performed using PEF treatments with voltages of 1000, 4000 and 7000 V and with pulse numbers of 20 and 60 pulses in distilled water solvent. The ability and power of foam formation and emulsion formation index were measured to study the efficiency of saponin extraction, and the amount of total phenols and inhibition rate of radical 2, 2-diphenyl-1picrylhydrazyl (DPPH) were measured to study the anti-oxidant characteristics of extract and the obtained results compared to the traditional method. The results indicated that with increase in the intensity of field and pulses number both of saponin extraction amount and antioxidant characteristic were increased significantly and the amount of saponin extraction and phenolic compounds were more than traditional treating without applying any thermal processes.

Keywords: Acanthophyllum Root Pulsed Electric Fields, Saponin, Foaming Properties, Antioxidant Properties.

Introduction

The medicinal plants are a part of natural reserves and resources and several countries have such a source more or less but the type, number and variety of herbal species is different on the basis of geographical condition and position of each region (Blumenthal et.al., 2000). The scientific and technical advances during two recent decades increase the importance and constructive role of medicinal plants in providing human being's needs particularly in the field of medicine and treatment. Also, regarding the side effects of using the chemical compounds and medicines most of countries have turned to use natural compounds (Salem, 2005; Salem &Hossain, 2010).*Acanthophyllum* is a shrub that belongs to *Caryophyllaceae*family. Totlly, there is 61species of this plant all over the world that 33 species of them is grown in Iran and also 23 species of them are native of this region too (Ghaffari, 2004). On the basis of available references, most of these species are found in the eastern regions of Iran (Khorasan Province) and its adjacent areas (Afghanistan and Turkmenistan) (Schiman-Czeika, 1988; Huber-Morath, 1967). The root of acanthophyllum is a rich source of Saponin compounds in such a manner that it is considered as the most important and active compound in it, the Saponins and Saponin extracts with herbal origin have various uses in the food industry, e.g. in Iran and Turkey the extract of *acanthophyllum* and other similar plants are used as

Hassan Abhari*
Ph.D student, Department of food science and technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran.

Amir HosseinElhami Rad
Associate Professor, Department of food science and technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran

Hojat Karajian
Associate Professor, Department of food science and technology, Torbat Heydariyeh Branch, Islamic Azad University, TorbatHeydariyeh, Iran.

Abbas Abhari
Assistant Professor, Department of agronomy,Payame noor University,19359-4697,Tehran, I.R. Iran

***Email**: eh.abhari_63@yahoo.com

emulsifier in preparing some kinds of traditional sweet paste (Gaidi et.al.,2000; Gaidi et.al.,2004).Keyhani et al. (2011) examined theeffects of ultrasound on the characteristics of emulsion and foamig of edible extract of Chubak's plant. They reported that parameters such as sonication time, temperature, particle size and sonication rate on the emulsion formation index in A 24-hour period (E24) and the ability to form a foam (FH) had a significant effect.Oladzadabbasabadi et al (2016) studied influence of Chubak plant extract and egg white as high potential agents to generate foam for aeration and to introduce air bubbles into the texture of the grape juice during evaporation. The results show that the addition of the extraction Chubak extract and application of the evaporation process, together with mixing for 60 min at 80 0C improved color, viscosity and other physiochemical properties of grape juice samples.

The name of Saponin is derived from Greek root of (Soap). Saponins are glycosides with high molecule weight which consist ofone glucose section attached to Triterpenoid or steroid aglycone with Glycosidic bond (Hostettmann, 1995; Osbourn 1996a;Sun& Pan, 2006).The sugars which usually exist in saponin structures are Arabinose, Galactose, Glucose, Rhamnose, Xylose, Galacturonic Acid and Glucuronic Acid (Aminoddin&Chowdhyry, 1983).There are several witnesses about influences of these compounds in stimulating the immune system, increase membrane permeability (Gurfinkel&Rao, 2003), decrease cholesterol (Harwood et.al., 1993), disorder in protein digestion and vitamin absorption, hypoglycemia stimulation (Oleszek& Bialy, 2006) in human beings.Also Saponins obtained from various plants stop the growth of cancer cells (e.g. Colorectal Cancer) in laboratory conditions. Various compounds of saponin have emulsifier and antioxidant and also antifungal and antiviral activity (Oleszek& Bialy, 2006). Saponins take part in some of the protective mechanisms against free radicals (Hu et.al, 2002).Saponins are non-ionic natural Surfactants, because of having the surface tension reduction activity so they are used as the foam making materials, emulsifier in food materials and some nonfood materials such as soaps, lotions, shampoos and detergent gels widely (Balandrin, 1996).

There are various and common methods to extract from medicinal plants (such as extraction using solvent).In methods which heating is used in them, the cell wall become inactive under the influence of thermal treating and undesired compounds will enter the extract that in the next steps some complicated and costly procedures to separate these compounds from extract (Van der Poel et.al., 1998). Also using high temperature in extraction process causes to consume so significant energy, meanwhile it leads to decomposition and increase in the oxidation speed ofeffective compounds of extract and therefore causesa decrease in the efficiency. Regarding the above mentioned problems, currently researchers search for new methods of extraction to decrease the process time and organic solvents consumption and at the same time have suitable efficiency for extracting target compounds. During recent years some reports about possibility of using Pulsed electric field (PEF) in extracting various compounds from different parts of plants are published and this provide new chances and applications for food material industry (Vilkhu et.al., 2008).

Pulsed electric field technology consists of applying some pulses in short time (for about micro second) in strong electric field (0.1-50kV/cm) on the food material that is places between two electrodes at the room temperature.High intensity short time pulsed electric fields lead to cells electric decomposition and increased their permeability, this phenomenon is called Electroporation (Pourzaki&Mirzaee, 2008; Asavasanti.al., 2011). When the biological cell exposed to external electric field, potential difference between two sides of membrane will increase because of electric charge in membrane. The critical amount of electrical field intensity (0.2 – 1 V) is required to create membrane potential difference that causes to create reversible or irreversible pores in membrane (Chalermchat et.al, 2004). Reversible electroporation that keeps the cell life,is used in biological researches while microorganisms inactivation and mass transfer improvement in food industry need irreversible electroporation.When the intensity of external field is less than the critical value, the electroporation is reversible and makes possible to reconstruct the structure and function of cell membrane. This non-thermal method, with increase the permeabilityof cell membrane, has significant influence on inactivating microbes, press efficiency increase or increase the extraction from plant tissue and therefore will increase the efficiency of drying the food materials (Eshtiaghi& Knorr, 2002; Bazhal et.al, 2001; Wouters et.al., 2001; Lebovka et.al, 2007). The most important characteristics of this method toward thermal methods is thatfirst: the consistency and structure of cell is preserved because degradation of cell wall and membrane under the influence of electric fields is performed without any damage to other parts and contrary to the thermal method that leads to complete cell degradation. Secondly: change in the tissue properties particularly in the membrane of cell wall is in the way that intracellular materials exist from cell quickly andeasily and so destruction of micro-organisms and also extraction or drying the food materials are performed at minimum time, thirdly: the required energy for these procedures is so less than thermal procedures (Bouzrara&Vorobiey, 2000).

Luengo et.al. (2013) reported that using PEF method in comparison to the traditional method of extracting from orange pulp with field intensity of 1, 3, 5 and 7 kV/cm caused an increase in antioxidant activity of extract about 51%, 94%, 148% and 192%, respectively. Guderjan et.al. (2005) indicated thatextraction and retrieval of phytosterol from corn and iso-flavonoids from soybean using PEF procedure increased 32.4% and 20-21% respectively. Corralesa et.al (2008) studied extraction of bioactive compounds such as Anthocyanins a side product of grape using different techniques and indicated that using PEF method better and more extraction of Anthocyanin mono Glucosides is obtained.Delsart et.al. (2012) reported that applying pulsed electric field treatment on the Merlot skin permeability led to increase in polyphenols and anthocyanins.

Regarding the anxieties about toxicity effects of synthetic antioxidants such as butyl hydroxyanisole, butyl hydroxytoluene, butyl hydroxy quinone and Propyl Gallate, using them in food materials is limited.Searches for natural replacements of several antioxidants from plan sources are done because they also lead to increase the shelf life and therefore prevent pungency or hotness and bad taste of food (Zou et.al, 2012)..In this research, PEF technology is used as a novel method with no temperature process and high extraction efficiency. Different voltages and repetitions are used to extract biochemical compounds of *acanthophyllum*. The most important compounds of *acanthophyllum* include Saponin and total phenolic compounds, which are as foamier , emulsifier, and antioxidant compoundswill be measured, and finally are compared with traditional methods.

Materials and Methods

Materials

The root of *acanthophyllum* plant was gathered from mountainous regions located at 20 km distance of west of TorbatHeydariyeh, and the main characteristics of it recognized and specified by the Herbarium section of plant sciences research Institute of Ferdowsi University of Mashhad Unit: *Caryophyllaceae*family, *Acanthophyllum* type, *Glandulosum* species.

Methods

Preparing acanthophyllum extract

The *acanthophyllum* root, after collecting,was cleaned at first andits wood shell was separated then it became changed to smaller pieces by a hammer and became powder by a home mill (Pars Khazar, made in Iran). At first its fat was removed and taken using Soxhlet device and n-hexane solvent at 45 centigrade degree for 4 hours (Lacaille-Dubois et.al., 1993). Extraction from fat removed powder was performed using two traditional method (thermal) and pulsed electric fields technology. 1- The traditional method was in this way that at first the *acanthophyllum* root powder was weighted to 500g and was mixed to water with proportion of 1 to 3, then do boil for 3 hours and reach to one third of the extract. 2- In the second method at first the *acanthophyllum* root powder was weighted to 500g and was mixed to water with proportion of 1 to 6 and then they were treated by pulsed electric field device (Modulator PG, ScandiNova, Uppsala, Sweden) with different electric field intensities (1, 4, and 7 kV/cm) and two pulse level (20 and 60).

Determining the foaming index of acanthophyllum root extract (FH)

To perform this test at first each dried extract was mixed with two times distilled water and density of 10 mg/ml was obtained. Then 5 ml of this solution was mixed in a vertex device in a longitudinal direction intensively for 15 seconds and at last the foam height was measured after 1 minutes remaining at 25 centigrade degree (Ross & Miles, 2007).

Determining the Emulsion formation index of acanthophyllum root extract (E24)

To perform this test at first the density of 5mg/ml was prepared from each dried extract.In the next step 5 ml of this solution was added to the test tube that contains 5 ml liquid paraffin and then each tube was placed on the vertex device in longitudinal direction in high speed for 2 minutes to perform emulsion. In the end after keeping the test tubes in the stasis or static state and the laboratory temperature for 24 hours, the thickness of Emulsion layer that is placed between the oil layer and saponin solution was measured. (DehghanNoudehet.al., 2010).

Measurement the total phenolic compounds

The amount of total phenolic compounds of extract was studied through spectrophotometric method with Folin Ciocalteu's reagent (EsmaeilzadehKenariet.al., 2014) and results were stated on the basis of miliequivalents Gallic Acid per gram extract (figure 1).

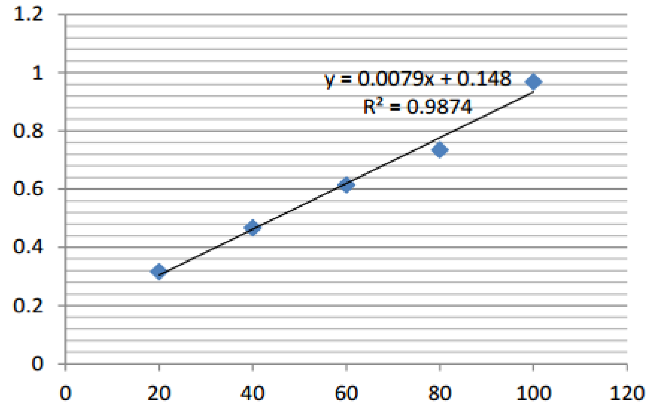

Fig. 1- the calibration curve of Gallic Acid in wavelength of 760 nm

Studying the free radical inhibition (DPPH)

The free inhibition test is performed based on the Blois method (1958). In this manner that0.3 ml of each extract was added to 3.7 ml of methanol DPPH solution (6 * 10^5mol/l) and the resulted mixture was mixed intensively. After 30 minutes placing it in the dark room in the room temperature, the samples optical absorption in wavelength of 517 nm against control sample was read. All of these steps in the case of TBHQ (100 ppm density)are performed as the standard and control antioxidant (methanol DPPH solution that I prepared in addition to relative solvents) and the percent of free radical inhibition DPPH was done according to equation 1.

Equation 1:

Free radical inhibition percentage = $\frac{control\ sample\ absorption - sample\ absorbtion}{control\ sample\ absorption}$ ×100

Quantitative measurement of Saponins with spectrophotometry method

In each step of extraction 0.01 ml of it was poured in the test tube and vacuumed in the oven until complete evaporation of water. When they get cold, 5 ml of 0.7% vanillin reagent in 65% Sulfuric Acid was added to each tube. This reagent is unstable and should prepare freshly. The mixture that exists in the tubes was mixed intensively using vertex device and was placed in hot water bath with temperature of 60±1 centigrade degree for one hour. Then the reaction was stopped in the ice water bath for 10 minutes and at last the absorption of reaction mixture was measured at 473 nm wavelength (Ebrahimzadeh&Niknam, 1998). The standard Saponin (Merck) was used to draw the standard curve.The standard curve was drawn in the range of 0 to 400 micrograms in ml. The saponin content of extract is evaluated using the standard curve (figure 2) and is calculated in terms of dry weight percentage.

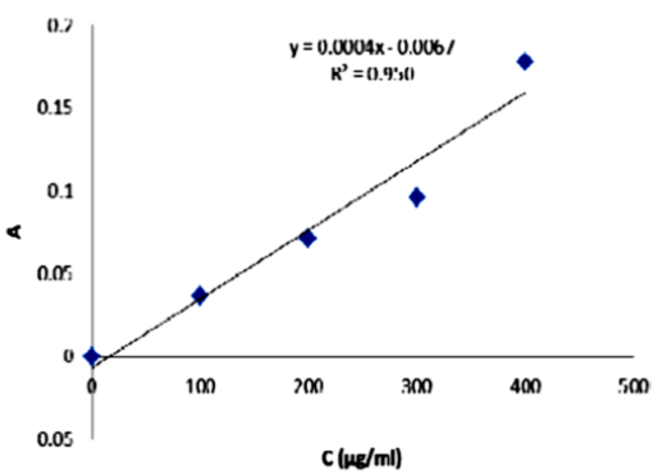

Fig. 2- The standard curve of saponin in 473 nm wavelength

The statistical design and data analysis

All the tests were conducted in the completely randomized design in 6 repetitions. Analysis and evaluation (Anova) was performed using the linear model (G.L.M) and the SPSS-18 statistical software in the probability level of 5% and Duncan's multiple range testswere conducted to confirm the difference between means.

Results and Discussions

Thefoaming index of acanthophyllum root extracts (FH)

The influence of PEF pretreatment on the ability of foam formation of *acanthophyllum* root extract was significant for both of electric field intensity and pulse numbersat the 5% probability level. With increase in the electric field intensity, as it shown in figure 3, the foam height has an ascending trend. Also with increase in the pulse number the foaming height has an ascending trend too. The ascendingtrend of foaming height expresses the increase in the saponin amount as the foam making compound in extract**.**

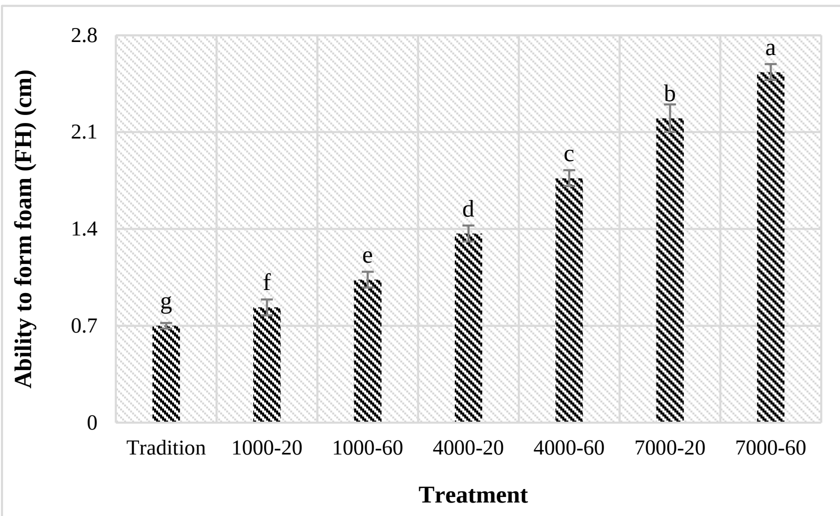

Fig. 3- The influence of electric field intensity and pulse numbers on the foam formation characteristics of *acanthophyllum*root extract

This finding can be a confirmation on this point that during the process of extraction from *acanthophyllum* root using pulsed electric field, extraction of desired compounds (saponins) is happened faster. In this regard it can be said that generally applying pulsed electric field causes physical break down of cell wall and membrane. This phenomenon accelerates the process of solvent penetration into the cells andfacilitates theprocess of mass transition between tissues and transition of particles from cell into the solvent therefore the extraction process will be ended in shorter time (Chen et.al., 2006).Bazhal et.al. studied the influence of pulse increase on degradation of herbal cell and tissues and indicated that with increase in the field intensity and pulse number the amount of cell degradation will increase too (Bazhal et.al., 2001). These results were similar to the researches of Luengo et.al.(2013) about the influence of electric field on improvement of polyphenol compounds extraction in orange pulp and Xue et.al.(2015) about extracting valuable compounds (polysaccharides, proteins and polyphenols) from white button mushroom (*Agaricusbisporus*) using pulsed electric field.

Emulsion formation index of acanthophyllum root extract (E24)

The influence of pulsed electric field intensity and pulse numbers on the emulsion formation index of *acanthophyllum*root extract in 5% probability level was significant. As it is seen in figure 4, with increase of electric field intensity and pulse numbers, the emulsion formation index has ascending trend too.The ascending trend of emulsion formation index indicates the extraction efficiency of this technology in comparison with traditional extraction method.

Since with increase in the electric field intensity and pulse numbers the membrane degradation will increase too therefore solvent penetration into the cell will increase and at last compound extraction will be done with more speed and in lower temperature. There are moreamounts of phenolic compounds such as saponin in the extract and at last the amount of the amount of emulsion formation characteristics of *acanthophyllum* root extract that is prepared using pulsed electric field has ascending and upward trend in comparison with traditional method. Meanwhile in traditional method because of temperature increase the activity of emulsion formation compounds such as saponins was degraded and stopped (Luengoet.al., 2013).

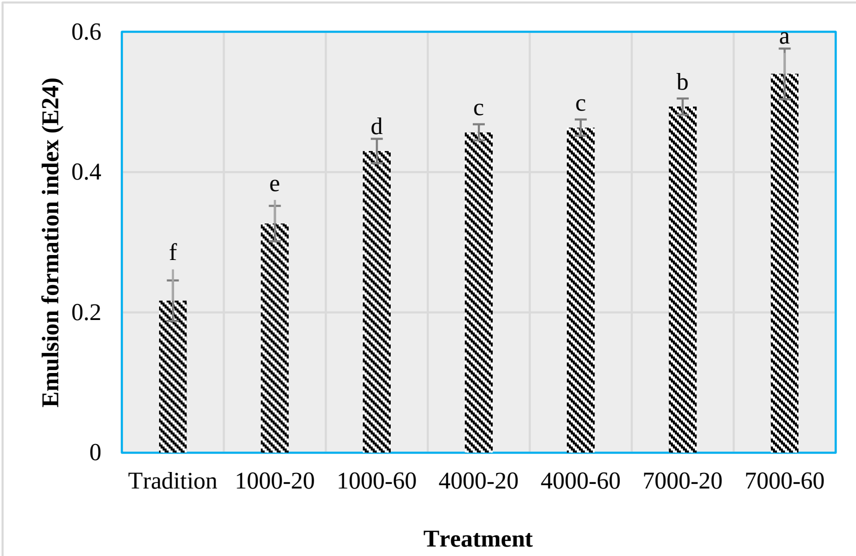

Fig. 4- The influence of electric field intensity and pulse numbers on the emulsion formation characteristics of *acanthophyllum* root extract

Total phenolic compounds

The influence of PEF pretreatment was significant on the amount of total phenolic compounds in *acanthophyllum* root extract for both of electric field intensity and pulse numbers on 5% probability level. With increase in the electric field intensity and pulse number in comparison to traditional method, as is shown in figure 5, the amount of total phenolic compounds had ascending or upward trend. The ascending or upward trend of total phenolic compound amounts in the extract indicates an increase in the amount of saponin and phenolic compounds and extraction efficiency in this technology compared with traditional extraction method.

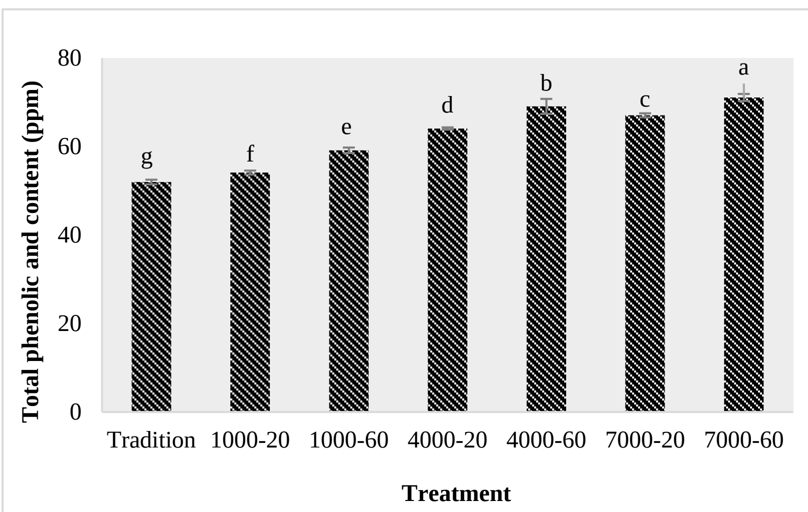

Fig. 5- The influence of electric field intensity and pulse numbers on the characteristics of total phenolic compounds in *acanthophyllum* root extract

The phenolic compounds are a great group of herbal secondary metabolites that their antioxidant ability is resulted from existence of hydroxyl groups in their structures.Nowadays utilization of natural phenols in the food industry is increased. Because these compounds delay the oxidative decomposition of lipids and therefore will improve and increase the quality and nutritional value of food materials (Muandaet.al., 2011).

Increase in the amount of total phenolic compounds of extract is due to the influence of electric field and pulse numbers on the permeability of cell wall membrane and its break down and therefore releasing the polyphenol compounds from cells (Luengo et.al, 2013). Rombaut et.al. (2015) indicated that polyphenols extraction from grapes powder increases with increase of the electric field intensity and procedure temperature. There are similar results from other researches about applying PEF treatment in extracting polyphenol from side products of grape (Boussettaet.al., 2012) and complete grape (Puértolas et al., 2010) during wine making.Also treating the juices by PEF process causes an increase in the polyphenols density in the grape and apple pulp juice (Grimi et.al, 2011; Grimiet.al., 2009; Jaeger et.al., 2012).

The main advantages of PEF technology toward other methods for improve and increase the polyphenols extraction from orange peel through press is that this process doesn't need to dewater the sample and water is used as the solvent. On the other side, the process time is too short and at microsecond extent and the inlet energy for permeability is very low (about 0.06-3.77 kilojoule/kg) without significant increase in temperature (Luengo et.al, 2013).

DPPH Free radical inhibition activity

The influence of PEF pretreatment on the amount of DPPH free radical inhibition activity in *acanthophyllum* root extract was significant in the probability level of 5% for both of electric field intensity and pulse numbers.Withincrease in the electric field intensity and pulse numbers in comparison with traditional method, as it is shown in figure 6, the total DPPH free radical inhibition activity amount had an ascending or upward trend. The ascending and upward trend of DPPH free radical inhibition activity of extract indicates an increase in the amount of saponin and phenolic compounds and extraction efficiency in this technology in comparison with traditional method of extraction.

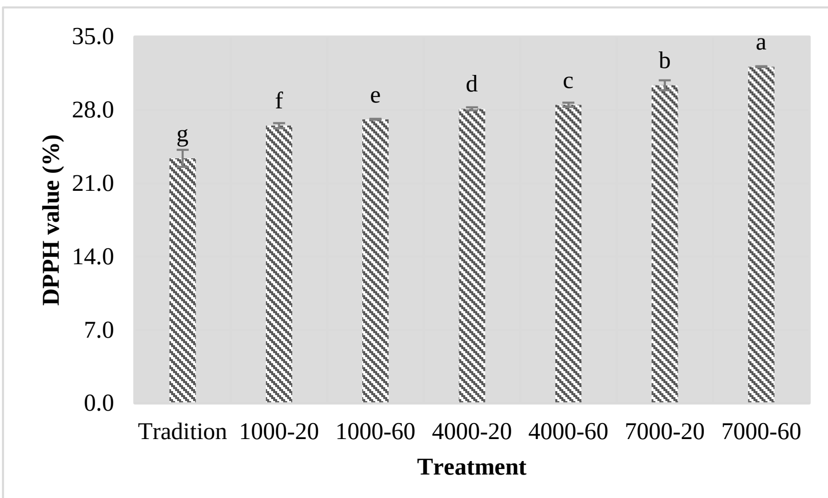

Fig. 6- The influence of electric field intensity and pulse number on the characteristics of DPPH free radical inhibition activity of *acanthophyllum* root extract

The herbal extracts, due to having phenolic compounds, have antioxidant activity and high capacity for donating Hydrogen or electron or free electron atoms (Demirciet.al., 2007). Use of DPPH stable radical is one of the reliable, exact, easy and economical methods with high repeatability that is used in laboratory conditions to study the antioxidant properties of herbal essences and extracts (Dordevic&Petrovic, 2007; Zouet.al., 2012). With increase in density or hydroxylation of phenolic compounds, the DPPH free radical inhibition activity will increase and it is defined as antioxidant activity (Ferrereset.al., 2007).Therefore, in this test the antioxidant activity of extracts are expressed on the basis of decreasepercentage in the absorption of DPPH solutions in presence of phenolic extracts toward the without extract solutions (Sanchez-Moreno et.al., 1999).The reason of high level of DPPH free radical inhibition in PEF extraction method in comparison to traditional method is high amount of phenolic compounds in them. Increase in density of phenolic compounds directly increases the ability of various extracts in inhibiting the free radicals.Increase in the amount of total phenolic compounds of extract is due to the influence of electric field and pulse number on the permeability of cell wall membrane and its break down and consequently releasing the polyphenol compounds from cells (Luengoet.al., 2013). In higher densities of phenolic compounds, the probability of donating Hydrogen to the free radical and thus the inhibitorypower of extract will increase, because of increase in the number of hydroxyl groups in the reaction environment (Jung et.al., 2006).

Extraction of Triterpenoid saponins

The influence of PEF pretreatment on the amount of Triterpenoid saponins of *acanthophyllum* root extract was significant in the probability level of 5%, for both of electric field and pulse numbers. As it is shown in figure 7, with increase in the electric field intensity and pulse numbers in comparison with traditional method, the amount of Triterpenoid saponins had an ascending or upward trend.The ascending or upward trend of Triterpenoid saponins amount in the extract expresses an increase in the amount of saponins and phenolic compounds and extraction efficiency in this technology compared to traditional method of extraction.

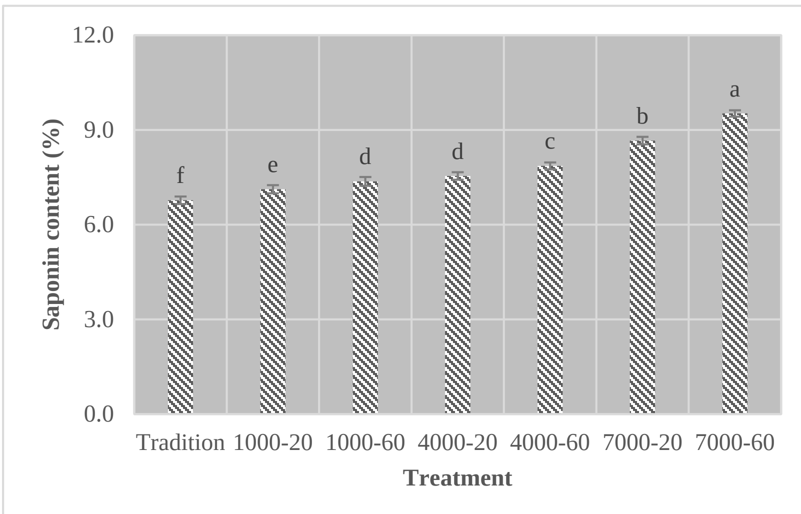

Fig. 7- The influence of electric field and pulse numbers on the amount of Triterpenoid saponins of *acanthophyllum* root

Cheng-Wen Lu et.al. (2016) studied the influence of PEF in combination with commercial enzyme on extracting total saponin from Ginseng root, the results indicated that because of increase in the permeability of membrane under the PEF process (15 Kv/cm and 10 pulses), both of extraction speed and total saponin extraction efficiency increased compared to traditional methods. Therefore, with increase in the field intensity and pulse numbers the quantitative amount of saponin will increase in the extract and in the meanwhile the extraction temperature will not increase compared to traditional method and consequently it leads to preserve saponin and non-decomposition of saponin.

Conclusion

Applying pulsed electric field technology in extracting compounds is a new challenge for food industry. Based on experimental findings, the effective parameters on extraction with PEF method is mainly the electric field intensity, pulse number, working time and temperature.Some characteristics of obtained extracts using PEF extraction method, such as foaming height, emulsion formation, polyphenol, saponin amount and DPPH radical inhibition activity were considered. The amount of polyphenols in *acanthophyllum* root extract will increase with an increase in the electric field intensity and pulse numbers. Characteristics such as foaming height, emulsion formation and saponin amount that all express saponin as the main compound of *acanthophyllum* root extract, will increase with an increase in the pulse number and electric field intensity. Generally, PEF process causes degradation of *acanthophyllum* root cells and the degradation influence of *acanthophyllum* root membrane is dependent to the field power and pulse number. Also in treatment with PEF,the extraction time is significantly lower than traditional (thermal) treatment. The mass transfer (releasing molecules and ions) will increase in usual and normal heating with PEF method with increase in the field intensity. Another important point is that extraction using PEF method with possibility of preparing *acanthophyllum* root extract in lower time and temperature, prevents the thermal damage of saponins and consequently destroying their functional characteristics particularly emulsion formation and foam making characteristics which are required in processing some of food materials).

References

Aminoddin, A., &Chowdhyry, A. R., 1983.Production of diosgenin in somatic callus tissue of Dioscoreadeltoidea .Planta Media. 48, 92-93. doi: 10.1055/s-2007-969894.

Asavasanti, S., Ristenpart, W., Stroeve, P., & Barrett, D.M., 2011.Permeabilization of Plant Tissue by MonopolarPulsed Electric Field: Effect of Frequency. Journal of Food Science, 76, No. 1. doi:10.1111/j.1750-3841.2010.01940.x.

Balandrin, M. F. 1996. Commercial utilization of plant-derived saponins: an overview of medicinal, pharmaceutical, and industrial Applications. Advances in Experimental Medicine and Biology, 404: 1-14. doi: 10.1007/978-1-4899-1367-8_1 .

Bazhal, M. I., Lebovka, N. I., &Vorobiev, E., 2001. Pulsed electric field treatment of apple tissue during compression for juice extraction. Journal of Food Engineering, 50(3), 129-139.doi:10.1016/S0260-8774(00)00235-1.

Blumenthal M, Goldberg A, Brinckmann J. 2000. Sage leaf. In: Blumenthal M, Goldberg A, BrinckmannJ,Eds. Herbal medicine. Newtown: Integrative Medicine Communications; 330-332.

Boussetta, N., Vorobiev, E., Le, L. H., Cordin-Falcimaigne, A., &Lanoiselle, J. L. 2012.Application of electrical treatments in alcoholic solvent for polyphenols extraction from grape seeds. LWT - Food Science and Technology, 46(1), 127–134. doi:10.1016/j.lwt.2011.10.016.

Bouzrara, H., &Vorobiev, E., 2000. Beet Juice Extraction by pressing and pulsed electric fields. International Sugar Journal, 102-1216: 194–200.doi:non.

Chalermchat, Y., Fincan, M., &Dejmek, P. 2004. Pulsed electric field treatment for solid–liquid extraction of red beetroot pigment: mathematical modelling of mass transfer. Journal of Food Engineering, 64(2), 229-236. doi:10.1016/j.jfoodeng.2003.10.002.

Chen, F., Sun, Y., Zhao, G., Liao, X., Hu, X., Wu, J., & Wang, Z. 2006.Optimization of ultrasound – assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using HPLC–MS. UltrasonicsSonochemistry, 14: 767–778. doi:10.1016/j.ultsonch.2006.12.011.

Cheng-Wen Lu , Yong-Guang Yin , Qing-Yu Yu ,(2016). Optimized Extraction of Ginsenosides from Ginseng Root (*Panax ginseng* C.A. Meyer) by Pulsed Electric Field Combined with Commercial Enzyme . Food Processing And Preservation , Volume 41.doi:10.1111/jfpp.12766.

Corralesa, M., Toepflb, S., Butza, P., Knorrc, D., Tauschera, B., 2008. Extraction ofanthocyanins from grape by-products assisted by ultrasonics, high hydrostaticpressure or pulsed electric fields: a comparison. Innovative Food Science andEmerging Technologies 9 (1), 85–91.doi:10.1016/j.ifset.2007.06.002.

DehghanNoudeh, GH.,Sharififar, F., Khatib, M., Behravan, E., &AhmadiAfzadi, M. 2010. Study of aqueous extract of three medicinal plants on cell membrane-permeabilizing and their surface properties. African Journal of Biotecnology, 9 (1): 110-116.doi:10.5897/AJB09.1403.

Delsart, C., Ghidossi, R., Poupot, C., Cholet, C., Grimi, N., Vorobiev, E., Milisic, V. &Peuchot, M.M., 2012.Enhanced extraction of phenolic compounds from merlotgrapes by pulsed electric fieldtreatment. American Journal of Enology andViticulture 63 (2), 205–211. doi**:** 10.5344/ajev.2012.11088.

Demirci. B, Kosar. M, Demirci. F. 2007. Antimicrobial and antioxidant activities of the essential oil of ChaerophyllumlibanoticumBoiss.etKotschy. Journal of Agriculture Food Chemistry, 105: 1512–1517. doi:10.1021/jf030698a.

Dordevic.S. &Petrovic. S. 2007. Antimicrobial, anti-inflammatory, antiulcer and antioxidant activities of Carlinaacanthifolia root essential oil. Journal of Ethno pharmacology. 109: 458–463. doi:10.1016/j.jep.2006.08.021.

Ebrahimzade, H. &Niknam, V., 1998.A revised spectrophotometric method for determination of triterpenoid saponins.Indian Drug. 35 (6), 379-381.doi:non.

Eshtiaghi, M. N., & Knorr, D., 2002. High electric field pulse pretreatment: potential for sugar beet processing. Journal of Food Engineering, 52(3), 265-272.doi:10.1016/S0260-8774(01)00114-5.

EsmaeilzadehKenari. R, Mohsenzadeh. F. &Amiri.Z., 2014. Antioxidant activity and total phenolic compounds of Dezful sesame cake extracts obtained by classical and ultrasound assisted extraction methods. Food science & nutrition,21, 54-62. doi:10.1002/fsn3.118.

Ferreres. F, Sousa. C, Valento. P, Seabra. R. M, Pereira. J. A. & Andrade. P. B. 2007. Tronchuda cabbage (Brassica oleracea L. var. costata DC) seeds: phytochemical characterization and antioxidant potential. Food Chemistry. 101: 549-558.doi:10.1016/j.foodchem.2006.02.013.

Gaidi, G., Miyamoto, T., Ramezani, M., and Lacaille-Dubois, M. A. 2000.Two new biologically active triterpene saponins from Acanthophyllum Squarrosum.Natural Products. 63(11), 1497-1502. doi:10.1021/np000212+.

Gaidi, G., Miyamoto, T., Ramezani, M., and Lacaille-Dubois, M. A. 2004.Glandulosides A-D, triterpene saponins from Acanthophyllum Glandulosum. Natural Products. 67, 1114-1118. doi:10.1021/np040001v.

Ghaffari S. M. 2004. Cytotaxonomy of some species of Acanthophyllum (Caryophyllaceae) from Iran. Biol. Bratislava. 59, 53-60.doi:non.

Grimi, N., Lebovka, N. I., Vorobiev, E., &Vaxelaire, J. 2009. Effect of a pulsed electric field treatment on expression behavior and juice quality of chardonnay grape. Food Biophysics, 4(3), 191–198. doi:10.1007/s11483-009-9117-8

Grimi, N., Mamouni, F., Lebovka, N., Vorobiev, E., &Vaxelaire, J. 2011. Impact of apple processing modes on extracted juice quality: Pressing assisted by pulsed electric fields. Journal of Food Engineering, 103(1), 52–61. doi:10.1016/j.jfoodeng.2010.09.019.

Guderjan, M., Töpfl, S., Angersbach, A., Knorr, D., 2005.Impact of pulsed electric fieldtreatment on the recovery and quality of plant oils.Journal of Food Engineering67 (3), 281–287. doi:10.1016/j.jfoodeng.2004.04.029.

Gurfinkel, D. M. &Rao, A. V. 2003. Soya saponins: the relationship between chemical structure and colon anticarcinogenic activity. Nutrition and Cancer, 47: 24-33. doi:10.1207/s15327914nc4701_3.

Harwood, H. J. Jr., Chandler, C. E., Pellarin, L. D., Bangerter, F. W., Wilkins, R. W., Long, C. A., Cosgrove, P. G., Malinow, M. R., Marzetta, C. A., Pettini, J. L., Savoy, Y. E. &Mayn, J. T. 1993. Pharmacologic consequences of cholesterol absorption inhibition: alteration in cholesterol metabolism and reduction in plasma cholesterol concentration induced by the synthetic saponin beta-tigogenincellobioside (CP-88818; tiqueside). Journal of Lipid Research, 34(3): 377-395. doi:10.1016/j.atherosclerosissup.2010.04.004

Hostettmann, K., and Marston, A., 1995. Saponins. (10), 267-268. Cambridge University Press, Cambridge, UK.

Hu, J., Lee, S. O., Hendrich, S. & Murphy, P. A. 2002.Quantification of the group B soyasaponins by high-performance liquid chromatography. Journal of Agriculture and Food Chemistry, 50(9): 2587-2594. doi**:** 10.1021/jf0114740.

Huber-Morath, A. 1967.Acanthophyllum. In: Davis PH. (ed.). Flora of Turkey and the East Aegean Islands.Vol. 2, Edinburg University Press. Edinburgh. pp. 175-177.

Jaeger, H., Schulz, M., Lu, P., & Knorr, D. 2012. Adjustment of milling, mash electroporation and pressing for the development of a PEF assisted juice production in industrial scale. Innovative Food Science & Emerging Technologies, 14, 46–60.doi:10.1016/j.ifset.2011.11.008

Jung. C. H, Seog. H.M, Choi IW, Park MW.& Cho HY. 2006. Antioxidant properties of various solvent extracts from wild ginseng leaves. LWT. 39: 266-274.doi:10.1016/j.lwt.2005.01.004.

Lacaille-Dubois, M., Hanquet, B., Rustaiyan, A., and Wagner, H. 1993.Squarroside A, a biologically active triterpene saponin from Acanthophyllum Squarrusom. Phytochemistry. 34(2), 489-495.doi:10.1016/0031-9422(93)80036-R

Lebovka, N. I., Shynkaryk, N. V., &Vorobiev, E., 2007. Pulsed electric field enhanced drying of potato tissue. Journal of Food Engineering, 78(2), 606-613.doi:10.1016/j.jfoodeng.2005.10.032.

Luengo, E., Álvarez, I., Raso, J., 2013. Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields.Innovative Food Science and Emerging Technologies, 17, 79–84. doi:10.1016/j.ifset.2012.10.005.

Muanda, F.N., Soulimani, R., Diop, B., &Dicko, A. 2011. Study on chemical composition and biological activities of essential oil and extracts from Stevia rebaudianaBertoni leaves. LWT-Food Science and Technology. 44(9): 1865-1872.doi:10.1016/j.lwt.2010.12.002.

Nazila Oladzadabbasabadi, Hojjat Karazhiyan , Vahid Keyhani,.2016 Addition of the Chubak extract and egg white on biophysical properties of grape juice during evaporation process. Journal of Food Process Eng. 10.1111; 1-13 . doi:10.1111/jfpe.12538.

Oleszek, W. & Bialy, Z. 2006. Chromatographic determination of plant saponins: an update (2002-2005). Journal of Chromatography A, 1112(1-2): 78-91. doi:10.1016/j.chroma.2006.01.037.

Osbourn, A. E., 1996a. Saponins and plant defense- a soap story. Trends in Plant Science. 1, 4-9. doi:10.1016/S1360-1385(96)80016-1

Pourzaki, A. &Mirzaee, H., Oct 2008. Pulsed Electric Field Generators in food processing. National Congress on Food Technology, 18th, Mashhad-Iran, 15-16.

Puértolas, E., López, N., Condón, S., Álvarez, I., &Raso, J. 2010.Potential application of PEF to improve red wine quality. Trends in Food Science and Technology, 21, 247–255. doi:10.1016/j.tifs.2010.02.002.

Rombaut, N., Savoire, R., Thomasset,B., Castello, J., Van Hecke, E. &Lanoisele, J.L. 2015. Optimization of oil yield and oil total phenolic content during grape seed cold screw pressing. Industrial Crops and Products. 63: 26-33.doi:10.1016/j.indcrop.2014.10.001.

Ross, J., & Miles, G. D., 2007. ASTM Standard Method D2243 –53, Philadelphia Pa., 2953, reapproved 2942.

Salem, M.L. &Hossain, M.S. 2010.Protective effect of black seed oil from Nigella sativa against murine cytomegalovirus infection.International Journal of Immunopharmacology. 22(9):729-740.doi:10.1016/S0192-0561(00)00036-9.

Salem, M.L. 2005.Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. International Immunopharmacology, 59(13):1749-1770.doi:10.1016/j.intimp.2005.06.008

Sanchez-Moreno. C, Larrauri. J. A. &Saura-Calixto. F. 1999. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Research International. 32:407-412.doi:10.1016/S0963-9969(99)00097-6.

Schiman-Czeika, H. 1988.Acanthophyllum. In: Rechinger KH. (ed.) Flora Iranica. Vol 2, Graz, Wien. pp. 135-163.

Sun, H. K., & Pan, H. J., 2006.Immunological adjuvant effect of Glycyrrhizauralensis saponins on the immune responses to ovalbumin in mice.Vaccine. 24 (11), 1914-1920.doi:10.1016/j.vaccine.2005.10.040.

Vahid Kayhani; Seyyed Ali Mortazavi, Mehdi Karimi; Hojat Karaghian; Zahra Shaykh al-Islami, 2011. Application of ultrasound waves in extraction of saponin compounds of Acanthophyllum glandulosum rootstock based on their emulsiveness and foaming properties. Peeling and Innovation in Food Industry. Dora 4, No. 4, Winter 1394, p. 325-342.doi:non.

Van der Poel, P. W., Schiweck, H., & Schwartz, T., 1998.Sugar Technology.Beet and Cane Sugar Manufacture. Berlin: Bartens, 88-107.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. 2008. Applications and opportunities for ultrasound assisted extraction in the food industry - a review. Innovative Food Science and Emerging Technologies, 9: 161-169. doi:10.1016/j.ifset.2007.04.014.

Wouters, P. C., Bos, A. P., &Ueckert, J., 2001.Membrane permeabilization in relation to inactivation kinetics of Lactobacillus species due to pulsed electric fields.Applied and Environmental Microbiology, 67(7), 3092-3101.doi: 10.1128/AEM.67.7.3092-3101.2001.

Xue, D. &Farid, M.F., 2015. Pulsed Electric Field Extraction of Valuable Compounds from White Button Mushroom (AgaricusBisporus), Innovative Food Science and Emerging Technologies, doi: 10.1016/j.ifset.2015.03.012. doi10.1016/j.ifset.2015.03.012.

Zou, T., Wang, D., Guo, H., Zhu, Y., Luo, X. & Liu, F., 2012. Optimization of microwave assisted extraction of anthocyanins from mulberry and identification of anthocyanins in extract using HPLC-ESI-MS. Journal of Food Science, 77(1): 46-50. doi: 10.1111/j.1750-3841.2011.02447.x.