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ORIGINAL PAPER

Filtered Molasses Concentrate from Sugar Cane: Natural Functional Ingredient Effective in Lowering the Glycaemic Index and Insulin Response of High Carbohydrate Foods

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Abstract An aqueous filtered molasses concentrate (FMC) sourced from sugar cane was used as a functional ingredient in a range of carbohydrate-containing foods to reduce glycaemic response. When compared to untreated controls, postprandial glucose responses in the test products were reduced 5–20 %, assessed by accredited glycaemic index (GI) testing. The reduction in glucose response in the test foods was dose-dependent and directly proportional to the ratio of FMC added to the amount of available carbohydrate in the test products. The insulin response to the foods was also reduced with FMC addition as compared to untreated controls. Inclusion of FMC in test foods did not replace any formulation ingredients; it was incorporated as an additional ingredient to existing formulations.

Filtered molasses concentrate, made by a proprietary and patented process, contains many naturally occurring compounds. Some of the identified compounds are known to influence carbohydrate metabolism, and include phenolic compounds, minerals and organic acids. FMC, sourced from a by-product of sugar cane processing, shows potential as a natural functional ingredient capable of modifying carbohydrate metabolism and contributing to GI reduction of processed foods and beverages.

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Abbreviations

CE	Catechin equivalent (non-specific measure of phenolic activity)
FMC	Filtered molasses concentrate
GI	Glycemic index (measurement of 2 h postprandial glycemic response relative to pure glucose)
II	Insulinemic index or response (measurement of 2 hour postprandial insulin response relative to glucose)
ORAC	Oxygen-radical absorbance capacity (measure of antioxidant activity)
pmol/L	Picomole per litre

Introduction

The increasing prevalence of metabolic syndrome in developed and developing populations and projected rates of obesity and diabetes are among the greatest health concerns in many countries [1]. In addressing these challenges, there is a growing interest in the demonstrated benefits of low glycaemic index (GI) diets as effective tools in addressing metabolic disorders and weight maintenance [2]. A meta-analysis suggests the benefits of low GI diets on medium-term glycaemic control among diabetic patients are comparable to those of known pharmacological agents which target hyperglycaemia [3].

Improved metabolic effects, including reduced glycemic response, have been attributed to crude plant extracts containing many naturally occurring compounds. Hsieh et al. [4]

found aqueous extracts of *Ajuga* species containing a range of phenolic compounds were effective in reducing glucose uptake *in vitro* and *in vivo.* Thompson et al [5] showed a correlation between increasing levels of polyphenols and reduction in GI for both healthy and diabetic individuals. Multiple modes of action for polyphenols moderating carbohydrate metabolism have been demonstrated *in vitro*, acting by inhibition of glycolytic enzymes as well as inhibiting or delaying intestinal glucose uptake [5, 6].

The minerals magnesium, calcium and potassium, abundant in molasses, may play a beneficial role in carbohydrate metabolism. Magnesium deficiency has been correlated with insulin resistance [7], calcium supplementation has been shown to increase insulin sensitivity [8] and low potassium levels have been associated with increased risk of developing diabetes, particularly in young African-Americans [9] and in otherwise healthy Japanese men [10]. Glucose and insulin responses can also be reduced in the presence of organic acids [11].

Molasses from sugar cane provides a ready source of plant derived compounds, including polyphenols, minerals and organic acids. This work describes the use of a filtered molasses concentrate (FMC) from sugar cane as a functional ingredient, observed to reduce both glycaemic and insulin responses in food and beverage matrices, assessed by accredited GI testing. Potential mechanisms for the observed effects are discussed. This study demonstrates the potential for FMC in the development of low GI foods and beverages as part of a low GI diet, contributing natural bioactive compounds and minerals.

Materials and Methods

Filtered Molasses Concentrate Syrup

Filtered molasses concentrate (FMC) used in this study is a crude extract made by a proprietary and patented aqueous filtration process from unrefined sugar cane mill molasses. Composition of FMC is provided in Table 1. Phenolic compounds present in FMC were analysed by LC-MS. The syrup was fractionated using ion exclusion chromatography. Fractions were diluted in mobile phase A (10 % aqueous acetonitrile with 0.1 % formic acid) and separated using a C18 column with a 105 minute gradient program. Known standards and literature data were used for preliminary identification. Compounds were confirmed by the pseudomolecular peak and characteristic fragmentation patterns detected under at least two different conditions: positive and negative ionisation or different in-source fragmentation voltages. [Gunter Kuhnle, personal communication]

Glycaemic Index, Insulin Index Testing

Control and test foods for GI and Insulin Index (II) testing were prepared, with FMC added extrinsically to test foods as a functional ingredient, with no replacement of other ingredients other than minor formula modifications to compensate for sugar and moisture contributed by FMC (Table 2). FMC was added with liquid ingredients ensuring even distribution.tgroup1

GI testing measures the glycaemic response to a standard quantity of carbohydrate, in this case 50 g. GI testing for this study was conducted by the accredited GI testing facility,

Table 1 Composition and nutrition information for filtered molasses concentrate (FMC)

Component	Average values	Minerals	Average values
Moisture, g/100 g	38.7	Sodium, mg/100 g	47
Energy, (calc) kJ/100 g	942	Calcium, mg/100 g	535
Protein(N x 6.25, g/100 g)	2.1	Iron, mg/100 g	7.3
Fat, g/100 g	0.9	Magnesium, mg/100 g	218
Sucrose, g/100 g	29.0	Manganese, mg/100 g	4.4
Glucose, g/100 g	5.4	Potassium, mg/100 g	2,350
Fructose, g/100 g	6.0	Zinc, mg/100 g	0.34
Total sugars, g/100 g	41.0	Phytochemicals	
Insoluble dietary fiber	0	Polyphenols[a], mg CE/100 g	Minimum 1,150
Soluble dietary fiber, g/100 g	0.9	Flavonoids[b], mg/100 g	398
Total carbohydrate (by difference, g/100 g)	53.0	ORAC Value[c] Vit E equivalent (total), μmol/100 g	19,970
Ash, g/100 g	6.1		
Organic acids, mg/100 g	110		

Reference methods for determination

[a] Polyphenols: [26]; [b] Flavonoids: [27]; [c] ORAC value: [28]. (*CE*=catechin equivalents, *calc*=calculated, *N*=nitrogen, *Vit*=vitamin)

Table 2 Foods tested for GI, II: Ingredients and approximate composition of test products shows the variety in type and amount of carbohydrate, and macronutrient composition

Food tested	Ingredients Test products also contain filtered molasses concentrate, FMC	Proximate composition, g/100 g				g CHO/ 100 g	Portion tested g	FMC added per 100 g	g FMC/ 100 g CHO
		Protein	Fat	Moisture	Dietary fiber (est)				
White bread	Flour, water, butter, salt, yeast, sugar	8	3	37	10.7	C: 41.3 T: 41.4	121.1 120.8	2.50 g	6.04
Glucose syrup	Glucose syrup	0.1	0	18.5	0	C: 81.4 T: 79.2	61.4 63.1	3.5 g	4.42
Fruit flavoured beverage	Water, sugar, citric acid, flavour, colour, sodium benzoate, sodium metabisulphite	0	0	93.5	0	C: 6.5 T: 6.1	769.2 819.7	0.22 g/ 100 ml	3.6
Energy bar	Soy protein isolate, peanut butter, corn syrup, inulin, fructose, sugar, rice starch, wheat germ, salt, high-fructose corn syrup, whey, vitamins, flavours, preservatives. Coating: sugar, palm kernel oil, cocoa, whey, nonfat milk, soy lecithin, flavours and preservatives.	11	13	19	5.3	C: 51.7 T: 49.9	96.7 100.2	2.0	4.01
HFCS	Fructose 55 %, glucose 40 %	0	0	22	0	C: 77.5 T: 80.3	64.5 62.3	3.3 g	4.16
Wheat flake cereal bricks	Wholegrain wheat, raw sugar, salt, barley malt extract, minerals (zinc gluconate, iron) vitamins (niacin, thiamin, riboflavin, folate)	12	1.5	8	11.5	C: 67 T1: 67.1 T2: 66.7	74.6 74.5 74.9	1.4 g 2.0 g	2.08 2.98

C=control product, *T*=test sample containing filtered molasses concentrate, *ND*=not determined, *CHO*=carbohydrate, *HFCS*=high fructose corn syrup, (est)=estimated

Sydney University's Glycaemic Index Research Service, Sydney, NSW, Australia according to the international standard GI test protocol (ISO/FDIS 26642:2010 Food products – Determination of the glycaemic index (GI) and recommendation for food classification).

The GI and II values of test foods were determined using healthy, non-smoking human volunteer subjects from the staff and student population of the University of Sydney. Test and control foods were tested by 10 subjects in a cross-over design. Age of the subjects ranged from 18 to 39 (average 26). Subject BMI ranged from 18.2 to 24.8 (average of 22.7).

This study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki. The experimental procedures used in this study were in accordance with international standards for conducting ethical research with humans and were approved by the Human Research Ethics Committee of Sydney University (approval number 08-2009/12029, valid August 13, 2009 – August 31, 2012). This study was performed between March 2011 and March 2012.

Statistical Analysis

A sample size calculation was done to detect a reduction in GI of 10 points from a starting GI of 65 (a mid-range value) and a standard deviation of 10 points. The two-sided alpha was set at 0.05 and the power at 80 %. The sample size requirement was a minimum of eight subjects; each individual test was conducted with a sample size of 10 subjects to allow for the possibility of outliers.

All individual subject GI and II values for each tested food were pooled (n=50) and analysed by a paired t-test comparing responses of product treated with FMC to untreated control products. Glucose and insulin responses were analysed by repeated measures ANOVA with time (7 points) and treatment (2 points; FMC presence or absence in product) as within-subject factors. The primary endpoints were differences in both glucose and insulin responses. Secondary endpoints were the time points at which glucose and insulin responses changed if the primary endpoint was significant. Significance was set at $p<0.05$. The wheat flake cereal tests were excluded from this analysis due to the absence of insulin response data.

Results and Discussion

Filtered Molasses Concentrate Reduces Glycaemic and Insulin Responses

Responses for GI and II to products tested are provided in Table 3. Reductions in GI and II due to FMC addition are calculated where possible.

Table 3 Addition of FMC reduces glucose, insulin responses. Addition of FMC reduces glycaemic index (GI) in all food matrices tested. Degree of GI reduction is dependent on ratio of FMC to available carbohydrate (see Fig. 1)

Food tested	GI±SEM	% GI reduction	II±SEM	% II reduction
White bread	C: 74±3 T: 59±6	- 20 %	C: 78±3 T: 67±6	- 14 %
Glucose syrup	C: 107±7 T: 93±9	- 13 %	C: 104±4 T: 87±7	- 17 %
Fruit flavoured beverage	C: 67±5 T: 58±3	- 13 %	C: 66±4 T: 56±3	- 13 %
Energy bar	C: 45±6 T: 40±4	- 11 %	C: 61±3 T: 63±3	- - 3.3 %
HFCS	C: 56±5 T: 50±3	- 10 %	C: 65±5 T: 58±3	- 11 %
Wheat flake cereal bricks	C: 76±5 T 1: 72±5 T 2: 70±4	- 5 % 8 %	ND ND ND	ND ND

C=control product, *T*=test sample containing filtered molasses concentrate, *SEM*=standard error of the mean, *ND*=not determined

The addition of FMC to the food products tested resulted in a universal reduction in GI. In comparing the reduction in GI in each individual study across the range of products tested, the percentage reduction due to addition of FMC was found to be dose-dependent, and directly proportional to the amount of FMC added *as a percentage of available carbohydrate content* of the food (Fig. 1). The high correlation (R^2= 0.922) of FMC to available carbohydrate on glucose response indicates FMC may have a direct effect on carbohydrate metabolism, despite containing approximately 40 % sugar. The observed dose–response correlation indicates FMC is effective in reducing the 2-h glucose response to a range of carbohydrate types. The correlation is consistent across test products including bread, with starch based carbohydrates, and glucose syrup containing simple carbohydrates, irrespective of fiber content.

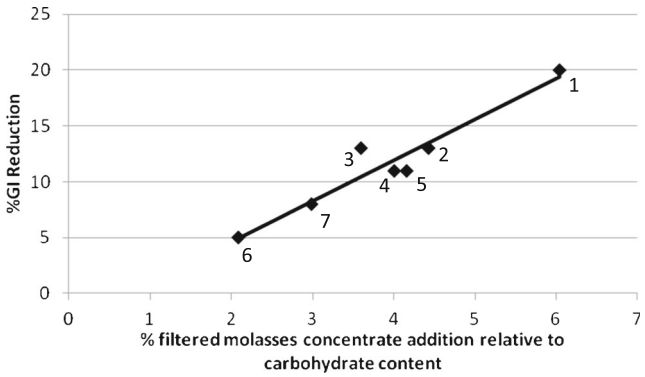

Fig. 1 Correlation of GI reduction to FMC addition, relative to carbohydrate content. As the amount of FMC added to the food matrix is increased (relative to carbohydrate content of the food), the glycemic index (GI) value is reduced (correlation R^2=0.922). Foods tested include: 1 - white bread, 2 – glucose syrup, 3 – fruit flavoured beverage, 4 – high fructose corn syrup (HFCS), 5 – energy bar, 6 – wheat flake cereal bricks (1.4 g/100 g), 7 – wheat flake cereal bricks (2 g/100 g)

The pooled individual subject data for all samples tested was also analysed for glucose and insulin responses over the 2-h test period (Fig. 2). Average responses for both glucose (GI) and insulin (II) of the treated products were found to be significantly lower (p<0.001) than their untreated controls using a paired Student's *t*-test (Figs. 2a, c).

Analysis of all 2-hour glucose response curves (Fig. 2b) over the test period using repeated measures ANOVA shows a significant treatment effect (p<0.005) and time by treatment effect (p<0.01). Glucose responses were significantly reduced at both 30 and 45 minutes (both p<0.01) for the treated products compared to the untreated controls.

Analysis of the combined insulin response curve (Fig. 2d) using repeated measures ANOVA shows a significant treatment effect (p<0.005). Insulin responses were significantly lower at 60 min (p<0.05) for the treated product compared to control, and were of borderline significance at the 30 min time point (p=0.05).

In most test products the observed reductions in glucose and insulin responses were comparable. In the case of the energy bar product, however, no reduction in insulin response was observed. The energy bar contained ingredients contributing varying types of carbohydrate, protein and fat. The insulin response values of the energy bar were 36 and 58 % higher than the corresponding GI value. Elevated insulin responses may have been due to soy and milk proteins [12] and/or fat content [13], all of which have been shown to be insulinogenic.

Potential Bioactive Components of Filtered Molasses Concentrate

While the active compounds in FMC are currently unknown, possible candidates are one or more

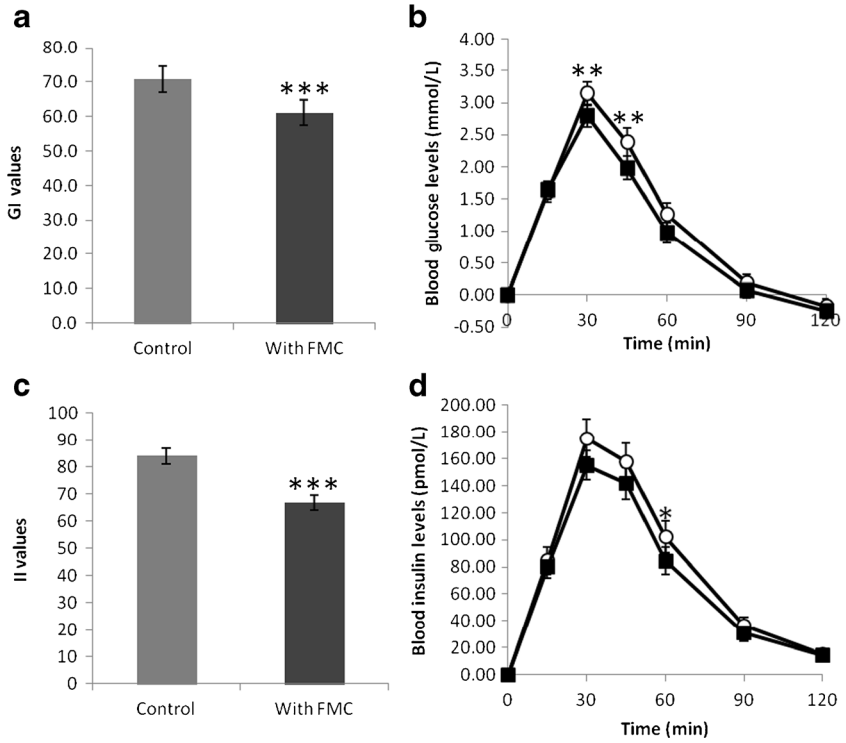

Fig. 2 Comparison of combined glucose and insulin responses to products tested at all addition levels; control product (no treatment) to product containing FMC. (**a**). Combined individual glycaemic index (GI) values (n=50) of white bread, fruit-flavoured beverage, energy bar, high fructose corn syrup (HFCS) and glucose syrup, control *vs.* FMC-fortified (**b**). Average combined individual 2-h glucose response change from baseline. Open circles, control products; closed squares, FMC-fortified products (**c**). Combined individual insulin index (II) values (n=50) of white bread, fruit-flavoured beverage, energy bar, HFCS and glucose syrup, control *vs.* FMC-fortified (**d**). Average combined individual 2-h insulin response change from baseline. Open circles, control products; closed squares, FMC-fortified products. Bars show standard error. *p<0.05, **p<0.01, ***p<0.001

phenolic compounds, minerals, organic acids, or a synergistic activity between several components. As the specific mechanism for bioactivity is as yet unknown, the bioactive levels of any particular compound, or combinations of compounds required, has yet to be determined.

The phenolic compound profile of FMC is complex, and current understanding of the bioactivity of the identified peaks is limited. Preliminary characterisation by LC-MS has identified phenolic compounds with known activities related to carbohydrate metabolism; details of these are provided in Table 4. Schaftoside, a prevalent phenolic compound in sugar

Table 4 Phenolic compounds found in FMC with known roles in carbohydrate metabolism. Schaftoside, a prevalent phenolic compound in sugar cane molasses was used as a quantification standard, but has no identified role in CHO metabolism

Phenolic compounds found in FMC using LC-MS	Quantification (μg/g)	Bioactivity					References
		Inhibition of carbohydrate digestion	Inhibition of glucose intestinal absorption	Increased insulin secretion/ content	Improved glucose uptake	Induction of hepatic glucokinase	
Schaftoside	1,900						
Orientin	340	x					[29]
Cyanidin-3-O-glucoside	330			x			[6]
Ferulic acid	250	x	x	x	x	x	[6]
Malvidin-glycoside	180	x					[30]
Diosmin	140			x			[31]
Epigallocatechin	100	x	x				[6]
p-coumaric acid	90	x					[6]
Vitexin	40	x					[32]

cane molasses, was used as a quantitative standard. Schaftoside has no reported activity on carbohydrate metabolism. The non-specific polyphenol content in FMC (1,150 mg CE/100 g) compares favourably to other rich sources of polyphenols such as coloured rice brans, raspberries, raisins and black pepper; the ORAC value of FMC is also comparable to other rich sources of antioxidants [14–16].

Some of the minerals found in FMC known to influence carbohydrate metabolism are present in quantities sufficient to contribute to dietary intake. For comparison, a 20 g quantity of FMC contains 10.7, 10.9 and 13.4 % of the U.S. FDA daily value for adults, for calcium, magnesium and potassium, respectively.

The inclusion of organic acids in foods results in reductions in postprandial responses for both glucose and insulin. While the amount of organic acid present in FMC is significantly lower than those studied by Liljeberg et al. [11] complementary effects on carbohydrate metabolism are possible.

Possible Effect of FMC on Glucose, Insulin Responses

Although the mechanism for the observed effects of FMC is not yet fully understood, the observed linear relationship between GI reduction and amount of FMC added is independent of carbohydrate type or presence of fiber (Fig. 1, Table 1). This suggests FMC may not be inhibiting enzymatic carbohydrate digestion. In support of this theory, an *in vitro* model study performed essentially as described by Munro et al [17] demonstrated FMC does not inhibit pancreatic digestion of pre-gelatinised starch under simulated gastrointestinal conditions, in a 50:50 ratio of starch to FMC. In addition, FMC did not inhibit *in vitro* activity of α-glucosidase or salivary or pancreatic α-amylase (unpublished results).

As digestive enzymes do not appear to be inhibited by FMC, and FMC addition does not increase insulin responses, the indicated action of FMC may be in inhibiting intestinal glucose transport and absorption. If this is the case, it could explain the similar magnitude of reduction in both glucose and insulin responses observed (excepting the energy bar). This theory is supported by research demonstrating aqueous extracts containing polyphenols and flavonoids reduce sugar absorption [18], or slow intestinal glucose transport by competition with receptor sites [19, 20]. As phenolic compounds have been shown to have varied effects on carbohydrate metabolism (Table 4), other mechanisms are also possible.

It remains possible that FMC has a role to play in directly moderating insulin response. Various flavonoids and phenolic acids have been shown in *in vitro* studies to increase the uptake of glucose into peripheral tissue cells [21]. They have been shown to enhance or bypass insulin signalling *via* numerous mechanisms: activation of insulin-dependent and-independent signalling pathways such as AMP kinase [21, 22] and phosphatidylinositol-3 kinase [23], mimicking insulin and activating the insulin receptor [24, 25]. Any or all of these mechanisms may result in decreased levels of insulin secreted from the pancreas.

The minerals calcium, magnesium and potassium found in FMC are known to affect insulin response, by increasing sensitivity with calcium supplementation in T2D and hypertensive patients [8], while long term observational studies of chronic deficiencies of magnesium [7] and low levels of dietary potassium are correlated to insulin resistance and increased incidence of developing diabetes [9, 10]. Although the acute effects are unknown, the presence of these minerals, alone or in combination with plant derived phenolic compounds in FMC, may be partly responsible for the observed reduction in glucose and/or insulin response in most of the food matrices tested.

Organic acids are also known to have acute effects on postprandial glucose and insulin responses [11]. Levels of organic acids present in FMC at typical application rates in foods are approximately 200-fold lower than that studied by Liljeberg et al [11] with similar reductions in glycemic response, suggesting any potential effect of organic acids would likely be synergistic in nature.

The observations in this study demonstrate that ingestion of FMC lowers postprandial glucose and insulin response to carbohydrate in acute studies. It is possible that chronic consumption of FMC may place less metabolic stress on pancreatic β-cells, and slow progression to insulin resistance. The effects of FMC on insulin response and insulin sensitivity require further exploration; additional studies will determine which of the components of FMC are responsible for the observed bioactivity, investigate chronic effects, and identify mechanisms of action.

Conclusions

Filtered molasses concentrate sourced from sugar cane molasses provides a natural, abundant and inexpensive source of valuable plant-derived phenolic compounds, minerals and organic acids. The work described here demonstrates FMC is effective in reducing glucose responses in a dose-dependent manner in acute human studies, as well as reducing insulin responses to carbohydrate. The work shows the potential for FMC as a natural functional ingredient for use in reducing glycaemic index and decreasing insulin response, as well as increasing the antioxidant potential and mineral content of carbohydrate-containing foods and beverages.

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Conflict of Interest The authors declare they have no conflict of interest.

Study subjects The authors declare this research study involved human subjects. This study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki. The experimental procedures used in this study were in accordance with international standards for conducting ethical research with humans and were approved by the Human Research Ethics Committee of Sydney University (approval number 08-2009/12029, valid August 13, 2009 – August 31, 2012). This study was performed between March 2011 and March 2012.

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