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Slow digestible starch in native pea starch (*Pisum sativum* L.) lowers glycaemic response with no adverse effects on gastrointestinal symptoms in healthy adults

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5 no adverse effects on gastrointestinal symptoms in healthy adults
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26 27 28 **1. Introduction** 29

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31 Since 1990, there has been a dramatic increase in the global prevalence of diabetes rising from
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33 211 million in 1990 to 476 million in 2017. By 2045, the prevalence is predicted to rise to 700
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35 million. Most of the cases nowadays are related to Type 2 diabetes as opposed to Type 1
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37 Diabetes which was the predominant case during the 1980's¹. Type 2 diabetes is now one of
38
39 the largest global public health concerns as it affects the health and the socio-economic
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41 development of millions of people. In 2019, the International Diabetes Federation (IDF)
42
43 estimated that total diabetes-related health expenditure will reach USD 760 billion. The United
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45 States of America is among countries with the highest expenditure with USD 9505 per person
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47 affected by diabetes per year². Although there are several factors that influence the development
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49 of type 2 diabetes, including both genetic and environmental, it is evident that the most
50
51 influential effects are through lifestyle and behavioural influences commonly associated
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53 with urbanization. These include consumption of unhealthy foods and inactive lifestyles
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55 alongside sedentary behaviour. Overconsumption of high fat, high sugar, hypercaloric diets and
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3 alcohol are the predominant dietary factors that affect the onset and development of Type 2
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5 Diabetes³.
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10 In this context, high glycaemic index (GI) carbohydrate consumption becomes problematic
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12 when they are rapidly digested in the small intestine. As a consequence, the post prandial
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14 glycaemia increases very high and very fast inducing a strong pancreatic insulin response that
15
16 when repeated over multiple meals, days, and years contributes to the onset of glucose
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18 intolerance, followed by insulin resistance and the associated increase in circulating insulin
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20 levels and finally the onset of type 2 diabetes. As carbohydrates are the main contributors to the
21
22 daily energy requirements of a balanced diet⁴, reducing their glycaemic impact ought to be
23
24 considered as a way of preventing and reducing prevalence of type 2 diabetes. High glycaemic
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26 impact caused by high GI carbohydrates or high GI foods are unhealthy by inducing an
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28 exaggerated insulin response followed by delayed hypoglycemia and reactive increases in free
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30 fatty acids (FFAs), which increase triglycerides and low-density lipoprotein-cholesterol while
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32 decreasing high-density lipoprotein (HDL)-cholesterol. The greater insulin responses may
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34 increase body fat and weight and lead to insulin resistance and to exhaustion of endocrine
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36 pancreatic function and insulin release. Predicting the glycaemic response and the insulin
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38 response of carbohydrate ingredients and foods is therefore of interest in the context of
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40 preventing Type 2 diabetes^{5,6}.
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49 In 1992, Englyst proposed a new carbohydrate classification according to their *in vitro*
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51 digestibility. The *in vitro* methodology allowed researchers to classify carbohydrates, especially
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53 starch, into three categories according to their ability to deliver glucose through the action of
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55 digestive enzymes: rapidly digestible starch (RDS), slow digestible starch (SDS) and resistant
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57 starch (RS) fractions⁷. The repeatability and reproducibility of the methodology were tested and
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3 the authors concluded that the SDS fraction content measured could identify slow release
4 carbohydrates and predict their slow and low glycaemic impact⁸⁻¹⁰. There is a strong inverse
5 correlation reported between the SDS content and the glycaemic response of foods¹¹. The
6 Englyst methodology thus serves to screen new food ingredients especially carbohydrates and
7 carbohydrate-rich food recipes in order to introduce to markets food innovations that can
8 prevent type 2 diabetes or be useful solutions for individuals with type 2 diabetes¹².
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10
11 Pea (*Pisum sativum L.*) is one of the most widespread and most cultivated annual legume crops
12 in temperate regions as it is an excellent source of protein (15-30% Dry Matter), starch (50%
13 Dry Matter) and micronutrients (zinc, iron and vitamins). Its richness in amylose - between 20.7
14 to 33.7% of the total starch - makes the pea starch rich in the slow digestible starch fraction and
15 contributes to a low impact on the post prandial glycaemia^{13,14}.
16

17
18 Therefore, this study aimed to assess the composition of pea starch and a recipe based on pea
19 starch to ascertain the different Englyst starch fractions, to measure the starch fractions content
20 and to correlate it with the effect on glycaemic index and gastrointestinal symptoms in healthy
21 individuals. In order to determine if pea starch can be qualified as a good source of slow
22 digestible carbohydrates, and its concomitant potential role in the prevention of type 2 diabetes,
23 a rapidly digesting carbohydrate maltodextrin and a recipe based on maltodextrin were used for
24 comparison.
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50 **2. Materials and Methods**

51 **2.1 Carbohydrates**

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3 Native pea starch (Pea Starch LN30®), Anhydrous glucose and Maltodextrin Glucidex®IT19
4 were processed by Roquette Frères (Lestrem, France). Maltodextrin IT19 is corn-based,
5
6 obtained by enzymatic process (Mean dextrose equivalent:18.4, average Mw: 9300).
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10 11 12 13 **2.2 Powder mix recipes**

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15 The composition and nutrition information for the pea starch-based recipe and the maltodextrin-
16 based recipe are given Tables 1 and 2.
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25 To prepare the powder mix recipes, all the ingredients were blended in a TURBULA® Blender
26 for 5 minutes. To prepare the pea starch-based final beverage, 46.7 g of the recipe was weighed
27 and combined with 230 ml of water in a shaker followed by shaking for 30 seconds. To prepare
28 the maltodextrin-based final beverage: 39.7 g of the recipe was weighed and added with 230
29 ml of water in a shaker followed by shaking for 30 seconds
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40 **2.3 *In vitro* testing by Englyst method**

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42 The starch profile of pea starch LN30® and Glucidex®IT19 maltodextrin was determined
43 according to an adaptation of the Englyst methodology⁷, with slight modifications. This
44 procedure allows to measure different fractions of starch: RDS (Rapidly Digestible Starch),
45 SDS (Slowly Digestible Starch) and RS (Resistant Starch) following enzymatic digestion.
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50 To prepare the enzyme solution I, 10 g of porcine pancreatin α -amylase (P7545 Sigma-Aldrich)
51 was suspended into 80 mL of osmotic water with magnetic stirring and then centrifuged for 10
52 minutes at 1500 x g. Enzyme solution II contained 3.7 mL of amyloglucosidase solution (A7095,
53 Sigma-Aldrich) and 4.3 mL osmotic water. Enzyme solution III was composed of 50 mg of
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3 invertase (I-4504, Sigma-Aldrich) mixed into 6mL of osmotic water for 10 minutes. The
4 enzymatic cocktail was obtained by mixing 54 mL of Enzyme solution I with 6 mL of Enzyme
5 solution II and 4 mL of Enzyme solution III.
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12 800 mg of starch sample was dissolved in 20 mL of rehydrated guar-gum acetate buffer solution
13 (2.5 g/L of guar gum, 0.1M of sodium acetate, pH 5.2, 4 mM CaCl₂) at 37°C under agitation.
14 After addition of 5 mL of enzymatic cocktail, the solution was incubated for 120 minutes at
15 37°C. Aliquots of the samples were taken at 0min, 20 min and 120min, added into ethanol
16 solution (66%) and centrifuged at 1500 g for 3 minutes. Glucose was measured within the
17 supernatant using glucose oxidase/peroxidase reagent (GOPOD). The results were expressed as
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26 RDS = (Glucose at 20 minutes – Glucose at 0 minutes) x 0.9; SDS = (Glucose at 120 minutes
27 – Glucose at 20 minutes) x 0.9 and RS = (Total starch – Glucose at 0 minutes) x 0.9)-
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31 (RDS+SDS).
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37 **2.4. *In vivo* Glycaemic index testing**

38 39 **2.4.1 Study design**

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42 A non-blind, repeat measure, crossover design trial was used to study the glycaemic index of
43 Pea Starch LN30®, Glucidex®IT19 Maltodextrin, a pea starch powder mix recipe and a
44 maltodextrin powder mix recipe against a reference of glucose. The participants acted as their
45 own controls and they were randomly assigned to test each product against the reference
46 glucose using a pseudo-random number generator [15]. The trial was conducted by the Oxford
47 Brookes Centre for Nutrition and Health at Oxford Brookes University, UK. Ethical approval
48 for the study was obtained from the University Research Ethics Committee (UREC) at Oxford
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3 Brookes University (UREC Registration No: 140806). All participants gave written informed
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5 consent prior to participation.
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8 **2.4.2 Participant recruitment and screening**

10 Recruitment of participants was made through announcements in lectures, posters distributed
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12 on notice boards throughout Oxford Brookes University and through personal networks.
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14 Sixteen healthy participants (seven male, nine female) were recruited from the staff and students
15
16 from Oxford Brookes University. Prior to recruitment, all potential participants completed a
17
18 brief health questionnaire and they were excluded from the study if they met any of the
19
20 following criteria: aged < 18 or > 60 years, pregnant or lactating, body mass index (BMI) ≥ 30
21
22 kg/m^2 , fasting blood glucose value > 6.1 mmol/l, known food allergy or intolerance, medical
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24 condition(s) or medication(s) known to affect glucose regulation or appetite and/or which
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26 influence digestion and absorption of nutrients, known history of diabetes mellitus or the use
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28 of antihyperglycaemic drugs or insulin to treat diabetes and related conditions, major medical
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30 or surgical event requiring hospitalization within the preceding 3 months, use of steroids,
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32 protease inhibitors or antipsychotics (all of which have major effects on glucose metabolism
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34 and body fat distribution). In addition, participants were excluded if they were unable to comply
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36 with experimental procedures or did not follow GI testing guidelines.
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44 **2.4.3 Anthropometric measurements**

46 Anthropometric measurements were made in the fasting state during the first session. Height
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48 was recorded to the nearest centimetre using a stadiometer (Seca Ltd, UK), with participants
49
50 standing erect with head positioned in the Frankfurt plane and without shoes. Body weight was
51
52 recorded to the nearest 0.1 kg, with participants wearing light clothing and no shoes. Body
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54 mass index (BMI) was calculated using the standard formula: $\text{weight (kg)/height (m)}^2$. Body
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3 fat percentage was measured using a body composition analyser (Tanita BC-418 MA; Tanita
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5 UK Ltd).

6 7 8 **2.4.4. Test foods**

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10 The Pea Starch LN30® (32.66 g), Glucidex® IT19 Maltodextrin (26.32g), the Pea Starch
11
12 LN30® powder mix recipe (46.7g), the Glucidex® IT19 Maltodextrin recipe (39.7g) and the
13
14 reference food (glucose-monohydrate 27.48g) were tested in equivalent carbohydrate amounts
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16 (25 g). All reference and test samples were mixed and served with 250 ml of water.
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20 21 **2.4.5 Protocol**

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23 The protocol used was carried out in accordance with ISO standards [16]. On the day prior to
24
25 the test, participants were asked to restrict their intake of alcohol and caffeine-containing drinks
26
27 and to restrict their participation in intense physical activity (e.g. long periods at the gym,
28
29 excessive swimming, running, aerobics). Participants were also told not to eat or drink after 9
30
31 pm the night before a test, although water was allowed, in moderation. The Pea Starch LN30®,
32
33 Glucidex®IT19 Maltodextrin and the powder mix samples were tested once in random order
34
35 on separate days and the reference food was tested three times over the study on separate days,
36
37 with at least a one-day gap between measurements to minimise carry over effects. Participants
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39 were studied in the morning before 10 am after a 12-hour overnight fast. They consumed the
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41 reference food/test samples at a comfortable pace, within 15 minutes and remained sedentary
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43 during each session.
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48 49 *Blood glucose measurements*

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51 Blood samples were taken at -5 min and 0 min before consumption of the reference food/test
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53 samples and the baseline value taken as a mean of these two values. The reference food/test
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55 samples were consumed immediately after this and further blood samples were taken at 15, 30,
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57 45, 60, 90, 120, 150 and 180 minutes after starting to eat. Blood was obtained by finger-prick
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3 using the Unistik®3 single-use lancing device (Owen Mumford). Prior to a finger-prick,
4
5 participants were encouraged to warm their hand to increase blood flow. Fingers were not
6
7 squeezed to extract blood from the fingertip as this may dilute with plasma. Blood glucose was
8
9 measured using the HemoCue Glucose 201 DM analyser (HemoCue® Ltd), which was
10
11 calibrated daily using control solution from the manufacturer.
12

13 14 15 *Calculation of GI*

16
17 The incremental area under the curve (iAUC) for blood glucose response was calculated
18
19 geometrically by applying the trapezoid rule ignoring the area beneath the baseline [17]. The
20
21 iAUC for each test sample for each participant was expressed as a percentage of the mean IAUC
22
23 for the reference food consumed by the same participant:
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$$25 \quad \text{GI} = (\text{IAUC test sample} / \text{IAUC reference food}) * 100$$

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28
29 The GI value of the test products was calculated as the mean for the whole group.

30 31 32 *Gastrointestinal Symptoms*

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34 The Gastrointestinal Symptoms Questionnaire (GSQ) comprised six items designed to assess
35
36 well-being and the intensity of gastrointestinal symptoms experienced by participants at the end
37
38 of each test session. For each question, participants marked their strength of feeling on a one-
39
40 hundred millimetre visual analogue scale (VAS). These lines were anchored at the left and
41
42 right ends with opposing labels, 'absent' and 'severe', respectively. The GSQ items were well-
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44 being (What is your general feeling of well-being now?), nausea, abdominal cramping,
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46 distension, flatulence and diarrhoea. VAS ratings were quantified by measuring in millimetres
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48 the distance between the left end of the scale and the marked point.
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2.4.6 Statistical analysis

Data are presented as mean, standard deviation (SD) and standard error of the mean (SEM) values. The ISO standards recommend a sample size of 10 participants to determine GI of food samples. A sample size of 14 was shown to have a power of 80% to detect a difference in glycaemic response of 19% in a previous study [18]. Therefore, in this study, 16 participants were tested. Data were analysed using the IBM Statistical Package for the Social Sciences 25 (SPSS Inc., Chicago, Illinois). Prior to statistical analysis, the normality of the data was tested using the Shapiro-Wilk statistic. A paired-T-test or Wilcoxon signed rank test was used to compare the change in blood glucose, well-being and gastrointestinal symptoms between Pea Starch LN30® vs Glucidex®IT19 Maltodextrin and the pea starch recipe vs. the maltodextrin recipe. Statistical significance was set at $p < 0.05$ for all tests.

3. RESULTS

3.1 *In vitro* testing by Englyst method

The amount of RDS, SDS and RS in Pea Starch LN30 were 16%, 30% and 54%, respectively while Glucidex®IT19 Maltodextrin showed higher RDS content (93%) and much lower SDS (1%) and RS (6%) (Table 3). The maltodextrin-based powder mix recipe showed a higher percentage of RDS (72%) compared to the pea starch-based recipe (17% RDS).

3.2. *In vivo* Glycaemic index testing

3.2.1 Participant characteristics

Sixteen participants were recruited of which two participants did not complete the study. One outlier with a GI value outside the range of ± 2 SD from the mean GI value was excluded as

per the ISO standards [16]. Table 4 presents the physical characteristics of the thirteen participants who completed the study.

3.2.2 Glycaemic response and glycaemic index

Fig.1 illustrates the mean blood glucose levels and mean change in blood glucose for reference glucose, Pea Starch LN30® and Glucidex®IT19 Maltodextrin. There was a significant difference ($p<0.05$) in the change in blood glucose between Pea Starch LN30® and Glucidex®IT19 Maltodextrin at 15, 30, 45, 90, 120, 150 and 180 minutes after consumption. The glucose response after Pea Starch LN30® was significantly lower ($p<0.05$) at 15 (-1.3mmol/l), 30 (-2.5mmol/l) and 45 min (-1.7mmol/l) compared to Glucidex®IT19 Maltodextrin. There was no difference at 60 min (-0.2mmol/l); however, the blood glucose levels increased sharply following Glucidex®IT19 Maltodextrin consumption and then dropped significantly lower than Pea Starch LN30® below the baseline at 90 (-0.7mmol/l), 120 (-0.6mmol/l), 150 (-0.5mmol/l) and 180 min (-0.4mmol/l). The actual peak blood glucose for pea starch was 4.9 mmol/l compared to 7.3 mmol/l for maltodextrin showing a reduction of 2.4 mmol/l.

Fig.2 illustrates the mean blood glucose levels and mean change in blood glucose for Pea Starch LN30® recipe and Glucidex®IT19 Maltodextrin recipe. The change in blood glucose after consumption of the Pea Starch LN30® recipe and Glucidex®IT19 Maltodextrin recipe was significantly different ($p<0.05$) at 15, 30, 45, 90, 120 and 180 minutes. The glucose response after Pea Starch LN30® recipe was significantly lower ($p<0.05$) at 15 (-1.1 mmol/l), 30 (-1.8mmol/l) and 45 min (-0.9mmol/l) compared to Glucidex®IT19 Maltodextrin recipe. There was no difference at 60 and 150 min (-0.3mmol/l); however, the blood glucose levels increased sharply following Glucidex®IT19 Maltodextrin recipe consumption and then dropped

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3 significantly lower than Pea Starch LN30® recipe below the baseline at 90 (-0.5mmol/l), 120
4 (-0.4mmol/l) and 180 min (-0.4mmol/l). The actual peak blood glucose for pea starch recipe
5 was 5.0 mmol/l compared to 6.6 mmol/l for maltodextrin recipe showing a reduction of 1.6
6 mmol/l.
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15 The iAUC 180 value (mean \pm SEM) for Pea Starch LN30® was significantly lower than the
16 Glucidex®IT19 Maltodextrin (24.8 ± 6.4 vs 106.2 ± 10.4 ; $p=0.001$). Similarly, the iAUC 180
17 (mean \pm SEM) value for Pea Starch LN30® recipe was significantly lower than The
18 Glucidex®IT19 Maltodextrin recipe (21.9 ± 4.1 vs 69.5 ± 9.2 ; $p=0.001$). The GI value (mean
19 \pm SEM) of Pea Starch LN30® (23 ± 6.6) and the Pea Starch LN30® recipe (18 ± 3.7) were both
20 low. The Glucidex®IT19 Maltodextrin had a high GI value (100 ± 8.6) and the Glucidex®IT19
21 Maltodextrin recipe (66 ± 8.1) had a medium GI value.
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3.3 Gastrointestinal Symptoms

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37 Table 5 shows the mean scores for well-being and gastrointestinal symptoms at the end of the
38 test sessions after consumption of Pea Starch LN30®, Glucidex®IT19 Maltodextrin, Pea Starch
39 LN30® recipe and, Glucidex®IT19 Maltodextrin recipe.
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44 There was no significant difference ($p>0.05$) in mean scores for well-being and gastrointestinal
45 symptoms after consumption of Pea Starch LN30® and Glucidex®IT19 Maltodextrin or
46 between the two recipes.
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4. Discussion

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57 Since 1980, diabetes has dramatically increased worldwide. 35 million cases were reported in
58 1985 [19] mostly type 1 diabetes in comparison to 463 million today, 90% being type 2 diabetes
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3 [20] . Among all factors evoked by scientific experts, the decrease in energy expenditure and
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5 increase in energy input has been described as those having the major impacts. At the same
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7 time, the dietary habits changed a lot in Western countries and in Asian countries moving from
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9 traditional meal to more processed or even ultra-processed foods. Those foods rich in fats,
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11 sugars and highly digested starchy ingredients induce a strong impact on the postprandial
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13 glycaemia.
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17 The scientific work done by Englyst in the 90s has made it possible to predict the speed of
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19 digestion of starchy foods and thus the impact on the post meal glycemia⁷. Three fractions were
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21 proposed according to the *in vitro* starch digestion : the RDS (Rapidly Digested Starch) fraction
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23 digested in 20 minutes, the SDS (Slowly Digested Starch) fraction digested in between 20 and
24
25 120 minutes and the RS (Resistant Starch) fraction that is not digested 2 hours after the
26
27 beginning of the experiment. A special focus on this RS fraction will be made afterwards.
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31 The objective of this study was to test if the native pea starch could be used to propose recipes
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33 with claims based on slow digestibility and low GI. The research has developed recipes of
34
35 powder mix for ready to drink beverages that can be consumed as healthy snack alternatives or
36
37 during sports sessions.
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41 In our experiment we confirmed that raw pea starch has a very low RDS fraction (16%). Potato
42
43 has been shown to have 6%, corn starch 23%, waxy maize 42% and wheat starch 43% RDS,
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45 respectively (unpublished). This weak digestibility can be explained by the semi-crystalline
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47 starch structure with concentric layers of amorphous and crystalline regions, the amylose
48
49 content, and the size of the granules in relation with surface characteristics. All these features
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51 confer resistance to amylase enzyme, which delays the glucose release from starch. These
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53 positive properties disappear with the cooking of starch, and in cooked starch, digestibility is
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55 very high due to the loss of amylase resistance ¹⁰
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3 This study shows that the native pea starch contains 30% SDS and 54% RS according to the *in*
4 *vitro* testing by Englyst method. This implies a high dietary fibre content in pea starch as RS is
5 known to act as dietary fibre. However, when determined with the current consensual resistant
6 starch AOAC 2002.02 methodology, [21] the dietary fibre content (represented by type 2
7 resistant starch) of the native pea starch [22] was determined as 11%. This suggests an
8 overestimation of RS by Englyst method, which may be attributed to the fact that the current
9 EU and CODEX dietary fibre definitions and approved methodologies for dietary fibre
10 determination came after the first publications by Englyst [7]. Therefore, it is recommended to
11 use AOAC protocol to precisely measure the RS fraction. With 11% resistant starch considered
12 as dietary fibre, meaning the starch fraction that is not at all digested in the upper part of the
13 small intestine, the slowly digested fraction of the pea starch can be estimated to be 73% (100
14 - 11% RS - 16% RDS). This explains the very weak postprandial glycaemic response which
15 might consequently trigger a very weak insulin response.
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19 The glycaemic response result reflects the slow digestion feature of native pea starch leading to
20 a very limited increase in blood glucose, with less than 0.5 mM/L increment. The shape of the
21 curve with a plateau between 30 and 60 minutes reflects the long-lasting digestion pattern of
22 the native starch in comparison to maltodextrin which induced a very high excursion of blood
23 glycemia that returns to baseline from 30 minutes after ingestion. With maltodextrin, there was
24 a hypoglycaemic period starting 75 minutes after ingestion and lasting more than 90 minutes,
25 which was not observed with the pea starch. This hypoglycaemic effect of maltodextrin may be
26 due to the long-lasting insulin production associated to its prolonged effects. We assume that
27 pea starch may not promote strong insulin response or even potentially negligible insulin
28 secretion as observed with the shape of the glycaemic response curve due to its very low RDS
29 content.
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3 In a study published in 2020, Petropolou and co-authors¹⁹ explained that the different glycemic
4 and insulinemic responses observed between two *Pisum sativum* lineages were due to their
5 relative amylose content conferring them different digestibility pattern. The lowest glucose
6 availability in the duodenal space was associated to the lowest gastric inhibitory polypeptide
7 (GIP) incretin release which may explain the lower insulin levels. According to Pilichiewicz *et*
8 *al.* (2007)²⁰ there is a positive correlation in between the duodenal space glucose concentration
9 and the blood insulin which supports the hypothesis that in our study the insulin excursion might
10 be lower with the pea starch and the pea starch recipe in comparison to high GI test foods..
11

12 In this study, the pea starch-based recipe elicited an attenuated glycaemic response compared
13 to the maltodextrin-based recipe. This effect may be attributed to the low RDS content and high
14 RS and SDS contents of pea starch identified during *in vitro* digestion. Previous studies have
15 reported ²¹⁻²³low glycaemic index values for foods that contained high SDS contents of 26 to
16 28 g/100g [23–25]. Food products developed using 25 to 30 g of a commercial SDS product (a
17 blend of tapioca flour and corn starch) have previously demonstrated a 50% reduction in GI
18 values compared to control products without SDS[18]. In this study, the reduction in GI was
19 more pronounced, potentially due to the presence of resistant starch and higher SDS content of
20 73% as mentioned earlier. Therefore, the recipe, which included 51% of pea starch consisted
21 of approximately 5.6 g/100g of resistant starch and 37 g/100g SDS.
22

23 Pea starch has reduced digestibility compared to starches in cereals and potatoes due to its
24 intermediate amylose content. Moreover, the starch granules are separated by cell wall
25 structures that reduces access to amylase enzymes [26]. The above characteristics may have
26 contributed to the low glycaemic index value of the pea starch recipe. The digestibility of
27 different pea starch components may change during cooking, thereby leading to an increase in
28 the RDS fraction[27]. However, the recipe used in this study is expected to have retained the
29 low RDS content due to the absence of thermal processing.
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3 The high percentage of SDS associated to the low glycaemic profile observed in the healthy
4 individuals are indications that pea starch is digested throughout the small intestine, from
5 duodenum to ileum where brush border enzymes and glucose transporters are located. The
6 glucose efflux from the intestine to liver takes place slowly and the portal glucose
7 concentrations may be much lower than with rapidly digestible and absorbable carbohydrates
8 like sugar, maltodextrins and high GI foods. The lower glucose blood influx may limit the
9 insulin secretion and the production of insulin by the pancreatic b-cells. As insulin inhibits fat
10 oxidation specially during exercise low GI foods allow an increased utilization of fats and
11 preservation of the glycogen stores. Wu and Williams²⁸ found that during exercise the overall
12 fat oxidation is higher after consuming a low GI bar compared to a high GI one. In this study,
13 a low insulin response was observed. The very low digestibility kinetics of the pea starch
14 attested by the shape of the glucose response seems to be adapted to sportsman and
15 sportswoman during endurance exercise. The slow glucose delivery associated to a low insulin
16 response may be profitable to people in preparation to endurance sessions and high-intensity
17 exercise ^{29,30}.

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37 Non-digestible carbohydrates such as resistant starch are known to cause gastrointestinal
38 discomfort due to gas production following its fermentation in the large intestine [28]. However,
39 in this study, there were no differences in the gastrointestinal symptoms following pea starch
40 product compared to the maltodextrin product. This is in agreement with Seewi et al (1999)
41 who reported that pea starch alone did not cause bloating or flatulence like pea flour which
42 consisted of oligosaccharides and fibre[29].Furthermore, this result supports our earlier
43 assumption on the presence of higher SDS content and lower RS content in pea starch. The
44 gastrointestinal tolerability of pea starch is beneficial for product development to promote
45 consumer acceptance. The SDS fraction is of importance because it indicates the weak
46 postprandial excursion of glucose, which may have long-term beneficial effects due to a weaker
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3 insulin response. Finally, the presence of carbohydrates at the end of the small intestine may
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5 trigger different neuroendocrine responses that may promote satiety feelings through the gut
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7 brain axis [30].
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10 A limitation of the study is that only 25 g available carbohydrate serving sizes were tested for
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12 pea starch and pea starch recipe; therefore, the effects cannot be generalised to higher doses of
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14 pea starch. Moreover, there may be further changes to the digestibility, glycaemic response and
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16 gastrointestinal response following thermal processing of pea starch, which was not explored
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18 in this study. Future studies may investigate the incorporation of lower doses of pea starch in
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20 different food products aimed at individuals at risk of type 2 diabetes or those looking for ready
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22 to drink products. Higher doses of pea starch may not be palatable as a raw ingredient.
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26 In conclusion, this study has demonstrated the presence of high SDS content in pea starch,
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28 which reduced postprandial glycaemic response compared to maltodextrin, which is a rapidly
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30 digested carbohydrate. Irrespective of the high levels of SDS, the pea starch recipe did not
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32 induce any negative gastrointestinal symptoms. Pea starch may therefore prove to be a
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34 beneficial ingredient in developing food products for improving glycaemic control without
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36 undesirable side effects.
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5. References

1. Lin X, Xu Y, Pan X, et al. Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. *Scientific Reports*. 2020;10(1):14790. doi:10.1038/s41598-020-71908-9
2. Williams R, Karuranga S, Malanda B, et al. Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*. 2020;162. doi:10.1016/j.diabres.2020.108072
3. Magkos F, Hjorth MF, Astrup A. Diet and exercise in the prevention and treatment of type 2 diabetes mellitus. *Nature Reviews Endocrinology*. 2020;16(10):545-555. doi:10.1038/s41574-020-0381-5
4. Buyken AE, Mela DJ, Dussort P, et al. Dietary carbohydrates: a review of international recommendations and the methods used to derive them. *European Journal of Clinical Nutrition*. 2018;72(12):1625-1643. doi:10.1038/s41430-017-0035-4
5. Wee MSM, Henry CJ. Reducing the glycemic impact of carbohydrates on foods and meals: Strategies for the food industry and consumers with special focus on Asia. *Compr Rev Food Sci Food Saf*. 2020;19(2):670-702. doi:10.1111/1541-4337.12525
6. Pfeiffer AFH, Keyhani-Nejad F. High Glycemic Index Metabolic Damage - a Pivotal Role of GIP and GLP-1. *Trends Endocrinol Metab*. 2018;29(5):289-299. doi:10.1016/j.tem.2018.03.003
7. Englyst HN, Kingman SM, Cummings JH. Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr*. 1992;46 Suppl 2:S33-50.
8. Englyst K, Goux A, Meynier A, et al. Inter-laboratory validation of the starch digestibility method for determination of rapidly digestible and slowly digestible starch. *Food Chem*. 2018;245:1183-1189. doi:10.1016/j.foodchem.2017.11.037
9. Englyst KN, Englyst HN. Carbohydrate bioavailability. *Br J Nutr*. 2005;94(1):1-11. doi:10.1079/bjn20051457
10. Zhang G, Hamaker BR. Slowly Digestible Starch: Concept, Mechanism, and Proposed Extended Glycemic Index. *Critical Reviews in Food Science and Nutrition*. 2009;49(10):852-867. doi:10.1080/10408390903372466
11. Vinoy S, Meynier A, Goux A, et al. The Effect of a Breakfast Rich in Slowly Digestible Starch on Glucose Metabolism: A Statistical Meta-Analysis of Randomized Controlled Trials. *Nutrients*. 2017;9(4). doi:10.3390/nu9040318
12. Garsetti M, Vinoy S, Lang V, Holt S, Loyer S, Brand-Miller JC. The Glycemic and Insulinemic Index of Plain Sweet Biscuits: Relationships to *in Vitro* Starch Digestibility. *Journal of the American College of Nutrition*. 2005;24(6):441-447. doi:10.1080/07315724.2005.10719489
13. Robinson GHJ, Balk J, Domoney C. Improving pulse crops as a source of protein, starch and micronutrients. *Nutr Bull*. 2019;44(3):202-215. doi:10.1111/nbu.12399

14. Dahl WJ, Foster LM, Tyler RT. Review of the health benefits of peas (*Pisum sativum* L.). *Br J Nutr.* 2012;108 Suppl 1:S3-10. doi:10.1017/S0007114512000852
15. Lieberman LS. Dietary, evolutionary, and modernizing influences on the prevalence of type 2 diabetes. *Annu Rev Nutr.* 2003;23:345-377. doi:10.1146/annurev.nutr.23.011702.073212
16. Xi Y, Xu PF. Diabetes and gut microbiota. *World J Diabetes.* 2021;12(10):1693-1703. doi:10.4239/wjd.v12.i10.1693
17. McCleary BV, McNally M, Rossiter P, et al. Measurement of Resistant Starch by Enzymatic Digestion in Starch and Selected Plant Materials: Collaborative Study. *Journal of AOAC INTERNATIONAL.* 2002;85(5):1103-1111. doi:10.1093/jaoac/85.5.1103
18. Birt DF, Boylston T, Hendrich S, et al. Resistant Starch: Promise for Improving Human Health. *Adv Nutr.* 2013;4(6):587-601. doi:10.3945/an.113.004325
19. Petropoulou K, Salt LJ, Edwards CH, et al. A natural mutation in *Pisum sativum* L. (pea) alters starch assembly and improves glucose homeostasis in humans. *Nat Food.* 2020;1(11):693-704. doi:10.1038/s43016-020-00159-8
20. Pilichiewicz AN, Chaikomin R, Brennan IM, et al. Load-dependent effects of duodenal glucose on glycemia, gastrointestinal hormones, antropyloroduodenal motility, and energy intake in healthy men. *American Journal of Physiology-Endocrinology and Metabolism.* 2007;293(3):E743-E753. doi:10.1152/ajpendo.00159.2007
21. Goux A, Breyton AE, Meynier A, et al. Design and Validation of a Diet Rich in Slowly Digestible Starch for Type 2 Diabetic Patients for Significant Improvement in Glycemic Profile. *Nutrients.* 2020;12(8):E2404. doi:10.3390/nu12082404
22. Goux A, Neufcourt L, Brack O, Atkinson F, Vinoy S. A high content of Slowly Digestible Starch decreases glycemic and insulinemic responses similarly in Asians and Caucasians. *Proceedings of the Nutrition Society.* 2020;79(OCE2):E495. doi:10.1017/S0029665120004437
23. Stewart ML, Wilcox ML, Bell M, Buggia MA, Maki KC. Type-4 Resistant Starch in Substitution for Available Carbohydrate Reduces Postprandial Glycemic Response and Hunger in Acute, Randomized, Double-Blind, Controlled Study. *Nutrients.* 2018;10(2):129. doi:10.3390/nu10020129
24. Goux A, Neufcourt L, Brack O, Atkinson F, Vinoy S. *A High Content of Slowly Digestible Starch Decreases Glycemic and Insulinemic Responses Similarly in Asians and Caucasians.* Vol 79.; 2020. doi:10.1017/S0029665120004437
25. Gourineni V, Stewart ML, Skorge R, Sekula BC. Slowly Digestible Carbohydrate for Balanced Energy: In Vitro and In Vivo Evidence. *Nutrients.* 2017;9(11):1230. doi:10.3390/nu9111230
26. Dahl WJ, Foster LM, Tyler RT. Review of the health benefits of peas (*Pisum sativum* L.). *Br J Nutr.* 2012;108 Suppl 1:S3-10. doi:10.1017/S0007114512000852

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2
3 27. Yao W, Liu C, Xi X, Wang H. Impact of Process Conditions on Digestibility of Pea Starch.
4 *International Journal of Food Properties*. 2010;13(6):1355-1363.
5 doi:10.1080/10942910903177368
6
7 28. Wu CL, Williams C. A Low Glycemic Index Meal before Exercise Improves Endurance
8 Running Capacity in Men. *International Journal of Sport Nutrition and Exercise*
9 *Metabolism*. 2006;16(5):510-527. doi:10.1123/ijsnem.16.5.510
10
11 29. Goodpaster B, Costill D, Fink W, et al. The Effects of Pre-Exercise Starch Ingestion on
12 Endurance Performance. *Int J Sports Med*. 1996;17(05):366-372. doi:10.1055/s-2007-
13 972862
14
15 30. Kaviani M, Chilibeck PD, Gall S, Jochim J, Zello GA. The Effects of Low- and High-
16 Glycemic Index Sport Nutrition Bars on Metabolism and Performance in Recreational
17 Soccer Players. *Nutrients*. 2020;12(4):982. doi:10.3390/nu12040982
18
19 31. Lockyer S, Nugent AP. Health effects of resistant starch. *Nutrition Bulletin*. 2017;42(1):10-
20 41. doi:10.1111/nbu.12244
21
22 32. Seewi G, Gnauck G, Stute R, Chantelau E. Effects on parameters of glucose homeostasis
23 in healthy humans from ingestion of leguminous versus maize starches. *Eur J Nutr*.
24 1999;38(4):183-189. doi:10.1007/s003940050060
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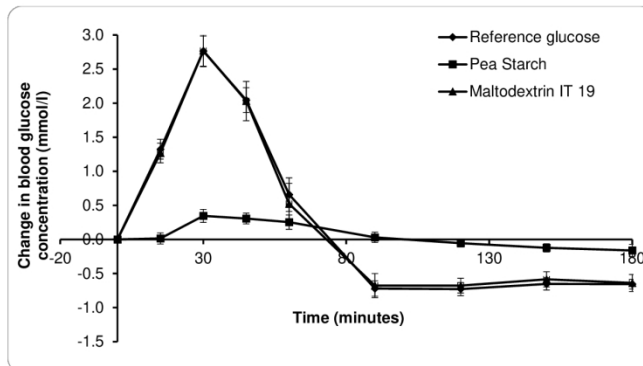


Fig.1.

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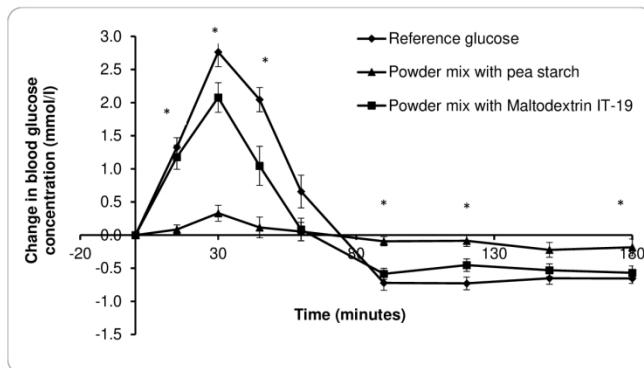


Fig.2.

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4 **Fig.1.** Glycaemic response curves for glucose, Pea Starch LN30® and IT 19 Maltodextrin. Data
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6 are presented as mean and SEM (*n* 13). *SEM*, *standard error of the mean*
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For Peer Review

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Fig.2. Glycaemic response curves for Pea Starch LN30® recipe and Glucidex®IT19 Maltodextrin recipe. Data are presented as mean and SEM (*n* 13). *SEM*, standard error of the mean

For Peer Review

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3 Slow digestible starch in native pea starch (*Pisum sativum* L.) lowers glycaemic response with
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5 no adverse effects on gastrointestinal symptoms in healthy adults
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11 Table 1: Composition of pea starch-based powder mix recipe and maltodextrin- based powder
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13 mix recipe
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Ingredients	Pea starch-based powder mix recipe (%)	Maltodextrin- based powder mix recipe (%)
GLUCIDEX® IT 19 maltodextrin	1,68	54,63
Pea Starch Pea Starch LN30®	51,34	--
Milk, whole, powder	23,90	23,90
NUTRALYS® pea protein	14,70	14,70
PREGEFLO®precooked waxy maize starch	2,80	2,80
NUTRIOSE®soluble fiber	2,21	2,21
Flavour, powder	1.097	1.097
Guar gum	1,30	1,30
Salt	0,77	0,77
Aspartame	0,137	0,137
Acesulfam K	0,068	0,068

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14 Table 2: Nutrition information for the pea starch-based powder mix recipe and the maltodextrin-
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16 based powder mix recipe
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Nutritional values	Pea starch-based powder mix recipe (Per 100g)	Maltodextrin-based powder mix recipe (Per 100g)
Energy value (kcal)	378	399
Total fat	7.7	7.6
of which, saturated fat	4.2	4.2
Carbohydrates, without fibre	57.4	62.5
of which, sugars	8.8	12.2
Fiber	3.0	3.0
Protein	18.6	18.6
Salt (sodium x2,5)	1.50	1.49

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14 **Table 3** : Starch profile of native Pea Starch LN30®, Glucidex®IT19 Maltodextrin, pea starch
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16 based powder mix recipe and maltodextrin based powder mix recipe
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Test products	Fractions (%)		
	<i>RDS</i>	<i>SDS</i>	<i>RS</i>
Pea Starch LN30	16	30	54
Maltodextrin IT 19	93	1	6
Pea starch powder mix	17	25	43
Maltodextrin powder mix	72	9	11

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11 **Table 4** Anthropometric characteristics (mean \pm SD) of the study population (n 13)
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Characteristics	Measurements
Age (y)	34.8 \pm 9.8
Height (m)	1.7 \pm 0.1
Weight (kg)	68.9 \pm 11.5
BMI (kg/m ²)	23.6 \pm 2.9
Fat mass (%)	26.7 \pm 11.4
Lean body mass (kg)	49.8 \pm 7.6

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Slow digestible starch in native pea starch (*Pisum sativum* L.) lowers glycaemic response with no adverse effects on gastrointestinal symptoms in healthy adults

Table 5: Mean (\pm SD) Gastrointestinal Symptoms Questionnaire scores (mm) after consumption of Pea Starch LN30®, Glucidex®IT19 Maltodextrin, Pea Starch LN30® recipe and Glucidex®IT19 Maltodextrin recipe (n 13)

Gastrointestinal symptoms	Pea Starch LN30®	Maltodextrin Glucidex®IT19	Pea Starch LN30® recipe	Maltodextrin Glucidex®IT19 recipe
Well-being	79.5 \pm 16.5	85.0 \pm 12.7	79.4 \pm 22.4	86.7 \pm 16.4
Nausea	0.0 \pm 0.0	0.2 \pm 0.8	0.1 \pm 0.3	0.2 \pm 0.6
Abdominal cramping	0.5 \pm 1.9	0.0 \pm 0.0	6.9 \pm 24.1	0.2 \pm 0.8
Distension	0.9 \pm 3.3	0.2 \pm 0.6	0.8 \pm 2.8	0.0 \pm 0.0
Flatulence	0.6 \pm 1.9	0.2 \pm 0.8	0.5 \pm 1.5	0.4 \pm 1.4
Diarrhoea	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0

SD, standard deviation