

The Canadian Society for Bioengineering
*The Canadian society for engineering in agricultural, food,
environmental, and biological systems.*



**La Société Canadienne de Génie
Agroalimentaire et de Bioingénierie**
*La société canadienne de génie agroalimentaire, de
la bioingénierie et de l'environnement*

Paper No. CSBE17037

Process Intensification of Phenolic Extraction from Plums

Andrea Di Nardo

School of Engineering, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada, N1G 2W1,
Email: dinardoa@uoguelph.ca

Jayasankar Subramanian, Ph.D.

Department of Plant Agriculture, University of Guelph, 4890 Victoria Avenue North, Vineland, Ontario,
Canada, L0R 2E0

Ashutosh Singh, Ph.D.

School of Engineering, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada, N1G 2W1,
Email: asingh47@uoguelph.ca

**Written for presentation at the
CSBE/SCGAB 2017 Annual Conference
Canad Inns Polo Park, Winnipeg, MB
6-10 August 2017**

ABSTRACT In Canada, more than \$31 billion worth of food waste is generated every year which has potential for significant environmental and social impacts. However, these wastes are rich in health promoting phytochemicals including phenolic compounds, flavonoids etc., which are low molecular weight plant secondary metabolites and are chemically very heterogeneous. Hence, valorization of agricultural and retail food wastes into nutraceutical compounds using conventional and novel extraction methods will generate more revenues for food producers, processors and retailers. But, conventional solid-solvent extraction methods, such as soxhlet and heat reflux, are associated with longer extraction times, high consumption of organic solvents, low energy efficiency and high operational and maintenance cost. Various novel extraction methods including microwave-assisted extraction (MAE), pulsed electric field (PEF), ultrasonication and supercritical fluid extraction (SFE) can be used as an alternative to conventional methods. Of all the aforementioned techniques MAE is a highly viable alternative. MAE utilizes microwave energy for rapid homogeneous heating of low volume of organic solvents and the biomatrix (eg. Organic food waste) to facilitate a synergistic combination of heat and mass transfer. This synergy results in volumetric dissipation of heat and

Papers presented before CSBE/SCGAB meetings are considered the property of the Society. In general, the Society reserves the right of first publication of such papers, in complete form; however, CSBE/SCGAB has no objections to publication, in condensed form, with credit to the Society and the author, in other publications prior to use in Society publications. Permission to publish a paper in full may be requested from the CSBE/SCGAB Secretary: Department of Biosystems Engineering E2-376 EITC Bldg, 75A Chancellor Circle, University of Manitoba, Winnipeg, Manitoba, Canada R3T 5V6 or contact bioeng@csbe-scgab.ca. The Society is not responsible for statements or opinions advanced in papers or discussions at its meetings.

pressure that enhances the overall extraction yield of phytochemicals from biomatrix and also provides selective extraction capabilities that lacks in other novel techniques. In the current study MAE technique is utilized for selective extraction of high value phenolic compounds from a genotype of Yellow European Plums, while reducing extraction time, solvent usage, and energy.

Keywords: Phenolics; antioxidants; microwave; extraction

INTRODUCTION Phenolic compounds are secondary plant metabolites found in fruits and vegetables. Polyphenols contribute to the colour, flavor, and oxidative stability of the food product (Pandey and Rizvi, 2009). Due to their ability to scavenge free radicals, polyphenols positively contribute to cellular processes in the human body and can potentially prevent diseases such as heart disease and certain types of cancer (Chun et al., 2003a; Nawaz et al., 2006). Therefore, adequate intake of foods containing phenolic compounds is essential in maintaining human health. Extraction of bioactive compounds from food waste can be utilized in industries such as functional foods, nutraceuticals, and pharmaceuticals.

Phenolic compounds are traditionally extracted using soxhlet extraction, also known as heat reflux extraction (HRE). HRE utilizes solvents such as methanol, ethanol, hexane, and water, coupled with heat and agitation, to extract compounds from food and plant sources (Singh and Orsat, 2015). HRE is frequently used as it is easy to implement and low in maintenance cost. However, conventional methods are associated with long extraction times, increased solvent usage, and low recovery of compounds due to high operating temperatures (Singh and Orsat, 2015). The use of toxic solvents also poses a risk to human and environmental safety. To mitigate these drawbacks of HRE, process intensification (PI) can be applied to improve process efficiency.

PI is the use of innovative technologies to reduce cost, improve safety, and minimize environmental impact of a conventional system (Aoune and Ramshaw, 1999). PI is constructed on four principles related to design control and optimization:

- Principle 1: Maximize the effectiveness of intra- and intermolecular events
- Principle 2: Give each molecule the same processing experience
- Principle 3: Optimize the driving forces at every scale and maximize the specific surface area
- Principle 4: Maximize the synergistic effects from partial processes

An intensified process utilizes one or more of the above mentioned principles while also utilizing the four functional PI domains: structural, energy, synergy, and time domain. Based on the definition of PI, and the current HRE process drawbacks, two PI approaches were chosen: microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). MAE is considered a PI due to its mechanism of volumetric heating, ensuring each molecule receives the same processing treatment. MAE is a novel extraction method which has been used to extract antioxidants and bioactive compounds from potato peels (Singh et al., 2011), grape seeds (Krishnaswamy et al., 2013), and asparagus (Zhang et al., 2009). UAE is another novel method which utilizes sound waves, with a frequency greater than 20 kHz, for extraction of flavonoids and polyphenols. UAE uses the method of expansion and compression cycles for extraction of polyphenols from plums (Kim et al., 2003), ginseng root (Kim et al., 2007), and green walnut husk (Xu et al., 2016). Both MAE and UAE utilize less solvent, and require less time and energy in comparison to HRE.

The objective of this study was to improve extraction yield, decrease extraction time, increase contact surface area, and reduce toxic solvent effects, by applying PI. This paper reports the effects of MAE and UAE on phenolic yield and antioxidant activity from Yellow European Plums (YEPs). A suitable PI approach will be chosen based on extraction efficiency, time, energy consumption, toxicity, and cost.

MATERIALS AND METHODS

Fruits and chemicals A genotype of YEP, obtained from Vineland Research and Innovation Centre (Vineland, Ontario, Canada), was studied. Folin-Ciocalteu reagent, sodium bicarbonate (NaHCO_3), gallic acid, neochlorogenic acid, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), formic acid, and ammonium formate were purchased from Sigma-Aldrich (Sigma-Aldrich Canada Co., Oakville, Ontario, Canada), whereas ascorbic acid, chlorogenic acid, methanol, sodium hydroxide, sodium acetate, hydrochloric acid (HCl), and ferric chloride were obtained from Fisher Scientific (Fisher Scientific Company, Ottawa, Ontario, Canada).

Sample preparation Plum samples were homogenized using a mortar and pestle. Two grams of the homogenized samples were mixed with 20 mL of the desired solvent, which then underwent various extraction methods.

Microwave extraction, ultrasound extraction, and heat reflux extraction Three experiments were conducted comparing HRE (Experiment 1) with MAE (Experiment 2), and UAE (Experiment 3) based on total phenolic content and antioxidant capacity. Experiment 1 was an evaluation of conventional HRE with two varying factors: solvent (water and methanol), and extraction temperature (50°C, 60°C, and 70°C). Prepared, fresh plum samples were placed in water baths at the appropriate temperatures for 90 minutes. Experiment 2 studied the effects of varying power level (10%, 20%, and 30%) and time (4, 8, and 12 minutes) for MAE. Prepared fresh samples, with water as the solvent, were placed in a conventional microwave oven (RCA Microwave Oven, RMW733, China) at the desired power level for a set amount of time. Experiment 3 studied UAE with one varying factor, time (30, 45, and 60 minutes). Prepared samples in water were placed in an ultrasonic water bath (Bransonic 220, Bransonic Ultrasonic Cleaner, Bransonic Cleaning Equipment Co., Shelton, Conn) at a set frequency of 50 kHz for the desired amount of time. After each experiment, the homogenates were centrifuged (Servall Enclosed Superspeed Centrifuge, Type SS-4, Norwalk, USA) at 10,000 rpm for 10 minutes. The supernatants were then filtered through a 0.45 μm filter and stored at 4°C until further analysis.

Determination of total phenolic content Total phenolic content was determined using a modified protocol by Singh et al. (2011). One millilitre of extract was mixed with 7.5 mL double distilled water, 0.5 mL Folin-Ciocalteu reagent, and 1 mL 5% NaHCO_3 . The mixture was incubated at room temperature, 23°C, for 90 minutes, and its absorbance was measured at 765 nm using a spectrophotometer (Biochrom visible spectrophotometer, Ultrospec 100 pro, Cambridge, England). Total phenolic content was calculated using a gallic acid curve (prepared in water and methanol), where the results were expressed as gallic acid equivalents (GAE) in mg per g of plum FW (fresh weight).

Quantification of phenolic compounds High performance liquid chromatography was used to quantify ascorbic acid, neochlorogenic acid, and chlorogenic acid. A Beckman Coulter System Gold instrument was used and consisted of a UV detector, set at 280 nm, a solvent pump, and a refrigerated autosampler, set at 12°C. Phenolic compounds were separated using a reverse phase C18 Gemini-NX (5 μm , 150 mm x 4.6 mm) column (Phenomenex, Inc., Torrance, CA, USA), fitted with a 4 mm x 3 mm Gemini-NX column guard (Phenomenex, Inc., Torrance, CA, USA). The mobile phase was composed of solvent buffer A (10 mM formic acid in water, pH 3.5, with NaOH) and buffer B (5 mM ammonium formate in methanol) with the following solvent gradient: 0–1 min 100% buffer A, 1–5 min 0–30% buffer B, 5–8.5 min 30–70% buffer B, and 8.5–14 min 70–100% buffer B. Twenty microliters of sample was injected using a flow rate of 1 mL/minute. Compounds were identified by chromatographic analysis of retention times, performed using 32 Karat Software (Version 1.3).

Ferric reducing antioxidant potential (FRAP) A modified method by Routray et al. (2014) was used to measure FRAP of the antioxidants in the extracts. Fresh FRAP reagent was prepared daily

using 300 mM sodium acetate buffer (pH 3.5), 10 mM TPTZ prepared in 40 mM HCl, and 20 mM ferric chloride mixed in a 10:1:1 volume ratio respectively. Twenty microliters of extract was mixed with 300 μ L of double distilled water and 2 mL of FRAP reagent, and incubated in a 37°C water bath for 30 minutes. Following incubation, the absorbance of the sample was measured in a microplate reader (BIO-RAD Microplate Reader, Model 3550) at 595 nm. FRAP activity was quantified using an ascorbic acid curve (prepared in water and methanol), where the results were recorded in terms of ascorbic acid equivalent (AAE) per g of plum FW.

Statistical analysis All experiments, each with their own experimental design and factors, were carried out and analyzed in duplicates. Total values for phenolic content and antioxidant assays are presented in this paper as the mean \pm standard deviation of the duplicates. Analysis of variance (ANOVA) and Tukey’s honestly significant difference (HSD) test were conducted for each experiment to determine the significance of each factor on phenolic content and antioxidant activity. All statistical design of experiments and analyses were conducted using JMP software version 11 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Total phenolic content Figure 1 illustrates the average total phenolic content for each experimental combination, with their standard deviation. It can be observed that MAE yielded the highest phenolic content followed by HRE and UAE. It can also be observed that with an increase in microwave power level, there is an increase in phenolic content. However, with an increase in power and time, there is a subsequent decrease in phenolic yield. For HRE, methanol was able to increase extraction yield, however there was no significant difference between extraction using methanol or water. Hence, water was chosen as the extraction solvent for MAE and UAE experiments. Utilizing water also improves process safety by mitigating the adverse environmental effects associated with the use of methanol, which aligns with the definition of PI.

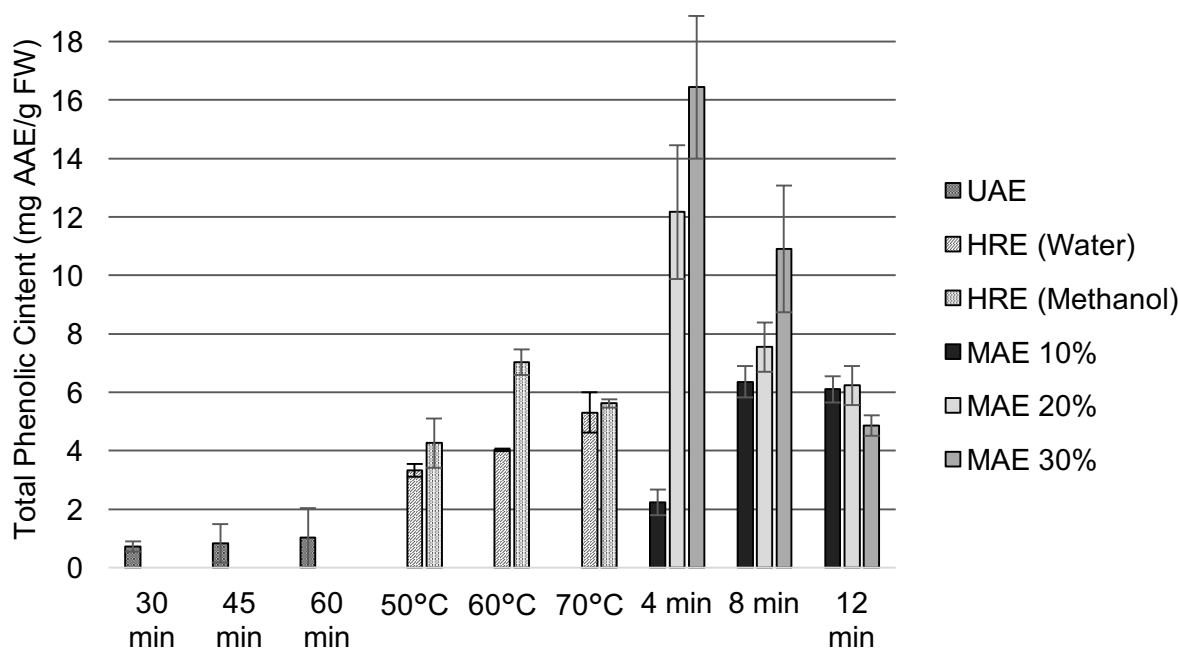


Figure 1. Total Phenolic content for Experiments 1, 2, and 3

Ascorbic acid, neochlorogenic acid, and chlorogenic acid were the three compounds quantified as they are the most predominant bioactive compounds in plums (Chun et al., 2003b; Piga et al., 2003;

Slimestad et al., 2009). Table 1 lists all experimental combinations and their respective concentration of each compound identified. For HRE and UAE, it can be observed that ascorbic acid was present in higher concentrations followed by chlorogenic acid and neochlorogenic acid. However, current literature reports that neochlorogenic acid is the most predominant compound in cherry plums (Miletić et al., 2013) and European plums (Slimestad et al., 2009), followed by chlorogenic acid and ascorbic acid. This trend can be attributed to neochlorogenic acid undergoing transformations in water. A study conducted by Dawidowicz and Typek, (2011) reported that neochlorogenic acid undergoes transformations in water, where it is often mistaken for new compounds. MAE extracts contained the highest neochlorogenic acid concentration followed by ascorbic acid and chlorogenic acid, which is consistent with trends reported in literature. For MAE, operating at 10% power for 8 minutes resulted in the highest concentration of compounds amongst all other combinations. For all power levels, operating for 8 minutes resulted in the highest concentration of compounds. For all power levels, increasing the time from 4 to 8 minutes resulted in an increase in concentration, where an increase from 8 to 12 minutes resulted in a further decrease in concentration. This could be attributed to prolonged exposure to the microwaves and the solvent choice (water). Water has an increased heating capacity in comparison to methanol and ethanol, during microwave extraction (Routray and Orsat, 2012). The prolonged exposure coupled with the increased heating effect may have led to the degradation of these compounds between 8 and 12 minutes.

Table 1. Ascorbic, neochlorogenic, and chlorogenic acid content in extracts for each Experiment

Extraction Method	Phenolic Compound ($\mu\text{g/g}$)		
	Ascorbic Acid	Neochlorogenic Acid	Chlorogenic Acid
HRE (Water)			
50°C	86.18	32.37	53.12
60°C	116.35	42.41	154.37
70°C	80.04	151.85	118.76
UAE			
30 min	93.04	34.77	49.72
45 min	83.33	16.82	39.85
60 min	104.95	20.27	86.03
MAE 10%			
4 min	86.35	15.95	40.64
8 min	192.82	276.25	132.47
12 min	60.09	271.66	130.34
MAE 20%			
4 min	104.64	335.52	106.10
8 min	106.93	449.07	97.02
12 min	83.36	224.25	76.80
MAE 30%			
4 min	34.68	96.60	38.66
8 min	104.46	70.83	79.92
12 min	68.06	86.25	57.03

Antioxidant activity Antioxidant activity in all extracts was evaluated by the reducing power of the ferric complex (Fe^{3+}) to ferrous complex (Fe^{2+}), in the presence of TPTZ. Figure 2 illustrates the average FRAP activity for all extracts, with their standard deviations. It can be observed that at 20% and 30% power levels, MAE resulted in higher FRAP activity than HRE and UAE. However, MAE at 30% for 4 minutes resulted in lower FRAP activity than UAE and HRE. Similar results have been

reported in that an increase in power level has led to a decrease in antioxidant potential in potato peels (Singh et al., 2011), blueberry leaves (Routray and Orsat, 2014), grape seeds (Krishnaswamy et al., 2013), and lemons (Papoutsis et al., 2016). UAE and HRE resulted in similar FRAP activity.

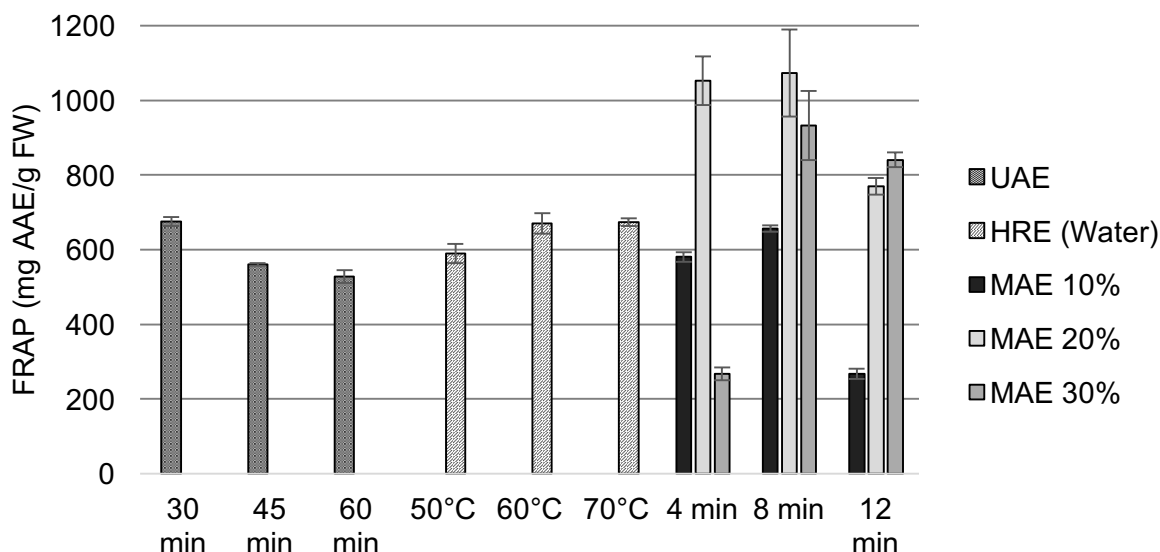


Figure 2. Ferric reducing antioxidant potential (FRAP) for Experiments 1, 2, and 3

Selection of PI approach Conventional HRE was evaluated against MAE and UAE with respect to extraction efficiency, time, and toxicity. MAE yielded the highest amount of phenolic compounds followed by HRE and UAE (Figure 1). With respect to time, MAE was able to increase phenolic yield in less time than both UAE and HRE, which were operated for approximately 20% longer. Toxicity is another important objective with respect to PI as less toxic solvents are safer for humans and the environment. The results in this study report that water is efficient in increasing extraction yield as it was used for both MAE and UAE. Based on the results in this study, it can be concluded that MAE is an effective extraction method over HRE and UAE with respect to the PI principles and domains. Hence, MAE was chosen as the appropriate PI solution to the bottlenecks of conventional extraction processes.

CONCLUSION Process intensification is the implementation of novel technologies to optimize process efficiency while also improving process safety. Conventional extraction methods require long processing times and often lead to denatured compounds due to high operating temperatures. Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) were suggested as PI approaches due to their mechanisms of volumetric heating and cavitation effect, which align with the principles of PI while also improving process yield. In this study, heat reflux extraction (HRE) was compared with MAE and UAE to determine which suggested PI could address the bottlenecks of HRE. It was observed that MAE yielded the highest concentration of phenolic compounds and antioxidants over UAE and HRE. Using water as the extraction solvent, MAE was also able to decrease the toxic risk of conventional solvents.

Acknowledgements. The authors would like to thank OMAFRA for a research grant through OMAFRA-UofG program to Dr. Subramanian and the support of Ontario Tender Fruit Marketing Board (OTFMB) and Niagara Peninsula Fruit and Vegetable Grower's Association (NPFVGA).

REFERENCES

- Aoune, A., Ramshaw, C., 1999. Process intensification: Heat and mass transfer characteristics of liquid films on rotating discs. *Int. J. Heat Mass Transf.* 42, 2543–2556. doi:10.1016/S0017-9310(98)00336-6
- Chun, O.K., Kim, D.O., Lee, C.Y., 2003a. Superoxide Radical Scavenging Activity of the Major Polyphenols in Fresh Plums. *J. Agric. Food Chem.* 51, 8067–8072. doi:10.1021/jf034740d
- Chun, O.K., Kim, D.-O., Moon, H.Y., Kang, H.G., Lee, C.Y., 2003b. Contribution of individual polyphenolics to total antioxidant capacity of plums. *J. Agric. Food Chem.* 51, 7240–7245. doi:10.1021/jf0343579
- Dawidowicz, A.L., Typek, R., 2011. The influence of pH on the thermal stability of 5-O-caffeoylquinic acids in aqueous solutions. *Eur. Food Res. Technol.* 233, 223–232. doi:10.1007/s00217-011-1513-x
- Kim, D.O., Jeong, S.W., Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* 81, 321–326. doi:10.1016/S0308-8146(02)00423-5
- Kim, S.J., Murthy, H.N., Hahn, E.J., Lee, H.L., Paek, K.Y., 2007. Parameters affecting the extraction of ginsenosides from the adventitious roots of ginseng (*Panax ginseng* C.A. Meyer). *Sep. Purif. Technol.* 56, 401–406. doi:10.1016/j.seppur.2007.06.014
- Krishnaswamy, K., Orsat, V., Gariépy, Y., Thangavel, K., 2013. Optimization of Microwave-Assisted Extraction of Phenolic Antioxidants from Grape Seeds (*Vitis vinifera*). *Food Bioprocess Technol.* 6, 441–455. doi:10.1007/s11947-012-0800-2
- Miletić, N., Mitrović, O., Popović, B., Nedović, V., Zlatković, B., Kandić, M., 2013. Polyphenolic content and antioxidant capacity in fruits of plum (*Prunus domestica* L.) cultivars “Valjevka” and “Mildora” as influenced by air drying. *J. Food Qual.* 36, 229–237. doi:10.1111/jfq.12035
- Nawaz, H., Shi, J., Mittal, G.S., Kakuda, Y., 2006. Extraction of polyphenols from grape seeds and concentration by ultrafiltration. *Sep. Purif. Technol.* 48, 176–181. doi:10.1016/j.seppur.2005.07.006
- Pandey, K.B., Rizvi, S.I., 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* 2, 270–8. doi:10.4161/oxim.2.5.9498
- Papoutsis, K., Pristijono, P., Golding, J.B., Stathopoulos, C.E., Bowyer, M.C., Scarlett, C.J., Vuong, Q. V., 2016. Enhancement of the total phenolic compounds and antioxidant activity of aqueous *Citrus limon* L. pomace extract using microwave pretreatment on the dry powder. *J. Food Process. Preserv.* 1–8. doi:10.1111/jfpp.13152
- Piga, A., Del Caro, A., Corda, G., 2003. From plums to prunes: Influence of drying parameters on polyphenols and antioxidant activity. *J. Agric. Food Chem.* 51, 3675–3681. doi:10.1021/jf021207+
- Routray, W., Orsat, V., 2014. MAE of phenolic compounds from blueberry leaves and comparison with other extraction methods. *Ind. Crops Prod.* 58, 36–45. doi:10.1016/j.indcrop.2014.03.038
- Routray, W., Orsat, V., 2012. Microwave-Assisted Extraction of Flavonoids: A Review. *Food Bioprocess Technol.* 5, 409–424. doi:10.1007/s11947-011-0573-z
- Routray, W., Orsat, V., Gariépy, Y., 2014. Effect of Different Drying Methods on the Microwave Extraction of Phenolic Components and Antioxidant Activity of Highbush Blueberry Leaves. *Dry. Technol.* 32, 1888–1904. doi:10.1080/07373937.2014.919002
- Singh, A., Orsat, V., 2015. Key Considerations in the Selection of Ingredients and Processing

Technologies for Functional Foods and Nutraceutical Products, in: Nutraceutical and Functional Food Processing Technology. Wiley Online Library, pp. 79–111. doi:10.1002/9781118504956.ch3

- Singh, A., Sabally, K., Kubow, S., Donnelly, D.J., Garipey, Y., Orsat, V., Raghavan, G.S. V, 2011. Microwave-assisted extraction of phenolic antioxidants from potato peels. *Molecules* 16, 2218–2232. doi:10.3390/molecules16032218
- Slimestad, R., Vangdal, E., Brede, C., 2009. Analysis of phenolic compounds in six norwegian plum cultivars (*Prunus domestica* L.). *J. Agric. Food Chem.* 57, 11370–11375. doi:10.1021/jf902054x
- Xu, M.H., Yang, X.Y., Fu, M.R., 2016. Combined Ultrasonic and Microwave Method for Juglone Extraction from Walnut Green Husk (*Juglans nigra*). *Waste and Biomass Valorization* 7, 1159–1166. doi:10.1007/s12649-016-9500-x
- Zhang, F., Yang, Y., Su., P., Guo, Z., 2009. Microwave-assisted extraction of rutin and quercetin from the stalks of *Euonymus alatus* (Thunb.) sieb. *Phytochem. Anal.* 20, 33–37. doi:10.1002/pca.1088