# Experimental study of antioxidant compounds extraction in red cabbage by PEF method

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## Abstract
This study was conducted to evaluate the effect of pulsed electric field (PEF) treatment on antioxidant extraction from red cabbage using acetic acid (10% v/v) as a solvent. Mashed cabbage was placed in a batch treatment chamber and subjected to PEF (3 kV/cm electric field strength; 60s pulse width and 50 pulses, 100s pulse width and 2 pulses). Effects of pulsed electric field (PEF) processing, Brix and electrical conductivityan of red cabbage were studied and compared with those of thermal processing. Extracted antioxidant concentrations were determined using Spectrometer. *Total antioxidant capacity* was determined by 0.6 ml of plant extract was added to 6 ml of the solution at acidic pH containing sulfuric acid (H2SO4; 0.6M) sodium phosphate (NaH2PO4, H2O; 28 mM) and the ammonium heptamolybdate ((NH4) 6MO7O24, 4H2O; 4 mM). The mixture is then incubated at 95 ° C for 90 min.

**Keywords**: antioxidant, extraction, pulsed electric field, red cabbage

## *Introduction*
Anthocyanins are a group of natural phenolic compounds found in plants. They are water-soluble pigments responsible for the blue, purple, and red color of plant fruits, flowers, and leaves (Strack and Wray 1993). They can be found in grapes, red currants, black currants, raspberries, strawberries, apples, cherries, red cabbage, red-flashed potato, radish, and aubergines (Henry 1996; Rodriguez-Saona et al. 1999). More than 250 different anthocyanins have been found in nature and 8 to 23 of them have been found in red cabbage (Markakis 1982; Baublis et al. 1994; Wu et al. 2006; Charron et al. 2007). The anthocyanin composition of red cabbage is very complex, but the major anthocyanins are based on a core structure of cyanidin-3-O-diglucoside-5-O-glycoside (Wu and Prior 2005). There has been an increased interest in red cabbage pigments as a source of natural food colorants since the mid-1970s (Salunkhe and Kadam 1998). Pigments from red cabbage are used widely in Japan and are available from European and Canadian companies. Applications for red cabbage colorants include reconstituted juices, beverages, jams (with pectin), fruits preparations, yogurts, confectionery, candies, dry mixes, chewing gum and a variety of sauces (Chigurupati et al. 2002). The daily consumption of anthocyanins in human diets has been estimated to be up to 200 mg/d (Wu and Prior 2005). Consumption of anthocyanin-rich fruit and vegetables and drinking of juice or wine have been associated with lower risk of coronary heart disease and cancer (Duthie et al. 2000). It also has been demonstrated that anthocyanins protect against DNA damage induced

by oxidative agents (Lazzè et al. 2003). The health benefits of anthocyanins are well known due to their antioxidant properties. The antioxidant and antihyperglycemic properties of red cabbage extract may offer a potential therapeutic source for the treatment of diabetes (Kataya and Hamza 2007). In contrast, some artificial colorants used in food processing have been shown to have adverse health effects (Damasceno 1988). For these reasons, consumer demand for natural colorants has been on the increase. Several technologies have been proposed for enhancing extraction of phytochemicals of plant and vegetable origins. Usaquén-Castro et al. (2006) used ultrasound to assist in extraction of polyphenols from red-grape residues. Microwave pretreatments improved extraction of anthocyanins in red raspberries (Sun et al. 2007). High-pressure extraction of anthocyanins from red grape pomace with carbon dioxide and cosolvents has been shown to increase their yield significantly (Mantell et al. 2003).
Antioxidant compounds have a very important role in health. The antioxidant is a compound that can counteract and mitigate the negative inpact of oxidants in the body. Various scientific evidence indicates that antioxidant compounds reduce risk of chronic disease such as cancer and coronary heart disease. The main character of the antioxidant compound is its ability to capture free radicals. Natural antioxidant compounds found in plants such as vitamin C, vitamin E, carotenoids, phenolic acids, polyphenols and flavonoids known to potentially reduce the risk of degenerative diseases. One of the plants that contain antioxidant activity is cabbage (Brassica oleracea L. var capitata L). Antioxidant compounds contained in cabbage, flavonoids, sterol/triterpenes. There are two types of cabbage, white and red cabbage. Cabbage is one vegetable consumed in lndonesia whether raw, steamed or boiled.[1]
Pulsed electric field (PEF) has been studied widely for microbial inactivation (Jin and Zhang 1999; Walkling-Ribeiro et al. 2009), enhanced juice extraction (Jemai and Vorobiev 2006; Lopez et al. 2008), and accelerating drying processes of fruits and vegetables (Ade-Omowaye et al. 2003; Shynkaryk et al. 2008). Applying PEF to biological cells above a threshold level can result in electroporation of the cell membrane or wall. This process leads to an increase in permeability of the cell walls and easier release of the intracellular contents (Knorr and Angersbach 1998). The degree of electroporation depends on many factors including product constituents, electric field intensity, type of pulse waveform, treatment time, and the pulse number (Jeyamkondan et al. 1999). PEF treatments in conjunction with fruit and vegetables processing were first reported in the mid-1900s (Flaumenbaum 1949). Enhanced juice yields from apples, carrots, beets, alfalfa, and other fruits and vegetables with PEF pretreatment have been reported (Gachovska et al. 2006). Chalermchat et al. (2004) used PEF to increase pigment extraction in red beet root. Gachovska et al. (2006) reported increased minerals extraction from alfalfa after PEF treatment. The use of PEF to enhance anthocyanin extraction in red cabbage has not been reported. In this study, the use of PEF treatment of red cabbage mash to enhance the release of antioxidants was evaluated. The objective of this study was to determine the effect of PEF treatment on the yield of antioxidants from red cabbage and to compare the temperature and light stability of the anthocyanin obtained by PEF-treated cabbage mash and control, nontreated mash. Pulsed electric fields is suitable technological option for pasteurization, able to preserve valued bioactive compounds in beverages. Over the last decade, PEF has attracted a significant interest from various food industries and found numerous applications .[2][3] Use of pulsed electric fields (PEFs) for inactivation of microorganisms is one of the more promising nonthermal processing methods. Inactivation of microorganisms exposed to high-voltage PEFs is related to the electromechanical instability of the cell membrane.[4]

### *Materials and Methods*

Fresh red cabbage was purchased from a local supermarket and stored at 4 °C for less than 2 d. The cabbage was removed from the refrigerator and held at room temperature (22 °C) for 2 h before processing. Preparation of the cabbage prior to PEF extraction included of cabbage chopping and discarding of the stems, which contained little pigment, and subsequent mashing with a domestic food processor for 1 min to obtain a homogenous mash without any addition of water. There was no measurable temperature increase during mashing. Approximately 1 g of the mash were placed in a PEF treatment chamber.

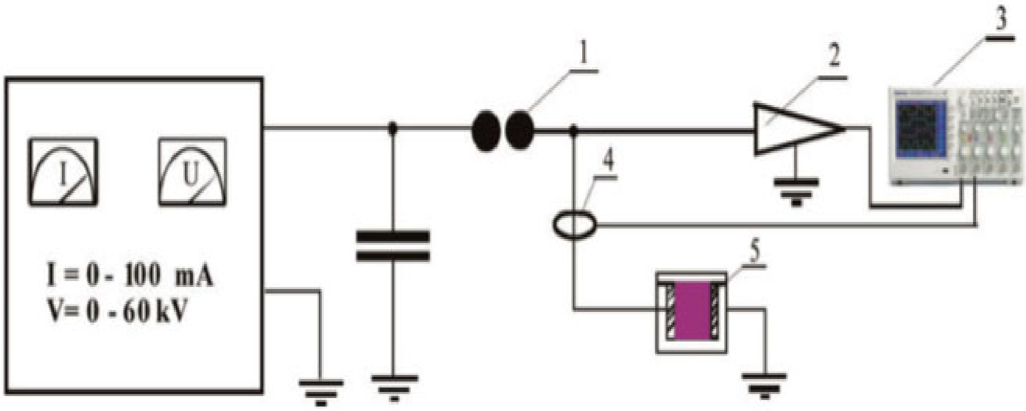


**Figure 1. Electrical circuit for pulsed electric field treatment of cabbage: 1 = spark gap switch; 2 = high voltage probe; 3 = oscilloscope; 4 = current probe; 5 = treatment chamber.**

### *PEF treatment*

PEF treatment of the cabbage was accomplished using an exponential decay PEF generator. The generator contained a DC power supply., a capacitor and a spark gap switch (Figure 1).
The energy stored in the capacitor was discharged through a parallel plate treatment chamber (Figure 2). The voltage applied to the chamber was measured with a voltage probe The chamber was made from 2 stainless steel electrodes separated by an insulation material . The distance between the electrodes was 3 cm . PEF treatment parameters of the cabbage samples were chosen based on preliminary experiments.[5]

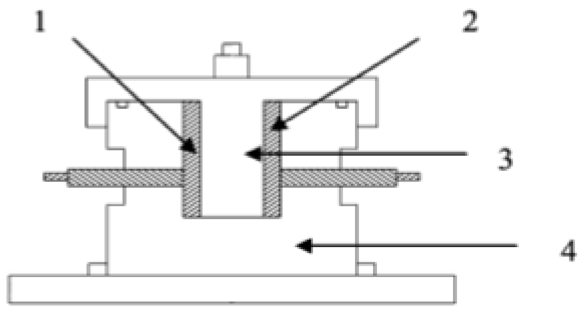


**Figure 2. Sketch of the pulsed electric field treatment chamber: 1 = high voltage electrode; 2 = ground electrode; 3 = sample; 4 = insulator/spacer.**

### *Thermally processed*

Red cabbage was held at 70 °C for 15min and 1hr in a ractor.The high-voltage pulse generator in PEF Processing Section were not activated during the thermal processing .
The effective parameters of the pulsed electric field method are shown in Table 1

**Table1. Effective Parameters in Pulse Extraction Process**

| Solvent | Sample shape | Temp | Time | Electric field | Frequency |
|---|---|---|---|---|---|
| Distilledwater | sliced up | 40 | 20 | 2/3 | 2 |
| methanol | Meshing | 50 | 40 | 3/11 | 50 |
| Ethanol | | 60 | 60 | 4 | |
| | | 70 | 80 | 4/6 | |
| | | | 100 | 5/6 | |
| | | | 120 | 7 | |
| | | | | 9/3 | |
| | | | | 14 | |

in this section and in Table 1 the effect of these factors on mass transfer rate, degradation index, energy consumption, extraction efficiency and weight loss percentage of extracted solution obtained by PEF method will be investigated. the number of pulses n was obtained from Eq. (1).

$$n = f \cdot t_{PEF} \qquad \text{Eq. (1)}$$

The PEF pulse duration was about $t_{PEF}$ = 20s , 40s , 60s , 80s , 100s and 120s and the pulses were applied with a repetition rate of 2 and 50 Hz, which was imposed by the generator.The specific energy input W (kJ/kg) was obtained from Eq. (2):

$$W = \frac{\sum_{i=1}^{n} W_{PEF}}{m} \qquad \text{Eq. (2)}$$

where WPEF is the pulse energy (kJ/pulse), and m is the mass of stevia leaves (kg). $W_{PEF}$ is determined from Eq. (3):

$$W_{PEF} = \int U \cdot I \cdot dt \qquad \text{Eq. (3)}$$

where U is the voltage (V) and I is the current strength (A).[6]

***Brix and Electrical conductivity***

The Brix of red cabbage was measured using a hand-held refractometer .The Electrical conductivity of red cabbage was measured using a Conductivity meter.[7]

***Natural sources of antioxidants***

Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2006). There are many synthetic antioxidants in use. It is reported, however, they have several side effects (Ito et al., 1983), such as risk of liver damage and carcinogenesis in laboratory animals (Gao et al., 1999; Williams et al., 1999; Osawa and Namiki, 1981). There is therefore a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants.[8]

***Total antioxidant capacity***

A dose of 0.6 ml of plant extract was added to 6 ml of the solution at acidic pH containing sulfuric acid (H2SO4; 0.6M) sodium phosphate (NaH2PO4, H2O; 28 mM) and the ammonium heptamolybdate ((NH4) 6MO7O24, 4H2O; 4 mM). The mixture is then incubated at 95 ° C for 90 min. After cooling to room temperature, the absorbance is measured at 695 nm. The total antioxidant activity is expressed as mg gallic acid equivalent per gram of dry matter (mg g-1MS EAG). [9][10]

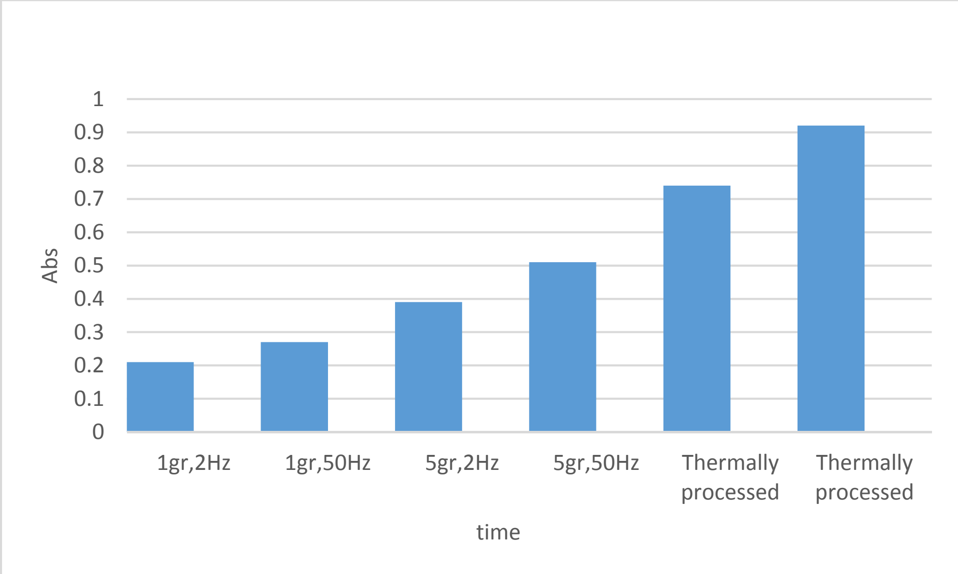


**Figure 3.Effects of thermal processing and PEF processing on red cabbage**

### *Conclusions*

In this study, the total antioxidant capacity of acetic acid extract (polar extract) of red cabbage leaves showed that this plant can be one of the potential sources of safer natural antioxidants. PEF processing was effective in inactivating endogenous microorganisms in red cabbage and extended the shelf life of red cabbage while reducing the degradation of the major qualities of orange juice including, flavor, and color.

PEF-processed red cabbage had a higher sensory preference on texture, flavor, and overall acceptability than thermally processed red cabbage.

The results from the analyses of volatile flavor, color, and sensory evaluation imply that PEF-processed orange juice had higher fresh qualities than those of the thermally processed red cabbage. It is commercially feasible to pasteurize orange juice by PEF technology.

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### *References*

[1] Published at The 2nd International Conference on Pharmacy and Advanced Pharmaceutical Sciences in Yogyakarta July 19-20, (2011).

[2] Baublis, A., Spomer, M. and Berber-Jimenez, M. D. ," Anthocyanin pigments comparison of extract stability", J Food Sci 59:1219–26, (1994).

[3] Chalermchat, Y., Fincan, M. and Dejmek, P. ," Pulsed electric field treatment for solid–liquid extraction of red beetroot pigment: mathematical modelling of mass transfer", J Food Engr 64(2):229–336, (2004).

[4] Ade-Omowaye, B. I. O., Rastogi, N. K., Angersbach, A. and Knorr, D. ," Combined effects of pulsed electric field pre-treatment and partial osmotic dehydration on air drying behaviour of red bell pepper", J Food Engr 60(1):89–98, (2003).

[5] Jeyamkondan, S., Jayas, D. S. and Holley, R. A. ," Pulsed Electric Field Processing of Foods: A Review", Journal of Food Protection: September 1999, Vol. 62, No. 9, pp. 1088-1096, ,(*1999*).

[6] Barba, F. J., Grimi, N. and Vorobiev, E. ,"Evaluating the potential of cell disruption technologies for green selective extraction of antioxidant compounds from Stevia rebaudiana Bertoni leaves", (2014).

[7] Min, S.,Jin, Z. T.,Min, S. K.,Yeom, H. and Zhang, Q. H. ," Commercial-Scale Pulsed Electric Field Processing of Orange Juice", (2003).

[8] Chanda, S. and Dave, R. ," In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview", (2009).

[9] Braca, A., Sortino, C., Politi, M.,Morelli, I. and Mendez, J. ," Anti-oxidant activity of flavonoids from Licania licaniaeflora", J Ethnopharmacol 79: 379381, (2002).

[10] Bors, W., Saran, M. and Elstner, E. F. ," Screening for plant antioxidants", In: Linskens HF, Jackson JF. eds. Modern Methods of Plant Analysis-Plant Toxin Analysis-New Series, Vol 13. Springer, Berlin, pp. 277-295, (1992).