Original Research

The Glycemic and Insulinemic Index of Plain Sweet Biscuits: Relationships to *in Vitro* Starch Digestibility

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Key words: biscuits, glycemic index, slowly available glucose (SAG), starch gelatinisation

Background: Among the various classes of processed starchy foods, wheat-based cereal products exhibit a wide range in glycemic and insulinemic responses. Understanding starch behavior during cooking and processing may help identify strategies that lower postprandial glycemia and insulinemia.

Objective: To determine the relationship between the *in vivo* glycemic index (GI)/insulinemic index (II) and *in vitro* digestibility and composition characteristics (rapidly available glucose and slowly available glucose, RAG and SAG respectively) of 24 plain sweet biscuits (cookies).

Methods: The products were commercially available and selected on the basis of their high starch content. *In vivo* responses (GI and II) were measured by standardised methods over 7 studies, with 12 subjects in each study (30 males, 42 females). *In vitro* digestibility characteristics were measured by the Englyst procedure.

Results: The observed GI ranged from 38 to 60 (low to moderate) with the majority between 40 and 50, and correlated strongly with the observed insulinemic index ($r = 0.76$, $P < 0.0001$). The digestibility profile of carbohydrates was significantly correlated to *in vivo* responses (SAG and GI: $r = -0.41$; $p = 0.04$; SAG and II: $r = -0.52$; $p < 0.01$; RAG and GI: $r = 0.5$; $p = 0.01$; RAG and II: $r = 0.34$; $p = 0.1$) and explained *in vivo* responses better than fat, protein and fiber content amongst this selection of plain sweet biscuits.

Conclusion: The findings indicate that plain sweet biscuits have a low GI and a moderate II and that these characteristics are correlated to *in vitro* starch digestibility and are dependent on the type of processing.

INTRODUCTION

Current dietary recommendations encourage the consumption of cereals and grains with high starch content. There is some concern, however, that refined starchy foods may cause postprandial hyperglycemia and hyperinsulinemia and be detrimental to people with impaired glucose tolerance or insulin secretory dysfunction [1,2]. In some countries, including the United Kingdom [3] and Australia [4], nutritional advice for people with diabetes specifically promotes the consumption of foods with a low glycemic index (GI). Although further research is necessary, low GI diets may also benefit the general population by preventing or delaying the development of diseases that are linked to insulin resistance [5]. In large-scale observational studies, diets with the highest average GI were associated with 1.3–2.0 times greater risk of type 2 diabetes [6], coronary infarct [7] and certain cancers [8,9]. Nevertheless, some studies have not confirmed these relationships [10,11]. The World Health Organisation [12] concluded that low GI diets are a possible factor helping in reducing the risk of obesity and type 2 diabetes.

Starchy foods should make up the largest part of total carbohydrate intake but differ greatly in terms of their impact on glycemia and therefore GI [13]. On the whole, legumes tend to have lower GI values while potatoes generally have higher values, irrespective of method of cooking. Intrinsic properties of the starch, such as chemical structure and hydration, as well as extrinsic factors such as fiber and fat content of the product, influence the rate of starch digestion [14,15]. Among the various classes of processed starchy foods, wheat-based cereal products exhibit a very wide range in GI [13]. Englyst *et al.* [16], for example, showed values ranging from as low as 28 to as high as 93 in a group of 23 products that included breakfast cereals, biscuits and bakery products. Moreover, the GI is often

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described as varying between and within food groups [17]. Understanding the reasons behind this variability, through differences in ingredients, composition and processing might lead to greater ability to formulate specifically products with a low GI.

In that study, plain sweet biscuit products, in particular, stood out as the category with the lowest average GI and the highest amount of slowly digested starch as defined by their *in vitro* methodology [16]. However, the range of GI in this category was still wide: 28–77. Specific processing steps in plain biscuit manufacture can hinder starch gelatinisation, helping to preserve the original properties of the native starch and thereby lower the GI. Despite this knowledge, the digestibility characteristics of cereal products and the relationship to glycemic impact have received insufficient study, particularly in the case of plain sweet biscuits (or cookies, the term used in the United States).

The objectives of the present study were therefore: 1) to measure the glycemic and insulinemic index of 24 plain sweet biscuits and 2) to evaluate the *in vitro* digestibility properties of the carbohydrate fraction which might be predictive of *in vivo* responses. The goal was to identify compositional and digestibility factors that influenced the final GI and insulinemic index of plain sweet biscuits.

MATERIALS AND METHODS

Twenty-four plain sweet biscuits manufactured by Danone Group or its subsidiaries in France, Belgium, Argentina, Malaysia, China and the Czech Republic were selected. Biscuits and/or cookies have a varied composition according to the relative amounts of sugars, fat and flour that are used, and according to the technology undergone by the dough (moulding, fermentation, extrusion, deposition etc.). In particular, starch content in biscuits ranges widely as a result of different levels of cereals incorporation in the recipe (ex: 10 g/100 g in some wafers; up to 60 g/100 g in some plain biscuits). We selected the richest in starch (and therefore in cereals), containing at least 25 g/100 g of starch. The interest of selecting biscuits with a high level of starch is based on previous data showing that process can modify the degree of starch gelatinisation, inducing a wide range of GI values [13,16]. Therefore, selecting high starch products allows to have a greater sensitivity when exploring the influence of process on GI. Composition and digestibility analyses and *in vivo* test on these products were run between 2001 and 2003. Fat and protein were determined using standard methods of the Association of Official Analytical Chemists [18]. Fiber, total sugars and fat quality were estimated from the individual components of the recipe using food composition tables.

In Vitro Studies

Englyst's method [19] was used to assess *in vitro* carbohydrate digestibility and allowed to evaluate the amount of rapidly available glucose (RAG), of slowly available glucose (SAG), total starch (TS), total fructose (free and deriving from sucrose) and total glucose (deriving from sucrose and free). The method is based on measurement of the glucose released from a test food during timed incubation with digestive enzymes under standardised conditions. This chemically-based classification estimates the amounts of glucose (from sugar and starch digestion) that are likely to become available for rapid or slow absorption from the small intestine. The physiological relevance of *in vitro* digestibility and its ability to identify types of carbohydrate that are important to health, are still being explored.

In Vivo Studies

In a series 7 separate studies, the glycemic index (GI) and insulinemic index (II) of each biscuit was determined by standardised GI methodology using glucose as the reference food as recommended by WHO and FAO [20]. Each study recruited 12 subjects (72 different subjects in total, 30 males, and 42 females) by advertisement from the student population of the University of Sydney. The mean (range) age and BMI of all subjects was 23.3 years (18–45) and 22.1 (19–25) kg/m^2 respectively. The protocol complied with the Helsinki Declaration of 1975 as revised in 1983 and was approved by the institutional ethics committee. Subjects gave written, informed consent.

A 50 g available carbohydrate portion of each biscuit was consumed with 250 mL water after a ≥10 hr overnight fast. Fingerprick blood samples (≥0.7 mL) were taken using an automatic sterile lancet device (Safe-T-Pro™, Boehringer Mannheim Australia, Castle Hill, NSW) from warmed hands at −5, 0, 15, 30, 45, 60, 90 and 120 min after the start of the meal. Blood samples were collected into 1.5 mL plastic microcentrifuge tubes coated with heparin (10 IU heparin sodium salt, Sigma Chemical Co., St Louis, USA), and were immediately centrifuged after collection at 12 500 g for 1 min. The plasma components of the samples were transferred into uncoated plastic tubes and stored at −20°C until assayed. Plasma glucose concentration was measured in duplicate using a Roche Hitachi 912® automatic centrifugal spectrophotometric analyser (Boehringer Mannheim, GmbH, Mannheim, Germany) employing a glucose hexokinase/glucose-6-phosphate dehydrogenase method (Roche Diagnostic Systems, Frenchs Forest, Australia). The mean intra-assay and inter-assay coefficients of variation were both below 3%. Plasma insulin was measured using a solid-phase radioimmunoassay (Coat-a-Count, Diagnostic Products Corporation, LA, USA). The mean intra-assay and inter-assay CVs were both below 5%. Cumulative changes in plasma glucose and insulin were quantified as the incremental area under the 120 min response curve and the GI and insulinemic index (II) were calculated as previously described [21]. When individual GI or II scores differed from the mean by more than two standard deviations, they were considered outliers and excluded from the dataset.

Statistical Analysis

Results are presented as mean ± SEM. To determine relationships between *in vitro* and composition data and *in vivo* responses, GI and II were matched for simple correlation and linear regression with the amount of nutrients and digestion fractions given in the glycemic index test (g per serving containing 50 g amount of available carbohydrate). The following criteria were selected: total glucose, total fructose, starch, total carbohydrates, dietary fiber, protein, fat, PUFA, MUFA, SFA (expressed in 50 g carbohydrate portion), % MUFA, % SFA, rapidly available glucose (RAG), slowly available glucose (SAG). SAS® statistical software version 8.02 was used.

RESULTS

The nutrient profile (g/serving used in the glycemic index test) of the 24 selected plain sweet biscuits is shown in Table 1. The content of the *in vitro* digestion fractions (g/serving used in the glycemic index test), GI and II data for each plain biscuit are shown in Table 2. Mean RAG content was 41.3 g per 100 g product (SEM 1.2), SAG was 14.9 per 100 g product (SEM 0.8) and SAG/starch was 32.4% (SEM 1.7). The mean GI was 48 (SEM 1.1) with a range from 38 to 60, with most of the plain biscuits being between 40 and 50 (Fig. 1A). The mean II was 56 (SEM 0.9) and ranged from 49 to 63, with the majority in the range 50–60 (Fig. 1B). GI and II for individual food products were highly correlated ($r = 0.76$; $p < 0.0001$).

Simple correlations between food composition data (total fructose, total glucose, total starch, fiber, protein, fat, PUFA, MUFA, SFA (expressed in g per 50 g carbohydrate portion), % MUFA and SFA over fat content, *in vitro* digestion fractions, SAG and RAG (expressed in g per 50 g carbohydrate portion) and *in vivo* data (GI and II) were tested. GI correlated positively with RAG ($r = 0.50$; $p = 0.01$, Fig. 2A) and with fiber ($r = 0.44$; $p = 0.03$) and inversely with protein ($r = -0.43$; $p = 0.03$), SAG ($r = -0.42$; $p = 0.04$), fat ($r = -0.49$; $p = 0.01$) and saturated fat ($r = -0.44$; $p = 0.03$). Similarly, II correlated positively with RAG ($r = 0.34$; $p = 0.10$) and fiber ($r = 0.40$; $p = 0.055$) and inversely with SAG ($r = -0.52$; $p < 0.01$, Fig. 2B).

DISCUSSION

In the 21st century the food industry is faced with the challenge of not only providing palatable foods but also formulating products with optimal nutritional properties that may beneficially affect public health. In the present study, our goal was to identify processing procedures and compositional factors that reduce the rate of starch digestion and thereby result in

Table 1. Nutrient Composition of the 24 Studied Plain Sweet Biscuits; Data Are Related to a Serving Containing 50 g of Available Carbohydrates (Used in the Glycemic Index)

	Svg g	Total fructose g	Total glucose g	Total sugars g	Starch g	Fiber g	Protein g	Lipids g	SFA g	MUFA g	PUFA g
1	69	9.4	8.9	19.5	29.5	2.9	4.6	11.4	5.1	4.2	2.1
2	69	6.7	6.6	14.3	36.3	4.7	5.2	8.4	2.5	3.8	2.1
3	72	9.3	9.3	20.4	30.5	4.7	4.7	11.8	3.2	5.8	2.7
4	69	8.2	7.8	18.4	31.5	5.4	4.5	7.6	3.3	3.2	1.1
5	72	8.5	8.6	18.8	32.2	3.8	5.2	12.5	3.4	6.1	3.0
6	72	10.7	10.8	23.4	25.1	4.3	5.0	11.4	5.8	4.3	1.4
7	72	9.3	10.4	20.3	27.5	3.1	4.5	12.5	6.1	4.7	1.7
8	68	6.9	7.3	15.3	31.3	1.9	5.1	10.2	2.8	4.8	2.7
9	69	9.0	8.7	18.6	31.0	2.9	4.7	10.1	3.0	4.6	2.6
10	73	7.8	8.0	17.1	31.2	4.0	5.3	13.2	6.7	4.9	1.7
11	67	8.8	9.0	19.1	31.3	2.8	4.6	9.9	2.9	4.6	2.5
12	61	7.1	7.2	15.7	34.4	1.7	3.7	7.5	3.5	2.9	1.1
13	72	6.9	6.2	16.1	32.3	1.7	8.2	9.6	4.7	3.6	1.3
14	67	7.3	7.1	15.8	33.1	2.4	4.7	8.8	5.6	2.6	0.7
15	66	7.0	7.0	15.5	34.8	2.2	4.5	8.5	4.0	3.3	1.2
16	70	7.9	7.9	15.1	32.2	2.9	5.2	10.9	4.1	5.0	1.7
17	62	6.9	6.8	15.1	35.0	4.3	4.6	3.9	0.5	2.5	0.9
18	72	10.1	9.9	22.3	25.7	3.6	5.3	11.3	5.9	4.7	0.7
19	70	8.1	7.7	19.2	29.1	1.6	5.6	11.6	5.6	5.2	0.9
20	69	7.9	8.0	16.5	31.7	3.1	5.6	10.4	5.1	4.0	1.4
21	70	6.3	6.2	13.8	35.2	2.4	5.8	12.0	6.3	4.1	1.6
22	69	8.3	8.4	17.5	31.8	2.5	5.6	11.7	6.8	3.5	1.4
23	70	6.3	6.4	14.6	35.4	2.4	6.4	11.8	7.1	3.3	1.4
24	63	6.6	6.4	14.8	37.0	1.9	5.0	7.6	4.9	2.1	0.6

* Total fructose and total glucose = respectively (free fructose + fructose from sucrose) and free glucose and glucose from sucrose.
Svg = serving, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

Table 2. Glycemic Index (GI) and Insulinemic Index (II) Values Based on 7 *in Vivo* Studies (12 subjects each) Using Glucose as a Food Standard (Glucose GI = 100), and *in Vitro* Digestibility Indices for the 24 Plain Sweet Biscuits Studied

n	GI ± SEM	II ± SEM	RAG g	SAG g	RDS g	SDS g	RS g	TS g	SAG/starch
1	48.0 ± 3.0	56.0 ± 1.0	27.8	9.3	18.8	9.3	1.2	29.4	31.6
2	58.0 ± 4.0	58.0 ± 4.0	34.2	7.8	27.6	7.8	0.9	36.3	21.4
3	56.0 ± 5.0	63.0 ± 5.0	25.8	12.5	16.5	12.5	1.5	30.5	41.0
4	55.1 ± 4.5	60.4 ± 2.9	28.7	9.7	20.8	9.9	0.9	31.6	30.9
5	44.0 ± 3.0	53.0 ± 3.0	26.3	13.1	17.7	13.1	1.3	32.2	40.8
6	47.0 ± 3.0	54.0 ± 3.0	25.6	9.2	14.8	9.2	1.2	25.1	36.4
7	51.5 ± 3.1	63.1 ± 4.3	27.5	7.3	17.4	9.8	0.3	27.5	26.5
8	46.0 ± 3.9	57.3 ± 2.8	28.8	8.4	21.4	8.7	1.1	31.3	26.9
9	45.0 ± 5.0	52.0 ± 4.0	28.6	9.9	19.8	9.9	1.3	31.0	31.9
10	50.9 ± 3.2	59.5 ± 3.2	31.4	6.3	23.3	6.3	1.5	31.2	20.3
11	43.0 ± 5.0	53.6 ± 2.0	28.3	10.7	19.3	10.7	1.2	31.3	34.2
12	48.0 ± 5.0	49.5 ± 4.0	30.5	9.9	23.3	9.9	1.2	34.4	28.7
13	44.2 ± 3.1	56.3 ± 3.4	30.5	6.8	24.3	6.8	1.2	32.3	21.0
14	49.6 ± 3.4	53.4 ± 2.6	26.9	11.9	19.8	12.0	1.3	33.1	36.0
15	60.0 ± 2.0	62.0 ± 3.0	27.8	12.4	20.9	12.4	1.5	34.8	35.7
16	46.8 ± 4.2	51.7 ± 2.4	26.2	12.7	18.3	12.7	1.2	32.2	39.4
17	55.1 ± 4.3	56.1 ± 3.1	32.2	8.7	25.4	8.7	0.8	35.0	25.0
18	47.9 ± 3.4	59.2 ± 2.2	26.0	9.0	16.1	8.8	0.9	25.7	35.1
19	45.9 ± 2.5	51.9 ± 2.9	25.8	10.5	18.1	10.5	0.6	29.1	35.9
20	47.0 ± 6.0	54.0 ± 4.0	27.0	12.3	19.0	12.3	0.6	31.8	38.7
21	43.0 ± 2.0	54.0 ± 2.0	29.3	11.7	23.1	11.7	0.5	35.3	33.2
22	38.0 ± 4.0	49.0 ± 4.0	23.3	15.8	14.9	15.8	1.1	31.8	49.5
23	41.0 ± 6.0	50.0 ± 3.0	24.3	15.7	17.9	15.7	1.8	35.4	44.2
24	54.0 ± 5.0	59.8 ± 4.0	36.9	5.2	30.5	5.2	1.3	37.0	14.0

In vitro data are expressed as g per serving containing 50 g of available CHO.

GI = glycemic index, II = insulinemic index, RAG = rapidly available glucose, SAG = slowly available glucose, RDS = rapidly digestible starch, SDS = slowly digestible starch, RS = resistant starch, TS = total starch.

lower postprandial glycemia and insulinemia. The major finding was that plain sweet biscuits as a group are characterised by having a low GI (63% of the sample was included in the 40 to 50 range) and moderate II (71% of the sample was included in the 50 to 60 range). In comparison to many other carbohydrate-containing foods, they have a high proportion of starch that is slowly digested and absorbed. Indeed, modern starchy foods tend to have higher GI values because the starch has been fully gelatinised and is therefore rapidly digested and absorbed [13,20]. Low fat starchy foods, in particular, including mashed potato (GI = 91), French baguette (GI = 95), Jasmine rice (GI = 109) and cornflakes (GI = 86), are digested quickly and have high GI values. However, even high fat starchy products (eg French fries, GI = 75) and high fiber breakfast cereals (eg shredded wheat, GI = 75) often have quite high GI values [13]. Thus the low GI of the plain biscuits cannot be explained simply by their fat or fiber content and associated effects on gastric emptying, but by the presence of starch which truly resists the action of alpha-amylases. In this form, starch not only reduces postprandial glycemia but also may exert sustained effect on satiety [22,23].

Our findings extend those of Englyst *et al.* [16] who studied 23 starchy cereal products (breakfast cereals, bakery products and crackers, and biscuits) but relatively few sweet biscuits. They reported that the biscuits group had the lowest GI values and the highest SAG content due to the presence of ungelatinised starch. They too found that the GI correlated positively with RAG ($r^2 = 0.54$) and negatively with SAG ($r^2 = 0.63$) and that these two factors explained more of the variability in GI than compositional factors such as the sugar, starch or fat content.

Importantly, we found a strong correlation between the GI and corresponding insulin response ($r = 0.76$, $p < 0.0001$). In general these two physiological measures of glucose metabolism have correlated well, showing correlation coefficients in the range of 0.70 to 0.88 [23–25,15]. Dairy products and chocolate-flavoured products, however, appear to be exceptions to the rule, tending to give significantly higher insulin responses than predicted by their GI [26,27]. Insulinotropic amino acids in milk proteins and free peptides in milk and chocolate are believed to be responsible but the clinical significance remains unclear.

In the case of plain sweet biscuits, the macronutrient composition of the biscuits explained relatively little of the observed variation in GI and II. Indeed higher fiber content, which might have been expected to lower the GI, was actually positively correlated. One explanation may be the fact that a dough high in fiber requires a high amount of water to be processed: this increases water availability for starch during cooking and therefore favours gelatinisation during baking.

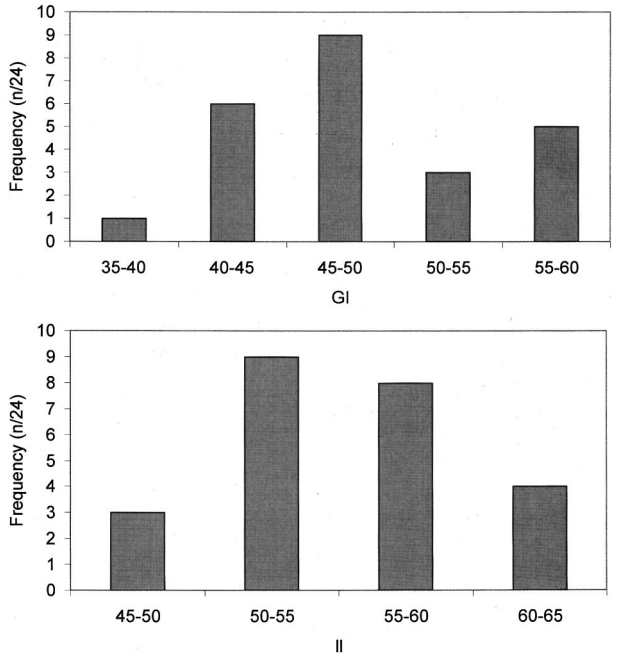

Fig. 1. Frequency histogram of GI (top, 1A) and II (bottom, 1B) in 24 plain sweet biscuits.

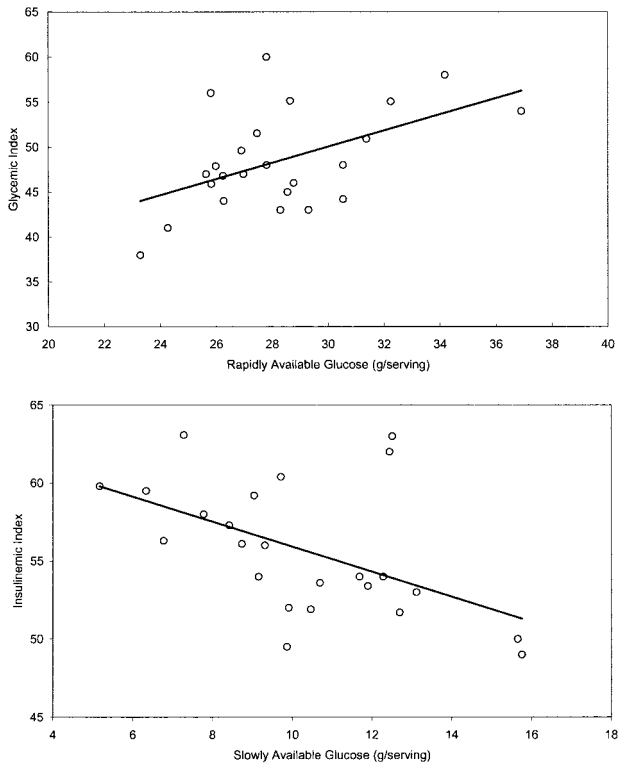

Fig. 2. Scatter plot between Glycemic Index and Rapidly Available Glucose (top, 2.A: $r = 0.504$, $p = 0.01$) and between Insulinemic Index and Slowly Available Glucose (bottom, 2.B: $r = -0.52$, $p < 0.01$). Linear trends represent linear regressions (2.A: $y = 0.9x + 23.1$; $R^2 = 0.25$; 2.B: $y = -0.8x + 63.9$; $R^2 = 0.27$).

One of our most important findings was that *in vitro* digestibility characteristics, specifically SAG and RAG, explained more of the variation in GI and II than the macronutrient composition of the plain biscuits. The inverse correlation between GI/II and SAG suggests that aiming for a high SAG content can be a strategy to lower the GI. Thus, processing conditions that decrease starch gelatinisation and therefore increase SAG need to be explored. Many parameters are currently known to affect the process of starch gelatinisation [28]. They include the processing parameters such as temperature, pressure and time, as well as factors that affect the physicochemical properties of the dough, such as water activity, fiber and kneading (mechanical manipulation). In addition, the nature of the raw ingredients will affect starch gelatinisation: the ratio of amylose to amylopectin, starch granule properties, degree of milling, type of wheat (soft/durum), and the level of damaged starch [17,29,30]. The amount and type of added sugars also has an important effect, because their ability to bind water reduces the amount of water available for starch gelatinisation [31]. Fat content has also been shown to lower the GI when present in high amounts, i.e. 40 g per serve but not low amounts (e.g. 15 g), as the case here [32]. In this study fat was correlated to GI, similarly as SAG correlation. This is in contrast with the results of a previous work that investigated a larger group of cereal products, exhibiting a wide range of GI, lipids and SAG [16] and where fat was less correlated to GI than SAG.

Plain biscuit-making involves the use of low-moisture doughs and short baking times and differs markedly from that of other cereal products. The low water activity availability reduces likelihood of swelling and gelatinisation of the starch granules, resulting in partially intact starch granules in the final product that are less susceptible to the action of amylolytic enzymes [33]. In plain sweet biscuits, the presence of sugars further limits water activity and thus the degree of starch gelatinisation [31]. Based on previous work, the high SAG levels of plain biscuits observed in the present work could be linked to the low extent of starch gelatinisation [16]. However, SAG content was even higher in the present study, averaging 32% of the total starch.

In contrast to plain biscuit manufacture, bread baking combines high moisture doughs, long resting periods and warm temperature conditions that lead to complete starch gelatinisation [33,34]. Similarly, the manufacture of many breakfast cereals and extruded food products incorporates high moisture conditions with mechanical shearing and very high temperature and pressure. If plain biscuits were to be manufactured under similar conditions, intact starch granules would likely disappear. Indeed, Englyst *et al.* [16] found that some biscuits had negligible quantities of SAG and a correspondingly high GI. These findings imply that if processing conditions are not carefully specified or controlled, even the process for plain biscuits will lead to high levels of fully gelatinised starch.

In conclusion, our findings indicate that it is possible to make low GI plain sweet biscuit products with the careful choice of ingredients and process parameters. Although further research is required, the slowly digested starch in plain biscuits may increase feelings of fullness and satiety, and reduce energy intake over the course of the day, as shown in other studies [23,35]. Attention to the overall nutritional profile of the products, including energy density, fiber, fat (quality and quantity) and micronutrient profile will still be important. Saturated fat and energy density could be decreased by replacing saturated fat with unsaturated fat, protein, starch, sugar and/or fiber, without compromising flavour and texture. Within a balanced diet, low GI plain sweet biscuits could therefore contribute to reducing the GI of the overall diet with consequent reduction in postprandial hyperglycemia and insulinemia.

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