Consumer acceptability and antidiabetic properties of flakes and crackers developed from selected native Australian plant species

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Consumer acceptability and antidiabetic properties of flakes and crackers developed from selected native Australian plant species

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Abstract Type 2 diabetes, linked to an unhealthy diet, is increasing in Australia. This study aimed to evaluate the acceptability of potential antidiabetic food preventatives followed by phyto-component detection by high-performance liquid chromatography analysis and glycaemic index (GI) estimation by *in vitro* enzymatic hydrolysis. Five flakes and a cracker were developed from *Acacia longifolia* seeds, *Typha orientalis* rhizomes and *Rhagodia candolleana* berries. Samples were tested for consumer acceptability against a commercially available flake and cracker (as controls) by 44 participants using a 9-point hedonic scale. Overall acceptability of 86.4% and 54.5%–65.9% was recorded against control and test flakes, while control and test crackers recorded 84.1% and 70.5%, respectively. The test cracker contained gallic acid (GA) and ρ-coumaric acid (PCA) with GI, 47.7 ± 1.3, whereas control cracker contained GA and had GI, 70.3 ± 2.5. These results indicate that the test cracker may have potential as an antidiabetic food preventative.

Keywords antidiabetic components, Indigenous foods, microbiological quality, nutritional profile, sensory testing, shelf life, starch hydrolysis.

Introduction

Food product development and/or improvement of pre-existing food varieties is often performed with the intent of improving the nutritional profile of the food product to help control some non-communicable diseases (Choudhary & Grover, 2012). Functional foods influence specific beneficial physiological functions in the body beyond their basic nutritional role (Granato *et al*., 2020). Their constituents support health, well-being or performance beyond regular nutrition, and food products of this nature are marketed and consumed for their value-added properties (Kasbia, 2005; Choudhary & Grover, 2012). It is estimated that approximately 3.5 million Australians will have type 2 diabetes mellitus (T2DM) by 2033 (Gulati *et al*., 2015; Hill *et al*., 2017) and food choice is a major contributing factor to this increase (Kaldor, Magnusson & Colagiuri, 2015). Indigenous Australians are reported to be 20 times more likely to have T2DM and twice as likely to be obese than other Australians (Titmuss *et al*., 2019). Frequent consumption of energy-dense foods has been linked to an increase in T2DM in Australia (Dendup *et al*., 2018), yet avoiding the temptation of eating foods that might worsen glycaemic control has been a major challenge in the management of this disease. These aspects in addition to food insecurity in rural areas are pushing research efforts towards the search for local healthier culturally acceptable food alternatives.

Dietary polyphenols may constitute possible strategies for mitigation of T2DM considering that they have a record of proven antidiabetic activities (Salim, 2014; Williamson, 2017; Deng *et al*., 2020). Australian Indigenous food plants that are rich in antidiabetic polyphenols and antioxidants are therefore possible sources of raw materials for the development of novel food products for people with T2DM. Some polyphenols in food, for example, may slow down the rate of carbohydrate digestion which is a means of regulating postprandial hyperglycaemia (Deng *et al*., 2020). Results of our previous study (Njume *et al*., 2020) indicated that edible portions of some native Australian plant species constitute a rich source of

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nutrients and antidiabetic polyphenols. These included seeds of *Acacia longifolia* subsp. *sophorae*, underground stems and rhizomes of *Typha orientalis* C. Presl and the glossy red berries of *Rhagodia candolleana* subsp. *candolleana*.

Acacia longifolia subsp. *sophorae* is commonly known as coast wattle and is a low-growing shrub or small tree (0.5–3 m) that may occasionally grow to a height of about 5 m (Entwisle *et al*., 1996). *Acacia longifolia* subsp. *sophorae* belongs to the family Fabaceae and is distributed along the Victorian volcanic plain, Victorian Riverina, Warrnambool plain, spreading in abundance along the coast (Entwisle *et al*., 1996). Its hard black seeds with brown fleshy arils, available late November to early April, have been used as food by members of the Australian Indigenous community for centuries (Brand-Miller & Holt, 1998). The seeds are consumed raw or roasted and sometimes used to make the local bread known as 'damper' (Maslin & McDonald, 2004). Ground seeds have more recently been added to chocolate, granola, ice creams and cakes as flavourings (Maslin & McDonald, 2004). The seed arils are rich in oils, mostly unsaturated fatty acids mainly in the form of linoleic or oleic acid (Brown, Cherikoff, & Roberts, 1987; Seigler, 2002; Njume *et al*., 2020).

Typha orientalis C. Presl. is a semi-aquatic perennial grass-like plant (2–4 m tall) commonly known in most Australian communities as reed or cattail (Gott, 1999). *Typha orientalis* produces thick white roots and rhizomes which are used as food by Indigenous Australians (Gott, 1999). It is an invasive plant species, spreading freely at the roots and growing through canals, ditches, reservoirs, cultivated fields, farms and ponds.

Rhagodia candolleana is a sprawling coastal halophytic shrub, about 2 m in height (Walsh, 1996). It belongs to the family Chenopodiaceae and produces small glossy dark red-purple attractive edible berries that are flattened and dimpled at the top and which upon touch/squeezing produces a red/purple juice that readily stains the hands. It is distributed along the Victorian volcanic plain, Bridgewater, Warrnambool and consumed by members of the Indigenous community (Walsh, 1996).

Although these three plant species have been relied upon as sources of food and in some cases primary health care for many centuries, there has been no scientific evidence linking them to the control and management of diseases such as obesity and T2DM, which now have a high rate of occurrence in the Indigenous community (Sainsbury *et al*., 2018; Titmuss *et al*., 2019). The level of acceptability of Indigenous foods produced from the selected species is also lacking. Having survived harsh environmental conditions under the wild Australian climate for many centuries, native plant species have accumulated bioactive components that may bestow unique tastes, flavours and aromas to food products developed from them (Sultanbawa *et al*., 2015), and some of these are yet to be experienced by the wider Australian community and the world at large. The objectives of this study, therefore, were to develop food products with edible portions of the selected species (*T. orientalis*, *A. longifolia* subsp. *sophorae* and *R. candolleana* subsp. *candolleana*), evaluate the products for consumer acceptability and estimate the glycaemic index of most acceptable product as a measure of its antidiabetic potential.

Materials and methods

Collection and selection of plant species

Plant material of the following species was collected and prepared according to our previously established procedures (Njume *et al*., 2020): *Rhagodia candolleana* subsp. *candolleana*, *T. orientalis* C. Presl and *A. longifolia* subsp. *sophorae* (Labill.) Court. In selecting plant species for preparation of food products, some requirements for antidiabetic food products (fibre/complex carbohydrate content, saturated/unsaturated fat and polyphenol content) that have been reported to play a role in the prevention and management of T2DM (Tutelyan *et al*., 2016; McMacken & Shah, 2017) were considered (Table 1). The selected plants (*T. orientalis*, *A. longifolia* and *R. candolleana*) displayed the highest amounts of dietary fibre and protein, contained low amounts of total fats, exhibited strong antioxidant capacities (Njume *et al*., 2020) and were able to inhibit either α-glucosidase and/or α-amylase *in vitro* (data

Table 1 Plant species selected for development of food product

Properties	Plant species		
	To	Al	Rc
Rich in dietary fibre/complex carbohydrates (≥10%)	O	O	
High protein content (≥10%)		O	
Low in saturated fats (≤10%)	O	O	O
Strong antioxidant capacity (≥0.12 mg/mL AAE)	O	O	O
Inhibition of at least 1 enzyme involved in carbohydrate metabolism (≥40%)	O		O
Rich in antidiabetic polyphenols (≥3 types)	O	O	
Low in simple sugars (≤50%)	O	O	
Rich in minerals (≥5 types)	O		O
TOTALS	7	6	4

To, *T. orientalis*; Rc, *R. candolleana* subsp. *candolleana*; Al, *A. longifolia* subsp. *sophorae*; AAE, ascorbic acid equivalence; O meets criterion; does not meet criterion. This table was extracted from nutritional information previously published for the plant species (Njume *et al*., 2020).

not shown). Edible portions of the selected species were developed into 5 varieties of flakes and one cracker at Victoria University (Melbourne Australia).

Production of flakes

Whole ground *Acacia* (*Acacia longifolia* subsp. *sophorae*) seeds were mixed with ground underground stems and rhizomes of Water Cumbungi (*Typha orientalis*), vanilla extract, *Acacia* gum, pinch of table salt and cold water. The mixture was stirred several times to form a paste. The paste was spread over a baking tray that had been previously lightly greased with cooking oil and lined with parchment paper (FW flakes). A second mixture of pressure-cooked ground *Acacia* seeds (with one-third of the seeds scarified) was made into a separate paste following the above steps (FR flakes). The paste was again spread over a baking tray that had been previously greased with cooking oil and lined with parchment paper. Two more pastes were prepared by mixing completely scarified pressure-cooked *Acacia* seeds which had been divided into 2 equal parts. To one part were added sea berry salt bush (*Rhagodia candolleana* subsp. *candolleana*) water extract, vanilla extract, *Acacia* gum, table salt and cold water (FES flakes). The other part was made into a similar paste but without addition of sea berry salt bush water extract (FE flakes). All the pastes were separately spread onto parchment paper-lined baking trays to the thinnest possible film by using a spatula. The pastes were baked at 120°C for 25 min and allowed to cool at room temperature for 10 min. The half-baked pastes were manually broken into triangular-rectangular flakes, inverted and baked at 100°C until crunchy. Equal portions of the different flakes were mixed together to form the mixed flake variety (FM flakes). The flakes were allowed to cool at room temperature, packaged and labelled with code numbers.

Production of crackers

Whole ground *Acacia* seeds were washed and mixed with underground stems and rhizomes of Water Cumbungi, vanilla extract, table salt and cold water to form the dough. The dough was transferred to a rolling parchment paper and covered with plastic wrap. A rolling pin was used to flatten the dough to a thin layer of desired thickness. The plastic wrap was removed and the dough cut into rectangular shapes by simply drawing lines on it with a sharp knife. The cut pieces of dough were carefully placed in a baking tray that had been previously lightly greased with cooking oil, lined with parchment paper and baked at 120°C for 30 min. The crackers were allowed to cool at room temperature for 10 min, inverted and baked again at 100°C until crispy. The crackers were allowed to cool at room temperature, packaged and labelled with code numbers.

Sensory evaluation of food products

A total of 44 participants who had given written informed consent (Victoria University Human Research Ethics Committee, approval number HRE19-161) performed the evaluation in a sensory room at Victoria University (Melbourne Australia). Participants were each given, at random 8 sachets (5 test flakes and 1 control flake; 1 test cracker and 1 control cracker) each containing 5 g of the food product (Fig. 1).

Considering that there were no products of *Acacia* seeds and rhizomes of *T. orientalis* in the supermarkets at the time of this research, the products selected for controls were based on matching where possible the

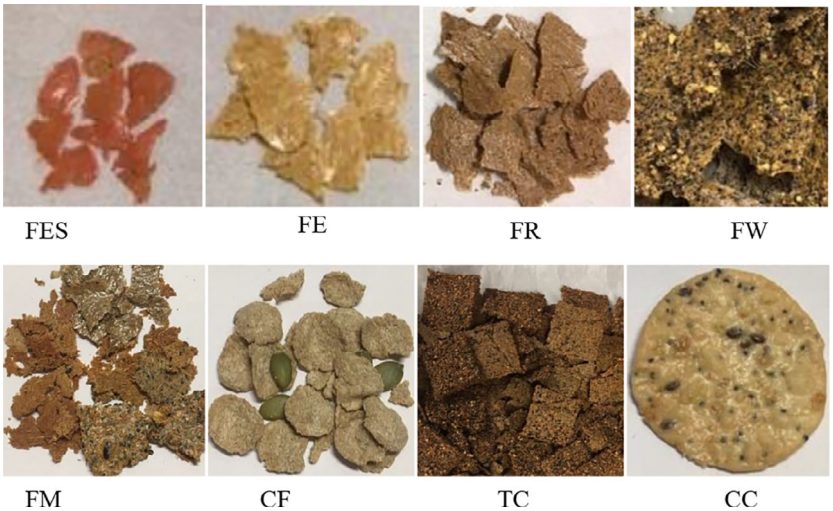

Figure 1 Test and control samples subjected to sensory evaluation. [Colour figure can be viewed at wileyonlinelibrary.com]

whole seed/fibre content of the test products. The products were served at room temperature under white light. Participants were provided with 50 mL of cold full cream milk, 1 spoon and 1 bowl to be used for the flakes. The sachets bore code numbers and were randomly handed to the participants. Each participant was given a pencil and 8 evaluation forms with the following parameters: colour, aroma, flavour, taste, aftertaste, texture, shape, appearance and general acceptability of the products according to previously established methods (Watts *et al.*, 1989; Bisla, Choudhary & Chaudhary, 2014). These methods included the rating of parameters by use of hedonic scale bearing acceptability ratings from 1 (lowest) to 9 (highest) as shown in Table 2.

Participants were requested to have a mouth rinse with tap water, pour one of the flakes into the bowl, note colour and appearance and add the desired quantity of milk in the bowl. Participants were requested to use the spoon to stir contents of the bowl before placing the product in their mouth. Panel members were requested to masticate the product at least 32 times, roll into a bolus and spit out into the container provided for that purpose. Panel members were requested to rate the product on the evaluation form and have another mouth rinse and non-salted crackers to cleanse the palate before proceeding to the next sample. For test and control crackers, panel members were requested to place a single cracker in the mouth, masticate about 32 times, roll into a bolus and spit out into the container provided for that purpose.

Preliminary storage stability assessment

Parameters of colour, texture, moisture, water activity and pH of test cracker (TC) and control crackers (CC) were studied weekly for a period of 35 days (with samples analysed on days 1, 7, 14, 21, 28 and 35) according to standard procedures (de Morais *et al.*, 2016). There were two groups of samples placed in zip-lock polypropylene bags. Each group contained vacuum-sealed (Multivac, Wolfertschwenden, Germany) and non-vacuum-sealed samples; group 1 samples (TC1 & CC1) were kept on an open shelf, whereas group 2 samples (TC2 & CC2) were kept in a dark cupboard. Colour (luminosity, chroma a* and chroma b*) was measured using a colorimeter (Konica Minolta Sensing, Tokyo, Japan) following the manufacturer's instructions.

Texture was measured using a texture analyser (TA plus, Stable Micro System, Godalming, UK) with a probe HDP/3PB (Stable Micro System) to determine hardness of crackers horizontally arranged on the platform (Stable Micro System). The probe height was calibrated to 11.2 mm, test speed set at 10 mm/s and sensitivity at 0.05N strength before testing. Three readings were performed for each sample on a weekly basis, and the mean and standard deviation was computed and recorded. Water activity (a_w) was determined using a water activity meter (Water Group, Pymble, NSW) and pH using a pH meter (Wissenschaftlich-Technische Werkstätten GmbH & Co. Weilheim, Germany) both according to the manufacturer's instructions. The moisture content of samples was determined by heating to constant weight and computing differences in weight before and after heating (AOAC, 2012). The temperature and relative humidity (RH) of the room were also recorded weekly.

Determination of microbial counts

Microbial counts were determined according to standard procedures (Shen & Zhang, 2017). Briefly, 1 g of each sample was homogenised in 9 mL sterile distilled water, appropriately diluted and inoculated onto nutrient agar plates, respectively, by pour plate technique (Shen & Zhang, 2017). The plates were incubated aerobically for 48 h, and the colonies enumerated using a colony counter. The colonies counted were recorded as cfu/mL alongside the standard deviation.

Nutritional composition of crackers

Total crude fat, total crude protein, total dietary fibre and total carbohydrates of test and control crackers were determined as previously described (Njume *et al.*, 2020). While the test crackers were made of coast wattle seeds and rhizomes of Water Cumbungi, the commercially sourced control crackers were made of brown rice, buckwheat flour, linseed, millet seeds, sunflower seeds, poppy seeds, yellow mustard seeds and quinoa seeds.

Plant component analysis by reverse-phase high-performance liquid chromatography (RP-HPLC)

One gram of test and control crackers was tested according to previous reports (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012; Njume *et al.*, 2020). The individual compounds were identified by

Table 2 Hedonic scale used in sensory evaluation of food products (Watts *et al.*, 1989; Bisla, Choudhary & Chaudhary, 2014)

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

comparing with the retention times of reference standards. Quantitative analysis was performed by calibration curves using the reference standards of gallic acid (GA), epigallocatechin (EPC), catechin (CH), epigallocatechin gallate (EPG), ρ-coumaric acid (PCA), ferulic acid (FA), luteolin and dihydroquinidine (DHQ) (Sigma-Aldrich, Castle Hill, Australia). Linearity was investigated in the range of 0–5 mg at five increasing concentrations. Intra-day analyses of the same solution containing all compounds tested were used to validate the precision of the chromatographic system according to previously published protocols (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012).

Total, resistant and digestible starch analysis

Two hundred milligrams of test and control crackers were tested in accordance with previously established procedures (Siddhuraju, & Becker, 2005; Barine, & Yorte, 2016), with modifications as previously reported (Njume *et al*., 2020). A standard solution of glucose (20, 40, 60, 80 and 100 mg/L) was prepared for the generation of a standard curve (plotting absorbance at 630 nm against concentration mg/mL, r- value = 0.999). The sample glucose concentration was obtained from the standard curve. The experiment was repeated twice, and the glucose measurements converted to starch by multiplying the percentage glucose concentration by 0.9 (Barine, & Yorte, 2016).

For determination of resistant starch (RS), 100 mg of the residue from the total starch analysis was tested as previously reported (Siddhuraju, & Becker, 2005; Barine, & Yorte, 2016). The experiment was repeated twice, and the glucose measurements converted to starch by multiplying the percentage glucose concentration by 0.9 (Barine, & Yorte, 2016). Digestible starch (DS) content was obtained by difference, i.e. TS minus RS (Siddhuraju, & Becker, 2005).

Determination of hydrolysis index (HI) and estimation of glycaemic index (GI)

The rate of digestion of starch in 2-g samples of test and control crackers and estimation of GI values were performed according to the method of Goni, Garcia-Alonso & Saura-Calixto *et al*., (1997) as modified by Siddhuraju & Becker (2005) and Barine & Yorte (2016). The concentrations of glucose in the supernatants of digested samples were determined by anthrone method as previously reported (Njume *et al*., 2020). The rate of starch digestion was expressed as percentage of TS hydrolysed over time intervals of 30-, 60-, 90-, 120-, 150- and 180-min incubation (Siddhuraju, & Becker, 2005). The hydrolysis index (HI) was derived from the ratio between the areas under the hydrolysis curve of the crackers and the reference control sample of glucose (Barine, & Yorte, 2016).

$$\text{Hydrolysis index (HI)} = \frac{\text{AUC of sample}}{\text{AUC of glucose}} \times 100 \text{(Goni, Garcia – Alonso, \& Saura – Calixto 1997)}$$

where AUC = area under the curve

$$\text{AUC} = C_\alpha(T_f - T_0) - (C_\alpha/k)[1 - \exp[-k(T_f - T_0)]],$$

where C_α corresponds to the concentration at equilibrium (T180), T_f is the final time (180 min), T_0 is the initial time (0 min), and k is the kinetic constant derived to be:

$$k = \frac{-\text{In}(1 - C/C_\alpha)}{t}; \; C = \text{Percentage of starch hydrolysed at time t.}$$

GI = 39.71 + (0.549 × HI). (Goni, Garcia-Diz, Manas, & Saura-Calixto, 1996).

Statistical analysis

All data including determinations for TS, RS, DS, GI values and quantities of polyphenols extracted, the 44 determinations for acceptability ratings, triplicate determinations for stability studies of colour, texture, moisture, water activity, pH and microbial counts were entered in IBM SPSS statistic software version 25 (IBM SPSS®, Chicago, IL). Means and standard deviations were computed, and multiple comparisons between means were performed by one-way analysis of variance test (ANOVA) followed by Turkey's post hoc test. Differences between means were considered significant at $P < 0.05$. Frequency distribution charts for various parameters on the hedonic scale were also generated from SPSS. Mean parameter ratings ≥6 on the 9-point hedonic scale, i.e. liking range, were considered acceptable (Prapasuwannakul, 2019).

Results

Sensory analysis

A total of 25 females and 19 males participated in the sensory evaluation, and while females found the flavour of the test cracker better than control ($P < 0.05$; data not shown), the males did not record any significant differences ($P > 0.05$; data not shown). None of the other parameters tested was found to be different between males and females, and as such data are presented as combined results from male and female participants. Except for aroma and mouth feel of the flakes developed with whole seeds (FW) and flakes developed with seeds containing 1/3 seed coat (FR), all the other varieties of flakes and crackers recorded ≥54.5% overall acceptability (Table 3). The control flake recorded the highest overall acceptability of 86.4%, followed by control and test crackers at 84.1% and 70.5%, respectively.

Table 3 Acceptability of different flake varieties and crackers

Product	Colour and appearance	Aroma	Flavour	Taste & aftertaste	Mouthfeel	Overall acceptability
Flakes						
FW	20 (45.5)	21 (47.7)	24 (54.5)	23 (52.3)	21 (47.7)	24 (54.5)
FR	28 (63.6)	21 (47.7)	23 (52.3)	23 (52.3)	24 (54.5)	26 (59.1)
FE	25 (56.8)	20 (45.5)	24 (54.5)	20 (45.5)	23 (52.3)	26 (59.1)
FES	30 (68.2)	25 (56.8)	31 (70.5)	24 (54.5)	22 (50)	26 (59.1)
FM	27 (61.4)	24 (54.5)	30 (62.2)	32 (72.7)	23 (52.3)	29 (65.9)
CF	40 (90.9)	22 (50)	35 (79.5)	36 (81.8)	37 (84.1)	38 (86.4)
Crackers						
TC	31 (70.5)	35 (79.5)	31 (70.5)	25 (56.8)	32 (72.7)	31 (70.5)
CC	42 (95.5)	32 (72.7)	38 (86.4)	38 (86.4)	41 (93.2)	37 (84.1)

Overall acceptability is computed based on ratings ≥6 on the hedonic scale with total number of participants = 44; data are presented as total number of participants (out of 44); values in brackets represent percentage of total participants.
FW, flakes made with whole seeds; FE, flakes made with scarified seeds; FES, flakes made with scarified seeds and aqueous extracts of sea berry salt bush; FM, mixture of all the flake varieties; CF, control flake; TC, test cracker; CC, control cracker; FR, flakes made with seeds containing 1/3 seed coat.

Ratings on the hedonic scale for sensory characteristics of different flakes ranged from 5.07 ± 1.99 to 8.59 ± 2.55, whereas the cracker ratings were 6.05 ± 1.61–7.71 ± 1.09 (Table 4). Except for mixed flake variety (FM), the overall acceptability of test and control crackers was higher than flakes ($P < 0.05$). Flakes made with scarified seeds and sea berry salt bush extracts had improved colour and appearance ratings of 6.25 ± 1.56 ($P < 0.05$) (Table 4), yet based on overall acceptability, these were not significantly different from the rest of the flakes ($P > 0.05$).

The frequency distribution for the overall acceptability of test and control crackers is displayed in Figs 2 and 3, respectively, showing that both products although different in ratings were highly acceptable as more than 30 participants in each case rated the products at ≥ 6 on the hedonic scale. Thus, the test cracker still presents marketable characteristics even though its overall acceptability of 70.5% was lower than the control.

Nutritional information of the most acceptable crackers

The test cracker contained mostly non-reducing carbohydrates, proteins, fats and higher amounts of total dietary fibre, whereas the control cracker contained high amounts of carbohydrates, half the amount of proteins, less fibre and more salt (Table 5). The total starch and digestible starch contents of the control cracker were higher than the test cracker ($P < 0.05$; Table 5), whereas the residual fraction of starch (resistant starch), resistant to enzyme hydrolysis was higher

Table 4 Mean acceptability ratings of different flake varieties and crackers

Product	Colour and appearance	Aroma	Flavour	Taste & aftertaste	Mouthfeel	Overall acceptability
Flakes						
FW	5.11 ± 2.25^a	5.86 ± 1.5	5.68 ± 2.07	5.43 ± 2.11^a	5.11 ± 2.12^a	5.36 ± 2.2^a
FR	6.16 ± 1.77^a	5.89 ± 1.54	5.66 ± 1.77	5.61 ± 1.67^a	5.43 ± 1.91^a	5.64 ± 1.78^a
FE	5.88 ± 1.85^a	5.46 ± 1.75	5.27 ± 1.91	5.07 ± 1.99^a	5.61 ± 1.85^a	5.48 ± 2.06^a
FES	6.25 ± 1.56^a	5.86 ± 1.62	6.09 ± 1.61	5.64 ± 1.69^a	5.61 ± 1.62^a	5.84 ± 1.83^a
FM	5.84 ± 2.03^a	6.11 ± 1.48	5.75 ± 1.79	5.75 ± 1.69^a	5.36 ± 1.92^a	5.79 ± 1.89^a
CF	7.34 ± 1.35	6.23 ± 1.87	8.59 ± 2.5	6.75 ± 1.59	6.96 ± 1.43	7.09 ± 1.39
Crackers						
TC	6.29 ± 1.69^a	6.41 ± 1.34	6.18 ± 1.89^a	5.97 ± 1.79^a	6.29 ± 1.59^a	6.05 ± 1.61^a
CC	7.71 ± 1.09	6.61 ± 1.91	7.21 ± 1.49	7.27 ± 1.45	7.25 ± 1.60	7.29 ± 1.61

Data are mean ± SD of 44 determinations for each attribute.
CC, control cracker; CF, control flake; FE, flakes made with scarified seeds; FES, flakes made with scarified seeds and aqueous extracts of sea berry salt bush; FM, mixture of all the flake varieties; FR, flakes made with seeds containing 1/3 seed coat; FW, flakes made with whole seeds; TC, test cracker.
[a]Represents significant difference to control sample, CF and CC for flakes and crackers, respectively.

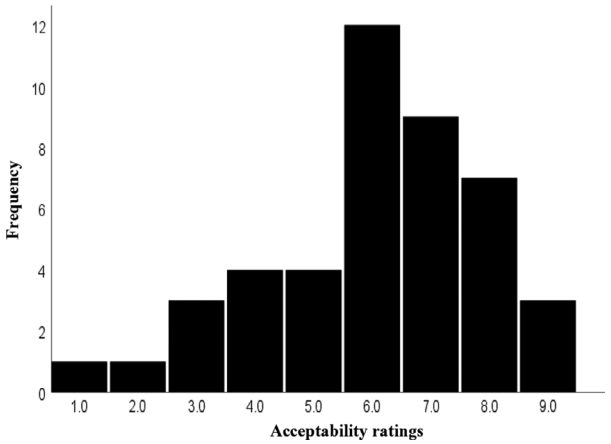

Figure 2 Frequency distribution for the overall acceptability of test crackers.

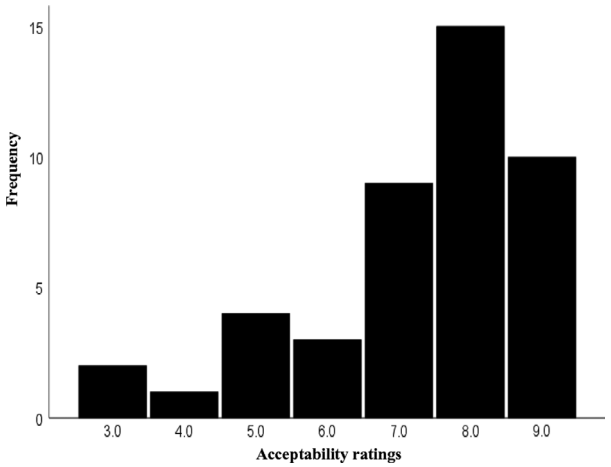

Figure 3 Frequency distribution for the overall acceptability of control crackers.

in the test cracker than control cracker ($P < 0.05$; Table 5). The estimated glycaemic indices (GI) for test and control crackers were 47.7 ± 1.3 and 70.3 ± 2.5, respectively. The amount of GA and PCA detected in the test cracker was 147 ± 21.1 mg/kg and 41.5 ± 11.3 mg/kg, respectively, whereas the control cracker recorded lower amounts of GA (18.1 ± 5.2 mg/kg) ($P < 0.05$; Table 5). PCA was not detected in the control cracker.

Preliminary stability findings

The test cracker which recorded highest overall acceptability of 70.5% compared to all test samples was subjected to further studies to determine stability and shelf life. Both test and control crackers remained stable and usable with no significant changes ($P > 0.05$) in texture, colour, moisture, water activity and pH during the 35-day study period (Table 6). The crackers did not record microbial growth from day 1 to day 35. The moisture content and water activity of all the crackers tested were ≤1.47% and ≤0.45, respectively. The temperature and relative humidity (RH) of the room were between 18°C and 19°C and 50% and 59%, respectively. The pH ranges for test cracker and control cracker were 5.21–5.30 and 6.16–6.23, respectively (Table 6).

Table 5 Nutritional information and physicochemical properties of crackers

Nutrients (g/100g)	Test cracker	Control cracker
Total carbohydrates	48.5 ± 0.5*	76.5 ± 2
Reducing sugar	2.5 ± 0.5*	10.1 ± 1
Proteins	18.3 ± 1*	9.2 ± 2.5
Total fats	12.7 ± 0.2*	6.7 ± 0.5
Saturated	3.5 ± 0.2*	<1 ± 0
Total dietary fibre	19.6 ± 0.5*	3.8 ± 1.5
Sodium	0.05 ± 0*	3.1 ± 0.2
Antidiabetic polyphenols (mg/kg)		
GA	147 ± 21.1*	18.1 ± 5.2
PCA	41.5 ± 11.3*	nd
Starch composition (%)		
Total starch	48 ± 0.5*	69.7 ± 5.9
Resistant starch	8.3 ± 1.2*	4 ± 1.5
Digestible starch	39.7 ± 1.5*	65.7 ± 2
Estimated GI	47.7 ± 1.3*	70.3 ± 2.5

Data are means of triplicate determinations ± SD.
GI, glycaemic index; nd, not detected.
*Significantly different to control cracker ($P < 0.05$).

Discussion

Despite the abundance of breakfast cereals in the shops and supermarkets, there are limited breakfast cereals that are suitable for people with T2DM as many are high in salt and simple sugars (Chepulis, Hill & Mearns, 2017; Pombo-Rodrigues *et al.*, 2017). The development of flakes, to be consumed as a breakfast cereal and crackers as a snack, was performed herein to improve the availability of such products for people with T2DM within Indigenous groups and in the wider Australian community. Food products developed from traditional Indigenous foods are noted for their unique taste, high nutritional value and functional properties that may offer health benefits and protection against T2DM, CVDs and cancer (Calinoiu & Vodnar, 2018; Adiamo *et al.*, 2020).

In this study, colour and appearance of some whole grain products produced from Indigenous items seem to have affected their overall acceptability. For example, flakes developed with whole grains (FW) were

Table 6 Preliminary stability assessment

Property (unit)	Time in days	Samples			
		TC1	TC2	CC1	CC2
Texture (N)	1	751.87 ± 8.78	762.87 ± 9.60	463.65 ± 13.8	463.65 ± 9.60
	35	756.21 ± 10.1	764.77 ± 0.32	459.78 ± 10.1	463.91 ± 11.07
Colour (Chroma*)	1	22.53 ± 0.38	22.53 ± 0.60	26.91 ± 0.55	26.31 ± 0.44
	35	22.35 ± 0.08	22.71 ± 0.32	26.81 ± 0.05	26.13 ± 0.33
Moisture (%)	1	1.2 ± 0	1.2 ± 0.05	1.36 ± 0.08	1.46 ± 0.05
	35	1.23 ± 0	1.2 ± 0	1.42 ± 0	1.5 ± 0
Water activity (a_w)	1	0.43 ± 0.01	0.43 ± 0.01	0.33 ± 0.02	0.33 ± 0
	35	0.43 ± 0	0.44 ± 0	0.35 ± 0.02	0.35 ± 0
pH	1	5.21 ± 0	5.21 ± 0	6.16 ± 0	6.16 ± 0
	35	5.22 ± 0	5.3 ± 0	6.23 ± 0	6.2 ± 0
Microbial counts (CFU/mL)	0	0	0	0	0

Data are means ± SD of triplicate determinations for days 1 and 35; data for days 7, 14, 21 and 28 were also measured, yet with no significant changes (data not shown).
CC1, control cracker kept on open shelf; CC2, control cracker kept in dark cupboard; TC1, test cracker kept on open shelf; TC2, test cracker kept in dark cupboard.

least acceptable in terms of colour and appearance (Table 3) and had the lowest ratings on the hedonic scale (Table 4) despite their richness in nutrients. For this reason, it was hoped that reduction or complete scarification of seeds in some flakes (FR, FE and FES) and addition of a colourant (betanidin 5-*O*-β-glucoside-rich sea berry salt bush water extract) would improve colour and overall acceptability. However, this was not the case as the acceptability of FR, FE and FES was not different from whole grain-containing flakes (Table 3). Instead, the test cracker which was also prepared with whole grains was more acceptable and recorded better ratings than most flake varieties (Table 4). It was however interesting to note that the mixed flake variety (FM) had a high overall acceptability of 65.9%, the highest among the flakes (Table 3).

The high ratings and acceptability of the control samples were not surprising as these were made with brown rice which are completely different in taste to the test product which was predominantly made with coast wattle seeds. This could have been a limitation in the selection of the control samples, since these were the closest to the test samples on the market, as there was no product with coast wattle seed. Overall, the test cracker had higher ratings than flakes and even though its acceptability was comparable to FM, it was selected for preliminary stability testing and further studies over the flakes because of its high overall acceptability of 70.5% (Table 3).

Preliminary stability studies were performed to provide information on longevity and shelf life of the product, yet parameters studied did not produce any significant changes after a 5-week storage on an open shelf and in a dark cupboard (Table 5). Generally, crackers maintain low moisture content, low water activity and are highly crunchy (Yilmaz & Karaman, 2017). These properties are not favourable to support most microbial growth. In fact, most micro-organisms require a water activity (a_w) of ≥0.88 to flourish in food (Leong *et al*., 2014; Yilmaz & Karaman, 2017). However, xerophilic moulds and some food-spoilage fungi are able to grow at a_w of 0.61–0.85 (Beuchat, 1983; Dagnas *et al*., 2017) and some fastidious species may require specific isolation techniques or enrichment media to enhance growth (Bonnet *et al*., 2020; Snyder, Churey & Worobo, 2019). It is worth mentioning that no visible mould growth was recorded during stability assessment studies. However, the use of a general purpose medium (nutrient agar) could have prevented our chances of isolating the organisms. Further inoculations of samples into different specific media selective for bacteria and fungi would improve the chances of isolating microbial contaminants during storage stability assessment studies. This would be helpful in assessing the effectiveness of storage and storage conditions, especially when dealing with a newly developed product. The stability of products colour, texture and pH at temperature of 18°C–19°C and RH of 50%–59% may be useful finding, especially within some remote Indigenous communities where longevity of the food product is highly desirable (Davy, 2016).

It is worth mentioning that all participants in the study were blinded to the nutritional components of the products and health benefits associated with them. Despite this blinding the sample was still highly acceptable, indicating that the commercial value of the food products is likely to increase with additional sensitization of their nutritional and health benefits within the community. Pink Himalayan salt, for example, has

a less salty taste, less sodium and is rich in minerals (Carapeto Brum, & Rocha, 2018). Despite the need for evidence to establish the health benefits, some people prefer it to regular table salt and this promotes its commercial value owing to its potential health implications (Reddy *et al*., 2015; Carapeto Brum, & Rocha, 2018). Similarly, some people may not like eating leafy vegetables due to perceptions of taste, yet a belief in their health benefits has been shown to increase consumption and commercial value of some fruits and leafy vegetables (Pollard, Kirk, & Cade, 2002). In a study conducted by Lockie *et al*., (2004), it was shown that Australians who consume organic foods rated them high in quality because of their naturalness, i.e. food free of artificial ingredients, pesticide and other chemical residues, preservatives, hormones and antibiotics. For this reason, preference and commercial value of organic products was higher than non-organic products (Lockie *et al*., 2004). It is logical therefore that the highly acceptable cracker (TC) and mix flake variety (FM) developed in this study are likely to have significant commercial value with additional sensitization of their potential health benefits.

It has been reported that heating and other food-processing techniques may affect polyphenolic content of food and profile of many ingredients even though many polyphenolic components have high boiling/sublimation points (Loncaric, Lamas, Guerra, Kopjar1, & Lores., 2018; Zeng, Ma, Li, & Luo, 2017). Despite their presence in the fresh plant material (Njume *et al*., 2020), this study did not detect any catechins in the test cracker, probably due to their instability at high temperatures (Loncaric, Lamas, Guerra, Kopjar1, & Lores, 2018; Zeng, Ma, Li, & Luo, 2017). It might be possible to reduce degradation of catechins during cooking by baking at temperatures lower than the 120°C that was used in this study (Su *et al*., 2003).

Gallic acid was detectable in the test and control crackers. Gallic acid is one of the most common phenolic antioxidant compounds (Gao, Hu, Hu, & Yang, 2019), and except for *R. candolleana*, it was detected in the fresh plant material of *T. orientalis* and *A. longifolia* (Njume *et al*., 2020). Its detection in the test cracker is of particular interest and not completely surprising as this antioxidant phenolic compound has been reported to be fairly stable with only 30% degradation occurring after heating at 100°C for 250 min (Volf, Ignat, Neamtu & Popa, 2014). Gallic acid's ability to inhibit diet-induced hyperglycaemia and hypertriglycaeridemia (Gandhi *et al*., 2014) would be a useful characteristic in the development of antidiabetic food preventatives. Its major mechanism of action has been attributed to its ability to reduce the size of adipocytes and protect pancreatic β-cells by inducing the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), a nuclear transcription factor that induces differentiation and insulin sensitivity in adipocytes (Gandhi *et al*., 2014; Kahkeshani *et al*., 2019). Its presence in food may also influence food properties in relation to microbial growth and absorption of other beneficial components in the gastrointestinal tract (Kardum, & Glibertic, 2018; Singh *et al*., 2019).

ρ-coumaric acid (4-hydroxycinnamic acid) was another useful antidiabetic component detected in the test cracker. With a thermal degradation temperature of 215°C (Contardi *et al*., 2019), the detection of PCA in the finished product after 30-min baking at 120°C was not unexpected, though not as high in quantity as gallic acid, PCA was also detected in the aqueous extracts of *T. orientalis* plant material (Njume *et al*., 2020). PCA has previously been reported to modulate the activity of enzymes involved in glucose metabolism, improve β-cell function and insulin action, stimulate insulin secretion and strengthen anti-inflammatory properties (Bahadoran, Mirmiran, & Azizi, 2013). PCA's antidiabetic capacity lies in its ability to decrease intestinal absorption of dietary carbohydrates by inhibiting α-amylase, α-glucosidase, β-glucosidase, thus delaying the digestion of starch or sucrose to glucose, delaying glucose absorption and protecting against postprandial hyperglycaemia (Bahadoran *et al*., 2013). The detection of PCA in the test cracker was an additional advantage over the control sample which only exhibited small quantities of GA and no presences of PCA.

With estimated GI value of 47.7, the test cracker may be classified as a low-GI food, which are known for their efficient postprandial glucose control, low insulin demand and improved satiety (Ratnaningsih, Harmayani, & Marsono, 2017). These properties alongside rich polyphenolic constituents could be helpful in managing T2DM within the Indigenous community. The GI of the test cracker was expected to be low because of the high fibre, high phenolic and resistant starch content of the plant species that were mixed together to prepare the product all of which can independently (Jenkins *et al*., 1987; Miller, Gabbay, Dillon, Apgar, & Miller, 2006; Zabidi, & Aziz, 2009; Bahadoran *et al*., 2013; Gandhi *et al*., 2014) impact the GI of the test cracker.

There is an unacceptable food-related health gap between Indigenous and non-Indigenous Australians, revealing an urgent need to improve food security for Indigenous peoples throughout the country (Davy, 2016). The evidence suggests that food security within the Australian Indigenous community is yet to be attained (Davy, 2016). While there is a high level of poverty within remote Indigenous communities (Altman, 2007), they could still be regarded as having available a rich habitat of native plant species, many

of which are underutilized and unknown to non-Indigenous Australians. Considering that food insecurity within the Indigenous community is associated with welfare dependency, low incomes and poverty (Booth & Smith, 2001), native plant resources that are rich in nutrients and dietary polyphenols and are readily available could be developed at industrial levels that could help improve income and alleviate poverty. Despite many government initiatives including creating outback stores in an attempt to close the social and economic divide (Davy, 2016), little has been done on the development of antidiabetic food preventatives. The development of some of the native plant species into acceptable food products for commercial purposes could be a huge opportunity for income generation and self-reliance within the Indigenous community, especially given the potential health benefits of the products.

Conclusion

Food products made with local Indigenous material are highly acceptable as more than 70% of the people who participated in the sensory evaluation rated the cracker at ≥6 in 5 of the 6 parameters on the hedonic scale. However, modifications and fortifications with different flavours may have potential to meet wider consumer demands. The cracker remained stable and unchanged with good physical and microbiological parameters after 35-day storage at room temperature in light and in the dark. Although the overall acceptability of the test cracker was interestingly high, there is a possibility to increase it even beyond 70.5% should the participants be aware of the potential health benefits of the product. The test cracker made of whole grains rich in proteins, fats, dietary fibre and slow-releasing carbohydrates could be likely protective against postprandial hyperglycaemia in people with T2DM. These characteristics were consistent with the low GI value of 47.7 recorded for the cracker. Phenolic antioxidant compounds of gallic acid and ρ-coumaric acid with known antidiabetic properties were still detectable in the test cracker after baking, possibly decreasing the GI even further and ultimately adding to the antidiabetic properties of the product. Animal/human intervention studies to determine the blood glucose response and long-term impact of the effects of the highly acceptable cracker would shed more light on its potential application as a functional food product against T2DM.

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Conflict of interest

The authors declare no conflict of interest.

Author Contribution

Collise Njume: Conceptualization (equal); Formal analysis (lead); Investigation (equal); Methodology (equal); Writing-original draft (lead); Writing-review & editing (equal). **Osaana Donkor:** Conceptualization (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal). **Todor Vasiljevic:** Conceptualization (equal); Methodology (equal); Writing-review & editing (equal). **Andrew J. McAinch:** Conceptualization (equal); Methodology (equal); Project administration (equal); Supervision (lead); Writing-review & editing (equal).

Ethical guidelines

A research permit to collect native species from Warrnambool, Victoria, Australia, was granted by the Department of Environment, Land, Water and Planning (Permit number 10008221). Collections were carried out in collaboration with Indigenous Elders in Warrnambool after obtaining their blessing to conduct the study. Approval to conduct product acceptability studies was granted by Victoria University Human Research Ethics Committee (VUHREC), approval number HRE19-161. Written informed consent was obtained from all participants.

Peer review

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Data Availability Statement

Research data are not shared.

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