Green Pulsed Electric Field-Assisted Extraction Method of Total Carotenoid Carrot Pulp Using Olive Oil as Solvent

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Abstract

Carrot (*Daucus carrota* L.) pulp as a by-product of carrot juice processing, containing a high content of total carotenoid. It was necessary for the extraction that can be used to other food products enriched by carotenoid. Green extraction method of total carotenoid from carrot pulp assisted by Pulsed Electric Field (PEF) which had been designed was well determined in this study. Extra virgin olive oil was applied as a substitute to organic solvent which was in line with green extraction concept. The oil based extraction process by PEF-assisted was compared not only with oil based extraction using both olive oil and crude palm oil without PEF-assisted but also compared with conventional solvent extraction using 96% ethanol as solvent. The present study has been determined that the PEF-assisted extraction method using olive oil as solvent had highest of total carotenoid extract (34.16+1.02 μg/g) when compared with other methods. This method under condition in carrot pulp-oil ratio was 1: 10 (w/v), treatment time was 10 second and electric field strength was 2.5kV/cm

Keywords: carrot pulp, green extraction, pulsed electric field, total carotenoid

INTRODUCTION

The composition of wastes emerging from vegetable food and agro industries are inexpensive and available in large quantities, which is characterized by a high dietary fiber content resulting with high water binding capacity and relatively low enzyme digestible organic matter [1]. Carrot pulp is a by-product obtained during carrot juice processing. The carotenoid content in carrot juice yield is only 60-70%, and even up to 80% may be lost with left over in carrot pulp [2]. As we know that carotenoid has been known to reduces the risk of chronic diseases (cardiovascular diseases, cancer, and so forth) [3-5].

The total carotenoid (TC) in carrots pulp is approximately of 2 mg/g of dry matter depending on the processing conditions [6], which has good residual amount of all the vitamins, minerals and dietary fiber [7]. The use of carrot pulp as by-product utilization in other enriched carotenoid food product will decrease the environmental load [8]. To obtain a high TC extract from carrot pulp, it is important to have a critical understanding of TC degradation during the extraction process, such as a well-known property of carotenoids is their ability to absorb light and oxygen which is once easy to oxidation process [9]. Thus, it suggests to extract these in fat-soluble compounds or extract with an edible oil, which makes them more bioavailable than carotenoids in a plant cellular matrix [10]. The other study reported that olive oil is also maintained carrot and tomato from carotenoid degradation and isomerization reactions [11].

However, solvents of petrochemical origin (hexane, methanol, ethanol, petroleum ether, and so forth) are now strictly regulated [12], and the heat is required to evaporate the solvent applied for conventional solvent extraction (CSE) that may damage some carotenoids [13]. Therefore, the industry is forced to use more alternative environmentally friendly solvent and new natural substance extraction processes or known as green extraction.

In recent years, PEF technology with low electric field strength (0.7-3.0 kV/cm) has been widely applied to increase yield of fruit juice and to improve the high extraction rate of important compounds such as antioxidants, colouring or flavor from inside of cell [14-19]. By using PEF technique to extraction process of carrot pulp, high efficiency and extraction rate of TC will be obtained. This is due to since electroporation on pulp formed, then the olive oil is expected to bind the carotenoid compound which regardless from inside the cell, considering that carotenoid and olive oil have the same degree of polarity. Therefore, the aim in present study is to extract of TC with fast and green PEF-assisted extraction

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technique using extra virgin olive oil (EVOO) as an alternative solvent. The oil base extraction(OBE) processed by PEF-assisted is compared not only with EVOO and CPO as solvent using magnetic stirrer (without PEF-assisted) but also compared with CSE using ethanol as solvent were carried out in terms of processing procedure and TC content.

MATERIAL AND METHOD

Materials, chemical and Reagents

Fresh carrots (*Daucus carrota* L.*)* was purchased from farmers in Batu City, East Java, Indonesia, harvested at the age of 3-4 month. Carrots washed with water and peeled with a thickness of 1 mm and the base and edge were cut. Then it fit into the juicer (*Oshiyama-Japan*). The juicers were used to separate the carrot juice and carrot pulp. The extra virgin olive oil (EVOO) was used in this study produced by *Aceites Borges Pont, S.A.U*. Tarrega-Spain.

The chemicals and reagents used in this study were ether, acetone, sodium sulfate, aluminum oxide, petroleum ether, and commercial total carotenoid were purchased in Panadia Laboratory Malang, Indonesia.

Pulsed electric Field Apparatus

The main apparatus in this study was PEF batch system which had been designed. The treatment chamber of PEF adopted from coaxial model and it was made of stainless steel as well as food grade material. PEF used in present study presented in Figure 1.

Figure 1. Pulsed Electric Field

PEF was also being equipped with control systems. It was specifically designed with electric field ranging from 1.5-5.06 kV/cm. The frequency from 1 to 3 kHz, the timer using *Omron H3CR* ranged from 0.05 second to 300 hours and pulse width produced of 160 µs. In present study, PEF was used to accelerate extraction rate of carrot pulp in electric field with the strength of 2.5 kV/cm and the treatment time of 10 seconds.

Total carotenoid analysis

The TC analysis reviewed by AOAC [20] method: First, the preparation sample obtained by adding 35 mL petroleum ether-acetone (1:1) solution to 5 g freshly carrot pulp. Second, cover flask and stir for 10 minutes in a magnetic stirrer. Third, allow residues to settle and decant thru sintered glass filter leaving most of the pulp in the flask. Next, rinse out sintered glass with the solvent and repeat extraction. Then, similarly filter as above and ultimately rinsing out flask and pulp. The result of this filtration was filtrate and pulp. After that, the pulp was discarded and the filtrate was making up volume to 100 mL and mix well.

The 25 mL of filtrate was taken and added to 25 mL distilled water and shake. Allow layers to separate and drain off lower layer. So it would produce sediment, the bottom layer was water-acetone phase, while at the top was the ether phase. Then, the ether phase was added Na_2SO_4 at a ratio of 5 to 10 g per 100 mL. Carefully transfer extract quantitatively to prepared column and apply gentle suction to facilitate running through the petroleum ether. Perform this operation in darkened room away from light. After that, add petroleum ether-acetone (10:1) solution to elute the TC band. Similarly apply gentle vacuum to speed up the process. Finally, make up volume of eluted total carotenoid to 25 mL with the petroleum ether-acetone (10:1) solution.

The process of making standard curve used stock solution of total carotenoid (5mg/mL), the weigh of 10 mg commercial total carotenoid. Then, it was dissolved in 2 mL of petroleum ether-acetone (10:1) solution. After processing, measure the absorbance at 450 nm and plot against amount of total carotenoid to get the slope value in mg total carotenoid/absorbance.

The absorption of the extracted carotenoid content were measured using a spectrophotometer (*Spectro 20D Plus RS-232C Labomed. Inc*.) at absorbance of 450 nm. The Beer–Lambert law was used to determine the carotenoid concentration from a calibration curve prepared using the carotenoid standard. The straight calibration curve of absorbance versus carotenoid concentration (µg/g) was reliant on the Beer–Lambert law. The TC calculation was presented as follows:

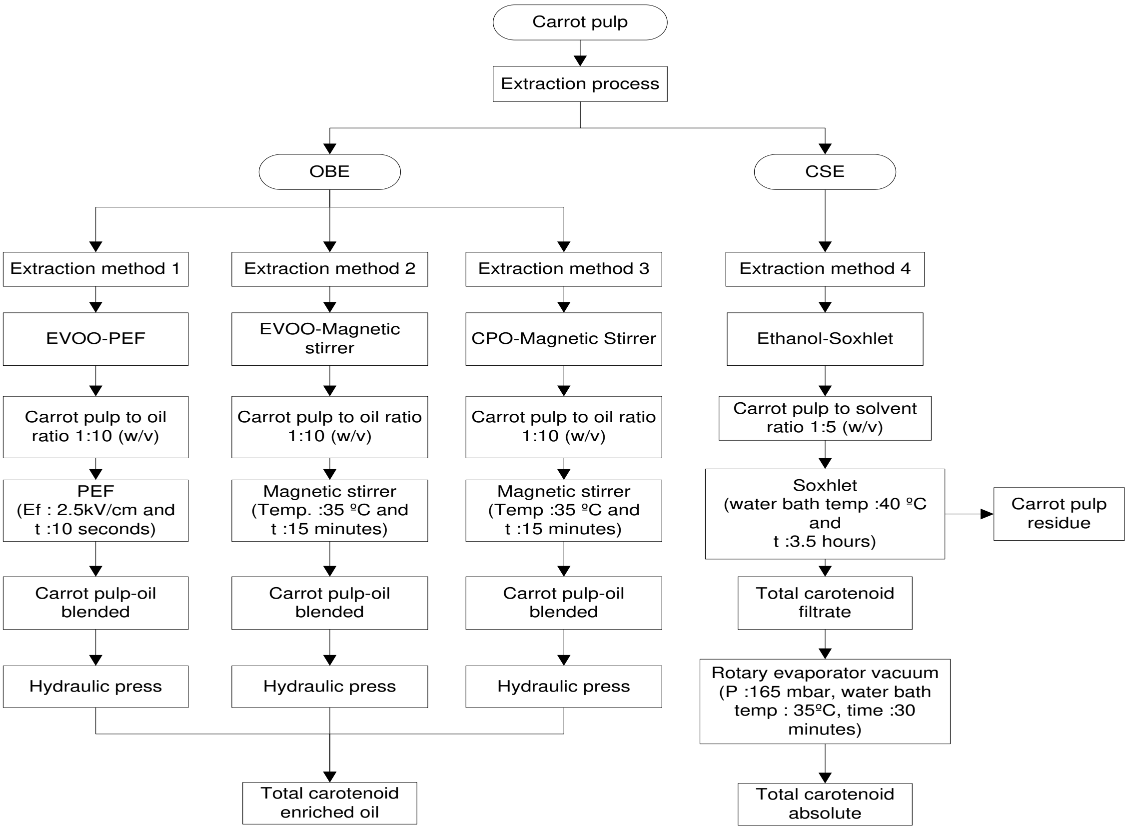


Figure 2. Extraction Procedures in Present Study
Note: OBE (Oil Base Extraction), CSE (Conventional Solvent Extraction), EVOO (Extra Virgin Olive Oil), CPO (Crude Palm Oil)

$$TC\ (\%) = \frac{X\left(\frac{mg}{ml}\right)}{w(mg)} x y(ml) x f x\ 100\%$$

Where *x* was the absorbance value of extract at 450 nm, *y* was the volume of sample, *w* was the weight of sample, and *f* was dilution factor. The conversion of TC to µg/g was to multiply the TC result (%) with 10^4 (µg/g).

Moisture content analysis

Determination of moisture content following AOAC [21] method: first, dry the empty dish and lid in the oven at 105°C for 3 hours and transfer into desiccators to cool. Weigh the empty dish and lid. After that weigh about 3 g of sample to the dish. Spread the sample to the uniformity. Place the dish with sample in the oven. Dry for 3 h at 105°C. After drying, transfer the dish with partially covered lid to the desiccators to cool. Reweigh the dish and its dried sample. The moisture content calculation was presented as follows:

$$\text{Moisture (dry matter) (\%)} = \frac{W_1 - W_2}{W_1}\ x\ 100\%$$

Where W_1 is weight (g) of sample before drying and W_2 is weight (g) of sample after drying.

Extraction Procedures

The TC extraction procedures from carrot pulp are divided into four extraction methods which are presented in Figure 2. Data were analyzed using analysis of variance. The TC enriched oil data of each method is given in histogram and standard errors of the mean. When significant differences were detected by the F-test, means were separated with LSD test at the 0.05 probability level.

RESULT AND DISCUSSIONS

Charateristic of Carrot Pulp

Carrot pulp was obtained after shredding by juicer from fresh carrots. One kg of fresh carrots may produce of 358 g carrot pulp by juicer. Furthermore, carrot pulp was used for the extraction process either with OBE or with CSE process. The pulp as by-product in the fruit and vegetables juice industry was becomes points to consider. Many juice industries simply dumping their pulp, even though it was keeping much of the much benefits such as other micro-content which is having beneficial to the body.

Table1. The Comparison of Carrot Pulp Composition with Other Study

Sources	Parameters	
	Moisture content (%)	Total carotenoid (µg/g)
Jamuna and Shyamala [22]	84.23 ± 0.18	40
Upadhyay *et al*. [23]	85.62	143.90
Present study	85.35 ± 0.23	104.64

This is in line with Singh et al. [2] who has conducted research on utilization of waste residues obtained during carrot juice extraction for the preparation of a value added product viz. carrot based condensed milk product (gazrella, an Indian sweetmeat).The utilization of carrot pulp as by-product in other enriched carotenoid food product will decrease the environmental load. In the present study the water content and TC of carrot pulp has investigated and compared with other study that given in Table 1.

Based on Table 1, the water content in present study is not much different both Jamuna & Shyamala [22] and Upadhyay et al. [23], but the TC of carrot pulp in present study had a lower content when compared with Upadhyay et al. [23] study(143.9 µg/g). This is presumably because the carrots used in the study had a different type. The fresh carrots used in Upadhyay et al. [23], has the TC of 552.50 µg/g, in contrast to carrots used in present study that has TC of 152.90 µg/g.

Whereas the present study has a higher of TC when compared with Jamuna & Shyamala [22] study. This is because carrot pulp left after juice extraction was dried in an oven at 50 ± 1°C and powdered using a lab grinder, then stored in air-tight jars under refrigeration at 4°C till use. The storage and drying process may lead to reduce the antioxidant compounds of fresh vegetables.

Green Extraction with EVOO as solvent

Several previous studies also were conducted carotenoids extraction by using a combination of organic solvent, but it takes some combination of the organic solvent to obtain the optimal results of carotenoid. These combinations conducted in order to the solvent can penetrate the thick layers of cell walls and dissolves carotenoid compound in it.

This obviously requires a lot of solvents, which are also expensive and ineffective such as carrot-solvent ratio of 1: 29 (w/v) [24] and 1: 8.12 (w/v) in which these solvent consist of acetone-methanol ratio of 9.37: 10 (v/v) [25]. This statement is supported by Mustafa et al. [26], that the conventional extraction methods of carotenoid, require a lot of organic solvents which are expensive, environmentally harmful, and require costly handling procedures.

Setting up the green extraction process in industrial production involves the use of alternative solvents which are non-flammable, biodegradable and non-toxic. Green extraction has now shown its benefits, not only in reducing risks to human health, but also protecting the environment in an economically beneficial manner in a sustainable world [12].

The green TC extraction using sunflower oil was determined by Li et al. [12] with Ultrasonic-assisted extraction (UAE) of 22.5 W/cm2, 40°C and sonication time of 20 min, may produce the higher TC (334.75 mg/L) when compared with CSE by using hexane as solvent for 60 minutes (321.36 mg/L). Their study explains that bio-refinering green techniques for extracting carotenoids from fresh carrots have various advantages in terms of cost, time, effort and results.

Edible vegetable oil has been reported to have potential as an alternative solvent or co-solvent in the extraction of desired compounds [27-30]. In addition, other study also reported that the edible oils such as olive oil as a solvent in extraction process may retain the stability of TC other than petroleum-based solvents [11].

Thus the EVOO is selected as alternative solvent in the present study. This applies because it is considered as an oil-based solvent which is non-polar oil and more stable in maintaining the carotenoid content of oxidation process. The addition of carotenoid in functional foods will have many advantages such as: carotenoid can be incorporated into an organic solvent such as edible oil, which makes the carotenoids become more bio-available than the carotenoid in plant cellular matrix [10].

Carrot Pulp Extraction

Carrot pulp has been known to have fairly high of the TC content. Based on several theories that have been proposed previously on the TC extraction of carrot pulp, then this study will explain the extraction process of carrot pulp by EVOO as solvent which in line with green extraction concept and compared with several methods. The first method is to extract the carrot pulp by mixing EVOO with PEF-assisted. Second method is carrot pulp-EVOO extraction without PEF-assisted, third, carrot pulp-CPO extraction using magnetic stirrer. Last method is carrot pulp extraction using soxhlet apparatus and 96% ethanol as a solvent. The TC results that can be extracted by using each of those methods are presented in Figure 3.

Carrot Pulp-EVOO with PEF-Assisted

The EVOO-carrot pulp mix was given the high voltage pulses by the PEF apparatus with electric field strength of 2.5 kV/cm and treatment time of 10 seconds. The PEF-assisted conducted to accelerate extraction process because when the electroporation of carrot pulp was occurred, the TC content within the cell will diffuse out and be able to bind by olive oil and keep it from TC oxidation process. In addition, the TC binding process is also effective, since the carotenoid and olive oil have the same level of polarity characteristic (non-polar compound). Thus, OBE was done to the recovery of TC in carrot pulp. Based on Figure 3, it can be seen that the TC extract which can be recovered from carrot pulp with different treatment is still quite low (below 50%) when compared with the TC content from freshly carrot pulp (104.64 μg/g).

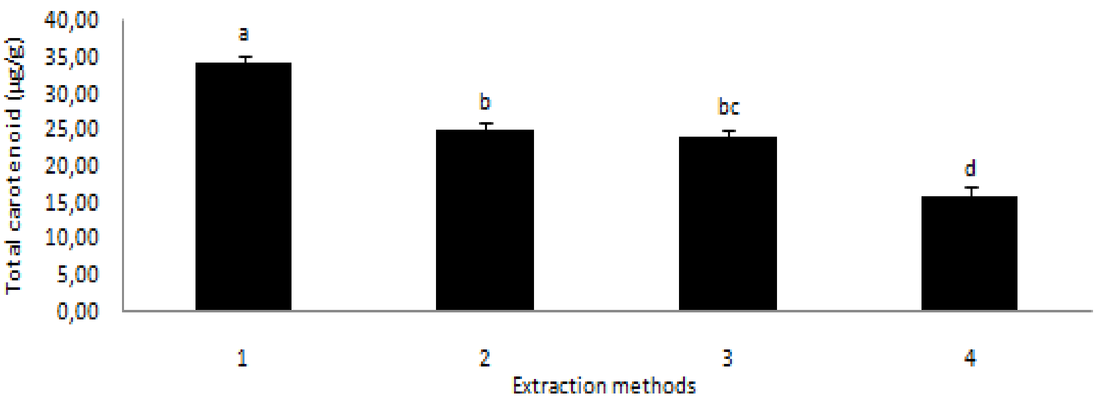
This is presumably because the factors that are important in extraction processes such as the solvent-material ratio, extraction time, and extraction temperature are not used as independent variables in present study, it just takes the best results from existing studies. Therefore, if there is one different factor, it will affect the extraction results obtained.

However, between the four extraction methods, the highest of TC extract was a mixture of carrot and EVOO using PEF-assisted which was capable of producing a TC of 34.16 $\pm$ 1.02 μg/g (Fig. 3) or were able to extract 32.64% from 100 g of freshly carrot pulp. Actually, the result of TC extract using PEF-assisted was 37.30 μg/g, but it was reduced by TC content in pure EVOO of 3.14 μg/g [31]. This was done to determine of TC extracted using OBE.

PEF-assisted extraction would make pores cells became much more and was irreversible, thus the TC which are in chromoplast would be easy to go out and dissolved into the EVOO. There are several potential advantages of using solid-lipid particles for delivering carotenoids into foods. First, incorporation of carotenoids into the core of core-shell solid-lipid particles, may offer a physical barrier for protecting carotenoids from aqueous pro-oxidants.

Second, the nature of the lipids used to create these particles may help to decrease the rate of oxidation reactions. Since more solid-like lipids are needed to form solid particles, it is likely that the lipids chosen for solid-lipid particle applications will be more saturated than fats commonly used in conventional emulsions [10]. In addition, TC compound in oil is useful in creating a high stability of TC such as lycopene [32].

Moreover, PEF-assisted extraction would also increase the volume of TC extract result (Table 2). Based on table 2, EVOO-PEF assisted method has a highest TC extract volume when compared with other methods. It was occurred since electroporation influence the big cell cavitations and release of intracellular compounds to EVOO.



Note:

1: Carrot pulp-EVOO with PEF-assisted
2: Carrot pulp-EVOO without PEF-assisted
3: Carrot pulp-CPO using magnetic stirrer
4: Carrot pulp-ethanol using Soxhlet Apparatus

Figure 3. Comparison of total carotenoid result by using different methods. Histogram values for each extraction method with the same notation are not significantly different at LSD 5%

Table 2. Mass Calculation in Each Extraction Methods

Extraction Methods	Solvent volume (mL)	Carrot pulp mass (g)	Treatment time (minutes)	Extract volume (mL)	Carrot pulp mass of extraction residue (g)
EVOO-PEF assisted	150	15	0.17	122	9.79
EVOO-without PEF-assisted	150	15	15	112	9.70
CPO-magnetic stirrer	150	15	15	110	9.81
Ethanol-Soxhlet	150	30	210	67*	29.88

Note:* = carrot pulp mass + solvent after throughout vacuum evaporator

Carrot pulp cytoplasm that exist within the cell which is come into EVOO influencing of high volumes of extract volume after the extraction process. In addition, when a mixture of oil and carrot pulp were compressed using a hydraulic press, the oil-pressed obtained some substances that cannot be homogeneous with olive oil. This substance believed to be water contained in the material, cytoplasm and other substances that are not soluble in oil.

The Carrot pulp-EVOO without PEF-Assisted

The second highest TC extraction in Fig. 3 was the carrot pulp-EVOO mix extraction without PEF-assisted of 24.81 $\pm$ 1.11 µg/g or able to extract at 23.71%. The TC result was also the reduction with TC content in EVOO. The lower results when compared with the first extraction method was presumably since the cells in the carrot pulp was still too strong and still protected the TC in the cell thus the heating in magnetic stirrer was still not able to break down the cells in the carrot pulp. Despite having a lower TC than PEF-assisted extraction method, the inclusion of TC in EVOO also brings a lot of benefits in the olive oil containing high antioxidant and its stability of the oxidation reaction. The EVOO can maintain the TC content than palm oil or other oils since the EVOO typical nature has high unsaturated fatty acids, especially high in oleic acid (C18: 1) that is equal to 83%. With the double-bonds less, causing oleic acid are more stable in the degradation, compared with linoleic acid (C18: 2) which has more double-bonds. The fatty oxidation reaction is influenced by the degree of unsaturation of fat, the configuration of the double bond, the degree of etherification, catalytic oxygen and temperature. As an example, entry linoleic acid (C18: 3) is more easily oxidized than linoleic acid (C18: 2).

This is in line with Bezbradica *et al*. [33], that the fatty acid composition of the solvent determines the rate of degradation of carotenoids. Triglycerides with a high content of linoleic acid are high susceptible to oxidation. Thus, the olive oil as a solvent extraction has higher stability in the degradation of carotenoids when compared with sunflower oil, soybean oil, grape seed oil and almond oil.

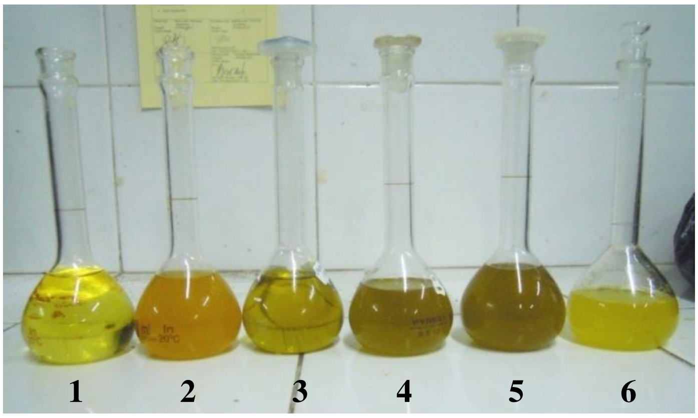
Carrot pulp-CPO Using Magnetic Stirrer

Based on Fig. 3, the extraction method using magnetic stirrer and CPO as a solvent may produce TC from carrot pulp of 23.98 $\pm$ 0.91 µg/g or be able to extract 22.92%. The reasons for using CPO solvent as a comparison since it is relatively cheaper than EVOO, thus when applied to the industry it will obviously affect in production costs. Both carrot pulp-EVOO without PEF-assisted and carrot pulp-CPO using magnetic strirrer have not significantly different at LSD 5% (Fig. 3). It indicates that the TC result both of this extraction methods are not significantly increase in enriched oil of TC.

However, based on the TC value, using CPO as solvent is actually have lower TC extract when compared with using EVOO as a solvent. Moreover, CPO enriched of TC will also have difficulty in determining the further processing of food product. It would be very ineffective when CPO enriched of TC is only used as cooking, since the oxidation process will occured rapidly during the frying process.

Carrot pulp-Ethanol using Soxhlet Apparatus

The reason for using ethanol 96% as a solvent since it was a common and safe solvent or generally recognized as safe (GRAS) solvent and has a positive net energy balance (NEB) [34,35]. Extraction of TC from carrot with 96% ethanol should be done at temperatures about 30-40°C to obtain a high extraction yield of carotenoid from carrot [36], but in present study was carried out at a temperature above 55°C. The selection of this temperature was still relatively safe for the extraction of TC that was carried out under a temperature of 60°C since at this temperature, a good release of carotenoid occured from the disturbed texture of the carrot [37]. Carrot pulp extraction using soxhlet method performed for 3.5 hours. This was done considering that a given temperature to evaporate the ethanol under the boiling point of ethanol itself (78°C), thus the extraction process with soxhlet method required more time.



Note:

1: Pure CPO
2: CPO enriched with extracted TC
3: Pure EVOO
4: EVOO enriched with extracted TC using magnetic stirrer
5: EVOO enriched with extracted TC using PEF-assisted
6: Ethanol enriched with extracted TC

Figure 4. The oil colors obtained by each extraction methods

The extraction process using soxhlet method and 96% ethanol as solvent may produce TC from carrot pulp was 15.61 ± 1.43 µg/g or be able to extract at 14.91% (Figure 2). The significant decrease in TC is presumably because ethanol has not fully dissolved in TC of carrot pulp. The temperature used to evaporate the ethanol was limited to a temperature of 55°C, whereas the boiling point of ethanol was 78°C. Thus, with a temperature of 55°C and 3.5 hours long, the soxhlet apparatus can vaporize a little ethanol drop to fall into carrot pulp. This would lead to TC content dissolved in ethanol was decreased. The TC extraction results in Figure 3 are supported by oil colors extraction results which can be seen in Figure 4.

Based on Figure 4, It can be seen that the TC extracted from the carrot pulp would create a turbid or make it into orange color both pure CPO and pure EVOO. This is in accordance with the Soujala [38] study, that the alpha and beta carotene is the major carotenoid pigment which leads to yellow and orange colors. The TC color which is produced on ethanol-soxhlet method has the brightest color (light yellow). This is presumably due to pure ethanol that has translucent colors, so that the extraction of the filtrate will be colored yellow. In addition to seeing the total carotene content of the low, it can be said that in the filtrate still contains a lot of ethanol.

The CPO color which is much of TC become more orange or red, while the EVOO which is containing a TC has rather turbid colors as color itself is not clear and has a darker color than pure CPO. But when it comparison between EVOO by magnetic stirrer homogenized treatment and PEF treatment, the EVOO color with PEF treatment has a darker color. This indicates that the presence of PEF-assisted may increase the percentage of the TC extraction in carrot pulp.

Conclusion

PEF-assisted extraction has been determined and had a highest of TC extract from carrot pulp when compared with CSE and other methods. Extra virgin olive oil also has been proven to be a green substitute for petroleum-based solvents like sunflower oil that can be safely used in extraction and in line with the green extraction concept. This technology was still carried out on a laboratory scale and potentially to be applied at an industrial scale.

References

[1] Serena A. and Bach Knudsen K.E. 2007. Chemical and physicochemical characterisat ion of co-products from vegetable food and agro industries. *Animal Feed Sci.and Technol.* 139: 109–124.

[2] Bohm V., Putpitasari-Nienaber N.L., Ferruzzi M.G., and Schwartz, S.J. 2002. Trolox equivalent antioxidant capacity of different geometrical isomers of alfa-carotene, beta-carotene, lycopene and zeaxanthin. *J. Agri. and Food Chem*., 50: 221–226.

[3] Krinsky, N.I. and Yeum, K.J. 2003. Carotenoid radical interactions. *Biochem. Biophys. Res. Commun*. 305: 754–760.

[4] Paolini, M., Abdel-Rahman, S.Z., Sapone, A., Peduli, G.P., Perocco, P., Can-telli-Forti, G., and Legator, M.S. 2003. β-Carotene: A cancer

chemopreventive agent or co-carcinogen. *Mutat. Res.* 543: 195–200.
[5] Stahl W., and Sies, H. 2003. Antioxidant activity of carotenoids. *Mol Aspects Med.* 24: 345–351.
[6] Kumar, N., Sarkar, B.C. and Sharma, H.K. 2010. Development and characterization of extruded product using carrot pulp, pulse powder and rice flour", *African J. Food Sci.* Vol. 4(11) : 703–717.
[7] Kumar, K. and Kumar, N. 2012. Development of vitamin and dietary fibre enriched carrot pulp and wheat flour based buns. *J. Pure and App. Sci.and Technol.* 2(1):107-115.
[8] Singh B., Panesar P.S. and Nanda, V. 2006. Utilization of carrot pulp for the preparation of a value added product. *World J. Dairy and Food Sci.* 1 (1): 22-27.
[9] Britton, G. 1995. Structure and properties of carotenoids in relation to function, *J.FASEB.* 9:1551–1558.
[10] Boon, C.S., Mc Clements, D.J., Weiss, J., and Decker, E.A. 2010. Factors influencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Sci. and Nutr.* 50: 515–532.
[11] Colle, I., Lemmens, L., Knockaert, G., Van Buggenhout, S., Van Loey, A., dan Hendrickx, M. 2013. Carotenoid conversion kinetics depend on the carotenoid type and on the food system. *Inside Food Symposium*, Leuven, Belgium, 9-12 April 2013.
[12] Li, Y., Fabiano-Tixier, A.S., Tomao, V., Cravotto, G., and Chemat, F. 2013. Green Ultrasound - assisted extraction of carotenoids based on the bio-refinery concept using sunflower oil as an alternative solvent. *Ultrasonics Sonochemistry*, 20: 12–18, available online at: www.elsevier.com/locate/ultson
[13] Lin, C.H. and Chen, B.H. 2005. Stability of carotenoids in tomato juice during storage. *Food Chem.* 90: 837–846.
[14] Knorr D., Angersbach A., Eshtiaghi M.N., Heinz V., and Dong-Un Lee D.U. 2001. Processing concepts based on high intensity electric field pulsed. *Trends Food Sci. Technol.* 12: 129–135.
[15] Bouzrara, H. and Vorobiev, E. 2003. Solid-liquid expression of cellular materials enhanced by pulsed electric feld. *J. Chem. Eng. Process* 42: 249–25.
[16] Vorobiev, E., and Lebovka, N.I. 2006. Extraction of intercellular components by pulsed electric felds. In: Raso J. and Heinz H. (eds) Pulsed electric field technology for the food industry - fundamentals and applications. Springer, New York, pp 153–194.
[17] Toepfl, S. 2006. Pulsed electric fields (PEF) for permeabilization of cell membranes in food and bioprocessing – applications, process and equipment design and cost analysis. PhD Dissertation. Prozesswissen-schaften der Technischen Universität Berlin. Jerman.
[18] Shamsi, K. 2008. Effects of pulsed electric field processing on microbial, enzymatic and physical attributes of milk and the rennet-induced milk gels. PhD Dissertation. School of Applied Sciences. College of Science, Engineering and Technology. RMIT University. Melbourne.
[19] Donsi, F., Giovana F., and Pataro, G. 2010. Applications of pulsed electric field treatments for the enhancement of mass transfer from vegetable tissue. *J. Food Eng. Rev.* 2:109–130.
[20] AOAC. 1975. Method of analysis of the association of official analytical chemists, 12th edition, Washington D.C.
[21] AOAC. 1999. Official Methods of Analysis of AOAC International, 16th edition. (Cunniff, P., ed.) Gaithersburg, MD.
[22] Jamuna, P. and Shyamala B.N. 2010. Nutritional content and antioxidant properties of pulp waste from daucus carota and beta vulgaris. *Mal. J. Nutr.* 16(3) :397 – 408.
[23] Upadhyay, A., Sharma H.K., and Sarkar B.C. 2008. Characterization and dehydration kineticsof carrot pomace. agricultural engineering international: The CIGR Ejournal. Manuscript FP 07 35. 10: 1-9.
[24] Wang, L. and Liu Y. 2009. Optimization of solvent extraction conditions for total carotenoids in rapeseed using response surface methodology. *J. Natural Sci*. 1: : 23-29.
[25] Ikawati, R. 2005. Optimization condition Extraction of Carotenoids from Carrots (*Daucus carrota* L.) Using Response Surface Methodology. *J. Agri. Technol*. 1(1): 14-22.
[26] Mustafa A., Leire M.T and Charlotta T. 2012. Pressurized Hot Ethanol Extraction of Carotenoids from Carrot By-Products. *Molecules*,(17) :1809-1818.
[27] Hamed S.F. 2006. Edible oil as an alternative solvent for extraction of antioxidant components from natural herbs, *J. App. Sci. Research* 2: 567–571.

[28] Sachindra N.M., and Mahendrakar N.S. 2005. Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. *Bioresource Technol.* 96: 1195–1200.
[29] Kang, C.D. and Sim, S.J. 2008. Direct extraction of astaxanthin from *Haematococcus* culture using vegetable oils, *Biotechnology Letters* 30: 441–444.
[30] Sun, M. and Temelli, F. 2006. Supercritical carbon dioxide extraction of carotenoids fromcarrot using canola oil as continuous co-solvent, Journal of Supercritical Fluids, 37: 397–408.
[31] Torres, M., Martínez, M., Pierantozzi, P., Albanese, M., Nasjleti, A., andMaestri, D. 2011. Contribution of Compositional Parameters to the Oxidative Stability of Olive and Walnut Oil Blends. *J. Amer. Oil Chem. Soc*. 88 (6):755-762.
[32] McClements, D. J. 2005. *Food Emulsions: Principles, Practices, and Techniques, 2nd ed*. New York, CRC Press.
[33] Bezbradica, D., Milic-AsKrabic, J., Petrovic, S.D., and Siler-Marinkovic, S. 2005. An investigation of influence of solvent on the degradation kinetics of carotenoids in oil extracts of *Calendula officinalis*. *J. Serb. Chem. Soc.* 70 (1): 115–124.
[34] Kim, S. and Dale, B.E. 2005. Environmental aspects of ethanol derived from no-tilled corn grain: nonrenewable energy consumption and greenhouse gas emissions. *Biomass Bio-energy,* 28: 475–489.
[35] Hill, J., Nelson, E., Tilman, D., Polasky, S., and Tiffany, D. 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels*. Proc. Natl. Acad. Sci.* 103: 11206–11210.
[36] Das, S. And Bera, D. 2013. Mathematical model study on solvent extraction of carotene from carrot. *Inter. J. Research in Eng. & Technol*. 2(9): 343-349.
[37] Fikselova, M. Stanislav Šilhár, Ján Marecek and Helena Francáková. 2008. Extraction of carrot (*Daucus carota* L.) carotenes under different conditions. *Czech J. Food Sci*. 26 (4): 268–274.
[38] Suojala T. 2000. Postharvest pre and development of carrot yield and quality. PhD Dissertation. Helsinki: Faculty of Agriculture and Forestry, University of Helsinki.