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- (71) Applicant (*for all designated States except US*): QUEEN BIOACTIVES PTY LTD [AU/AU]; 17-19 Wakefield Street, Alderley, Queensland 4051 (AU).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): LANG, Timothy, Ralston [AU/AU]; Unit 2, 1051 Pacific Highway, Pymble, New South Wales 2073 (AU). O'SHEA, Michael [AU/AU]; 14 Eastmere Street, Runcorn, Queensland 4113 (AU).
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(54) Title: METHOD OF LOWERING GLYCAEMIC INDEX OF FOODS

(57) Abstract: A method of delaying digestion by an animal or a human of carbohydrates in food, comprising administering an effective amount of one or more flavonoids to the animal or human in conjunction with the food, wherein the flavonoids are selected from luteolin, apigenin, tricetin and their pharmaceutically acceptable analogues and derivatives.

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Method of Lowering Glycaemic Index of Foods

5 The present invention relates to a method for lowering the Glycaemic Index (GI) of foods.

Glycaemic Index (GI) is a measure of how a given food affects postprandial blood sugar levels. It relates principally to foods that are high in carbohydrates, since proteins and fats have relatively little effect on blood sugar. GI values
10 indicate how quickly the carbohydrates in a given food are broken down in the intestine and converted to blood sugar. Surprisingly, the complex carbohydrates in some foods such as baked potatoes have a higher GI than refined white sugar. GI values are calculated in comparison with the assimilation of either glucose or white bread, which are given arbitrary GIs of
15 100. Note that the GI of white bread on the glucose scale is 70.

Glycaemic Load (GL) is an extension of the concept of GI. GL is calculated by multiplying GI by the carbohydrate content of a food, expressed in grams, and divided by 100.

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Recent studies have shown that the incidence of type 2 diabetes is on the rise, particularly throughout the Western World, but also in Africa and Asia. Studies have also shown that a diet with a high overall GL increases the relative risk of onset of type 2 diabetes by about 1.5 times, or by about 2.4 times when
25 accompanied by a low dietary fibre intake. The risk of coronary heart disease in diabetics has also been directly linked to dietary GL.

The GI, (and hence the GL) of foods may be reduced by slowing the conversion of carbohydrates into blood sugar. One way of doing this is to increase the viscosity of stomach fluids to slow the rate at which carbohydrates are digested.
30 Alternatively, controlling constriction of the pylorus can reduce the rate at which the stomach empties. The viscosity of stomach fluids may be increased by increasing the consumption of dietary fibre, whilst constriction of the pylorus is

achieved by the addition of low molecular weight organic acids, such as acetic acid (vinegar) or citric acid (lemon juice), to the diet.

Another means to lower GI is to inhibit the enzymes catalysing the break down of carbohydrate in the intestine. Two enzymes principally responsible for this
5 are α -glucosidase and α -amylase. The activity of these enzymes determines the rate at which glucose is produced from dietary polysaccharides and therefore the rate at which the glucose is absorbed into the blood.

Hyperglycemia is treated by lowering the GI of foods using the drug Acarbose.
10 This drug is a complex oligosaccharide which delays digestion of ingested carbohydrates, by inhibiting intestinal enzymes (α -glucosidases). Acarbose has maximal inhibitory activity against sucrase. Acarbose is also known to inhibit α -amylases.

15 Flavonoids are naturally occurring compounds found in plants. According to the present invention, some flavonoids have been found to exhibit inhibitory activity against α -glucosidases and α -amylases. Surprisingly, tests have shown that flavonoids and flavonoid analogues and derivatives isolated from the sugar cane plant exhibit inhibitory activity that exceeds that of Acarbose. Typically,
20 flavonoid derivatives obtained from vegetable sources such as sugar cane are flavonoid glycosides. As used herein the term "flavonoid" is taken to include both flavonoids per se and flavonoid analogues and derivatives.

Luteolin, apigenin and tricetin are all flavonoids that occur naturally in plants such
25 as wheat, alfalfa and sugar cane. As noted, the present invention relates to both these compounds and to their analogues and derivatives. In one embodiment, the present invention provides a method of delaying digestion by an animal or a human of carbohydrates in food, comprising administering an effective amount of one or more flavonoids to the animal or human in conjunction with the food,
30 wherein the flavonoids are selected from the group consisting of luteolin, apigenin, tricetin and their pharmaceutically acceptable analogues and derivatives. In a preferred embodiment of the invention, the one or more flavonoids are administered together with a nutritional fibre supplement.

Preferably at least 7 mg of flavonoid is administered for each 50.0g of carbohydrate in the food.

5 In a further embodiment of the invention, there is provided a pharmaceutical formulation suitable for oral administration, said formulation comprising an effective amount of one or more flavonoids selected from the group consisting of luteolin, apigenin, tricetin and their pharmaceutically acceptable analogues and derivatives, and one or more pharmaceutically acceptable excipients. The pharmaceutical formulation may contain other active ingredients, in addition to
10 lubricants, colouring and flavouring agents, anti-caking agents and fillers and excipients known to the art. The pharmaceutical formulation may be in the form of a tablet, dragee, pill or capsule provided with an enteric coating. Preferably, in addition to the one or more flavonoids, the pharmaceutical formulation contains a nutritional fibre supplement.

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In a further embodiment of the invention, there is provided a method of producing an oral pharmaceutical formulation for inhibiting intestinal enzymes, comprising combining an effective amount of one or more flavonoids selected from the group consisting of luteolin, apigenin, tricetin and their pharmaceutically
20 acceptable analogues and derivatives, with one or more pharmaceutically acceptable excipients. The one or more flavonoids may also be combined with other active ingredients. Preferably, the one or more flavonoids are combined with a nutritional fibre supplement.

25 In a further embodiment of the invention, there is provided a food product containing sufficient added amount of one or more flavonoids selected from luteolin, apigenin, tricetin and their nutritionally acceptable analogues and derivatives to reduce the glycaemic index of the food product. Preferably, the amount of added flavonoids contained in the food product is at least 7 mg per
30 50.0 g of carbohydrate in the food. Preferably, the food product also contains a nutritional fibre supplement.

In another embodiment of the invention, there is provided a method for lowering the glycaemic index of a carbohydrate-containing meal, comprising including as

part of the meal an effective amount of an extract of sugar cane containing tricin or its nutritionally acceptable analogues and derivatives. Preferably, the extract contains 5% tricin or tricin diglycoside. Preferably, the amount of extract ranges from 0.3g to 2.0g per 50.0g carbohydrate in the meal; more preferably the amount of extract is about 1.0g per 50.0 g carbohydrate. In a preferred embodiment, the extract is added to the meal during preparation. In a further preferred embodiment of the invention, a nutritional fibre supplement is also added to the meal.

10 In another preferred embodiment of the invention, there is provided a method for lowering the glycaemic index of a carbohydrate-containing meal, comprising including as part of the meal an effective amount tricin or its nutritionally acceptable analogues and derivatives. Preferably, the amount of tricin or its analogues or derivatives is at least 7 mg per 50.0 g of carbohydrate in the meal; more preferably it ranges from 15mg to 100mg per 50.0g carbohydrate in the meal; still more preferably the amount is about 50mg per 50.0 g carbohydrate. In a preferred embodiment, the tricin or its analogues or derivatives is added to the meal during preparation. In a further preferred embodiment of the invention, a nutritional fibre supplement is also added to the meal.

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Example A. *In Vitro* Assays.

In vitro assays have been conducted which demonstrate the effectiveness of luteolin, apigenin and tricin as inhibitors of digestive enzymes. Apigenin and luteolin were purchased from Sigma and tricin was obtained from the Bureau of Sugar Experiment Stations and supplied by Queen Bioactives Pty. Ltd. Solutions of the flavonoids in dimethylsulfoxide were made at a concentration of five mg/ml.

30 Alpha-glucosidase assay

Alpha-glucosidase Type 1 from bakers yeast, substrate (p-nitrophenyl-alpha-D-glucopyranoside) and a control inhibitor, castanospermine were purchased from Sigma. For the assay, enzyme dissolved in 50 mM acetate buffer, pH 4.5 was incubated for 30 min, at 37°C in the presence of substrate and in the presence

vs. absence of inhibitors (flavonoids or castanospermine at various concentrations). The reaction was carried out in 50 mM acetate buffer, pH 4.5. Final concentration of alpha glucosidase was 0.2 U/ml; final concentration of the substrate was 2 mM. Flavonoids concentrations ranged from 7.8 to 500 µg/ml and castanospermine was used at final concentration 1 µg/ml. The reaction was stopped by addition of 0.2 M Na₂CO₃ and absorbance was measured at 405 nm. Background absorbance (without enzyme) was subtracted for every flavonoid concentration used. The inhibitory activity was expressed as 100 minus relative absorbance difference between tested samples and controls.

10 **Alpha-amylase assay**

Alpha-amylase from porcine pancreas, alpha-glucosidase, glucoamylase and substrate (p-nitrophenyl-alpha-maltopentaoside) were purchased from Sigma. In the reaction, alpha-amylase (endo-type enzyme) hydrolysed the substrate into shorter chain glucosides and the auxiliary enzymes (exo-type enzymes) released the chromophore (p-nitrophenol) from the amylase-hydrolyzed maltopolysaccharides. For the assay, alpha-amylase (7.5 U/ml) was added to a substrate solution containing 1 mM p-nitrophenyl-alpha-maltopentaoside, 15 U/ml of alpha-glucosidase and 25 U/ml of glucoamylase in 50 mM HEPES buffer (pH 7.3) containing 3 mM CaCl₂ and 40 mM NaCl. The reaction was carried out for 15 min at 37°C in the presence vs. absence of flavonoids at concentrations ranged from 11.7 to 750 µg/ml. At the end of the incubation, 0.2 M borate, pH 9.8 was added to inactivate the enzymes and liberated p-nitrophenol was measured spectrophotometrically at 405 nm. The relative activity of alpha-amylase was calculated by subtracting reaction blanks containing the substrate, the two axillary enzymes and flavonoids at the same concentrations. The inhibitory activity was expressed as 100 minus relative absorbance difference between tested samples and controls.

In Vitro Results

30 The alpha-glucosidase assay was carried out in optimum conditions and the activity of the enzyme was reduced by 32% in presence of 1 µg/ml of castanospermine. As demonstrated in Figure 1 and in Table 1, addition of all three flavonoids to the reaction mixture resulted in a dose-dependent inhibition of the enzyme but tricin was more active than the other two flavonoids. IC₅₀

values (concentrations of flavonoids required to inhibit alpha-glucosidase by 50%) were 51.5 µg/ml, 59.4 µg/ml and 12.0 µg/ml for luteolin, apigenin and tricetin, respectively (Table 3). At concentration 1.6 mM (=454 µg/ml) all three flavonoids almost completely or completely inhibited alpha-glucosidase.

5

In the alpha-amylase assay, flavonoids were the only inhibitors used since they could reduce activity of both alpha-amylase and alpha-glucosidase (the control inhibitor would act only on alpha-amylase). As demonstrated in Figure 2 and in Table 2, all three flavonoids were effective in reducing the activity of alpha-amylase. Luteolin was the most active, inhibiting the enzyme completely even at the lowest concentration tested. The remaining two flavonoids inhibited alpha-amylase dose-dependently, with tricetin being more active than apigenin at lower doses. IC₅₀ values (concentrations of flavonoids required to inhibit alpha-amylase by 50%) were 5.8 µg/ml, 9.9 µg/ml and 16.2 µg/ml for luteolin, tricetin and apigenin, respectively (Table 3). At concentration 1.6 mM, all three flavonoids inhibited alpha-amylase completely (Table 3).

10

15

Discussion

The assays show dose-dependent inhibition of alpha-glucosidase and alpha-amylase by flavonoids tested in the study. The data additionally show that for all three flavonoids, concentrations lower than 1.6 mM (lower than 454 µg/ml) were sufficient to produce a substantial inhibition of alpha-glucosidase (70-100% inhibition achieved for concentrations 125-250 µg/ml) and that tricetin had much greater inhibitory potential than the other two flavonoids tested.

25

Similarly, lower than 1.6 mM concentrations of luteolin, apigenin and tricetin were sufficient to produce complete inhibition of alpha-amylase.

Example B. *In Vivo* Study

30

Subjects

A group of 10 healthy, non-smoking people, aged between 18-45 years was recruited from the staff and student population of the University of Sydney to participate in the study. People volunteering to participate in the study were

excluded if they were overweight, were dieting, had impaired glucose tolerance, were suffering from any illness or food allergy, or were regularly taking prescription medication other than oral contraceptive medication. A group of seven females and three males participated in the study. The average age of the group of study subjects was 22.3 years (range: 18.9-28.7 years) and the group's average body mass index (BMI) score was 21.5 kg/m² (range: 19.4-24.8 kg/m²). The BMI score is a measure of a person's weight in relation to their height. BMI values between 19-25 kg/m² are within the healthy weight range.

10 **Bioactive Extract**

A bioactive extract containing tricin diglycoside was used in the In Vivo study. The extract was obtained by:

1. 15 litres of dunder was obtained from CSR Sugar, Sarina, Queensland. (Dunder, (or vinasse), is a commercial by-product obtained after sugar cane molasses is fermented with yeast to convert sugars to ethanol).
2. 30 litres of 96% ethanol (EtOH) was added to the dunder, mixed and allowed to settle for 24 hours.
3. 35 litres of the dunder/ethanol solution was filtered using a stainless steel membrane filter to 0.1 micron. This resulted in 33 litres of permeate.
4. The permeate was evaporated to approximately 5 litres and allowed to settle for 24 hours at 4 °C. The permeate was then refiltered in preparation for chromatography.
5. Approximately 0.1% acetic acid by volume was added to the permeate before passing the solution over column chromatography (XAD resin). The column was then washed with one bed volume water (plus 0.1% acetic acid) and then eluted in order with 20%, 30% and 40% EtOH/water containing 0.1% acetic acid.
6. The 40% fraction was evaporated dry at low temperature. The fraction contained 68.95 grams total solids, of which 3.75g (5.4%) was analysed as an unidentified diglycoside of tricin.

Test Foods

Pure glucose sugar (Glucodin® powder, Boots Health Care Company, North Ryde, NSW, Australia) dissolved in 250 ml of water was used as the reference food, and was consumed by each of the 10 subjects on two separate occasions. The four test meals were consumed by each of the 10 subjects on one occasion only. The four test meals and the reference food were fed to the subjects in portions containing 50 grams of available carbohydrate. The weights and nutrient contents of the test portions of the reference food and the four test meals are listed in Table 4. For the purpose of this study, the bioactive extract was assumed not to provide any macronutrients. The extract contained 5 mg of tricin diglycoside per 100 mg extract.

Each portion of the reference food was prepared the day before required by fully dissolving 50 grams of pure glucose sugar in 250 ml of hot water in a heatproof plastic glass, which was then covered with airtight plastic wrap and stored overnight in a fridge. The next morning, each portion of the reference food was taken from the fridge shortly before being served to a subject together with 250 ml of plain water. The required portions of the four test meals were prepared shortly before being served to the subjects. The wheat-based cereal was weighed into a large china bowl and served to the subjects together with a glass of 185.1 grams of reduced-fat milk, a glass of 250 ml of water, and a small plastic container of the bioactive extract. The subjects were also given a spoon and were instructed to consume everything that was served to them at a comfortable pace within 12 minutes. The subjects were free to decide how they would consume the bioactive extract. Some subjects added the extract to the cereal and milk, whereas others poured some water into the extract's container and consumed it like a drink.

Experimental Procedures

Using standard methodology to determine a food's GI value, a portion of the food containing 50 grams of available carbohydrate is fed to 10 healthy people in the morning after they have fasted for 10-12 hours overnight. A fasting blood sample is obtained and then the food is consumed, after which additional blood samples are obtained at regular intervals during the next two hours. In this way, it is possible to measure the total increase in blood sugar produced by that food

over a two-hour period. The two-hour blood glucose (glycaemic) response for this test food is then compared to the two-hour blood glucose produced by the same amount of carbohydrate in the form of pure glucose sugar (the reference food: GI value of glucose = 100%). Therefore, GI values for foods are relative measures (ie. they indicate how high blood sugar levels rise after eating a particular food compared to the very high blood sugar response produced by the same amount of carbohydrate in the form of glucose sugar).

In this study, the 10 subjects consumed the reference food on two separate occasions and each of the four test meals on one occasion only. Therefore, each subject completed six separate test sessions for this study. For each subject, the reference food was consumed at both the first and last test sessions, and the four test meals were consumed in random order in between.

The day before each test session, the subjects were required to refrain from consuming alcohol the whole day and to abstain from unusual levels of physical activity or food consumption. The night before each test session, the subjects ate a regular evening meal based on a carbohydrate-rich food (other than legumes) and then fasted for 10 hours overnight.

The next morning they reported to the research centre in a fasting condition. A fasting finger-prick blood sample (0.5 ml) was first collected from each subject using a sterile automatic lancet device (Safe-T-Pro®, Boehringer Mannheim GmbH, Mannheim, Germany). After the fasting blood sample was obtained, the subjects were given a fixed portion of the reference food or a test meal, which they consumed together with 250 ml of plain water at a comfortable pace within 12 minutes. A stopwatch was started for each subject as soon as they started eating. The subjects were required to consume everything that was served to them, after which they were required to remain seated at the research centre and refrain from any additional eating or drinking during the next two hours. Additional finger-prick blood samples were taken 15, 30, 45, 60, 90 and 120 minutes after eating had commenced. Therefore, a total of seven blood samples were collected from each subject during each two-hour test session.

Measurement of blood glucose responses

For each subject, the concentration of glucose in the plasma component of each of the seven blood samples collected during each two-hour test session was analysed in duplicate using a glucose hexokinase enzymatic assay (Roche Diagnostic Systems, Sydney, Australia) and an automatic centrifugal spectrophotometric analyser (Roche/Hitachi 912®, Boehringer Mannheim GmbH, Mannheim, Germany). Using the average plasma glucose concentration for each blood sample, a two-hour blood glucose response curve was then constructed for this subject. The area under this two-hour blood plasma glucose response curve (AUC) was then calculated in order to obtain a single number, which expresses the total increase in blood glucose in that subject as a result of ingesting that test meal during the two-hour experimental period. A glycaemic index (GI) value for this test meal was then calculated for that person by dividing the two-hour blood glucose AUC value for the test meal by their average two-hour blood glucose AUC value for the reference food and multiplying by 100 to obtain a percentage score.

$$\text{GI value for test meal (\%)} = \frac{\text{Blood glucose AUC value for the test meal}}{\text{Average AUC value for the equal-carbohydrate portion of the reference food}} \times 100$$

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In this way, a GI value for each test meal was calculated for each of the 10 subjects in the study. The final reported GI value for each test food is the average of the 10 individual subjects' GI values. Due to differences in body weight and metabolism, blood glucose responses to the same food vary between different people. The use of the reference food to calculate GI values reduces the variation between the subjects' blood glucose results to the same food arising from these natural differences. Therefore, the GI value for the same food varies less between the subjects than their glucose AUC values for this food.

30

In Vivo Results

The average two-hour blood glucose values for the 50 gram carbohydrate portions of the reference food (glucose sugar) and the four test meals shown as the change in blood glucose from the fasting baseline level are set out in Table

4 (excluding outliers' results). The reference food produced the largest blood sugar glucose response, followed by the control meal (cereal + (0g bioactive extract tricin diglycoside)). The addition of 0.3g bioactive extract (15 mg of tricin diglycoside) to the cereal test meal did not affect the peak blood glucose concentration at 15 minutes, but resulted in lower blood glucose levels at every time point thereafter. The 1.0 and 2.0 g doses of bioactive extract (50 and 100 mg doses of the tricin diglycoside) resulted in slightly lower peak blood glucose concentrations at 15 minutes, but there is little difference in their overall glycaemic effect.

10

Glycaemic Index values

The GI value for each test meal varied among the 10 people who participated in the study. This variation in GI values for the same meal between people is normal and is due to a number of factors, such as the different rates at which the subjects ingested the foods, different physical activity and dietary habits, and genetic differences in carbohydrate metabolism. It is standard scientific practice that if any individual subject's GI value for a test food is either greater than the group mean (average) value plus two standard deviations (StDev) or less than the group mean value minus two StDev then that value is classified as an outlier value or unusual observation and removed from the datasheet. One outlier value was found among the 10 subjects' GI values for the test meals with 50 and 100 mg of the bioactive compound. Therefore, the final GI values for these two test meals is the average of nine subjects' GI values. The mean $\pm$ standard error of the mean (SEM) GI values for the reference food and the four test meals are listed in Table 6.

Conclusions

Using glucose as the reference food (GI = 100), foods with a GI value of 55 or less are currently considered to be low-GI foods (Brand Miller et. al. The glycaemic index solution for optimal health - the new glycaemic revolution (revised edition), Hodder, Sydney 2003). Foods with a GI value between 56-69 have a medium GI rating, and foods with a GI value of 70 or more are high-GI foods. Therefore, in this study, the meal of wheat-based cereal and reduced-fat

milk with no added bioactive extract, the control test meal, was found to have a high GI value. The wheat-based cereal meal with 15 mg of added bioactive extract was found to have a medium GI value, and meals with 50 and 100 mg of added bioactive extract were found to have low GI values. The results of this study suggest that the tricin diglycoside in the extract added to the test meals can effectively reduce the glycaemic response to a high-GI meal. The addition of just 0.3 grams of the bioactive extract (containing approximately 15 mg of tricin diglycoside) reduced the GI value of the control meal by 15 units (21%), causing the meal's average GI value to drop from the high to the medium GI category. The addition of one gram of bioactive extract (50 mg of tricin diglycoside) reduced the GI value to the control meal by 26 units (37%), making it a low-GI meal, on average. The largest dose of bioactive extract (100 mg tricin diglycoside) reduced the GI value to the control meal by an average of 18 units (25%). Therefore, increasing the dose of bioactive compound from 50 to 100 mg did not result in a further reduction of glycaemia.

It will be appreciated that various alterations, modifications and/or additions may be introduced to the invention described herein without departing from the spirit or ambit of the invention.

Table 1
Inhibition of alpha-glucosidase by flavonoids luteolin, apigenin and triclin.

Flavonoid conc. μg/mL	Luteolin		Apigenin		Tricin	
	% control	% inhibition	% control	% inhibition	% control	% inhibition
0	100.0	0.0	100.0	0.0	100.0	0.0
7.8	81.4	18.6	73.5	26.5	97.7	0.3
15.6	60.2	39.8	74.3	25.7	26.4	73.6
31.3	65.8	34.2	68.4	31.6	15.6	84.4
62.5	45.7	54.3	50.4	49.6	6.4	93.6
125	17.9	82.1	29.5	70.5	0	100.0
250	19.1	80.9	0.0	100.0	0	100.0
500	0.0	100.0	0.0	100.0	0	100.0

5

Table 2.
Inhibition of alpha-amylase by flavonoids luteolin, apigenin and triclin

Flavonoid conc. μg/ml	Luteolin		Apigenin		Tricin	
	% control	% inhibition	% control	% inhibition	% control	% inhibition
0	100.0	0.0	100.0	0.0	100.0	0.0
11.7	0.0	100.0	81.0	19.0	40.9	59.1
23.4	0.0	100.0	0.0	100.0	18.2	81.8
46.9	0.0	100.0	0.0	100.0	0.0	100.0
93.8	0.0	100.0	0.0	100.0	0.0	100.0
187.5	0.0	100.0	0.0	100.0	0.0	100.0
375.0	0.0	100.0	0.0	100.0	0.0	100.0
750.0	0.0	100.0	0.0	100.0	0.0	100.0

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Table 3.
Summary of alpha-glucosidase and alpha-amylase inhibition by luteolin, apigenin and tricetin

5

Enzyme	IC ₅₀ concentration (µg/ml)			% inhibition at flavonoid concentration 1.6 mM (454 µg/mL)		
	Luteolin	Apigenin	Tricetin	Luteolin	Apigenin	Tricetin
Alpha-Glucosidase	51.5	59.4	12.0	96	100.	100
Alpha-amylase	5.8	16.2	9.9	100	100	100

Table 4
The weights and nutrient content of the test portion of the reference food and the four test meals, calculated using manufacturer's data

10

Food	Portion Size (g)	Energy (kJ)	Protein (g)	Fat (g)	Available Carbohydrate (g)	Sugars (g)	Fibre (g)
Reference Food (glucose sugar)	50g glucose 250 ml water	800	0.0	0.0	50.0	50.0	0.0
Wheat-based cereal with reduced-fat milk (control meal)	60.0g cereal 185.1g milk	1200	14.4	4.3	50.0	11.5	6.4
Wheat-based cereal with reduced-fat milk + 15 mg tricetin diglycoside	60.0g cereal, 185.1g milk, 0.3g extract	1200	14.4	4.3	50.0	11.5	6.4
Wheat-based cereal with reduced-fat milk + 50 mg tricetin diglycoside	60.0g cereal, 185.1g milk, 1.0g extract	1200	14.4	4.3	50.0	11.5	6.4
Wheat-based cereal with reduced-fat milk + 100 mg tricetin diglycoside	60.0g cereal, 185.1g milk, 2.0g extract	1200	14.4	4.3	50.0	11.5	6.4

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Table 5

Change in mean plasma glucose concentration from fasting baseline level (mmol/L)

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TIME (min)	Glucose reference food (average of two meals)	Control Meal (nil bioactive extract)	0.3g bioactive extract	1.0g bioactive extract	2.0g bioactive extract
0	0	0	0	0	0
15	2.68	1.26	1.04	0.95	1.17
30	4.17	3.09	3.15	2.89	2.88
45	3.56	2.85	2.20	2.07	2.62
60	2.48	1.87	1.19	1.25	1.59
90	0.66	0.42	0.15	-0.10	0.16
120	-0.45	0.08	-0.09	-0.24	-0.30

Table 6

The mean SEM GI values for the four test meals and the reference food.

10

Test Food	GI Value (%)	GI Category
Test meal with 50 mg tricin diglycoside	46 ± 5	Low
Test meal with 100 mg tricin diglycoside	54 ± 4	Low
Test meal with 15 mg tricin diglycoside	57 ± 7	Medium
Test meal with 0 mg tricin diglycoside	72 ± 7	High
Reference food (glucose)	100 ± 0	High

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CLAIMS:

- 5 1. A method of delaying digestion by an animal or a human of carbohydrates in food, comprising administering an effective amount of one or more flavonoids to the animal or human in conjunction with the food, wherein the flavonoids are selected from luteolin, apigenin, tricetin and their pharmaceutically acceptable analogues and derivatives.
- 10 2. A method according to claim 1 wherein the amount of flavonoids administered is at least 7mg per 50.0g of carbohydrates in the food.
- 15 3. A method according to claim 1 or claim 2 wherein the flavonoids are derived from sugar cane.
4. A method according to any one of claims 1 to 3 wherein the flavonoids include tricetin and its analogues and derivatives.
- 20 5. A method according to any one of claims 1 to 4 wherein the flavonoid is tricetin or a tricetin analogue or derivative.
6. A method according to any one of claims 1 to 5 wherein the flavonoids are administered prior to the food.
- 25 7. A method according to any one of claims 1 to 5 wherein the flavonoids are administered with the food.
8. A method according to any one of claims 1 to 5 wherein the flavonoids are administered after the food.
- 30 9. A method according to claim 7 wherein the flavonoids are incorporated into the food.

10. A method according to any one of claims 1 to 9 wherein the flavonoids are administered in conjunction with a nutritional fibre supplement.
11. A pharmaceutical formulation suitable for oral administration comprising an effective amount of one or more flavonoids selected from luteolin, apigenin, tricin and their pharmaceutically acceptable analogues and derivatives and one or more pharmaceutically acceptable excipients.
12. A formulation according to claim 11 wherein the flavonoids are derived from sugar cane.
13. A formulation according to claim 11 or claim 12 wherein the flavonoids include tricin and its pharmaceutically acceptable analogues and derivatives.
14. A formulation according to any one of claims 11 to 13 wherein the flavonoid is tricin or a pharmaceutically acceptable tricin analogue or derivative.
15. A formulation according to any one of claims 11 to 14 wherein the formulation includes a nutritional fibre supplement.
16. A method of producing an oral pharmaceutical formulation for inhibiting intestinal enzymes, comprising combining one or more flavonoids selected from luteolin, apigenin, tricin and their pharmaceutically acceptable analogues and derivatives with one or more pharmaceutically acceptable excipients.
17. A method according to claim 16 wherein the flavonoids are derived from sugar cane.
18. A method according to claim 16 to claim 17 wherein the flavonoids include tricin or its pharmaceutically acceptable analogues and derivatives.
19. A method according to any one of claims 16 to 18 wherein the flavonoid is tricin or a pharmaceutically acceptable tricin analogue or derivative.

20. A method according to any one of claims 16 to 19 wherein the formulation also includes a nutritional fibre supplement.
21. A food product containing carbohydrates which also contains sufficient
5 added flavonoids selected from luteolin, apigenin, tricetin and their nutritionally acceptable analogues and derivatives to reduce the glycaemic index of the food product.
22. A food product according to claim 21 wherein the amount of added
10 flavonoids contained in the food product is at least 7mg per 50.0g of carbohydrate in the food.
23. A food product according to claim 21 or claim 22 wherein the food product also contains a nutritional fibre supplement.
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24. A food product according to any one of claims 21 to 23 wherein the flavonoids are derived from sugar cane.
25. A food product according to any one of claims 21 to 24 wherein the
20 flavonoids include tricetin or its analogues and derivatives.
26. A food product according to any one of claims 21 to 25 wherein the flavonoid is tricetin or a tricetin analogue or derivative.
- 25 27. A method of lowering the glycaemic index of a carbohydrate-containing meal comprising including as part of the meal an effective amount of an extract of sugar cane containing tricetin or its nutritionally acceptable analogues and derivatives.
- 30 28. A method according to claim 27 wherein the extract contains at least 5% tricetin diglycoside.
29. A method according to claim 27 or claim 28 wherein the extract is obtained by:

- a. mixing one part dunder with two parts 96% ethanol and allowing to settle for 24 hours;
- b. filtering the dunder/ethanol mixture at 0.1 micron to obtain a permeate;
- 5 c. concentrating the permeate by evaporation to approximately 15% of its initial volume, and refiltering the concentrate;
- d. adding approximately 0.1% by volume acetic acid to the filtered concentrate and passing the resulting mixture through a chromatography column loaded with XAD resin;
- 10 e. washing the chromatography column with one bed volume water containing 0.1% v/v acetic acid, followed by successive elution with 20%, 30% and 40% v/v ethanol in water solutions containing 0.1% v/v acetic acid; and
- 15 f. collecting the 40% fraction and evaporating to dryness at low temperature to obtain a sugar cane extract containing about 5% tricin diglycoside.

30. A method according to any one of claims 27 to 29 wherein the amount of extract ranges from 0.3 g to 2.0 g to 50.0 g of carbohydrate in the meal.

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31. A method according to any one of claims 27 to 30 wherein the amount of extract is about 1.0 g per 50.0 g of carbohydrate in the meal.

32. A method according to any one of claims 27 to 31 wherein the extract is added to the meal during preparation of the meal.

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33. A method of lowering the glycaemic index of a carbohydrate-containing meal which comprises including as part of the meal an effective amount of tricin or its nutritionally acceptable analogues or derivatives.

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34. A method according to claim 33 wherein the amount of tricin or tricin analogue or derivative is at least 7 mg per 50.0 g of carbohydrate in the meal.

35. A method according to claim 33 or claim 34 wherein the amount of tricin or tricin analogue or derivative ranges from 15 mg to 100 mg per 50.0 g of carbohydrate in the meal.

5 36. A method according to any one of claims 33 to 35 wherein the amount of tricin or tricin analogues or derivatives is about 50 mg per 50.0 g of carbohydrate in the meal.

10 37. A method according to any one of claims 33 to 36 wherein the tricin or tricin analogues or derivatives are added to the meal during preparation of the meal.

35. A method according to claim 27 or claim 33, substantially as described with reference to Example B.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU03/01001

A. CLASSIFICATION OF SUBJECT MATTER												
Int. Cl. ⁷ : A23L 001/29; A61K 031/352; A61P 001/00												
According to International Patent Classification (IPC) or to both national classification and IPC												
B. FIELDS SEARCHED												
Minimum documentation searched (classification system followed by classification symbols) See electronic database below												
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched See electronic database below												
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPIDS & FSTA keywords: luteolin, apigenin, tricin, glucosidase, amylase, carbohydrate, food CA & MEDLINE keywords: luteolin, apigenin, tricin, glucosidase, amylase, carbohydrate												
C. DOCUMENTS CONSIDERED TO BE RELEVANT												
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.										
X	Patent Abstracts of Japan, JP 07-061927 A (LOTTE CO LTD) 7 March 1995 See whole abstract	1-35										
X	Patent Abstracts of Japan, JP 2002-010753 A (MARUKIN CHUYU CO LTD) 15 January 2002 See whole abstract	1-35										
X	SHIMURA et al. Inhibitory Effects of Flavonoids on Lipase. Nippon Shokuhin Kogyo Gakkaishi. 1994, vol. 41, no. 11, pages 847-850 See whole document	1-35										
☒ Further documents are listed in the continuation of Box C ☒ See patent family annex												
* Special categories of cited documents: <table border="0"> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	"P" document published prior to the international filing date but later than the priority date claimed	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention											
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone											
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art											
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family											
"P" document published prior to the international filing date but later than the priority date claimed												
Date of the actual completion of the international search 24 September 2003		Date of mailing of the international search report 8 OCT 2003										
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929		Authorized officer KAREN TAN Telephone No : (02) 6283 2277										

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU03/01001

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIM et al., Inhibition of Alpha-glucosidase and Amylase by Luteolin, a Flavonoid. Biosci. Biotechnol. Biochem. 2000, vol. 64, no. 11, pages 2458-2461 See whole document	1-35
P, A	SAMPSON et al. Flavonol and Flavone Intakes in US Health Professionals. Journal of The American Dietetic Association. 2002, vol. 102, no. 10, pages 1414-1420 See whole document	1-35
A	Derwent Abstract Accession No. 19056C/11, Class D13 E24, JP 5 5013-771 A (SAN EI CHEM IND KK) 30 January 1980 See whole abstract	1, 4, 5, 11, 13, 14, 16, 18, 19, 21, 25-28, 33-37

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU03/01001

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
JP 2002-010753	
JP 07-061927	
JP 55013771	
END OF ANNEX	