

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

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ABSTRACT

The thick root of *Acanthophyllum bracteatum* is a rich source of Saponin compounds as it is considered the main compound in it, the existed Saponin in the *Acanthophyllum bracteatum* 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 the extraction of Saponin and total phenolic compounds from root of *Acanthophyllum bracteatum*. After removing and taking far 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-1 picrylhydrazyl (DPPH) were measured to study the antioxidant 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 bracteatum* root, Pulsed electric fields, Saponin, Foaming properties, Antioxidant properties.

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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, Hossain and Salem 2010). *Acanthophyllum bracteatum* is a shrub that belongs to *Caryophyllaceae* family. Totally, there is 61 species 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 (Gaffari 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 1988, Huber 1967). The root of *Acanthophyllum bracteatum* 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 bracteatum* and other similar plants are used as emulsifier in preparing some kinds of traditional sweet paste (Gaidi et al. 2000, Gaidi et al. 2004). Keyhani et al. (2011) examined the effects of ultrasound on the characteristics of emulsion and foaming 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 h period (E 24) and the ability to form a foam (FH) had a significant effect. Nazila 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°C improved color, viscosity and other physico-chemical 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 of one glucose section attached to Triterpenoid or steroid aglycone with Glycosidic

bond (Hostettmann and Marston 1995, Osbourn 1996, Sun and Pan 2006). The sugars which usually exist in Saponin structures are Arabinose, Galactose, Glucose, Rhamnose, Xylose, Galacturonic Acid and Glucuronic Acid (Aminuddin and Chowdhury 1983). There are several witnesses about influences of these compounds in stimulating the immune system, increase membrane permeability (Gurfinkel and Rao 2003), decrease cholesterol (Harwood et al. 1993), disorder in protein digestion and vitamin absorption, hypoglycemia stimulation (Oleszek and 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 and 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 non-food 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 of effective compounds of extract and therefore causes 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 apply-

ing some pulses in short time (for about micro second) in strong electric field (0.1–50 k V/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 and Mirzaee 2008, Asavastani et 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 micro-organisms 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 permeability of 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 and Knorr 2002, Bazhal et al. 2001, Wouters et al. 2001, Lebovka et al. 2007). The most important characteristics of this method toward thermal methods are that first: 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 and easily 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 (Bouzzara and Vorobiev 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 that extraction and retrieval of phytosterol from corn and iso-flavonoids from soybean using PEF procedure increased 32.4% and 20–21% respectively. Corrales 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 hydroxyquinone and Propyl Gallate, using them in food materials is limited. Searches for natural replacements of several antioxidants from plant 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 bracteatum*. The most important compounds of *Acanthophyllum bracteatum* include Saponin and total phenolic compounds, which are as foamer, emulsifier and antioxidant compounds will be measured and finally are compared with traditional methods.

MATERIALS AND METHODS

Materials

The root of *Acanthophyllum bracteatum* plant was gathered from mountainous regions located at 20 km distance of West of Torbat Heydariyeh 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 bracteatum* type, *Glandulosum* species.

METHODS

Preparing Acanthophyllum bracteatum extract

After collecting the *Acanthophyllum bracteatum* root cleaned its 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 h (Lacaille et al. 1993). Extraction from fat removed powder was performed using two traditional method (thermal) and pulsed electric fields technology. The traditional method was in this way that as first the *Acanthophyllum bracteatum* root powder was weighted to 500 g and was mixed to water with proportion of 1 to 3, then do boil for 3 h and reach to one third of the extract. In the second method at first the *Acanthophyllum bracteatum* root powder was weighted to 500 g and was mixed to water with proportion of 1 to 6 and then they were treated by pulsed electric field device (Modulator PG, Scandinavia, 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 bracteatum 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 and Miles 2007).

Determining the emulsion formation index of Acanthophyllum bracteatum root extract (E 24)

To perform this test at first the density of 5 mg/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 h, the thickness of emulsion layer that is placed between

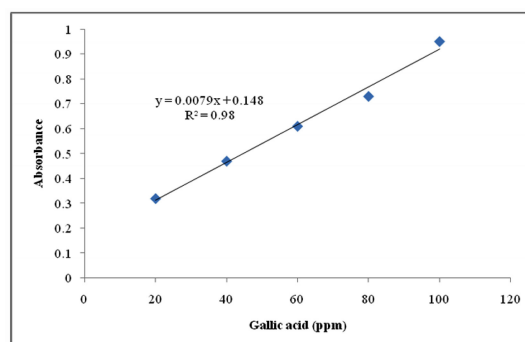


Fig. 1. The calibration curve of Gallic Acid in wavelength of 760 nm.

the oil layer and Saponin solution was measured (Dehghan et 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 (Esmailzadeh et al. 2014) and results were stated on the basis of mili equivalents Gallic Acid per gram extract (Fig. 1).

Studying the free radical inhibition (DPPH)

The free inhibition test is performed based on the Boiss method (1958). In this manner that 0.3 ml of each extract was added to 3.7 ml of methanol DPPH solution (6×10^5 mol/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 :

$$\text{Free radical inhibition percentage} = \frac{\text{control sample absorption} - \text{sample absorption}}{\text{control sample absorption}} \times 100$$

Quantitative measurement of Saponins with spectrophotometry method

In each step of extraction 0.01 ml of it was poured in

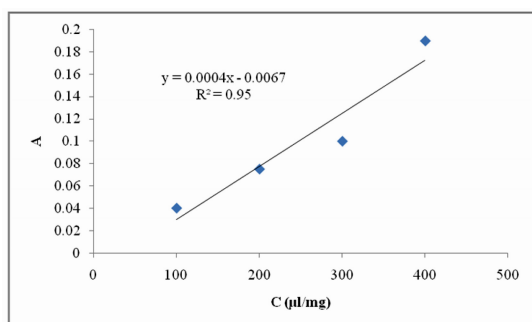


Fig. 2. The standard curve of Saponin in 473 nm wavelength.

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 vortex 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 and 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 mg in ml. The Saponin content of extract is evaluated using the standard curve (Fig. 2) and is calculated in terms of dry weight percentage.

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 (GLM) and the SPSS-18 statistical software in the probability level of 5% and Duncan's multiple range tests were conducted to confirm the difference between means.

RESULTS AND DISCUSSION

The foaming index of *Acanthophyllum bracteatum* root extracts (FH)

The influence of PEF pretreatment on the ability of foam formation of *Acanthophyllum bracteatum* root

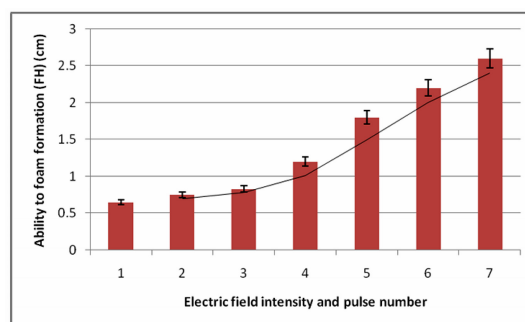


Fig. 3. The influence of electric field intensity and pulse numbers on the foam formation characteristics of *Acanthophyllum bracteatum* root extract (1 to 7, 1: Control, 2: Voltage 100 with 20 replication, 3: Voltage 100 with 60 replication, 4: 400-20, 5: 400-60, 6: 700-20 and 7: 700-60 respectively).

extract was significant for both of electric field intensity and pulse number sat the 5% probability level. With increase in the electric field intensity, the foam height has an ascending trend. Also with increase in the pulse number the foaming height has an ascending trend too. The ascending trend of foaming height expresses the increase in the Saponin amount as the foam making compound in extract (Fig. 3).

This finding can be a confirmation on this point that during the process of extraction from *Acanthophyllum bracteatum* 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 and facilitates the process 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. (2001), 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. 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 (*Agaricus bisporus*) using pulsed electric field.

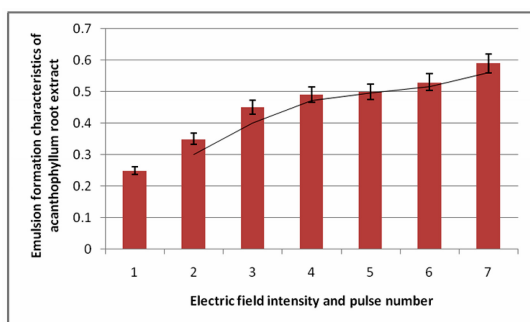


Fig. 4. The influence of electric field intensity and pulse numbers on the emulsion formation characteristics of *Acanthophyllum bracteatum* root extract (1 to 7, 1: Control, 2: Voltage 100 with 20 replication, 3: Voltage 100 with 60 replication, 4: 400-20, 5: 400-60, 6: 700-20 and 7: 700-60 respectively).

Emulsion formation index of *Acanthophyllum bracteatum* root extract (E 24)

The influence of pulsed electric field intensity and pulse numbers on the emulsion formation index of *Acanthophyllum bracteatum* root extract in 5% probability level was significant. 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 (Fig. 4).

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 more amounts of phenolic compounds such as Saponin in the extract and at last the amount of the amount of emulsion formation characteristics of *Acanthophyllum bracteatum* 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 (Luengo et al. 2013).

Total phenolic compounds

The influence of PEF pretreatment was significant on

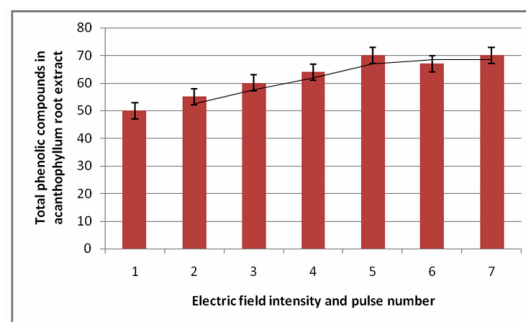


Fig. 5. The influence of electric field intensity and pulse numbers on the characteristics of total phenolic compounds in *Acanthophyllum bracteatum* root extract (1 to 7, 1: Control, 2: Voltage 100 with 20 replication, 3: Voltage 100 with 60 replication, 4: 400-20, 5: 400-60, 6: 700-20 and 7: 700-60 respectively).

the amount of total phenolic compounds in *Acanthophyllum bracteatum* 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, 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 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 (Muanda et al. 2011). Increase in the amount of total phenolic compound 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 prod-

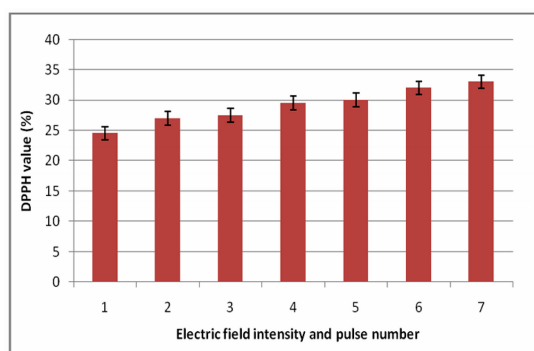


Fig. 6. The influence of electric field intensity and pulse number on the characteristics of DPPH free radical inhibition activity of *Acanthophyllum bracteatum* root extract (1 to 7, 1: Control, 2: Voltage 100 with 20 replication, 3: Voltage 100 with 60 replication, 4: 400-20, 5: 400-60, 6: 700-20 and 7: 700-60 respectively).

ucts of grape (Boussetta et 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, Grimi et 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 bracteatum* root extract was significant in the probability level of 5% for both of electric field intensity and pulse numbers. With increase in the electric field intensity and pulse numbers in comparison with traditional method, 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 herbal extracts, due to having phenolic compounds, have antioxidant activity and high capacity for donating hydrogen or electron or free electron atoms (Demirci et 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 and Petrovic 2007, Zou et 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 (Ferrerres et al. 2007). Therefore, in this test the antioxidant activity of extracts are expressed on the basis of decrease percentage in the absorption of DPPH solutions in presence of phenolic extracts toward the without extract solutions (Sanchez 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 (Luengo et al. 2013). In higher densities of phenolic compounds, the probability of donating hydrogen to the free radical and thus the inhibitory power 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 bracteatum* root extract was significant in the probability level of 5%, for both of electric field and pulse numbers. 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 ex-

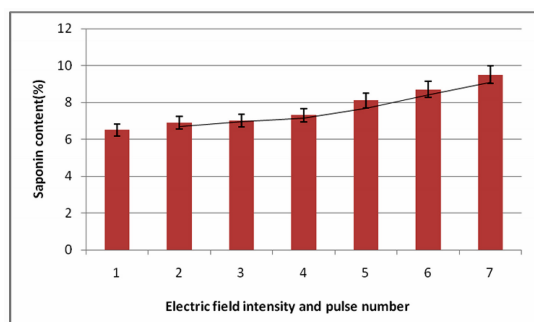


Fig. 7. The influence of electric field and pulse numbers on the amount of Triterpenoid Saponins of *Acanthophyllum bracteatum* root (1 to 7, 1: Control, 2: Voltage 100 with 20 replication, 3: Voltage 100 with 60 replication, 4: 400-20, 5: 400-60, 6: 700-20 and 7: 700-60 respectively).

presses an increase in the amount of Saponins and phenolic compounds and extraction efficiency in this technology compared to traditional method of extraction (Fig. 7).

Cheng 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 bracteatum* 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 bracteatum* root extract, will increase with an increase in the pulse number and electric field intensity. Generally, PEF process causes degradation of *Acanthophyllum bracteatum* root cells and the degradation influence of *Acanthophyllum bracteatum* 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 bracteatum* 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.

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