Phenolic Compounds in Potato Skin: Source of Variation and Prebiotic Property

Abstract

Eight varieties of potato tubers were tested and compared for the total phenolic compound contents and prebiotic activities of their skin extracts on *Bifidobacterium longum* CSCC 5089. Factors, such as origins, tuber sizes and skin colours, were investigated for any effect on the phenolic compound contents. A significant difference ($p < 0.00001$) of the contents was observed among potato varieties. Origins ($p = 0.234596$, power = 0.9748207) and sizes ($p = 0.173474$, power = 0.9958413) of the potatoes were not significant factors when investigated individually. For the skin colours, pairs of L* and a* ($r = – 0.878318$, $p = 0.004134$, critical $p = 0.008333$), also a* and the contents, ($r = – 0.849283$, $p = 0.007662$, critical $p = 0.010000$), showed significantly negative correlations. While the correlations were insignificant for the pair of L* and the contents ($r = 0.714970$, $p = 0.046252$, critical $p = 0.012500$, power = 0.5744392), as well as any pair involving b*. Regarding the prebiotic activities, no significant difference ($p = 0.878254$, power = 0.0501455) was concluded among potato varieties.

Keywords

Potatoes, Phenolic compounds, Prebiotics, *Bifidobacterium*

1 Introduction

Being the fourth most important agricultural commodity, after maize, rice and wheat, over the globe, potato tubers are produced in more than 150 countries across continents, with the highest production ranked from Asia, Europe to Americas [1].

Belonging to the genus *Solanum*, potatoes are diversified into up to thousand species [2]. Among numerous potato varieties, potato skin colour is one of the characteristics for classification. Potato skin can be identified white, cream, yellow, russet, red, blue, red parti-coloured or blue parti-coloured [3].

Raw potato skin [4] contains significant amount of nutrients, including carbohydrate, dietary fibre, protein, minerals (e.g. potassium, phosphorus, calcium, magnesium, sodium, iron) and vitamins (e.g. folate, ascorbic acid, niacin). Additionally, phenolic compounds, especially phenolic acids, are found concentrated in potato skin [1,5,6,7].

Phenolic compounds are a group of antioxidant phytochemicals, chemically classified into phenolic acids, flavonoids, flavonols, flavones, isoflavones, flavanones, anthocyanins, flavanols, stilbenes and lignans [8]. Potential benefits of the compounds have been reported to prevent and help recover from various organ damage, to act against cancer development, and to lower risk of metabolic syndrome [9,10]. Phenolic compounds from various sources have also demonstrated both selective properties of pro-probiotic and anti-pathogenic bacteria [11,12,13], acting as a prebiotic.

Bifidobacterium is a probiotic, which confers a health benefit on the host when administered live in adequate amounts. The bacterium can enhance resistance to intestinal infection with a physical barrier and can improve the immune response regarding allergic inflammation [14,15]. Throughout the lifetime, from infancy to senescence, *Bifidobacterium* inhabits predominantly in the microbial flora of a healthy human [15], with a dynamic composition of the species [16]. Within a human body, *Bifidobacterium* is usually concentrated in the large bowel [16,17]. Among the species, *B. longum* is the most prevalent one [18], also found inhabiting during both infancy and adulthood [16].

In this research, phenolic compound contents in potato skin were evaluated among great varieties of the produce available in the market for any significant difference. Characteristics such as skin colours, sizes and origins are the facets of comparison. Besides, prebiotic properties of the phenolic compounds extracted from various potato skins on *B. longum* were investigated *in vitro*.

2 Materials and Methods

2.1 Potatoes

Eight varieties of potato tubers with labelled origins were purchased in Hong Kong. The skins of the tubers were all perceived yellow. Four categories of tuber sizes were assigned to the potatoes based on visual perception. The characteristics are listed in *Table 1*.

Table 1: Characteristics (origins and tuber sizes) of potato varieties

Potato variety	Origin	Tuber size
A	China	Medium
B	United States	Large
C	Japan	Tiny
D	Australia	Small
E	China	Large
F	Japan	Medium
G	Australia	Small
H	Australia	Small

2.2 Potato skin sample collection and extraction

First of all, samples of potato skin powders had to be obtained [19]. After being washed off soils and impurities on the surface with tap water, the potatoes were rinsed with pure water. Skins were peeled manually with great care to avoid additional flesh. The skins were vacuum packaged by a vacuum sealer MS-1160 at –20 ℃ until processed to freeze dry. Freeze dried skins were then milled into powder by a blender KJ-8118. Secondly, phenolic compounds in the skin powders were extracted and maintained in various media [20]. For each gram of potato skin powder, 25 mL pure methanol was used to extract the phenolic compounds for 24 hours at room temperature. After that, the mixtures were filtered, with the filtrates collected as ‘potato skin extracts in methanol’. Methanol in the extracts was waited to vaporize in clean shallow containers at room temperature under atmospheric pressure until their weights had become constant. The resultant extracts were mixed with 0.9 % saline solution with 10 % dimethyl sulphoxide (DMSO) in the ratio of 1 mg to 10 μL for ‘potato skin extracts in saline DMSO solution’.

2.3 Potato skin sample colourimetry

Colours of potato skin powders were tested by a colourimeter CR-400 in CIELAB colour space [20]. Three values, L*, a*, b*, were collected for each sample. L* indicates lightness against darkness, a* indicates redness against greenness, whereas b* indicates yellowness against blueness. The colourimetry was performed in triplicate for each variety.

2.4 Folin-Ciocalteu antioxidant assay

Antioxidant strengths, hence total phenolic compound contents, in potato skin samples were tested in the method of Folin-Ciocalteu antioxidant assay [21]. In each set of experiment, 125 μL ‘potato skin extract in methanol’ was reacted with 500 μL Folin-Ciocalteu reagent and 500 μL 10 % sodium carbonate solution for 30 minutes. The absorbance at 765 nm of the reacted mixture was then measured by a Smartspec Plus Spectrophotometer. Nine replicates were tested for each variety. A standard curve of linear regression was also generated using gallic acid of up to 0.14 mg/mL under the same protocol.

2.5 *Bifidobacterium longum*

B. longum CSCC 5089 was the probiotic strain used in this research. The frozen strain was activated on MRS agar at 37 ℃ for 24 hours twice and cultivated in fresh MRS broth in the ratio of 100 µL to 10 mL at 37 ℃ for 18 hours weekly. All growth media were supplemented with 0.05 % L-cysteine hydrochloride. The population of the *B. longum* used was determined to be 2.4×10^9 CFU/mL.

2.6 Prebiotic activity assay

For each sample, 100 µL 'potato skin extract in saline DMSO solution' was administered to 10 mL MRS broth with the live probiotic, resulting a mixture with 1 mg/mL potato skin extract and 0.1 % DMSO. Controls were produced by substituting 0.9 % saline solution with 10 % DMSO for 'potato skin extracts in saline DMSO solution'. Three replicates were prepared for each supplement group. The mixtures were then incubated at 37 ℃ for 18 hours. To quantify the probiotic populations in the mixtures after incubation, viable plate counting was performed. The mixtures were diluted with 0.9 % saline solution by 10^{-7}, of which 100 µL was spread on MRS agar. The agar plates were then incubated at 37 ℃ for 36 hours.

3 Results and Discussion

3.1 Total phenolic compound (TPC) content

In this research, TPC contents in potato skin samples were evaluated as gallic acid equivalents in the method of Folin-Ciocalteu antioxidant assay.

3.1.1 Gallic acid equivalent (GAE) standard curve

A standard curve intercepting the origin, shown in *figure 1*, was generated by linear regression using gallic acid of up to 0.14 mg/mL. The regression equation was found to be $y = 9.8723x$, where x is referred to GAE concentration in mg/mL, while y is referred to absorbance at 765 nm. The regression had $p < 0.00001$ and $R^2 = 0.99265$. This equation was used to determine the GAE of potato skin samples later.

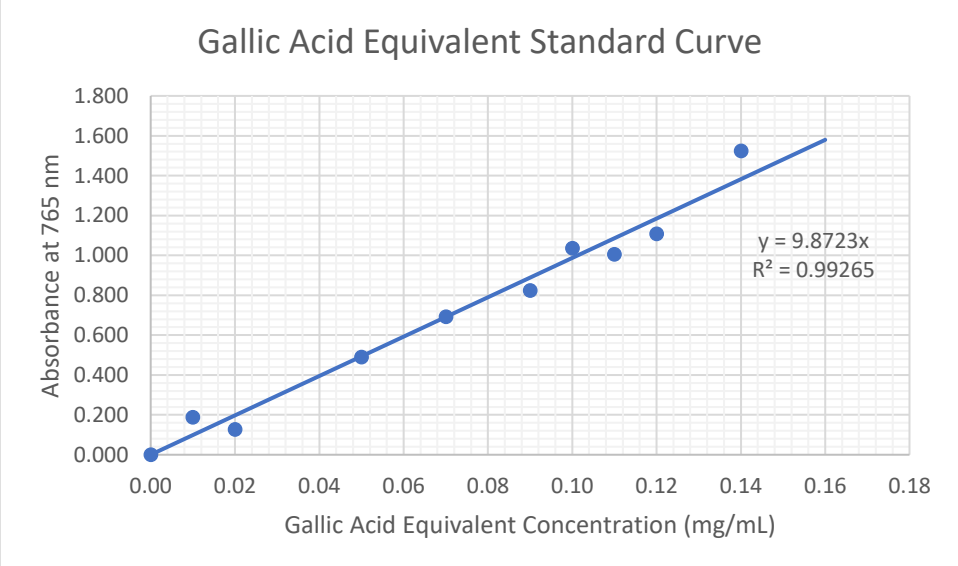

Figure 1: Gallic acid equivalent standard curve

3.1.2 Effect of potato varieties

Antioxidant, hence TPC, contents in potato skin samples were evaluated using the regression equation of the standard curve. The unit was converted to mg GAE / g potato skin. Since heteroscedastic data was obtained, Kruskal-Wallis test and Dunn's test were conducted to observe any significant difference of TPC contents among and between potato varieties respectively. From Kruskal-Wallis test, a significant difference ($p < 0.00001$) among varieties was observed. Detailed results on the GAE and of Dunn's test are shown in *Table 2* and illustrated in *Figure 2*. If not specified, error bars in all figures in this report indicate the 95 % confidence intervals using Student's *t* distribution due to small sample sizes. Between the varieties bearing the same small letter, there was no significant difference ($p > 0.05$) in gallic acid equivalents. Since a significant difference of TPC contents in the skin samples was observed among potato varieties, potential factors of such a difference were further investigated.

Table 2: Potato varieties on gallic acid equivalents

Potato variety	Gallic acid equivalent (mg/g)		Note
	Mean	S.D.	
A	4.0244	0.3975	a,b
B	3.9744	0.5167	b
C	3.0335	0.7387	c
D	1.5847	0.9326	d
E	3.0017	0.6445	c
F	3.5917	0.7515	b,c
G	4.4710	0.1553	a
H	4.0987	0.3873	a,b

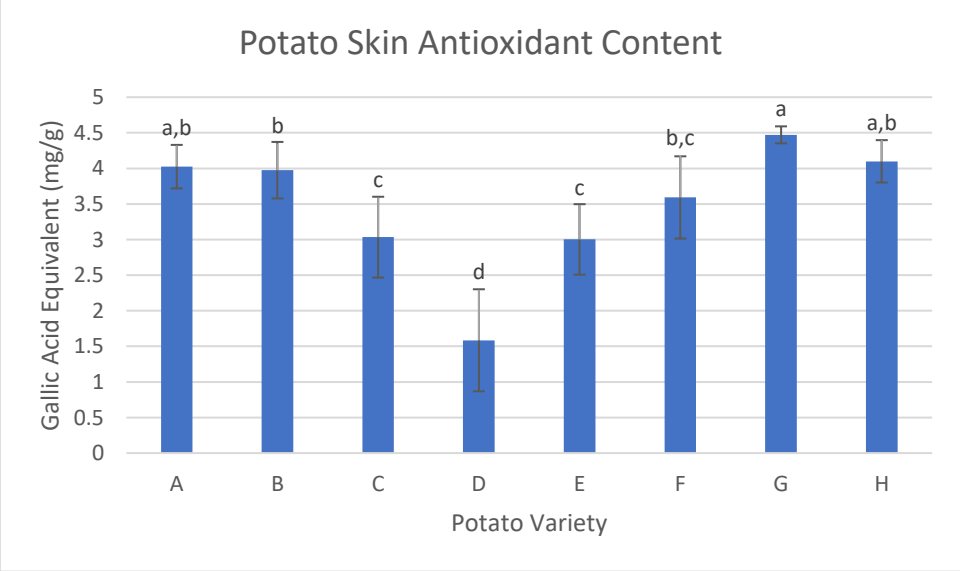

Figure 2: Potato varieties on skin antioxidant content

3.1.3 Effect of potato origins

In order to investigate the effect of potato origins on TPC contents in the skin samples, the varieties were placed into four groups according to the origins. Since heteroscedastic data was obtained among groups, Kruskal-Wallis test was conducted to observe any significant difference of TPC contents among potato origins. Insignificant difference (p = 0.234596, power = 0.9748207) was observed. Detailed results are illustrated in *Figure 3*.

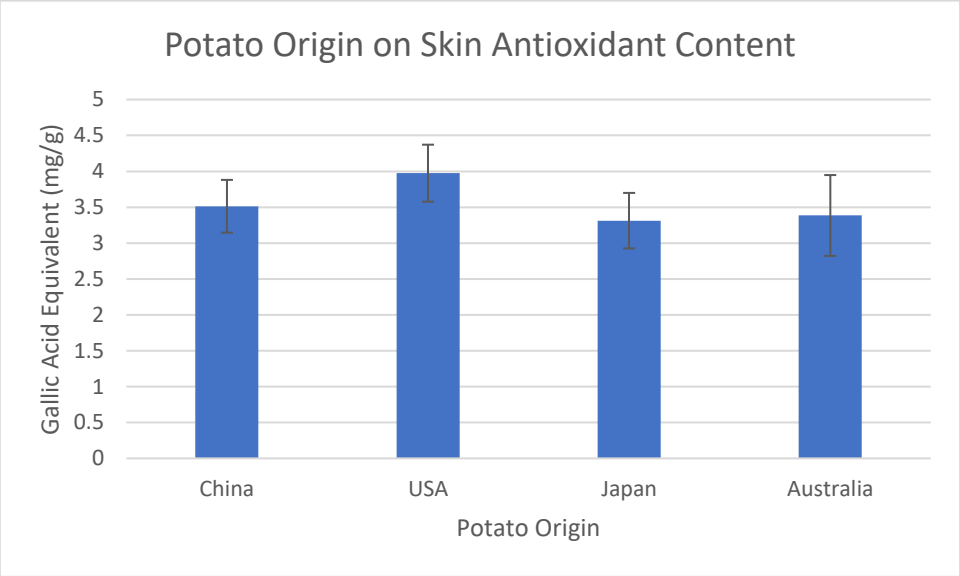

Figure 3: Potato origins on skin antioxidant contents

3.1.4 Effect of potato sizes

According to the sizes, the potato varieties were placed into four groups to investigate their effect on TPC contents in the skin samples. Since heteroscedastic data was obtained among groups, Kruskal-Wallis test was conducted to observe any significant difference of TPC contents among potato sizes. Insignificant difference (p = 0.173474, power = 0.9958413) was observed. Detailed results are illustrated in *Figure 4*.

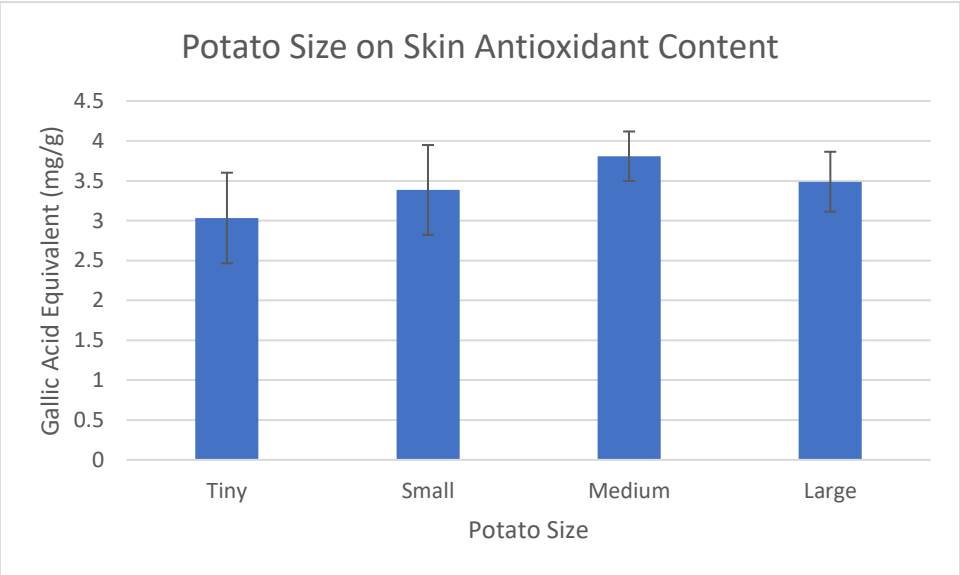

Figure 4: Potato sizes on skin antioxidant contents

3.1.5 Effect of potato skin colours

To investigate the effect of colours on TPC contents in the skin samples, Pearson's product-moment correlation analysis following by sequential Bonferroni correction was conducted among L*, a*, b* and GAE. The detailed results are shown in *Table 3*. As marked '*' under 'power' in *Table 3*, significant correlations were detected in the pairs of L*-a* and a*-GAE. By the correlation between L* and a*, it was indicated that the lighter was also the greener the potato skin colour. Whereas by the correlation between a* and gallic acid equivalent, it was indicated that the greener the potato skin colour, the richer the TPC content. To the direct interest of this research, the inverse relationship between a* and GAE is illustrated in *Figure 5*. However interestingly, the pair of L*-GAE did not show a significant correlation in this research. Given the power, a larger sample size may be adopted in a later research to minimize the probabilities of accepting false null hypotheses for the pairs without significant correlation. Besides, although some significant correlations were already observed using each of the varieties wholly as a data point in this research, potatoes within the same variety may be tested individually in a later research to avoid influences by other factors.

Table 3: Correlations among CIELAB values and antioxidant contents of potato skin

Pair	*r*	*p*	Critical *p*	Power
L* - a*	–0.87832	0.004134	0.008333	*
L* - b*	0.53435	0.172521	0.016667	0.1409770
L* - GAE	0.71497	0.046252	0.012500	0.3158663
a* - b*	–0.21081	0.617673	0.025000	0.0412402
a* - GAE	–0.84928	0.007662	0.010000	*
b* - GAE	0.205548	0.625398	0.050000	0.0760210

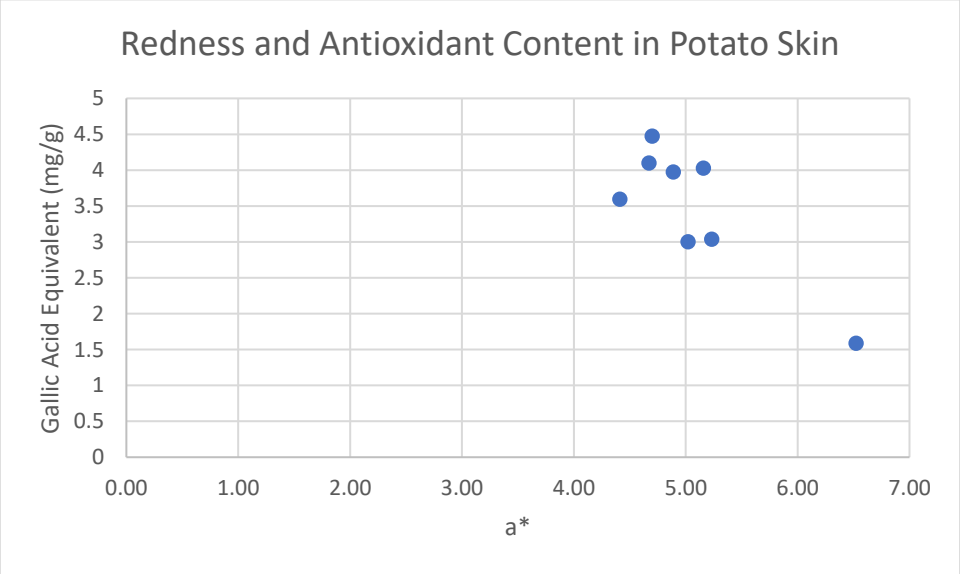

Figure 5: Relationship between redness (a*) and antioxidant contents in potato skins

3.1.6 Combination of factors

As the potential factors were investigated individually in this research, other factors might have exerted some influences and induced a large variance within each group. This reduces the sensitivity of the tests. Therefore, it is recommended that in a later research, varieties with designated combinations of characteristics should be selected so that multiple-way analysis of variance (ANOVA), or non-parametrically Scheirer-Ray-Hare test, can be applied. The tests should be able to analyse each single factor more sophisticatedly, without receiving much disturbance from other factors.

3.2 Prebiotic activity

The growths of *B. longum* supplemented with potato skin extracts of different varieties and a control were evaluated in the prebiotic activity assay. Numbers of colonies formed in the final agar plates were counted and converted into colony forming units (CFU). Since heteroscedastic data was obtained among groups, Kruskal-Wallis test was conducted to observe any significant difference of skin prebiotic activities among potato varieties. Insignificant difference (p = 0.878254, power = 0.0501455) was observed. Detailed results are illustrated in *Figure 6*. The control is labelled 'X'. Given the extremely low power and enormous variances within groups, a larger sample size should be involved in a later research for a valid conclusion.

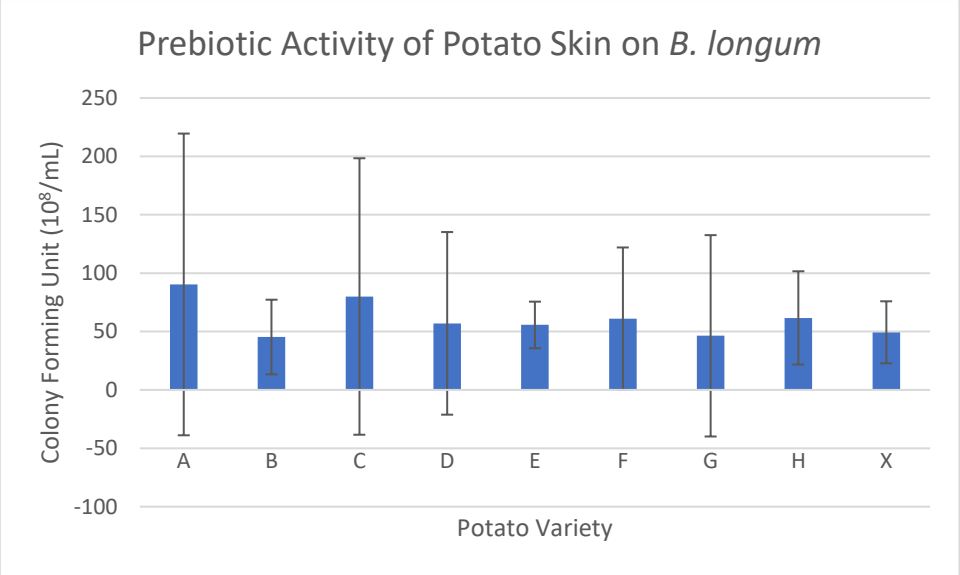

Figure 6: Growths of *B. longum* under potato skin extracts of different varieties and control

4 Conclusion

In this research, total phenolic compound contents in potato skins among potato varieties were significantly different. Regarding the factors for such a difference, neither origins nor sizes had a significant effect on its own. While for the skin colours, L* and a* were detected a significantly negative correlation. Meanwhile, a* and the contents also had a significantly negative correlation. However, L* and the contents, as well as b* with any value, were not proven any significant correlation. In terms of prebiotic activities of potato skin extracts on *B. longum*, no conclusion supported by statistically significant results could be drawn in this research.

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