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Recovery of Functional Horticultural Ingredients Using Cost Effective and Commercially Viable Methods

Johannes Bartusch[1], Jing Zhou[1] and Zaid Saleh[1,2 +]

[1]Plant and Food Research Limited, Private Bag 92169, Auckland, New Zealand
[2] The University of Auckland, Private Bag 92019, Auckland, New Zealand

Abstract. The recovery of plant ingredients for functional food products has become an important value in our society. The aim of this study was to develop an extraction condition for extractable phenolic compounds, which is cost efficient, food-safe, commercially feasible and able to transfer to pilot-plant scale experiment. Fruits, including apples and berries, and vegetables, including celery and potatoes, were lyophilised (freeze-dried) and extracted in the first laboratory scale step. All plants were separated into skin, flesh and seeds. The second step transferred the laboratory experiments into pilot plant experiments. Folin-Ciocalteu assay and HPLC were used in the first step for analysis and just the Folin-Ciocalteu assay in the second step. It was found that the pre-treatment of the fruit has an influence on the yield and the stability of extractable phenolics.

Keywords: Extraction, phenolics, fruits, vegetables.

1. Introduction

The food industry is split in many categories which generate waste by creating valuable bio-products, bio fuels or food ingredients [1]. All these waste or by-products could be used for other purposes. Apple skin, flesh and seeds are by-products of the beverage industry, including apple juice and apple cider. This waste has been used for a variety of products such as pectin recovery [2], animal feed [3]-[5], citric acid production [6], ethanol production [7] and enzyme production [8], [9]. The waste products can be used as commercial product or as raw materials and has become more important in the food industry over the last few years [10]. The benefits of using waste or by-product are reducing raw material cost, processing cost and eliminating the waste cost. The extraction is an important step for recovering compounds with health benefits. It is necessary to isolate and identify the compounds. The ingredients are split in major compounds, which include carbohydrates, protein, fibre (Pectin) and lipids, among other minor compounds such as minerals and phytochemicals. The phytochemicals might be phenolic acids or polyphenolics [11]. The extracts from the dried and fresh samples were compared. The different extraction processes and conditions have been compared including extraction solvent, temperature, pH value, enzyme treatment. The lab scale experiments were firstly carried out for identifying the best condition and best ingredients resources after which one ingredient was selected for the scale up study. Different extraction conditions and processing equipments have been used and compared.

2. Raw Materials

CentriVap® concentrator, pH-Meter, spectrophotometer, plate reader, Liquid chromatography-Mass Spectrometry, milli-Q water, ethanol, methanol, ortho-Phosphoric acid 85%, enzyme Depol 740L, High Temperature Antioxidant Extraction Rig, screw press, colloidal mill, standard solid ring mill, freeze-dryer, raw materials of fruits and vegetables including celery, potato (red and brown), blackberry and blackcurrant, Granny Smith and Royal Gala apples.

+ Corresponding author. Tel.: + (64-9-925 7221); fax: + (64-9-925 8627).
E-mail address: (zaid.saleh@plantandfood.co.nz).

3. Method

The extraction variables of pH, solvent and enzyme concentration for the pilot scale experiments were selected according to the laboratory scale experiments. The extracts had been analysed using the Folin-Ciocalteu assay (prior with solid phase extraction (SPE)) and High Performance Liquid Chromatography (HPLC. The lab scale experiments were carried out in two steps. The first step was to get the best extraction conditions using all fruits and vegetables. The second step was to prove if these experiments were repeatable, which carried out by small selections of fruits and vegetables. The samples were prepared, frozen, freeze-dried and ground. The powdered samples were extracted, with an extraction ratio of 1:10 between sample and solvent. The samples are centrifuged by 4300 rpm for 5 minutes in order to get the supernatant for analysis. The pilot plant experiments were all carried out in the extraction rig with 2L of solvent. All the experiments were subjected to enzyme and non-enzyme treatment. The extractions were carried out at 60°C or 50°C for 20min. After the extraction, the extracts were collected and centrifuged at 4,400 rpm for 5 minutes. The trials with enzyme were carried out with the concentration of 2% or 5% with pH value of 3 at 50 °C, because the enzymes were sensitive to the temperature. The trials without enzyme were with pH3 or non-pH change. Water and 50% Ethanol were used as extraction solvents. Between 1-3 ml of ortho-Phosphoric acid was added to adjust the pH value to pH3.

4. Results

Fruits and vegetable were separated into skin/ leaves and flesh/ stem, except the berries as they were processed as pomace. The fruits always had a higher amount of total extractable polyphenolic content than the vegetables for both dried and fresh samples. For the dry weights, berries had higher polyphenolic content than apple skins. However, it was the opposite when the results were converted to fresh weight. This might be due to the higher water content in berries. The apple skins also had a higher amount of polyphenolic content compare to the flesh. However, the potato flesh showed higher phenolic content in comparison to skin. For all other vegetables, the pH adjustment did not have significant influence on the Folin results. The extraction conditions were better with a lower pH for berries and apple skin as shown in Figure 1. For all other vegetables, the pH adjustment did not have significant influence on the Folin results.

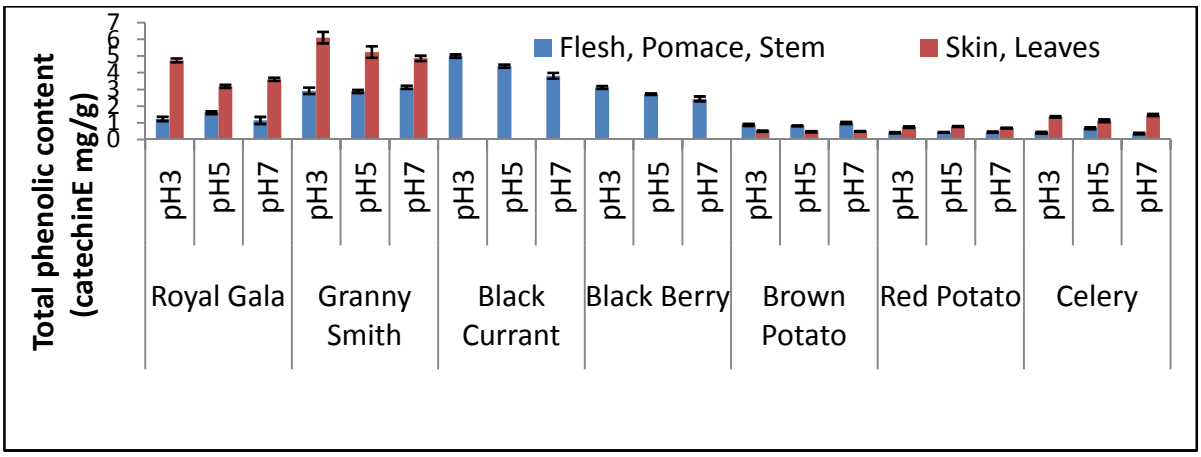

Fig. 1: Extraction with 50% EtOH for 35min, 20°C and different pH for fresh sample.

The time did not have a big influence. Fig. 2 shows that the differences between the changed times were not significant. Phenolic compounds were extracted in high proportions after the 20 minute mark. Therefore, 20 minutes was chosen for the remaining experiments.

For all fruits including berries, Royal Gala skin and flesh and Granny Smith skin and flesh, red potato skin and celery leaves, 50% ethanol was the best solvent to use. Water was the best extraction solvent for potato flesh as well as brown and red potato (Figure 3). There seemed to be no significant influence of the solvent on the total extractable polyphenolic content of celery stems and red potato skin. For most fruit and vegetables in this study, 50% ethanol was the best solvent therefore 50% ethanol was used for further

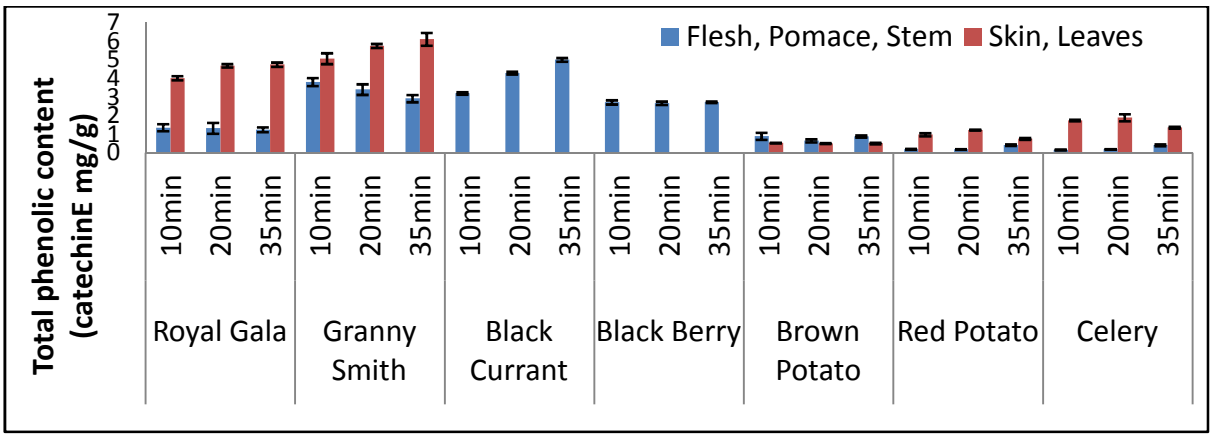

Fig. 2: Extraction at 20°C, pH 3 with 50% EtOH at different times for fresh samples.

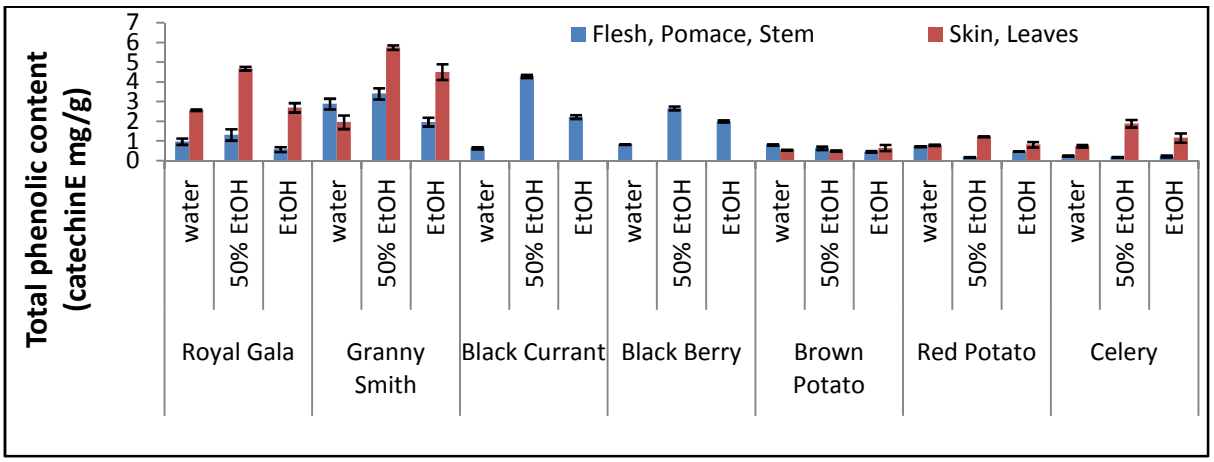

Fig. 3: Extraction at 20min, 20°C, pH 3 with different solvents for fresh fruit.

experiments. For all fruits, potato skin and flesh and celery stem, 60°C is the best temperature. The temperature had the biggest influence on the phenolic compounds and their extractions (Figure 4). The total extractable phenolic content of celery leaves had the best results at 40°C. According to these results, 60°C was selected for remaining experiments.

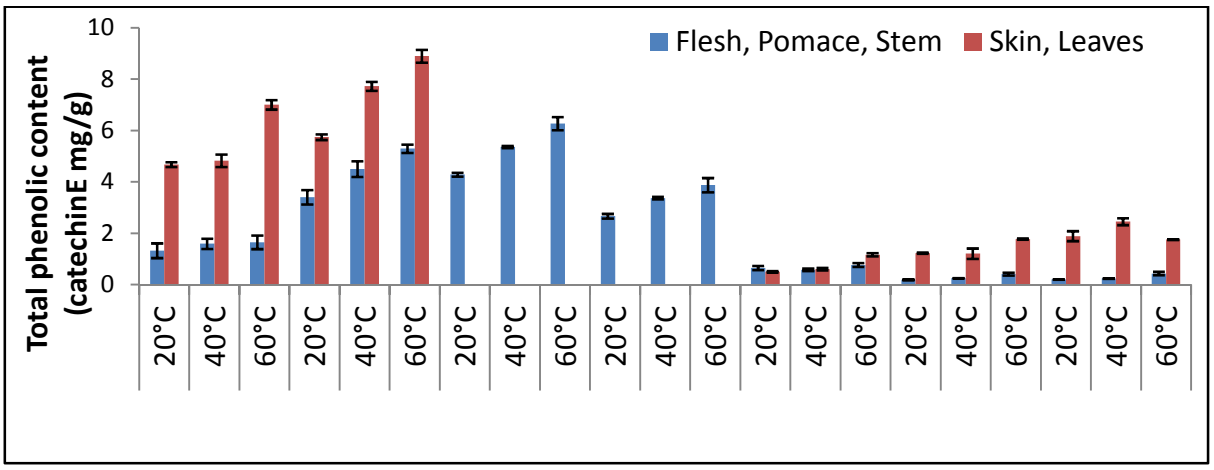

Fig. 4: Extraction at 20min, pH 3, 50% EtOH at different temperatures for fresh fruit.

HPLC results show that apple skin, including Royal Gala and Granny Smith had higher amounts of phenolic compounds including epi-catechin, apigenin 7-glucoside, rutin and phoridzin. The amounts of chlorogenic acid were 1.021 mg/g for Royal Gala flesh and 0.818 mg/g for Granny Smith flesh. The yields of chlorogenic acid in apple skins were 0.546 mg/g for Royal Gala skin and 0.240 mg/g for Granny Smith skin. Royal Gala apple had higher amounts of phenolic compounds from the major phenolics. Chlorogenic acid is

present in Granny Smith, Royal Gala and celery. Letuolin has not been detected from the apples and in celery no epi-catechin, apigenin 7-glucoside, rutin, epi-catechin, phoridzin were detected (Figures 5 and 6).

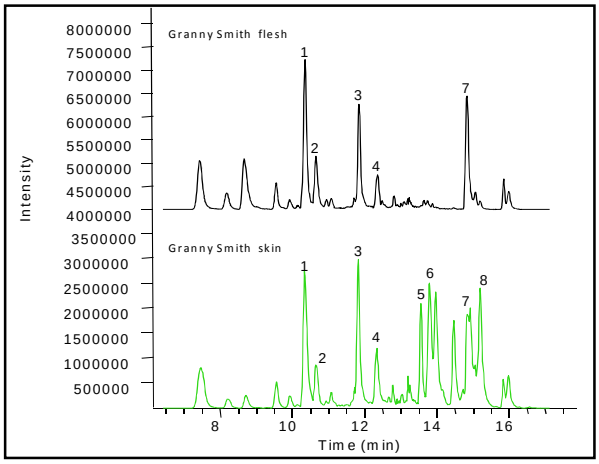

Fig. 5: High performance liquid chromatography (HPLC) chromatograms for Granny Smith apple. Possible phenolics. Peaks: 1. Procyanidin-B2 (dimer) 2. Catechin 3. Procyanidin (trimer) 4. Procyanidin (tetramer). 5. Quercetin-3-rutinoside 6. Quercetin-3-O-glucoside 7. Phloretin-2-O-xylo-glucoside 8. Quercetin-3-rhamnoside.

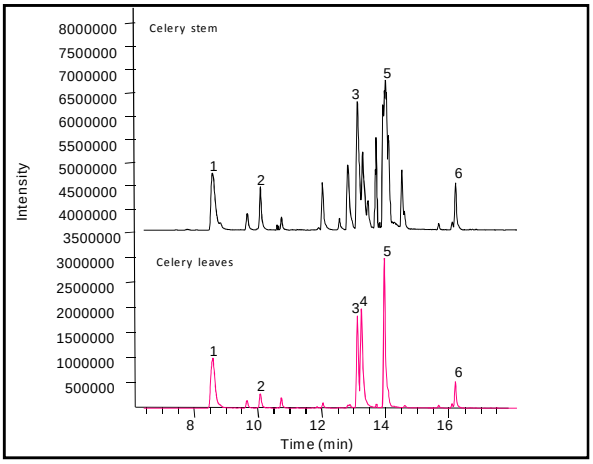

Fig. 6: High performance liquid chromatography (HPLC) chromatograms for celery. Possible phenolics. Peaks: 1. 5-CQA (caffeoyl quinnic acid; chlorogenic acid) 2. 5-O-p-coumaroylquinic acid 3. Luteolin-ferruloyl-acetylglucoside 4. Apigenin-7-apiosylglucoside 5. Apigenin-molonyl sambubiose 6. unknown

The amount of total extractable phenolics from the pilot plant trial is illustrated in Figures 7. It shows the results of the non-enzymic trial at 60°C and 20 minutes extraction time. A lower amount of polyphenolic extracts were processed via the water extraction in comparison to the 50% ethanol extractions. The extraction under pH3 was more efficient than with no pH adjustment. Granny Smith skin and core showed the highest phenolic content. The yield of extracted phenolics from pomace was higher than the yield of phenolics from flesh, probably because of the pomace including flesh and skin. The skin had higher polyphenolic content as compared to the flesh. The results from pilot plant experiments were different compared with the laboratory scale experiments. The no pH adjustment extraction of the total extractable polyphenolic content was more efficient than pH3. This was different to the dried samples. The 50% ethanol extraction was more efficient than the 100% water extraction according Folin-Ciocalteu results. The highest amount of phenolics obtained was from the pomace extract. The wet samples had obtained better results. The pH treatment did not have significant influence on the extraction of wet samples. The concentration of enzymes had an influence of the extractable phenolic compounds on skin&core but had no significant influence on pomace and flesh. It seemed that enzymes were mostly used to destroy the cell wall to give better pressing results.

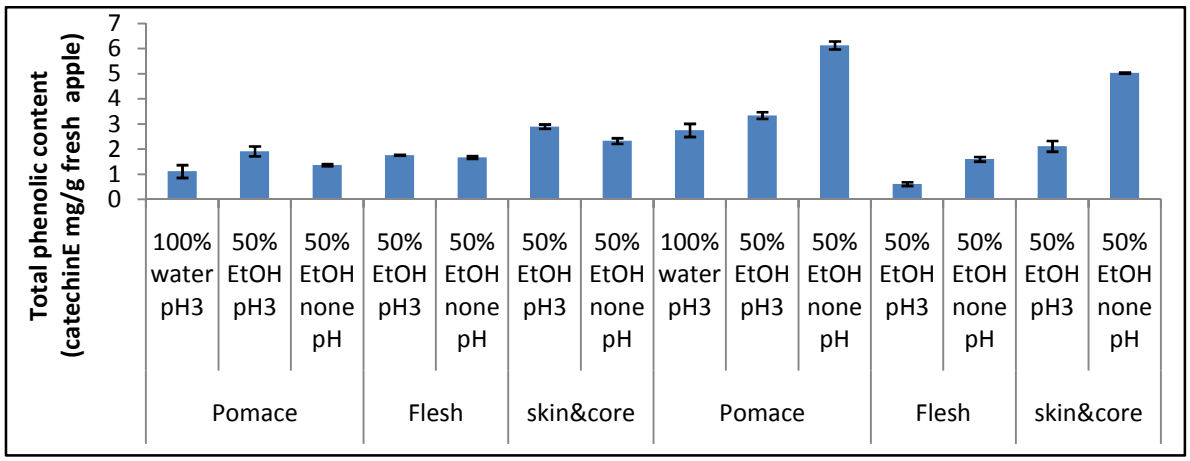

Fig. 7: Pilot plant non-enzymatic extraction at 60°C, 20 minutes.

5. Conclusion

The best conditions for fruits have been found are 20 minutes, 60°C, pH3 and 50% ethanol as extraction solvent. The vegetables did not show any significant effect when the pH was adjusted. The temperature and time were fixed at 60°C and 20 minutes, except for the celery leaves. For celery leaves the best temperature is 40°C. The best extraction solvent was changed between pure water for potato flesh and 50% ethanol for potato skins and celery leaves and stems. The pilot plant experiments were carried out with Granny Smith apples by 20 minutes and 60°C for the experiments with no enzyme treatment and 50°C for experiments with enzyme treatment. The non-enzymic treatment experiments were changed by pH value, including pH3 and no pH adjustment, solvent, including 100% water and 50% ethanol, and with dried samples and fresh samples. The dried experiments showed the same results as the laboratory scale experiments. The biggest amount on extractable phenolic compounds was found in fresh apple pomace. The fresh pre-treatment were generally the best way to prepare the apples. The fresh experiments have found that the pH adjustment does not have a significant influence on the extractable phenolic compounds. The freeze-drying is good for making the samples non-perishable. The experiments have shown that if the samples are directly being processed, drying is unnecessary. The contact with oxygen when preparing the fresh samples might cause the degradation of polyphenolic compounds. A short treatment with heat might be good to stop the oxidation. The experiments could be processed with steam for couple of seconds (maybe 30 seconds). Fresh samples processed at 60°C, 20 minutes, 50% ethanol and no enzyme and pH pre-treatment are the best way to get higher amounts on phenolic compounds.

6. Acknowledgment

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7. References

[1] Casazza, A. A., Aliakbarian, B., Mantegna, S., Cravotto, G., & Perego, P. Extraction of phenolics fom Vitis vinifera wastes using non-conventional techniques. *Journal of Food Engineering*, 2010, **100**: 50-55.

[2] Schieber, A., Hilt, P., Streker, P., Endress, H.U., Rentschler, C., & Carle, R. A new process for the combined recovery of pectin and phenolic compounds from apple pomace. *Innovative Food Science and Emerging Technologies*, 2003, **4**: 99-107.

[3] Sehm, J., Lindermayer, H., Dummer, C., Treutter, D., & Pfaffl, M.W. The influence of polyphenol rich apple pomace or red-wine pomace diet on the gut morphology in weaning piglets. *Journal of Animal Physiology and Animal Nutrition*, 2007, **91**: 289-296.

[4] Joshi, V.K., Gupta, K., Devrajan, A., Lal, B.B., & Arya, S.P. Production and evaluation of fermented apple pomace in the feed of broilers. *Journal of Food Science and Technology*, 2000, **37**: 609-612.

[5] Bucic-Kojić, A., Planinić, M., Tomas, S., Bolić, M., & Velic, D. Study of solid-liquid extraction kinetics of total polyphenols from grape seeds. *Journal of Food Engineering*, 2007, **81**: 236-242.

[6] Hang, V. K. & Woodams, E. E. Apple Pomace. A potential substrate for citric acid production by Aspergillus niger. *Biotechnology Letters*, 1984, **6**: 763-764.

[7] Hang, V. K., Lee, C.Y., Woodams, E.E., & Cooley, H.J. Production of alcohol from apple pomace. *Applied and Environmental Microbiology*, 1981, **42**: 1128-1129.

[8] Berivic, M. & Ostroversnik, H. Production of Aspergillus niger pectolytic enzymes by solid state bioprocessing of apple pomace. *Journal of Biotechnology*, 1997, **53**: 47-53.

[9] Favela-Torres, E. Volke, S. T., & Viniegra, G. G. Production of hydrolytic depolymerising pectinases. *Food Technology and Biotechnology*, 2006, **44**: 221-227.

[10] Wijngaard, H.H., & Brunton, N. The optimisation of solid-liquid extraction of antioxidants from apple pomace by response surface methodologoy. *Journal of Food Engineering*, 2010, **96**: 134-140.

[11] Corinstein, S., Haruenkit, R., Poovarodom, S., Park, S.-Y., Vearaslip, S., Suhaj, M., Ham, K.-S., Heo, B.-G., Cho, J.-Y., & Jang, H.G. The comparative characteristics of snake and kiwi fruits. *Food and Chemical Toxicalogy*, 2009, **47**: 1884-1891.