

## Original Article

# Australian sweet lupin flour addition reduces the glycaemic index of a white bread breakfast without affecting palatability in healthy human volunteers

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The addition of some legume ingredients to bread has been associated with effects on glycaemic, insulinaemic and satiety responses that may be beneficial in controlling type 2 diabetes, cardiovascular disease and obesity. However, the effect of Australian sweet lupin (*Lupinus angustifolius*) flour (ASLF) is unknown. This investigation examined the effect of adding ASLF to standard white bread on post-meal glycaemic, insulinaemic and satiety responses and palatability in healthy subjects. Using a randomised, single-blind, cross-over design, 11 subjects consumed one breakfast of ASLF bread and two of standard white bread  $\geq 7$  days apart after fasting overnight. Each breakfast also included margarine, jam, and tea with milk and contained 50g available carbohydrate. On each test day, blood samples were taken after fasting, then several times over 2 hours post-prandially, and analysed for plasma glucose and serum insulin. Subjects rated breakfast palatability and perception of satiety, in the fasting state and over 3 hours post-prandially, after which food intake from an *ad libitum* buffet and for the rest of the day was recorded. Incremental areas under the curves for glucose, insulin and satiety, glycaemic index, insulinaemic index and satiety index were calculated. ASLF addition to the breakfast reduced its glycaemic index (mean  $\pm$  SEM; ASLF bread breakfast =  $74.0 \pm 9.6$ . Standard white bread breakfast = 100,  $P=0.022$ ), raised its insulinaemic index (ASLF bread breakfast =  $127.7 \pm 12.0$ . Standard white bread breakfast = 100,  $P=0.046$ ), but did not affect palatability, satiety or food intake. ASLF addition resulted in a palatable breakfast; however, the potential benefits of the lowered glycaemic index may be eclipsed by the increased insulinaemic index.

**Key Words:** lupin, glycaemic index, insulinaemic index, satiety, palatability, white bread.

## Introduction

Diabetes, cardiovascular disease (CVD) and obesity are highly prevalent in most Western countries, with diet exerting a significant role in the aetiology of these conditions.<sup>1-3</sup> Although the regular consumption of legumes is promoted by health authorities in most Western countries as a means of reducing the risks of such diseases,<sup>4-6</sup> consumption levels of legumes remain low due to the general perception that legumes are “the poor man’s meat”,<sup>6</sup> lack sensory appeal, are inconvenient to prepare and are associated with excessive flatulence.<sup>6,7</sup>

Legumes are often generalised as being low glycaemic index (GI) foods, with lentils and chickpeas being good examples of available carbohydrate-rich legumes with a low-GI;<sup>8</sup> however, other legumes such as soybeans and lupin contain very little available carbohydrate and therefore are not appropriately categorised as low-GI foods. It has been suggested that the consumption of a diet rich in high-GI foods (i.e. higher glycaemic load) increases the risk of type 2 diabetes, CVD and obesity;<sup>1, 9-11</sup> however, the clinical application of GI remains controversial for the prevention and treatment of these conditions.<sup>1,9,10,12</sup> Post-

meal studies have shown a direct correlation between GI and insulinaemic index (II) of foods,<sup>13,14</sup> and the resultant lowered II is thought to be of benefit in the prevention of type 2 diabetes and CVD.<sup>9</sup> There is also some suggestion that low-GI foods have a more satiating effect than higher-GI foods,<sup>15,16</sup> which may have implications for obesity control and prevention.<sup>9</sup>

Australian sweet lupin (*Lupinus angustifolius*) (ASL) is a legume grown in large quantities in Australia. It is considered underutilised as a human food source, being mostly used as animal feed.<sup>17</sup> Australian sweet lupin flour (ASLF) is an ingredient produced by finely milling the split dehulled kernels of ASL. ASLF is noted for being pale yellow in colour and is reported as being slightly beany in flavour;<sup>17,18</sup> however, the incorporation of up to 10% of ASLF in bread products has been shown to be palatable in

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consumer sensory trials.<sup>19</sup>

ASLF is rich in protein and dietary fibre and contains a wealth of phytochemicals such as oligosaccharides,<sup>20</sup> phytic acid,<sup>18</sup> tannins<sup>21</sup> and saponins,<sup>22</sup> which may act as hypoglycaemic agents<sup>17,23-25</sup> when combined in available carbohydrate-rich foods such as bread. A purified dietary fibre ingredient extracted from the kernels of ASL has been shown to have a beneficial effect on post-meal insulinaemic response when added to bread<sup>26</sup> and to increase post-meal satiety response when used as a fat-replacer in sausage patties.<sup>27</sup>

Since little or no information on the post-meal effects of ASLF is available, the aim of the present study was to examine the effect of ASLF addition to standard white bread on glycaemic, insulinaemic and satiety responses and on palatability in the post-meal setting in healthy human volunteers.

## Materials and methods

### Subjects

Eleven healthy subjects (nine male and two female) were recruited through posted notices and direct personal communication in Melbourne, Australia. After giving informed signed consent, volunteers were screened for suitability using a health questionnaire. Exclusion criteria were: cigarette smoking; pregnancy; allergy to any food ingredients used in the study or to legumes such as soy and peanuts; history of cardiovascular disease; diabetes or gastrointestinal disease; use of medications known to affect dependent variables of the study; excessive consumption of alcohol; and not being regular breakfast eaters. Subjects' mean age  $\pm$  SEM was  $31.6 \pm 1.8$  y (range 25–45 y) and mean body mass index  $\pm$  SEM was  $24.7 \pm 0.8$  kg/m<sup>2</sup> (range 20.9–28.6 kg/m<sup>2</sup>). The study was approved by the Deakin University Ethics Committee and complied with the Helsinki Declaration of 1975, as revised in 2000.

### Experimental design and products

Each subject consumed an ASLF bread breakfast once and a standard white bread breakfast twice. Breakfasts were consumed on different mornings, at least 7 days apart, after an overnight fast of 10–12 h. Subjects were blinded to which test breakfast they were receiving and breakfasts were randomised to counterbalance any order effect. To avoid the “second meal effect”,<sup>15</sup> subjects were asked to adhere to a list of high-GI foods for the evening meal prior to the tests and no food or drink except water was permitted after the evening meal. Subjects were requested to abstain from alcohol consumption and excessive levels of exercise on the day prior to each test day.

On each test day, upon arrival at the laboratory, subjects were required to rate their perception of satiety and have an indwelling catheter inserted into a vein in the cubital fossa through which a 5 mL fasting blood sample was collected. The catheter was kept patent with sterile 0.9% saline solution. Subjects were given a 50 mL glass of water to drink in its entirety in order to cleanse their palate and then consumed a test breakfast within 10 min, during which time they were required to rate its palatability. Subjects were again required to rate their

perception of satiety at 10, 25, 40, 55, 85, 115 and 175 min after the beginning of the test breakfast. Blood samples were collected through the in-dwelling catheter at 15, 30, 45, 60, 90 and 120 min after breakfast began. No other food or drink was allowed to be consumed until 175 min after the beginning of the test breakfast when a set menu buffet, containing a range of lunch and snack options, was served to the subjects and a weighed record of the *ad libitum* food and drink consumed was undertaken. After leaving the laboratory, subjects were required to keep a weighed food record of all food and drink consumed for the remainder of the day.

The ASLF derived from *Lupinus angustifolius* (cv. Merritt) was supplied by Food Science Australia (Werribee, Victoria, Australia). Wheat flour (unbleached bakers flour) was sourced from Laucke Mills (Strathalbyn, South Australia, Australia). The compositions of both the ASLF and the wheat flour as provided by the suppliers are given in (Table 1). The ASLF ingredient was considerably higher in protein, dietary fibre and fat levels when compared with the wheat flour ingredient; however, the wheat flour contained an extra ~68 g/100 g of available carbohydrate. The ASLF and standard white breads were manufactured at a semi-commercial scale at the Golden Bread Basket (Morwell, Victoria, Australia). An ASLF incorporation rate of 10% wheat flour replacement in standard white bread was selected based on earlier consumer sensory studies conducted by our laboratory<sup>19</sup> that indicated a palatable laboratory-scale product could be made at this level without the need for additional gluten. A standard recipe white bread acted as the control. The ASLF bread was made by adding ASLF and additional water (to compensate for the higher water binding ability of the ASLF) to the standard white bread. The standard white bread was prepared according to the following recipe: 375g fresh yeast, 100g “improver”, 200g salt, 300g canola oil, 10.2kg bakers' white wheat flour and 5.3kg water. The ASLF bread recipe included 1.0kg ASLF (displacing 1.0kg of bakers' white wheat flour) and 700g extra water. Both the ASLF bread and the standard white bread were manufactured under similar conditions. The nutrient composition of the experimental bread was directly analysed using standard procedures based on the AOAC methods.<sup>28</sup>

**Table 1.** Compositional data on wheat flour and ASLF<sup>a</sup> ingredients

	Wheat Flour	ASLF <sup>a</sup>
Energy (kJ/100g) <sup>b</sup>	1416	981
Protein (g/100g) <sup>c</sup>	11.9	41.8
Available carbohydrate (g/100g) <sup>c</sup>	68.8	0.9
Total dietary fibre (g/100g) <sup>c</sup>	2.7	41.5
Soluble dietary fibre (g/100g) <sup>d</sup>	1.1 <sup>4</sup>	11.0
Insoluble dietary fibre (g/100g) <sup>d</sup>	1.6 <sup>4</sup>	30.5
Fat (g/100g) <sup>c</sup>	1.2	6.9

<sup>a</sup>Australian sweet lupin (*Lupinus angustifolius*) flour; <sup>b</sup>Calculated using Atwater factors; <sup>c</sup>Analysis based on the methods of the AOAC;<sup>28</sup> <sup>d</sup>Calculated based on proportions of soluble/insoluble dietary fibre in regular wheat flour.<sup>29</sup>

Each of the breakfasts consisted of 3 – 4 slices of toasted ASLF or standard white bread, spread with 6g low-fat margarine and 20g low-joule apricot spread. In addition, a cup of decaffeinated tea with 30g skim milk was provided. The ASLF bread breakfast contained approximately 7.7g of the ASLF ingredient. The composition of the test breakfasts were calculated using FoodWorks version 3.01, build 472 (Xyris Soft-ware, Brisbane, Australia) using AusNut database (All Foods, Rev. 14, Food Standards Australia New Zealand, Canberra, Australia). The database was supplemented with the direct analysis of the experimental foods and manufacturers' information for foods not found on the database. The ingredients used in both of the test breakfasts and their compositional profiles are given in Table 2. The two test breakfasts contained equal amounts of available carbohydrate. However, due to the differences in composition of the ASLF and wheat flour, the ASLF breakfast was higher in protein, fat and total dietary fibre and therefore also higher in energy than the standard white bread breakfast. The study therefore investigated the overall effect of the addition of ASLF to bread and could not distinguish the effect of single nutrient components. All breakfasts were adjusted to an equal weight by adjusting the amount of water in the tea in order to remove the potential for the weight and volume of the test breakfast to influence satiety.<sup>30</sup>

**Table 2.** Ingredients and macronutrients contents of standard white bread and ASLF<sup>a</sup> bread breakfasts

	Standard white bread breakfast <sup>b</sup>	ASLF <sup>a</sup> bread breakfast <sup>c</sup>
Ingredients		
Bread (g)	98	115
Margarine (g)	6	6
Jam (Low Joule) (g)	20	20
Milk (Skim) (g)	30	30
Tea (g)	197	180
Water (g)	50	50
Total weight (g)	401	401
Macronutrients/Energy		
Energy (kJ) <sup>d</sup>	1243	1338
Protein <sup>e</sup>	9.2	12.8
Fat <sup>e</sup>	6.4	7.3
Total dietary fibre <sup>e</sup>	2.8	4.9
Available carbohydrate <sup>e</sup>	50.0	50.0

<sup>a</sup>Australian sweet lupin (*Lupinus angustifolius*) flour; <sup>b</sup>Containing 0g ASLF; <sup>c</sup>Containing ~ 7.7 g ASLF; <sup>d</sup>Calculated using Atwater factors; <sup>e</sup>Analysis based on the methods of the AOAC.<sup>28</sup>

### Blood samples

Blood was collected into both fluoride ethylenediamine tetraacetic acid (FI-EDTA) tubes and plain tubes. FI-EDTA tubes were centrifuged immediately at 1260 g for 20 min at 4 °C and the resulting plasma stored at –70 °C until analysed. Plain tubes were allowed to coagulate for 1 h at ~20 °C prior to centrifugation at 1260 g for 20 min at 4 °C, after which the resulting serum was stored at –70 °C until analysed.

### Glucose and Insulin

Plasma glucose was measured by enzymatic colorimetric methods using diagnostic kits (Roche, Mannheim, Germany) on a Hitachi 704 autoanalyser (Tokyo, Japan). Accuracy and precision of plasma glucose analyses was confirmed against quality control standards Precinorm U and Precipath U (Roche, Mannheim, Germany). Serum insulin was measured in duplicate by radioimmunoassay using a Linco Human Insulin Specific RIA Kit (Linco Research Inc., MO, USA).

### Perceptions of satiety

Perceptions of satiety were rated using a 15 cm structured graphical scale marked with a far left anchor of “Extremely hungry” followed by anchors 2.5 cm apart of “Hungry”, “Semi-hungry”, “No particular feeling”, “Semi-satisfied”, “Satisfied” and “Extremely satisfied”.<sup>31</sup> Subjects were asked to mark a position anywhere along the scale that matched their perception of satiety. Subjects' ratings were converted to a numerical score based on distance in millimetres from the far left anchor of the scale. The perception of satiety scores for both the standard white and the ASLF bread breakfasts were both adjusted and presented per 1000 kJ, as suggested by Holt and co-workers.<sup>31</sup>

### Glycaemic, insulinaemic and satiety calculations

Incremental glycaemic, insulinaemic and satiety scores for each breakfast were calculated by subtracting each subject's fasting scores from their scores at each post-breakfast time point. Incremental glycaemic, insulinaemic and satiety responses for each breakfast were calculated as each subject's area under the curve of incremental score versus time from the start of breakfast using above-baseline trapezoidal calculation.<sup>32</sup> The GI of the ASLF bread was calculated as the incremental glycaemic response after the ASLF bread breakfast expressed as a percentage of the same subject's incremental glycaemic response after the standard white bread breakfast. The individual subject's resulting GI values were then averaged. The II and SI of the ASLF bread were calculated in the same manner.

### Sensory analyses

Appearance, flavour, texture (in mouth) and general acceptability of the ASLF and the standard white bread breakfasts were rated using a 15 cm structured, graphical, hedonic scale marked with a far left anchor of “Extremely unacceptable” followed by anchors 2.5 cm apart of “Very unacceptable”, “Unacceptable”, “Neither acceptable or unacceptable”, “Acceptable”, “Very acceptable” and “Extremely acceptable”.<sup>19</sup> Subjects were asked to mark a position anywhere along the scale that matched their perception. Subjects' ratings were converted to a numerical score based on distance in millimetres from the far left anchor of the scale.

### Post-meal food intake

Weighed food records were completed by subjects to determine the energy consumed during both the *ad libitum*

buffet and the rest of each test day. Subjects received both verbal and written instructions on how to accurately weigh and record all food and drink consumed. Weighed food records were analysed using FoodWorks version 3.01, build 472 (Xyris Software, Brisbane, Australia) using AusNut database (All Foods, Rev. 14, Food Standards Australia New Zealand, Canberra, Australia). The database was supplemented with manufacturers' information for foods not found on the database.

### Statistical analyses

Normality of variables was evaluated using Kolmogorov-Smirnov tests. All data from the two standard white bread breakfasts were averaged. A paired samples t-test, or 2-related samples non-parametric test for non-normal data was used to compare the standard white bread and ASLF breakfasts for incremental glycaemic, insulinaemic and satiety responses, post-meal food intake during the *ad libitum* buffet and the rest of the test day and the palatability parameters. A one sample t-test was used to compare the GI, II and SI for the ASLF bread breakfast compared to the standard white bread breakfast (GI, II and SI = 100). All statistical analyses were conducted using SPSS software, Release 11.5 (SPSS Inc, Chicago, IL, USA). In all analyses,  $P < 0.05$  was considered significant. Data are expressed as means  $\pm$  SEMs.

## Results

### Plasma glucose response to test breakfasts

The incremental glycaemic response to the standard white bread and ASLF bread breakfasts is shown in (Fig. 1). The peak incremental glycaemic concentration for both the standard white bread and ASLF bread breakfasts was seen at 30 min after the start of breakfasts. At this time point there was a trend ( $P = 0.068$ ) towards a higher incremental glucose concentration after the standard white bread breakfast compared to the ASLF bread breakfast. The mean incremental glucose concentration fell below zero (baseline) value at 75 min after the start of the standard white bread breakfast and ~55 min after the start of the ASLF breakfast. The GI (mean  $\pm$  SEM) of the ASLF bread breakfast was  $74.0 \pm 9.6$ , which was significantly lower ( $P = 0.022$ ) than that of the standard white bread breakfast (GI = 100). The GI of the ASLF bread breakfast is 52 when recalculated using glucose as the standard.<sup>8</sup>

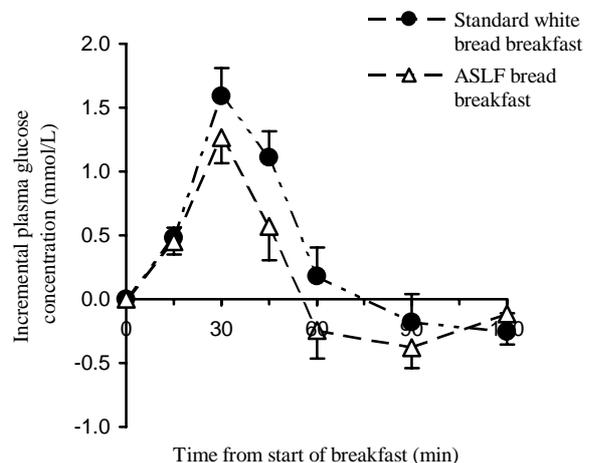
### Serum insulin response to test breakfasts

The incremental insulinaemic response to the standard white bread and ASLF bread breakfasts is shown in (Fig. 2). The peak incremental insulinaemic concentration for both the standard white bread and ASLF bread breakfasts was seen at 30 min after the start of the breakfasts. At this time point the mean incremental insulin concentration after the ASLF bread breakfast was a higher value than after the standard white bread breakfast but the difference did not reach statistical significance ( $P > 0.05$ ). Unlike the incremental glucose levels, the incremental insulin levels did not return to zero (baseline) within the 120 min post-meal test period. The II (mean  $\pm$  SEM) of the ASLF bread breakfast was  $127.7 \pm 12.0$ , which was significantly

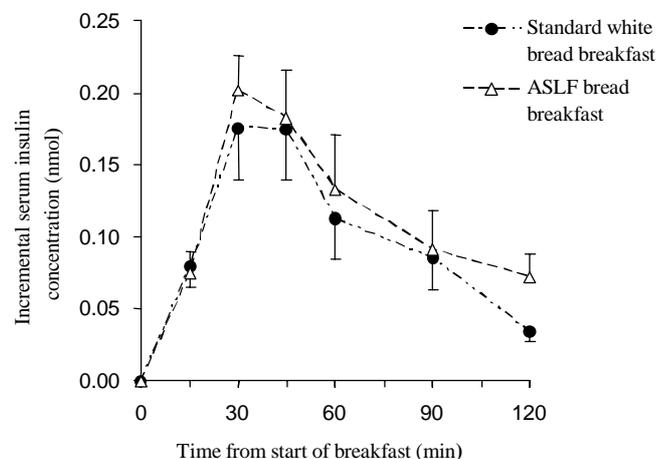
higher ( $P = 0.046$ ) than that of the standard white bread breakfast (II = 100).

### Perception of satiety response to the test breakfasts

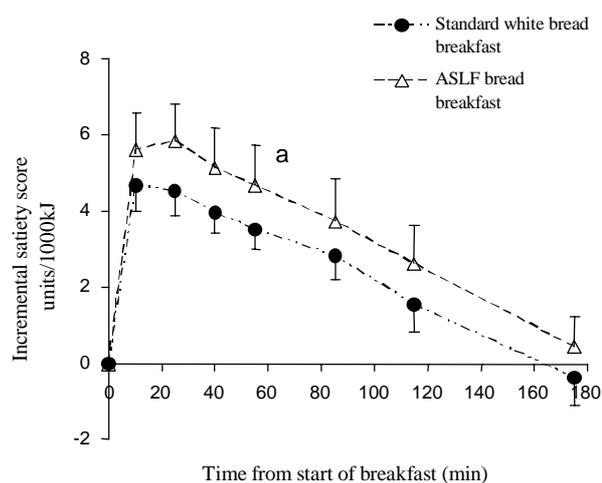
The incremental perception of satiety response (per 1000 kJ) to the standard white bread and ASLF bread breakfasts is shown in (Fig. 3). The peak incremental satiety score for the standard white bread breakfast was seen at 10 min after the start of the breakfast, while for the ASLF bread breakfast it was seen at 25 min. At 25 minutes, there was a trend ( $P = 0.066$ ) towards a higher incremental perception of satiety score after the ASLF bread breakfast than the standard white bread breakfast. The mean incremental perception of satiety score for the standard white bread breakfast fell below zero (baseline) after approximately 160 min, whereas the ASLF breakfast perception of satiety response did not reach zero within the 175 min post-meal period. The SI (mean  $\pm$  SEM) of the ASLF bread breakfast was  $134 \pm 29$  per 1000kJ, which was not significantly different ( $P = 0.259$ ) to that of the standard white bread breakfast (SI=100 per 1000kJ).



**Figure 1.** Incremental glycaemic response (mean  $\pm$  SEM) to standard white bread and Australian sweet lupin (*Lupinus angustifolius*) flour (ASLF) bread breakfasts ( $N = 11$ ).



**Figure 2.** Incremental insulinaemic response (mean  $\pm$  SEM) to standard white bread and Australian sweet lupin (*Lupinus angustifolius*) flour (ASLF) bread breakfasts ( $N = 11$ ).



**Figure 3.** Incremental satiety response adjusted for energy (mean  $\pm$  SEM) to standard white bread and Australian sweet lupin (*Lupinus angustifolius*) flour (ASLF) bread breakfasts ( $N = 11$ ).

**Table 3.** Sensory acceptability ratings (mean  $\pm$  SEM) of standard white bread and ASLF<sup>a</sup> bread breakfasts<sup>b</sup>

	Standard white bread breakfast	ASLF <sup>a</sup> bread breakfast
Sensory parameters		
Appearance	10.70 $\pm$ 0.27	10.93 $\pm$ 0.36
Flavour	11.46 $\pm$ 0.42	11.79 $\pm$ 0.29
Texture (in mouth)	10.92 $\pm$ 0.43	11.23 $\pm$ 0.44
General acceptability	11.42 $\pm$ 0.37	11.63 $\pm$ 0.36

<sup>a</sup>Australian sweet lupin (*Lupinus angustifolius*) flour; <sup>b</sup>there were no significant differences between the breads for any of the sensory parameters.

### Post-meal food intake

The energy consumed during the *ad libitum* buffet directly after the post-meal period (mean  $\pm$  SEM) for the standard white bread and ASLF bread breakfasts was 4340  $\pm$  360 kJ and 4550  $\pm$  550 kJ, respectively, which did not differ significantly ( $P = 0.584$ ). The energy consumed during the rest of the day after the *ad libitum* buffet (mean  $\pm$  SEM) for the standard white bread and ASLF bread breakfasts was 6231  $\pm$  636 kJ and 5427  $\pm$  573 kJ, respectively, which again did not differ significantly ( $P = 0.309$ ).

### Palatability of test breakfasts

The results of the sensory evaluation of the two test breakfasts are presented in (Table 3). There were no significant differences between the two breakfasts in terms of acceptability of appearance, flavour, texture (in mouth) and general acceptability. Both breakfasts were rated above 10 (representing "Acceptable" on the line scale) for all sensory parameters.

### Discussion

Lupins are a highly valued animal feed but have been underutilised as a human food despite being a rich source of protein, dietary fibre and a range of other potentially beneficial phytochemicals. Lupins are now receiving

international interest as a future source of food ingredients<sup>33,34</sup> that could be used to enhance the nutritional profile of existing food products.

In the current study, the addition of the 7.7 g of ASLF to the breakfast resulted in a glycaemic index that was lowered to a value comparable to those of some fibre enriched breads.<sup>8</sup> In fact the ASLF bread breakfast would be considered "low-GI".<sup>35</sup> The reduced GI of the ASLF bread may have been a result of its higher protein content stimulating a higher insulin response. The reduced GI of the ASLF bread could also have been due, in part, to the actions of the various phytochemicals and the dietary fibre found in ASLF, which possibly slowed the starch digestion and glucose absorption processes.<sup>36-38</sup> A modified crumb structure of the ASLF bread product as a result of the high water binding properties of its constituent dietary fibre<sup>39</sup> may also have influenced starch digestibility. Other potentially hypoglycaemic phytochemicals such as oligosaccharides, phytic acid, tannins and saponins may have played some role in the reduced GI; however, their levels were not determined and therefore the extent of their effect is unknown.<sup>17,23-25</sup> Further investigation is required to establish which components within ASLF contributed to the reduced GI of the ASLF bread breakfast and by what mechanisms they acted.

The addition of the ASLF ingredient resulted in an increased II for the ASLF bread breakfast, which is not consistent with the effect found with some other legume ingredients such as *Deterarium senegalense* flour and guar gum, which, when added to food, have resulted in reduced glycaemic and insulinaemic responses.<sup>37,38,40</sup> This result in the present study also contrasts the results of a previous study that demonstrated a beneficial hypo-insulinaemic effect of ASL kernel fibre when added to bread,<sup>26</sup> although the fibre dose was lower in the present study. Proteins are known regulators of insulinaemic response, both in the presence of a carbohydrate load and independent of glucose.<sup>41</sup> The higher II may partly be explained by the fact that the ASLF breakfast contained an additional 3.6g of protein. However, higher levels of protein may be required to significantly increase insulin secretion, though the amount may depend on the type of protein present. Protein from peanut butter (a legume product) for instance, has demonstrated a greater effect on glycaemic response than protein from cheese.<sup>42</sup> Brand-Miller and co-workers<sup>43</sup> have shown that another legume ingredient, cocoa (*Theobroma cacao*) powder, also stimulates insulin secretion. This insulinogenic effect has been partly attributed to specific amino acids, including arginine, found at high levels in cocoa.<sup>44</sup> ASL protein is rich in arginine, which makes up  $\sim$ 12% of the total amino acid content,<sup>18</sup> and therefore this amino acid may have contributed to the raised II of the ASLF bread breakfast. Calbet and MacLean have determined that the combined administration of glucose and peptide hydrolysates of both the pea (legume) and whey protein stimulate a synergistic release of insulin.<sup>45</sup> Their results suggest that the insulin response was mainly determined by the plasma concentration of both phenylalanine and glucose. Phenylalanine makes up  $\sim$ 4% of ASL protein<sup>18</sup> and therefore may also have contributed to the raised II of the ASLF bread breakfast. Another suggested stimulant of insulin

secretion is stearic acid,<sup>46</sup> which makes up ~4% of ASLF oil content<sup>18</sup> and therefore may partially contribute to the insulinaemic effect. Despite these possible explanations, further research is needed to confirm the hyperinsulinaemic effect of ASLF observed in the present study and to establish the mechanism leading to this effect.

A plausible explanation for the reduced GI of the ASLF bread breakfast could be the effect of glucose-independent stimulation of insulin secretion leading to greater clearance of blood glucose and therefore lowered GI. This proposed mechanism is supported by the findings that protein induced-hyperinsulinaemia stimulated by pea and whey peptide hydrolysates can lead to mild hypoglycaemia.<sup>45</sup>

The lack of effect of ASLF addition on satiety and energy intake in the present study contrasts with previous findings that the addition of lupin kernel fibre to sausage patties increased perception of satiety in men<sup>27</sup> and whole lupin kernels reduced feed consumption in pigs.<sup>47</sup> In addition, dry beans have been shown to increase cholecystokinin (a biochemical marker of satiety) postprandially.<sup>48</sup> The small number of subjects in the present study may not have provided sufficient statistical power to detect differences in satiety response. Since there are inconsistencies in the outcomes of studies investigating the satiating effect of lupin, further studies focussing specifically on satiation power of ASLF appear warranted.

The palatability of the ASLF bread breakfast compared favourably with that of the standard white bread breakfast. All palatability parameters for the ASLF bread exceeded the "Acceptable" point on the line scale and surpassed the score recorded for laboratory-manufactured bread with a similar ASLF incorporation rate in an earlier study in which the bread was served alone and untoasted.<sup>19</sup> In the present study, toasting and assessment of palatability as part of a more "real-life" breakfast may have disguised the noted bitter taste detected by 17% of the panellists in the earlier study.<sup>19</sup> In addition, the semi-commercial scale manufacturing of the product in the present study could have resulted in improved palatability.

The addition of ASLF to standard white bread when included as part of a breakfast did not reduce breakfast palatability or effect satiety and food intake, but did lower the GI and raise the II of the breakfast. The interesting but contrasting effects on GI and II of incorporating ASLF into a breakfast do not allow unequivocal conclusions of any potential health benefits of ASLF to be made. Longer-term studies are now required to further investigate the effect of ASLF in the human diet on risk factors for diabetes, CVD and obesity.

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