CAFEi2016-117

Valorizing guava (*Psidium guajava* L.) seed: Its prebiotic potential in comparison with winter wheat (*Triticum aestivum* L.) and red bean (*Vigna angularis*)

Y.P. Chang[a] and L.Y. Tan

Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, Malaysia.

Abstract

Guava processing industry produces peels, seeds and decanter, which can be valorized for other applications. This provides an economical solution for solid waste management and reduces environmental pollution problem at the same time. Guava seed is rich in dietary carbohydrates which may be a source of prebiotics that enhances the growth of probiotics in the digestive tract, and improves the immune system of the host. Seed germination-induced carbohydrate metabolism may alter the prebiotics content and activity. Seeds of guava (*Psidium guajava L.*), together with other seed-type staple food like winter wheat (*Triticum aestivum L.)* and red bean (*Vigna angularis*) were germinated and analyzed. Inulin-type fructan content was determined enzymatic-colorimetrically while the seed extracts were tested on the probiotic Lactobacillus rhamnosus for prebiotic activity score. Germination induced a decrease in fructan content but raised the prebiotic activity score for all the seed samples. The extract of germinated guava seed showed approximately a five-fold increment in prebiotic effects. Further research is warranted to probe further the effects of germination on the feed value of under-utilized seeds.

Keywords: fructan content, germination, guava seed, prebiotic activity score, red bean, winter wheat

INTRODUCTION

Guava fruit is an exotic tropical fruit which is rich in dietary fibre and exhibits high antioxidant capacity (Jiménez-Escrig et al., 2001). Approximately 3.49 million metric tons of guava fruit were produced globally, in 2009-2010 (Kumar et al., 2010). Fruit and cereal processing has increased considerably during the last 25 years due to the increased in demand as a result of consumers' awareness on the health-promoting effects of fruits and cereal fibres (Federici et al., 2009). The mass processing of fruit and vegetable generates great amount of waste and by-products such as peel, pomace, seeds, etc. Guava seed core take up about 30% of the whole fruit and are often discarded after processing of guava fruit. Nevertherless, guava seed is rich in dietary carbohydrates (Jiménez-Escrig et al., 2001). Current trend in valorizing by-products from agro-based industry and agronomic sector views the dietary carbohydrate in these by-products as a source of functional ingredient (Rodríguez et al., 2006). Finding ways to make use of this functional ingredient would prevent the disposal of the plant-based by-products, subsequently alleviates the problem of food waste (FAO, 2011).

Dietary carbohydrates are often regarded as energy storage material. However, some oligosaccharides and certain products of carbohydrate metabolism possess prebiotic potential (Rodríguez et al., 2006) which can modify the composition of gut microbiota. By

definition, prebiotics are non-digestible carbohydrates that can selectively enhance the growth and stimulating the activity of some groups of beneficial bacteria (probiotics) origin

---

[a] E-mail: changyp@utar.edu.my

from the colonic flora, the prebiotics are subsequently fermented into short chain fatty acids. This prebiotic-probiotic interaction results in risk reduction of both intestinal and systemic pathologies, and the host's health can be maintained or improved (Sarbini and Rastall, 2011). Commercial prebiotics have been included in the formulation of animal feed to enhance the immune system in order to reduce the usage of in-feed antibiotics (M'Sadeq et al., 2015).

Fructooligosaccharides, inulin, fructan and galactooligosaccharides are well-known prebiotics. They are present naturally in root vegetables including Jerusalem artichokes, burdock, chicory, leeks, and onions (Sarbini and Rastall, 2011). Cereal and legumes are seed-type staple food but report on its prebiotic content and prebiotic activity is scanty. While saccharides (xylans, mannans, arabinogalactans, pectins and/or their respective oligomeric products) derived from agricultural waste of agro-industry by-products such as corn cob, rice husk, fruit waste also possess similar prebiotic effects (Gullón et al., 2009). Treatments include fermentation, heat treatment, enzymatic hydrolysis and acid hydrolysis may alter prebiotic contents of plant materials. In our previous work, germination reduced some anti-nutritional compounds significantly in guava seed (Chang et al., 2014). Therefore changes in the seeds' prebiotic content may occur due to germination-induced nutrient mobilization. In addition, composition variations among seeds of fruit, cereal and legume can result in distinct trends of carbohydrate metabolism during germination. Thus, this study aims to evaluate prebiotics potential in the seeds of guava (*Psidium guajava* L.), winter wheat (*Triticum aestivum* L.) and red bean (*Vigna angularis*) before and after germination to explore the potential of guava seed as a source of prebiotics in comparison with common cereal and legume.

MATERIALS AND METHODS

Seed sample preparation

Fresh guava seed cores were obtained from a local fresh-cut fruit supplier and the seeds were separated according to Chang et al. (2014). Red bean and winter wheat were purchased from another local supplier. Seed samples were washed and soaked in distilled water at ~25°C for 8 h to terminate seed dormancy. The drained seeds were surface-sterilized with 1% sodium hypochlorite and rinsed with distilled water. A portion of seeds were directly air-dried prior to further analysis while others were germinated (6-8 g per Petri dish) on some sterilized wet cotton in 9 cm-diameter Petri dishes. Germination time was 24 h for winter wheat; 50 h for red beans; and 14 days for guava seeds before the commencement of seedling growth. Seed samples were then air-dried at ~25°C for overnight, ground and kept in air-tight containers for further analysis.

Determination of moisture content

The moisture content of the seed samples was determined by following the AOAC official method 934.06 (2005).

Quantitation of prebiotic content

The prebiotic content in the form of fructan polysaccharide of the seed samples was quantified enzymatic-colorimetrically using the Fructan Assay Kit K-FRUC 5/2008 (Megazyme international Ireland Ltd.). The content of fructan in ground seed samples was expressed in % dry weight basis (db).

Isolation of carbohydrate fraction

Seed carbohydrates were extracted thrice using 80% ethanol (v/v) at a ratio of 1:4 (w/v) based on the method of Farrant et al., (2003). All extracts were combined and evaporated at 50°C to dryness in a rotary evaporator (Heidolph, Hei-VAP) prior to prebiotic activity assay.

Prebiotic activity assay and estimation of prebiotic score

Lactobacillus rhamnosus used as the probiotic strain was isolated from commercial probiotics LACTO-5 ™; while *Escherichia coli* as the enteric strain was obtained from the Collection of Faculty of Science, Universiti Tunku Abdul Rahman. Prebiotic activity assays on the sample extracts were performed according to Huebner et al. (2007) as shown schematically in Figure 1. Seed carbohydrate extracts, analytical grade fructo-oligosaccharide and inulin, was tested respectively. The prebiotic activity score was estimated using the following Equation 1:

Prebiotic activity score

$$= \left[\frac{(probiotic \log O.D. \; on \; the \; prebiotic \; at \; 24\,h - probiotic \log O.D. \; on \; the \; prebiotic \; at \; 0\,h)}{(probiotic \log O.D. \; on \; glu\cos e \; at \; 24\,h - probiotic \log O.D. \; on \; glu\cos e \; at \; 0\,h)}\right] - \left[\frac{(enteric \log O.D. \; on \; the \; prebiotic \; at \; 24\,h - enteric \log O.D. \; on \; the \; prebiotic \; at \; 0\,h)}{(enteric \log O.D. \; on \; glu\cos e \; at \; 24\,h - enteric \log O.D. \; on \; glu\cos e \; at \; 0\,h)}\right] \quad (1)$$

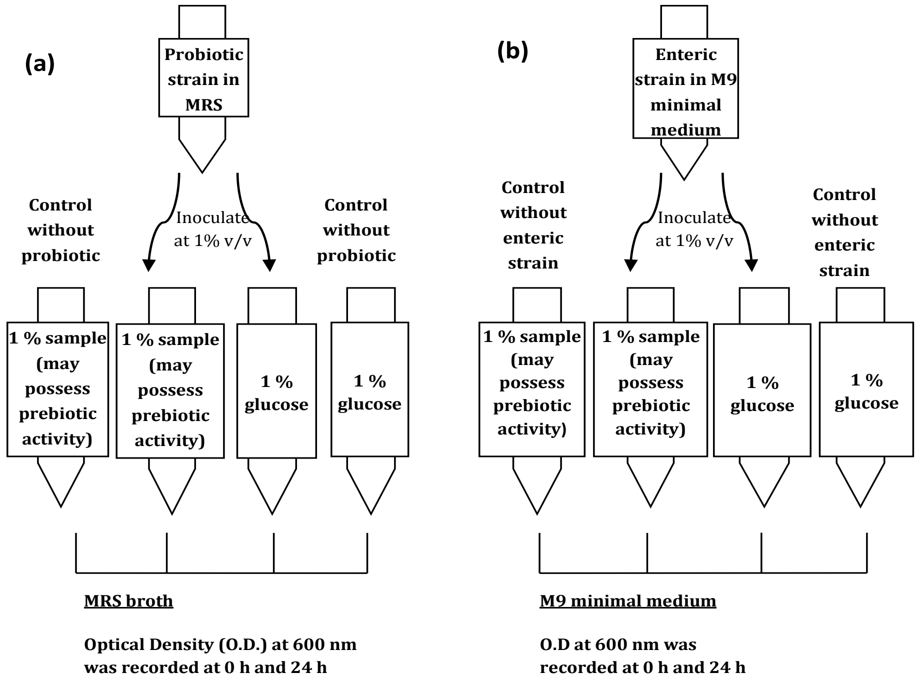
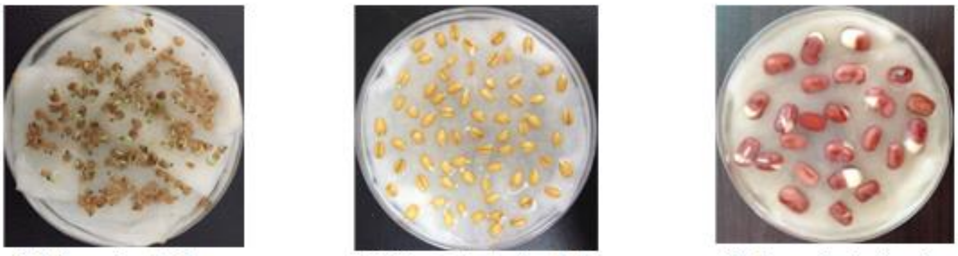


Figure 1. Schematic illustration of prebiotic activity assay set-up: (a) Cultivation of probiotic strain with MRS broth and (b) cultivation of enteric strain with M9 minimal medium

RESULTS AND DISCUSSION

The sizes of both red beans and winter wheat increased slightly after germination. At the end of the germination period, radicles developed and broke through the seed coating. The size of guava seeds did not increased after germination probably due to its rigid outer coat. It became darker in color at the end of the germination period (Figure 2). Figure 3 shows the levels of fructan in the ungerminated and germinated seeds. The fructan contents followed the order of guava seed<winter wheat<red bean. The fructan content in guava seeds were 10 fold-lower than those of red bean. Winter wheat contained 1.1% fructan which was within the range of 0.7 to 2.9% as reported by other researchers (Verspreet et al., 2015). The fructan content decreased in all the germinated seeds probably due to its utilization as part of the carbohydrate reserves to fuel metabolism during seed germination (Pollock and Lloyd, 1994).

(a) Guava seeds
(c) Winter wheat grains
(e) Red beans



(b) Germinated guava seeds
(d) Germinated winter wheat
(f) Germinated red beans

Figure 2. Physical appearance of the seed samples.

Although fructan are well-known prebiotics (Kelly, 2008), there are other substances in the seeds which may act as prebiotics too. Thus, the prebiotic activity score was tested to evaluate the feed value of the seeds.

Figure 4 shows the prebiotic activity score of different types of seed extracts, fructo-oligosaccharides and inulin. Before germination, the prebiotic activity scores for all the seeds were above 1.5 followed the order of guava seed<red bean<winter wheat. Despite its lower fructan content (Figure 2), winter wheat extract showed a much higher prebiotic activity score (9.2) when compared to red bean extract (2.8). This confirmed that there are other substances in the seed extracts which acted as prebiotics. Both common prebiotics like inulin and fructo-oligosaccharide showed the lowest activity which was approximately 1.06 and 1.22, respectively. These scores were in agreement with other published data on various probiotic's growth supported by the commercial prebiotics (0.5 to 1.25) (Huebner et al., 2007). Generally, the seed samples showed higher prebiotic activity score compared with those of inulin and fructo-oligosaccharide. This implies that seed extracts contained a combination of carbohydrates (Zamski, 1995) rather than solely fructan, which were able to support the growth of *L. rhamnosus*. Germination induced an increase in the prebiotic activity score for all the seeds studied. The prebiotic activity scores increased to values above 6, followed the order of red bean<guava seed<winter wheat. Guava seeds function as prebiotic showed the greatest activity increase of 5.3 fold. While winter wheat showed the least changes in activity with only one-fold of increment. Germination mobilizes the reserves in seeds through seed enzymes' action. Cereals and legumes contain: Phytase (Sung et al., 2005), proteases (Vijaylaxmi, 2013), lipase (Barros et al., 2010) and carbohydrate-degrading enzymes (Bialecka and Kepczynski, 2010). The breakdown of the nutrient reserves to simpler substances in the seeds may have improved the feed value of the seed itself.

CONCLUSIONS

The following conclusions can be drawn from the study:

- The seeds of guava, winter wheat and red bean consisted of fructan polysaccharide over a range of 0.27 to 2.5%. It followed the order: guava seed<winter wheat<red bean. Fructan content decreased in all the seeds after germination.
- However, the prebiotic activity score in the seed extracts were distinctly higher as compared to those of inulin and fructo-oligosaccharides, the two common commercial prebiotics. It followed the order: guava seed<red bean<winter wheat. There were

carbohydrates other than fructan in the seeds that raised the prebiotic activity scores. Germination induced more liberation of these substances in guava seeds which enhanced the growth of probiotics to 5.3-fold while germinated winter wheat showed only a one-fold increment in the prebiotic activity score.

- Thus, further research is warranted to investigate the effects of germination on feed values of under-utilized seeds in order to transform a plant-based waste to an animal feed.

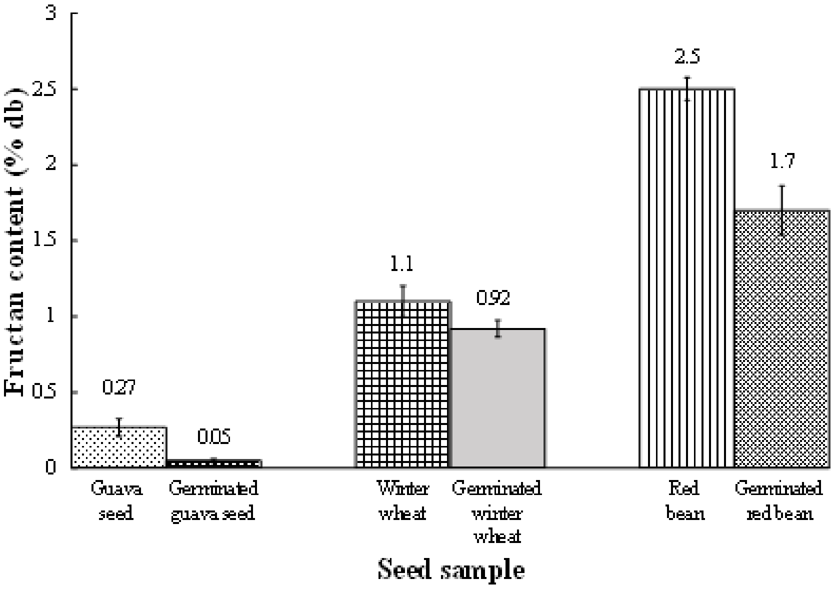


Figure 3. Fructan content of non-germinated and germinated seeds of guava, winter wheat and red bean (values represent mean±SD, n=4).

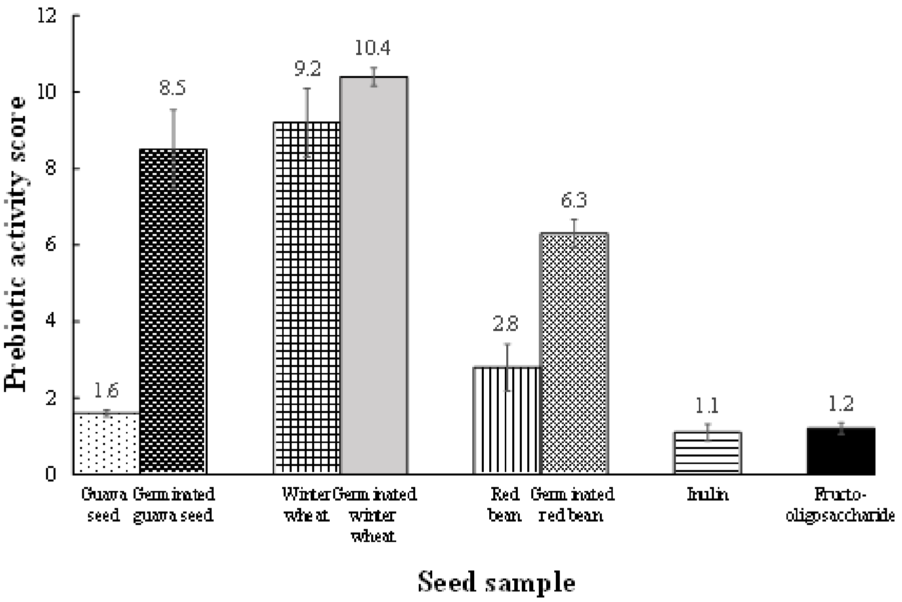


Figure 4. Prebiotic activity score of ungerminated and germinated seeds of guava, winter wheat and red bean in comparison with inulin and fructo-oligosaccharide (values represent mean±SD, n=3).

ACKNOWLEDGEMENTS

The authors thank Universiti Tunku Abdul Rahman for the support given to this research through the FYP scheme (FYP0514/CYP).

Literature cited

AOAC. (2005). Official Methods of Analysis 934.06, 18th edition. Washington, D.C.: Association of Official Analytical Chemists.

Barros, M., Fleuri, L.F., and Macedo, G.A. (2010). Seed lipases: Sources, applications, and properties – A review. Braz. J. Chem. Eng. *27*, 15-29 https://dx.doi.org/10.1590/S0104-66322010000100002.

Bialecka, B., and Kepczynski, J. (2010). Germination, α-, β-amylase and total dehydrogenase activities of amaranthus caudatus seeds under water stress in the presence of ethephon or gibberellins A_3. Acta Biol. Cracov., Ser. Bot. *52*, 7-12 http://dx.doi.org/10.2478/v10182-010-0001-0.

Chang, Y.P., Tan, M.P., Lok, W.L., Pakianathan, S., and Supramaniam, Y. (2014). Making use of guava seed (*Psidium guajava* L): The effects of pre-treatments on its chemical composition. Plant Foods Hum. Nutr. *69*, 43-49 http://dx.doi.org/10.1007/s11130-013-0396-3.

FAO. (2011). Global food losses and food waste – Extent, causes and prevention. Rome. http://www.fao.org/docrep/014/mb060e/mb060e.pdf.

Farrant, J.M., Bailly, C., Leymarie, J., Hamman, B., Côme, D., and Corbineau, F. (2003). Wheat seedlings as a model to understand desiccation tolerance and sensitivity. Physiol. Plant. *120*, 563-574 http://dx.doi.org/10.1111/j.0031-9317.2004.0281.x.

Federici, F., Fava, F., Kalogerakisc, N., and Mantzavinosc, D. (2009). Valorisation of agro-industrial by-products, effluents and waste: concept, opportunities and the case of olivemill waste waters. J. Chem. Technol. Biotechnol. *84*, 895-900 http://dx.doi.org/10.1002/jctb.2165.

Gullón, P., Gullón, B., Moure, A., Alonso, J.L., Domínguez, H., and Parajó, J.C. (2009). Manufacture of prebiotics from biomass sources. In Prebiotics and Probiotics Science and Technology, D. Charalampopoulos and R. A. Rastall, eds. (New York: Springer), 535-539.

Huebner, J., Wehling, R.L., and Hutkins, R.W. (2007). Functional activity of commercial prebiotics. Int. Dairy J. *17*, 770-775 http://dx.doi.org/10.1016/j.idairyj.2006.10.006.

Jiménez-Escrig, A., Rincón, M., Pulido, R. and Saura-Calixto, F.(2001). Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. J. Agric. Food Chem. *49*, 5489-5493 http://dx.doi.org/10.1021/jf010147p.

Kumar, B., Mistry, N.C., Singh, B., and Gandhi, C.P., (2010). Indian horticulture database 2010. Gurgaon, India: National Horticulture Board, Ministry of Agriculture, Government of India. nhb.gov.in/area-pro/database-2011.pdf.

M'Sadeq, S.A., Wu, S., Swick, R.A., and Choct, M. (2015). Towards the control of necrotic enteritis in broiler chickens with in-feed antibiotics phasing-out worldwide. Anim. Nutr. *1*, 1-11. http://doi:10.1016/j.aninu.2015.02.004.

Pollock, C.J., and Lloyd, E.J. (1994). The metabolism of fructans during seed development and germination in onion (*Allium cepa* L.) New Phytol. *128*, 601-605 http://dx.doi.org/10.1111/j.1469-8137.1994.tb04023.x.

Rodríguez, R., Jiménez, A., Fernández-Bolanõs, J., Guillén, R., and Heredia, A. (2006). Dietary fibre from vegetable products as source of functional ingredients. Trends Food Sci. Technol. *17*, 3-15. http://dx.doi.org/10.1016/j.tifs.2005.10.002.

Sarbini, S.R., and Rastall, R.A. (2011). Prebiotics: Metabolism, structure and function. Funct. Food Rev. *3*, 93-106 http://dx.doi.org/10.2310/6180.2011.00004.

Sung, H.G., Shin, H.T., Ha, J.K., Lai, H.-L., Cheng, K.-J., and Lee, J.H. (2005). Effect of germination temperature on characteristics of phytase production from barley. Bioresource Technol. *96*, 1297-1303 http://dx.doi.org/10.1016/j.biotech.2004.10.010.

Verspreet, J., Dornez E., Ende, W. V. D., Delcour, J. A., and Courtin, C. M. (2015). Cereal grain fructans: Structure, variability and potential health effects. Trends Food Sci. Technol. *43,* 32-42 http://dx.doi.org/10.1016/j.tifs.2015.01.006.

Vijaylaxmi. (2013). Biochemical changes in cotyledons of germinating mung bean seeds from summer and rainy seasons. Ind. J. Plant Physiol. *18*, 377-380 http://dx.doi.org/10.1007/s40502-013-0014-7.

Zamski, E. (1995). Transport and accumulation of carbohydrates in developing seeds: The seed as a sink. In Seed Development and Germination, J. Kigel and G. Galili, eds. (New York: Marcel Dekker Inc,), 25-44.