THE CANADIAN SCIENCE FAIR JOURNAL

ARTICLE

Phytochemical Cocktail – Waste Utilization of Inedible Dragon Fruit Peel

Jason Chen

Age 17 | Vancouver, British Columbia

CWSF Challenge award - Senior | Excellence award - senior gold medal | Entrance Awards from Carleton ($4000), Dalhousie ($5000), UBC ($4000) UManitoba ($5000) UOttawa ($4000) Western ($4000)

Dragon fruit, or pitaya, is a structurally unique plant indigenous to the Americas. While dragon fruit pulp is usually consumed entirely, its peel is often disposed as organic waste. The objective of this project was to test the various bioactivities and applications of phytochemicals from commonly wasted dragon fruit peel, which both adds value to dragon fruit and minimizes its associated waste disposal problems. Phytochemicals in dragon fruit peel were extracted using an ethanol-water mixture and the metal chelating activity of the extract was evaluated as an assessment of dragon fruit peel bioactivity. The phytochemical extract was used to inhibit tyrosinase in shrimp, the enzyme responsible for melanosis, by chelating its copper cofactor. Furthermore, the interactions among the three major phytochemical groups (betacyanins, polyphenols, and flavonoids) in dragon fruit peel were studied for both metal chelating and antioxidant activities. Lastly, a 72-h solid-state fermentation of dragon fruit peel was performed using a commercial *Aspergillus oryzae* strain and naturally present microorganisms in dragon fruit peel to convert conjugated phytochemicals into free, extractable forms. The contents of betacyanins, polyphenols, and total flavonoids were monitored every 24 h during fermentation. The results indicated that the extract possessed a significantly high metal chelating activity of 94%. A synergistic interaction among betacyanins, polyphenols, and flavonoids was identified for metal chelating activity for all combinations of the three main dragon fruit peel phytochemicals groups, while an antagonistic interaction was observed for antioxidant activity. Lastly, betacyanin content degraded considerably during the solid-state fermentation, resulting in a light-coloured extract, while the final polyphenol and flavonoid contents increased during the 72-h fermentation.

INTRODUCTION

Phytochemicals are bioactive, non-nutritive chemicals found in plants and are commonly associated with health benefits (Cheynier, 2012). Hylocereus undatus, also known as dragon fruit, has a pulp that has been shown to possess antioxidant and anti-proliferative activities (Wu et al., 2006). However, there is limited research on the bioactive properties of its peel phytochemicals. Furthermore, the study carried out by Kim et al. (2011) showed that dragon fruit peel (DFP) was 5-6 times more concentrated in phytochemicals than its flesh. The global trade value of dragon fruit in 2016 was 4.9 billion US dollars, with an average price of 5.63 US dollars per kilogram (Tridge, 2018). Based on these figures, an estimated 870,000 tons of dragon fruit were produced globally in 2016. Given that non-edible DFP accounts for approximately 20% of the whole fruit, around 174,000 tons of peel waste was generated in 2016. DFP waste is currently discarded as organic waste, which contributes to pollution, landfill, and strong odors. Identifying the various bioactivities of DFP phytochemicals may increase the market value and applications of DFP, transforming it from waste into commodity.

Numerous studies have been carried out to evaluate the metal chelating activity of natural antioxidants from plants (Liu et al., 2015; Bouriche et al., 2011). However, DFP, which is rich in phytochemicals with phenolic and carboxyl groups that can bind to metal ions (Pereira et al., 2009; Bala et al., 2007), has not been studied for its metal chelating ability. These natural metal chelating agents can inhibit enzymes with metal ion cofactors, such as tyrosinase, the enzyme that causes enzymatic browning. Through its metal chelating and antioxidant activities, DFP phytochemicals can be used as a food preservative against both enzymatic browning and oxidation of food. In addition, natural phytochemicals have greater consumer appeal and are safer than synthetic preservatives. The DFP phytochemicals can be categorized into betacyanins, flavonoids, and polyphenols based on their chemical structures (Priatni et al., 2015; Kim et al., 2011). These three main groups of DFP phytochemicals can be further divided into subsets of individual phytochemicals. For instance, betanin, isobetanin, gomphrenin I, isogomphrenin I, and other derivatives are categorized under the class of betacyanins and are all present in DFP (Garcia-Cruz et

al., 2017). Distinct groups of phytochemicals in DFP may interact with each other when combined in solution, exhibiting synergistic, antagonistic, or no effect for separate bioactivities. Interactions among antioxidants in green tea, berries, and other plants have already been investigated (Luis et al., 2018; Colon and Nerin, 2016; Yin et al., 2012), while DFP phytochemicals remain unstudied. Identifying the interactions among DFP phytochemicals may allow for more efficient optimization when combining different phytochemicals to deliver higher bioactivities with less phytochemical usage.

It is known that phytochemicals naturally occur in both free and conjugated forms in plant cells (Howard, 2008). Solvents can readily extract free forms of phytochemicals, which are usually suspended in the cytosol. In contrast, conjugated phytochemicals, which are commonly bound to cell wall polysaccharides and proteins, are associated with low extraction yield and no bioavailability (Eprilliati & Ginjom, 2012). A majority of the studies have only focused on optimization of extraction conditions (e.g. temperature, extraction time, and solvent) and application of engineering-based technologies (e.g. microwave-assisted processing) to enhance extraction yield of free-form phytochemicals (Zhang et al., 2018). Few studies have addressed converting conjugated phytochemicals in DFP into free forms through removing the polysaccharide and protein fractions bound to phytochemicals. Solid-state fermentation by *A. oryzae,* which are able to hydrolyze polysaccharides and proteins, is a potential low-cost biological strategy to achieve a higher bioactive phytochemical yield.

Therefore, the objective of my project is to test the effectiveness and application of phytochemicals from DFP as metal chelating agents to study the interactions amongst DFP phytochemicals in chelating and antioxidant activities, and to evaluate the feasibility of applying *A. oryzae* to convert non-extractable conjugated phytochemicals into extractable free forms.

HYPOTHESES

i) If phytochemicals in DFP are able to chelate metal ions, then the phytochemical extract can potentially be applied to inhibit tyrosinase, the enzyme responsible for enzymatic browning in shrimp, by depriving the enzyme of its copper cofactor through metal chelation.

ii) If phytochemicals in DFP interact with each other for metal chelating and antioxidant activities, then the combination of phytochemicals will result in lower (antagonistic) or higher (synergistic) activities than the sum of their individual activities.

iii) If *A. oryzae* or natural microorganisms in DFP can effectively hydrolyze polysaccharide and protein that are conjugated to phytochemicals, then more free forms of phytochemicals can be extracted.

MATERIALS AND METHODS

Materials, Chemicals, and Reagents

Dragon fruits (*Hylocereus undatus*), white shrimp, and active dry *A. oryzae* (Angel Yeast, Yichang, China) were purchased from T&T Supermarket (Richmond, BC, Canada). All chemicals and reagents were purchased from Sigma-Aldrich (Oakville, ON, Canada) and TCI (Portland, OR, USA).

DFP Pre-treatment and Extraction

DFP was collected and ground by a food processor (Elemental 13-Cup, Cuisinart) for 5 seconds, followed by 30-min extraction by 50% (v/v) ethanol with an extraction ratio of 1:4 (w/v) at 30°C. The supernatant was collected from 10-min centrifuge at 4000 rpm and concentrated by a rotary evaporator (R-200, Buchi) to remove ethanol.

Metal chelating activity assay

Metal chelating activities of the extract and a betanin solution with the same betanin equivalent content as the extract were measured according to the ferrous-ferrozine method used by Lee et al. (2008) with a few modifications. Betanin, the most abundant pigment in DFP (Rebecca et al., 2010), contains three carboxyl groups (shown in Fig. 6(A)), which have high binding strength to metal ions (Bala et al., 2007). Thus, betanin was selected for comparison with the DFP extract.

Ferrozine is able to bind to ferrous ions in a complexation reaction to form a complex which has a unique absorbance at 562 nm. Metal chelating agents in the solution can compete with ferrozine to bind ferrous ions, which results in less ferrous-ferrozine complex being formed, as indicated by a reduction in absorbance at 562 nm. The reduction in absorbance indicates the level of metal chelating activity, which was calculated as shown in Equation (1). The activity difference was assessed by Analysis of Variance (ANOVA) followed by Tukey's test ($p<0.05$).

$$\text{Metal chelating activity (\%)} = \frac{(A0 - Asample)}{A0}\ x\ 100$$

Where A_0: Absorbance of control blank with water;
A_{sample}: Absorbance of sample

Application of DFP Extract on White Shrimp

Six fresh shrimp were soaked in DFP extract for 30 min, stored at 25°C overnight, and boiled in water for 5 min. Shrimp soaked in water was used as control. Shrimp heads were collected for comparison of melanosis.

Interaction among DFP phytochemicals

Betanin, hesperetin, and tannic acid were selected as the representatives of betacyanins, flavonoids, and polyphenols, respectively, based on their presence and abundance in DFP (Yong et al., 2017; Kongkachuichai et al., 2010; Rebecca et al., 2010). The metal chelating and free radical scavenging activities of individual, two combined, and three combined phytochemicals were evaluated.

The metal chelating activity was measured with the same assay as in 4.3. The free radical scavenging activity was determined by the DPPH-based assay used by Lee et al. (2005) with a few modifications. DPPH is a free radical which has a unique absorbance at 517 nm. Neutralization of DPPH by antioxidants results in a reduction of absorbance at 517 nm. The percentage of free radical scavenging activity is calculated using the following equation:

$$\text{Free radical scavenging activity (\%)} = \frac{(A0 - Asample)}{A0} * 100$$

Where A_0: Absorbance of control blank with water;
A_{sample}: Absorbance of sample

Solid-State Fermentation by *A. oryzae* & Phytochemical Characterization

75 g of ground DFP was weighed in baking pans and blended with 0.4 g dry active *A. oryzae*. No inoculation was applied for the control group. Sampling at 24 h, 48 h, and 72 h was carried out in triplicate. Phytochemicals were extracted and characterized.

Betacyanin content was measured according to Priatni et al. (2015) and presented as betanin equivalent. Total flavonoid and polyphenol levels were determined according to Kim et al. (2011) and expressed as rutin equivalent and gallic acid equivalents, respectively.

RESULTS

Metal Chelating Activity of Phytochemical Extract from DFP & its Application for Shrimp Preservation

As shown in Fig. 1, metal chelating activity of 94% was determined for the DFP extract, which was significantly higher than 17% of the betanin only solution. According to the mechanism of the metal chelating assay used in this study, metal chelating activity of 94% elucidated that 94% ferrous ions loaded for the analysis were chelated by the DFP extract.

The high metal chelating activity of the DFP extract indicated that it can potentially be applied to chelate metal ion cofactors of certain enzymes. Thus, the phytochemical extract was used to inhibit enzymatic browning in shrimp by binding the dinuclear copper catalytic center of tyrosinase (Fig. 2). As shown in Fig. 3, water-treated shrimp developed severe enzymatic browning, while black spots were seldom observed for the DFP extract-treated shrimp.

Interactions Among DFP Phytochemicals

According to Fig. 4(A), all the combined groups of the three phyochemicals had higher metal chelating activity than the sum of the individual activities, illustrating synergistic interactions among betanin, hesperetin, and tannic acid. Fig. 4 (B) shows that the free radical scavenging activity of the combined groups was lower than the sum of the individual activities, indicating antagonistic interactions among the three phytochemicals.

Solid-state Fermentation of DFP by *A. oryzae*

Fig. 5(A) showed that betacyanins were degraded substantially during 72-h fermentation. Up to 70% of betacyanins reduction was observed for both the control and *A. oryzae* groups after 24-h fermentation. Overall, the *A. oryzae* group had faster betacyanin degradation rate than the control group during the first 48-h fermentation. In Fig. 5(B), total flavonoid content decreased dramatically for the first 24 h; then it increased after 48-h fermentation. Polyphenol content, as shown in Fig. 5(C), was found to gradually increase during fermentation. Overall, there was no significant difference of the phytochemical content change between the control and *A. oryzae* groups.

DISCUSSION

Metal Chelating Activity of Phytochemical Extract from DFP

In addition to betacyanins, the other two main phytochemical groups in the DFP extract are flavonoids and polyphenols

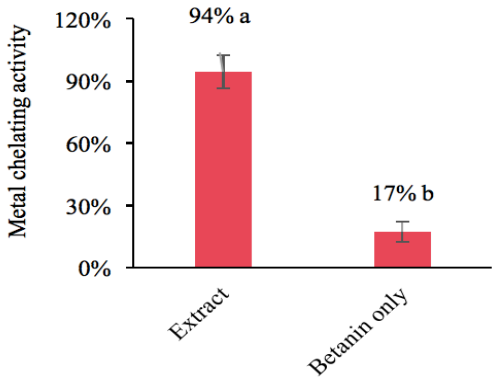

Figure 1. Metal chelating activity of DFP extract and betanin.

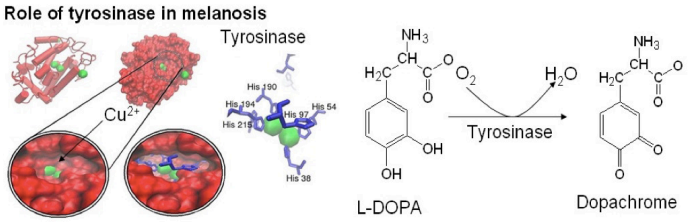

Figure 2. Role of copper cofactor in enzymatic browning of shrimp caused by tyrosinase (Murphy, 2018; Byszewska & Kanska, 2014).

Figure 3. Comparison of shrimp treated by water and DFP extract.

which are both phenolic compounds, contain hydroxylated aromatic rings (Swanson, 2003). The chemical structures of hesperetin and tannic acid, representatives of flavonoids and polyphenols in DFP, were shown in Fig. 6(B) and 6(C). The aromatic rings and phenolic hydroxyl groups, which are structural features of phenolic compounds, contribute to the excellent hydrogen-donating and antioxidant properties of phenolic substances. Similarly, the interactions between the hydroxyl groups and π-electrons of the adjacent benzene rings in phenolic compounds also contribute to their metal chelating ability (Pereira et al., 2009). The metal chelating activities of flavonoids and polyphenols have been tested from extracts of other plants (Hider et al., 2001; Sanchez-Vioque et al., 2013; Cherrack et al., 2016). For instance, the study of Cherrack et al. investigated and elucidated the metal chelating properties of natural flavonoids, such as quercetin, catechin, and rutin, towards iron, copper, and zinc ions. Therefore, the significantly higher metal chelating activity of the DFP extract than the betanin

only solution was likely due to the presence of other phenolic phytochemicals, such as flavonoids and polyphenols.

Application of DFP Extract for Shrimp Preservation

As shown in Fig. 3, enzymatic browning was hardly observed for the DFP extract-treated shrimp, which was likely due to the effective inhibition of tyrosinase caused by phytochemicals chelating the copper cofactor.

Enzymatic browning, or melanosis, is a major cause of food deterioration and is primarily caused by enzymes. Polyphenol oxidases which are responsible for enzymatic browning in foods catalyze polymerization of o-quinones, resulting in production of dark pigments. Tyrosinase is a polyphenol oxidase, naturally present in many fruits, vegetables, and seafood. In the presence of oxygen, tyrosinase is able to catalyze the oxidation of phenols and catechols into their respective quinones. These quinones are then easily polymerized into melanin, which results in food browning in seafood (Solomon et al., 1996) (shown in Fig. 2). Shrimp, which is a major seafood consumed worldwide, can experience severe melanosis during postmortem handling and storage when exposed to oxygen (Gomez-Guillen et al., 2005). An effective alternative to the traditional sulfite treatment, which is associated with adverse health effects, is highly desirable for prevention of melanosis in shrimp. The results obtained in this study have provided a proof-of-concept for utilizing the DFP phytochemicals as a potential low-cost treatment for minimizing melanosis in shrimp.

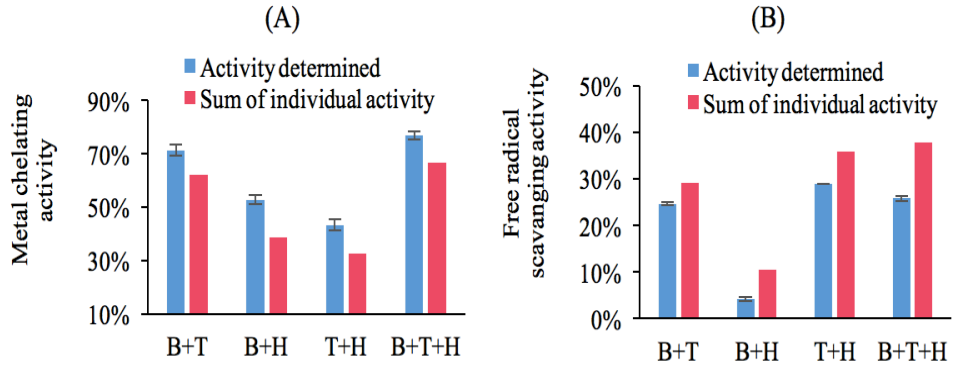

Figure 4. Interactions among phytochemicals*. (A) Synergistic interactions for metal chelating activity; (B) Antagonistic interaction for free radical scavenging activity.
*B - betanin; H - hesperetin; T - tannic acid.

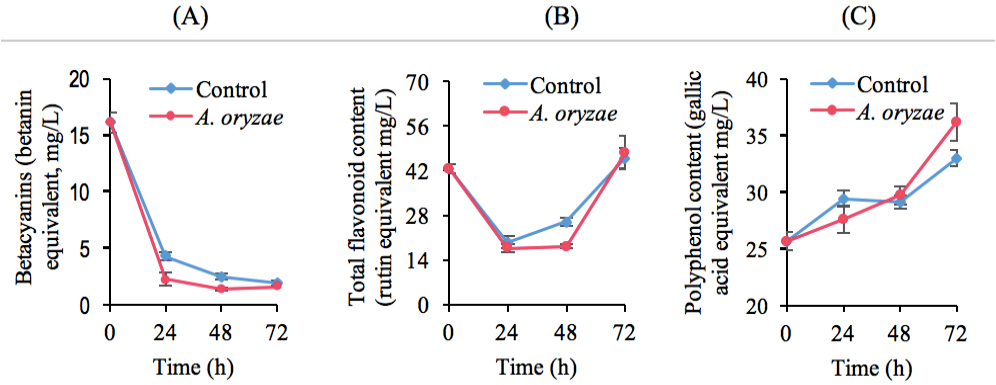

Figure 5. Contents of betacyanins, total flavonoids, polyphenols during 72-h fermentation. (A) Betacyanin content; (B) Total flavonoid content; (C) Polyphenol content in fermented DFP.

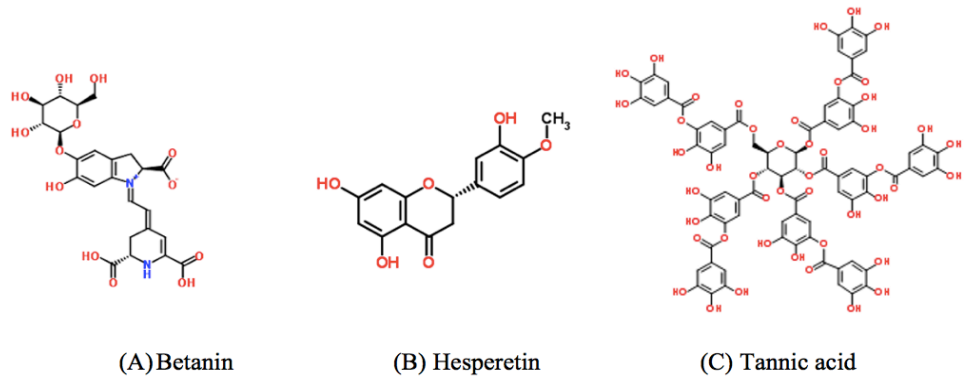

Figure 6. Chemical structures of betanin, hesperetin, and tannic acid.

Tyrosinase catalyzes the oxidation of ortho-diphenols when its substrate binds onto the dinuclear copper center at the tyrosinase catalytic site (see Fig. 7). Specialized "Caddie" proteins carry copper ions to the catalytic site of tyrosinase, where two copper ions each become coordinated by three histine ligands and two catalytic oxygen atoms. One oxygen atom acts as a catalytic base and removes the proton (H^+) from the hydroxyl groups of the substrates, resulting in a negatively charged oxygen ions attached to the ortho-diphenols. The negative oxygen ions attach onto tyrosinase by binding onto the sixth coordination site of the copper cofactors. Metal chelating agents, such as DFP phytochemicals that contain abundant phenolic hydroxyl groups capable of binding metal ions (Fig. 6), can possibly compete with the phenolic substrates to bind onto the sixth coordination site of copper ions, thereby preventing enzymatic browning. Future experimentation of enzyme kinetics using Lineweaver-Burk plots can be used to identify the kinetic parameters and determine the specific inhibition mechanism (competitive, non-competitive, and uncompetitive) of DFP phytochemicals on tyrosinase.

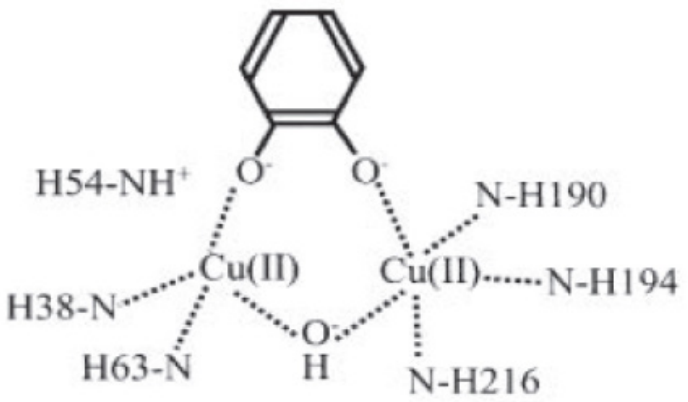

Figure 7. Interaction between ortho-diphenol and active site of tyrosinase.

Interactions Among DFP Phytochemicals

Metal chelation is a stabilizing effect, whereby separate ligands of the metal chelating agent form two or more coordinate covalent bonds with a single metal ion (IUPAC, 1997). A possible mechanism for the synergistic effect observed in this study is that phytochemicals of different molecular sizes can form more stable complexes with ferrous ions. The ferrous ion has a coordination number of six, indicating that metal chelators must form six coordinate covalent bonds with the ion in order to effectively form a stable complex. Relatively large chelating agents may have limited flexibility in binding onto all six coordination sites of the ferrous ion. For instance, when one tannic acid molecule (as shown in Fig. 6) binds onto a ferrous ion, it may limit the accessibility of the remaining binding sites for other tannic acid molecules. However, when betanin, a phytochemical with smaller molecular size and denticity, is also added into the solution, these betanin molecules may be able to form coordinate covalent bonds with sites that other tannic acid molecules cannot access. When acting in conjunction, phytochemicals of different molecular sizes may be able to more effectively bind onto all six binding sites of ferrous ions, resulting in a synergistic effect.

This antagonistic interaction among betanin, hesperetin, and tannic acid can potentially be explained by hydrogen bonding between hydroxyl groups of phytochemicals, resulting in reduced numbers of hydroxyl groups available to donate hydrogen atoms to neutralize free radicals (Luis et al., 2018). Luis et al. (2018) have comprehensively studied the interactions between nineteen major bioactive polyphenols of berries for their antioxidant activities. Antagonistic interaction was identified between the majority of phenolic compounds tested, such as quercetin and syringic acid, ferulic acid and resveratrol, quercetin and naringin, rutin and ferulic acid, which is consistent with the results shown in this experiment.

Solid-state Fermentation of DFP by *A. oryzae*

The considerable reduction of betacyanins during fermentation shown in Fig. 5(A) was possibly attributed to degradation caused by oxidation and hydrolysis by glucosidases from microorganisms (Herbach et al., 2006). Many naturally occurring fungi and yeasts and a few bacteria secrete glucosidases (Sorensen et al., 2013), which could result in deglycosylation of betacyanins by attacking the glucose moiety. In addition, Aspergillus has high glucosidase-producing ability and possibly contributed to the degradation of betacyanins during the solid state fermentation (Sorensen et al., 2013). In fact, the faster betacyanin degradation of the *A. oryzae* group during the first 48-h fermentation was likely due to greater glucosidase expression by *A. oryzae* as compared to the natural microorganisms in the control group. Betacyanins contribute to the red coloration of DFP; the substantial betacyanin degradation resulted in a light-coloured solution, which yields more potential applications in the Food and Cosmetic Industries without the bright purple color limitation of DFP phytochemicals.

In Fig. 5(B), the decrease of the total flavonoid content during the first 24 h was possibly because the rate of degradation of flavonoids, due to oxidation and temperature, was greater than its rate of release from conjugated forms. Flavonoid content increased after 48-h fermentation, indicating that the rate of release from conjugated forms is greater than the rate of degradation. The gradual increase of polyphenol content throughout the fermentation, as shown in Fig. 5(C), was likely due to a consistently higher releasing rate than degradation rate. Overall, there was no significant difference between the control and *A. oryzae* groups. Kunnika and Pranee (2011) has applied a commercial enzyme called Pectinex® Ultra SP-L to degrade polysaccharides in the flesh and peel of red dragon fruit to release more bioactive compounds. It was found that higher total phenolic and flavonoid contents were obtained due to enzymatic hydrolysis of polysaccharides. In addition to the polysaccharide-conjugated phytochemicals, solid-state fermentation adopted in this study also targeted to improve the extractability and bioavailability of protein-conjugated phytochemicals.

A. oryzae, also known as koji in Japan, is commonly used to make traditional Asian foods, such as soy sauce, miso, and alcoholic beverages such as sweet rice wine, baijiu, and sake. The frequent use of *A. oryzae* for fermentation of these complex food matrices is largely due to its ability to secrete various enzymes, such as amylase, glucoamylase, β-glucosidase, endo-proteinases, and exo-peptidases (Nakadai & Nasuno, 1988; Machida, 2002), which are able to hydrolyze many proteins and polysaccharides. Therefore, *A. oryzae* is a good option for the hydrolysis of polysaccharides and proteins bound to the conjugated phytochemicals to release more free forms. As shown in Fig. 5, natural microorganisms in DFP (control) were as effective as *A. oryzae* in degrading betacyanins and releasing flavonoids and polyphenols during 72-h fermentation. Solid-state fermentation by natural microorganisms in DFP does not require any additional cost for microorganism inoculation, which is a major advantage over the fermentation by *A. oryzae*. However, over-growth of food pathogens and accumulation of microbial toxins are potential safety issues associated with solid-state fermentation by natural microorganisms. Comprehensive studies on naturally existing food pathogens in DFP are critical for application of natural microorganisms for solid-state

fermentation of DFP.

CONCLUSIONS

This work proposes an approach for waste utilization of inedible DFP through extracting a high-value phytochemical cocktail, which was shown to have strong metal chelating ability. The phytochemical cocktail was shown to minimize enzymatic browning of shrimp, providing a proof-of-concept for its future application in the food industry. The synergistic interaction for metal chelating activity and antagonistic interaction for antioxidant activity among phytochemicals can support future recipe development for applications that combine various phytochemicals. Apart from betacyanins, most non-extractable phytochemicals were shown to be released by *A. oryzae* and the natural microorganisms in DFP. The degradation of betacyanins resulted in a light-coloured phytochemical cocktail without red coloration, which has less impact on food appearance when used as a preservative; this may lead to industrial applications, such as in the food and cosmetic industries.

ACKNOWLEDGEMENTS

Thank you to Yiqiong Jin (Renaissance Bioscience) for her guidance and Dr. Darren Gao (University of British Columbia) for his support in obtaining chemicals and access to the UBC chemistry lab.

REFERENCES

Bala, T., Prasad, B.L.V., Sastry, M., Kahaly, M.U., Waghmare, U.V. (2007). Interaction of different metal ions with carboxylic acid group: a quantitative study. *J. Phy. Chem. A., 111*(28), 6183-6190.

Bouriche, H., Meziti, H., Senator, A., Arnhold, J. (2011). Anti-inflammatory, free radical-scavenging, and metal chelating activities of Malva parviflora. *Pharm. Biol., 49*(9), 942-946.

Byszewska, W., Kanska, M. (2014). Studies on enzymatic oxidation of 3′,4′-dihydroxy-l-phenylalanine to dopachrome using kinetic isotope effect methods. J. Radioanal. *Nucl. Chem., 299*(3), 1373-1378.

Cherrak, S.A., Mokhtari-Soulimane, N., Berroukeche, F., Bensenane, B., Cherbonnei, A., Merzouk, H., Elhabiri, M. (2016). In vitro antioxidant versus metal ion chelating properties of flavonoids: a structure-activity investigation. *PLoS One, 11*(10): e0165575. doi:0.1371/journal.pone.0165575

Cheynier, V. (2012). Phenolic compounds: from plants to foods. *Phytochem. Rev., 11*, 153-177.

Colon, M., Nerin, C. (2016). Synergistic, antagonistic and additive interactions of green tea polyphenols. *Eur. Food Res. Technol., 242*, 211-220.

Epriliati, I., Ginjom, I.R. (2012). *Bioavailability of Phytochemicals, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health,* Dr Venketeshwer Rao (Ed.), ISBN: 978-953-51-0296-0, InTech, Available from: http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-innutrition-and-health/bioavailability-of-phytochemicals

Garcia-Cruz, L., Duenas, M., Santos-Buelgas, C., Valle-Guadarrama, S., Salinas-Moreno, Y. (2017). Betalains and phenolic compounds profiling and antioxidant capacity of pitaya (Stenocereus spp.) fruit from two species (S. pruinosus and S. stellatus). *Food Chem., 234,* 111-118.

Gomez-Guillen, M.C., Martinez-Alvarez, O., Llamas, A., Montero, P. (2005). Melanosis inhibition and SO2 residual levels in shrimps (Parapenaeus longirostris) after different sulfite-based treatments. *J. Sci. Food Agric., 85*(7), 1143-1148.

Herbach, K.M., Stintzing, F.C., Carle, R. (2006). Betalain stability and degradation – structural and chromatic aspects. *J. Food Sci., 71*(4), R41-R50.

Hider, R.C., Liu, Z.D., Khodr, H.H. (2001). Metal chelation of polyphenols. *Methods Enzymol., 335,* 190-203.

Howard, L. (2008). Chapter 18 Processing techniques and their effect on fruit and vegetable phytochemicals. Improving the Health-Promoting *Properties of Fruit and Vegetable Products,* P449-472. Woodhead Publishing.

IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. *Blackwell Scientific Publications, Oxford (1997).* XML on-line corrected version: http://goldbook.iupac.org (2006-) created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. ISBN 0-9678550-9-8. https://doi.org/10.1351/goldbook.

Kim, H., Choi, H.K., Moon, J.Y., Kim, Y.S., Mosaddik, A., Cho, S.K. (2011). Comparative antioxidant and antiproliferative activities of red and white pitayas and their correlation with flavonoid and polyphenol content. *J. Food Sci., 76,* C38-C45.

Kongkachuichai, R., Charoensiri, R., Sungpuag, P. (2010). Carotenoid, flavonoid profiles and dietary fiber contents of fruits commonly consumed in Thailand. *Int. J. Food Sci. Nutr., 61*(5), 536-548.

Kunnika, S., Pranee, A. (2011). Influence of enzyme treatment on bioactive compounds and colour stability of betacyanin in flesh and peel of red dragon fruit Hylocereus polyrhizus (Weber) Britton and Rose. *Int. Food Res. J., 18*(4), 1437-1448.

Lee, C.H., Yang, L., Xu, J.Z., Yeung, S.Y.V., Huang, Y., Chen, Z.Y. (2005). Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chem., 90,* 735-741.

Liu, H., Cao, J., Jiang, W. (2015). Evaluation and comparison of vitamin C, phenolic compounds, antioxidant properties and metal chelating activity of pulp and peel from selected peach cultivars. LWT – *Food Science and Technology, 63,* 1042-1048.

Luis, A., Duarte, A.P., Pereira, L., Domingues, F. (2018). Interactions between the major bioactive polyphenols of berries: effects on antioxidant properties. *Eur. Food Res. Technol., 244,* 175-185.

Machida, M. (2002). Progress of *Aspergillus oryzae* Genomics. *Adv. Appl. Microbiol., 51*, 81-106.

Matoba, Y., Kumagai, T., Yamamoto, A., Yoshitsu, H., Sugiyama, M. (2006). Crystallographic evidence that the dinuclear copper center of tyrosinase is flexible during catalysis. *J. Biol. Chem., 281*(13), 8981-8990.

Murphy, K. at English Wikipedia - Transferred from en.wikipedia to Commons., Public Domain, https://commons.wikimedia.org/w/index.php?curid=3406229

Nakadai & Nasuno. (1988). Culture conditions of Apergillus oryzae for production of enzyme preparation. *J. Ferment. Technol., 66*(5), 525-533.

Pereira, D.M., Valentao, P., Pereira, J.A., Andrade, P.B. (2009). Phenolics: from chemistry to biology. *Molecules, 12,* 2202-2211.

Priatni, S., Pradita, A. (2015). Stability study of betacyanin extract from red dragon fruit (Hylocereus polyrhizus) peels. *Procedia Chem., 16*, 438-444.

Rebecca, O.P.S., Boyce, A.N., Chandran, S. (2010). Pigment identification and antioxidant properties of red dragon fruit. *Afr. J Biotechnol., 9*(10), 1450-1454.

Sanchez-Vioque, R., Polissiou, M., Astraka, K., Mozos-Pascual, M., Tarantilis, P., Herraiz-Penalver, D., Santana-Meridas, O. (2013). Polyphenol composition and antioxidant and metal chelating activities of the solid residues from the essential oil industry. *Ind. Crops Prod., 4,* 150-159.

Solomon, E. I., Sundaram, U. M., and Machonkin, T. E. (1996). Multicopper oxidases and oxygenases. *Chem. Rev., 96*(7), 2563-2606.

Sorensen, A., Lubeck, M., Lubeck, P.S., Ahring, B.K. (2013). Fungal Beta-Glucosidases: A Bottleneck in Industrial Use of Lignocellulosic Materials. *Biomolecules, 3*(3), 612-631.

Swanson, B.G. (2003). Tannins and polyphenols P5729-5733. *Encyclopedia of Food Sciences and Nutrition* (Second Edition). Academic Press.

Tridge. (2018). *White dragon fruit.* Retrieved on 29th April, 2018 from https://www.tridge.com/intelligences/dragon-fruit/production

Wu, L.C., Hsu, H.W., Chen, Y.C., Chiu, C.C., Lin, Y.I., Ho, J.A. (2006). Antioxidant and antiproliferative activities of red pitaya. *Food Chem., 95*, 319-327.

Yin, J., Becker, E., Andersen, M., Skibsted, L. (2012). Green tea extract as food antioxidant. Synergism and antagonism with α-tocopherol in vegetable oils and their colloidal systems. *Food Chem., 135,* 2195-2202.

Yong, Y.Y., Dykes, G., Lee, S.M., Choo, W.S. (2017). Comparative study of betacyanin profile and antimicrobial activity of red pitahaya and red spinach. *Plant Foods Hum. Nutr., 72*, 41-47.

Zhang, Q., Lin, L., Ye, W. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine, 13,* 20. https://doi.org/10.1186/s13020-018-0177-x

ABOUT THE AUTHOR

Jason Chen, currently attending the 11th grade at St. George's School, is thrilled to attend the Canada Wide Science Fair. In school, Jason is part of the STEM club, which hosts weekly meetings and presentations for young, passionate scientific minds. Jason's passion for molecular biology and organic chemistry also compelled him to participate in a variety of science competitions: he competed in the Greater Vancouver Regional Science Fair for the past four years, and ranked 14th in the national Canadian Biology Olympiad. Jason is very grateful for this opportunity, and looks forward to sharing his project with the future young generation of scientists. Jason Chen's project focuses on dragon fruit peel extracts. Dragon fruits are structurally unique, savory plants indigenous to the Americas. The objectives of this project were to extract chemically active compounds from dragon fruit peels, test the various properties of the peel chemicals, and propose an alternative method of extracting chemicals using microorganisms. The results showed that dragon fruit chemicals have many unexplored merits and can potentially be used as a food preservative.