Bioactive Carbohydrates and Dietary Fibre 28 (2022) 100318

Contents lists available at ScienceDirect

Bioactive Carbohydrates and Dietary Fibre

journal homepage: www.elsevier.com/locate/bcdf

Bread with a high level of resistant starch influenced the digestibility of the available starch fraction

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ARTICLE INFO

Keywords:
Bread
Resistant starch
Glycaemic response
Insulinemic response
Glycaemic index
Satiety

ABSTRACT

Eating foods in which available starch was replaced by resistant starch (RS) causes lower glucose and insulin responses. There is insufficient evidence for the effects of RS when the total available carbohydrates content remains constant. The present study aimed to evaluate the effects of bread with a high amount of RS3, compared to control bread. The effects of RS on glycaemia, insulin response, appetite visual analogue scale (VAS) scores were examined. It was also determined the glycaemic index (GI) of both loaves of bread. In a single-blind, crossover study, 37 nondiabetic adults consumed equivalent samples of a test bread with a high amount of RS (HRS - 2.4%) or a conventional bread (REF - 0.8%), with a washout period of at least one week. Postprandial glucose and VAS scores were measured at fasting, 15, 30, 45, 60, 90 and 120 min after the meal. Insulin response was measured at fasting and 30 min. HRS bread presented a significantly lower postprandial glucose response at 60, 90 and 120 min ($P \leq 0.05$). Incremental AUC glycaemia response showed significantly lower values for HRS bread (127.15 ± 71.54 mmol*min/l) as well as higher satiety scores, compared to REF bread (153.77 ± 80.38 mmol*min/l); $t(36) = 2.234$; $P = 0.016$. HRS bread showed a GI = 60 much lower than the REF bread. The higher amount of RS causes a significantly lower postprandial glucose response. These results shows that RS3 influenced the digestibility of the available starch fraction. No differences were observed at insulin response. RS is related to higher satiety scores.

1. Introduction

Resistant starch (RS) is defined as the sum of starch and starch degradation products not absorbed in the small intestine of healthy individuals (Englyst et al., 1996). RS is classified into five categories based on the mechanism that contributes to the digestion resistance (Englyst et al., 1996; Haralampu, 2000; Higgins & Brown, 2013). The most recent definition of dietary fibre includes RS as one of its components (CodexAlimentarius, 2009; European Commission, 2008). RS presents physiological benefits similar to soluble fibre and has a positive impact on the intestinal tract health. (Haralampu, 2000). RS resists to digestion in the small intestine where it is hydrolysed by amylases from the gut microbiota. The resulting glucose is slowly fermented into carbon dioxide, methane, hydrogen and short chain fatty acids (SCFA). SCFA include butyrate, acetate and propionate being the responsible components for the beneficial effects of RS (Nugent, 2005).

The effects of different categories and doses of RS on glucose and insulin responses were the aim of numerous studies However, there is a lack of consensus and the results are hard to interpret and compare: test and control meals composition are different, presence/absence of other macronutrients (proteins and/or fats), cooking/processing conditions of the starch and total dietary fibre content. Whereas some researchers report decreased postprandial glycaemic and/or insulinemic responses, other reports no change or a small change that may not be physiologically relevant. Granfeldt et al. have suggested that the RS content influences the digestibility of the available starch fraction (Granfeldt et al., 1995). Other studies were conducted using different categories of RS and non-similar experimental conditions (Al-Mana & Robertson, 2018; Bodinham et al., 2010; Hallstrom et al., 2011; Haub et al., 2010; Klosterbuer et al., 2012). The obtained results are not consistent and lead to the question about the physiological effects of the different RS categories (Robertson, 2012). A recent systematic review and meta analysis

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https://doi.org/10.1016/j.bcdf.2022.100318
Received 23 November 2021; Received in revised form 31 March 2022; Accepted 31 March 2022
Available online 9 April 2022
2212-6198/Published by Elsevier Ltd.

concluded that the effect of resistant starch on fasting plasma glucose was significant compared with digestible starch (Xiong et al., 2021).

Increasing the RS content causes an improvement in the total fibre content and some authors refer that it also influences the appetite scores and increases satiety (Amini et al., 2021; Higgins, 2004; Nugent, 2005).

The European Food Safety Authority (EFSA) recognizes the beneficial effects of RS on the glycaemic response (EFSA, ID 681 health claim). The claimed effect is "healthy blood glucose/sugar levels". According EFSA, high carbohydrate baked foods should contain at least 14% of total starch as RS in replacement to digestible starch (EFSA Panel on Dietetic Products, 2011). However, the change of the digestible starch fraction cannot be used to assess RS as a true functional component.

The present study aimed to evaluate the short-term effects of bread with a high amount of RS3 in comparison to a control bread with low RS, keeping constant the amount of available carbohydrates, in nondiabetic adults. The bread was developed in a previous study and the increase in RS content resulted only from the breadmaking technique, no RS supplier ingredient was added (Amaral et al., 2016). The effects of RS on glycaemia and insulin response and appetite visual analogue scale (VAS) scores were examined. It was also determined the glycaemic index (GI) of the bread containing a high level of RS and the control bread.

2. Materials and methods

2.1. Tested breads

Two different bread products were prepared: one with a high amount of RS (HRS) and the other as a conventionally reference bread (REF) (see composition in Table 1). The tested bread portions provided corresponding to 50 g of available carbohydrates. HRS bread is based on 80% white wheat flour and 20% maize flour baked at long time/low temperature conditions and the formula was developed in a previous study (Amaral et al., 2016). REF bread is a conventionally baked bread (200°C/30 min) from white wheat flour. The main difference between the samples was essentially the RS content: REF portion (88 g) contained 0.7 g of RS while HRS portion (102 g) contained 2.5 g of RS. The amount of RS in the breads was determined using the AOAC method 2002.02 (McCleary et al., 2002) using a Megazyme Kit (Megazyme International Ireland Ltd).

2.2. Participants characteristics

Thirty-seven (n = 37) nondiabetic adults were recruited from the academic population at the Polytechnic Institute of Beja, Portugal, via e-mail advertisement. Eligible subjects were men and women aged 18–65 years, with a BMI≥18.5 kg/m^2, weight stable over the past 3 months and with normal fasting blood glucose. Exclusion criteria were as follow the history of gastrointestinal disease or endocrine disorders, use of medications that interfere with glucose or lipid metabolism, hospitalization in the last 3 months, pregnant or lactant women, smoker, individuals who did not regularly consume breakfast and vegetarians. Measurements of height and weight were obtained, and Body Mass Index (BMI) was calculated. Participants were assigned into two groups: **N** (normal weight) – BMI<25.0 kg/m^2 and **O** (overweight/obese) – BMI≥25.0 kg/m^2. All subjects had HbA1c levels <6.5%. The study was approved by the Centro Hospitalar Lisboa Norte/Faculdade de Medicina Ethics Commission. Written informed consent was obtained from all subjects before the start of the study.

Table 1
Nutritional composition of test breads (%).

COMPOSITION	REF g/100g	REF g/portion	HRS g/100g	HRS g/portion
Protein	7.5	6.6	5.5	5.6
Lipids	0.04	0.04	0.03	0.03
Available carbohydrates	57.1	50.3	48.8	49.8
Moisture	34.2	30.1	43.1	44.0
RS	0.8	0.7	2.4	2.5

REF – reference bread; HRS – bread with a high amount of resistant starch.

2.3. Experimental design

The study was a randomized, subject-blind, balanced crossover study that investigated the acute effects of HRS and REF breads on appetite, glucose and insulin levels. Subjects were instructed to avoid strenuous exercise, drinking alcohol or eating meals particularly rich in fiber in the day before each test. Subjects were asked to standardize their intakes by eating the same type of meal before all test days and maintain their regular lifestyle throughout the entire study. On two separate occasions, subjects arrived at the laboratory after an overnight fast of 12 h. Each visit lasted approximately 2 h and, between visits, there was a washout period of at least one week. Capillary fasting blood samples were taken at time 0. The test portion bread was served with 250 ml of water and the subjects were told to finish it within 12–15 min. During the test, the subjects were not allowed to eat or drink anything else. Postprandial glucose was measured by finger-prick capillary blood sampling at 15, 30, 45, 60, 90 and 120 min timed after the beginning of the bread intake. Glucose values were measured directly using a glucometer OneTouch Verio (LifeScan-Johnson & Johnson). Individuals completed visual analogue scales (VAS) to assess satiety at fasting and 15, 30, 45, 60, 90 and 120 min after the beginning of the bread intake. Samples for insulin analysis were collected by finger prick before (0) and 30 min after the meal to a Multivette® Blood Collection System (Sarstedt). They were centrifuged for 10 min/4000 rpm and the serum was then frozen at −18°C until analysis. The insulin content was determined in the serum with an enzyme immunoassay method (Insulin ELISA kit ME E−0900 – Labor Diagnostika Nord). The procedures for determining the glycaemic index (GI) in breads were based on the methodology described by (Brouns et al., 2005; FAO/WHO, 1998; Wolever et al., 2008). For this determination, two more sessions were considered for the volunteers with BMI<25.0 kg/m^2 to obtain the glucose measurements for the reference food (50 g of anhydrous glucose). These sessions were separated by a one-week period.

2.4. Visual analogue scales

Satiety was evaluated using four (4) VAS previously validate (Flint et al., 2000). VAS combines a question followed by a 100 mm horizontal line with words anchored at each end, describing the extremes. Questions of the validated VAS were the following with the words in brackets attached to each of the endings: "How satisfied do you feel?" (I am completely empty/I cannot eat another bite); "How hungry do you feel?" (I am not hungry at all/I have never been hungrier); "How full do you feel?" (Not at all full/Totally full) and "How much do you think you can eat?" (Nothing at all/A lot). Subjects completed VAS, in each moment, by putting a mark over the line to express, to each question, the sensation of distance from the expressions of the endings. Quantification was made by measuring the distance, in millimetres, from the left end of the line to the mark.

2.5. Calculations and statistical analysis

Data were expressed as means ± SD (standard deviation). The incremental area under the curve (iAUC) was calculated for glucose response (0–120 min) and each of the questions of the VAS, for each subject and sample test, using the trapezoid model and using the fasting level as the baseline. The glycaemic index (GI) of the REF and HRS breads were calculated from the 120 min incremental postprandial area for glucose using 50 g of anhydrous glucose as the reference (GI = 100) according to (Wolever, 2013). All statistical analyses were carried out using SPSS 23.0 for Windows. The conditions for statistical analysis application, namely data normality and homogeneity of variance were evaluated, respectively, with Shapiro-Wilk test and Levene test. All normalized data were analyzed using independent sample *t*-test to compare groups N and O and paired samples *t*-test to comparing REF bread and HRS bread. For all non-normalized data, the statistical analysis was made using nonparametric tests: Mann-Whitney test to comparing groups N and O and Wilcoxon matched-pair to comparing treatments. When no significant differences were found between groups, data were considered together. Statistical significance was taken as $P \leq 0.05$.

3. Results

Twenty-six women and eleven men participated in this study (22–59 ages). N group consists of 16 individuals (14 women/2 men) and O group includes 16 subjects (8 women/8 men) with BMI up to 29,9 kg/m^2 and 5 subjects (4 women/1 man) with BMI higher than 29,9 kg/m^2.

3.1. Glycaemia

The comparison of the blood glucose levels between the two groups did not differ significantly; as a result, glycaemia values of both groups were analyzed together. Fig. 1 represents the postprandial glucose response curve for all the subjects. It was observed that the peak blood glucose for HRS bread occurred at 30 min, from which it decreased quickly and markedly. For REF bread the maximum value was reached at 45 min followed by a less marked decrease. The comparison of means between the two treatments, in each evaluation time, allowed to observe significant differences at 60 min (P = 0.041), 90 min (P = 0.001) and 120 min (P = 0.006). At 30 and 45 min, the differences were not statistically significant. Statistical analysis of individual iAUC glycaemia response showed significantly lower values for HRS bread (127.15 ± 71.54 mmol*min/l) compared to REF bread (153.77 ± 80.38 mmol*min/l); t(36) = 2.234; P = 0.016.

3.2. Insulin response

Table 2 shows the insulin level at fasting and 30 min after both treatments and for the two subject groups. The comparison of data between N and O groups showed no significant differences. Joint analysis of data showed a slightly higher insulin increase after HRS bread ingestion compared to REF bread (39.69 *vs* 37.37), not statistically significant, t (24) = -0.374, P = 0.356.

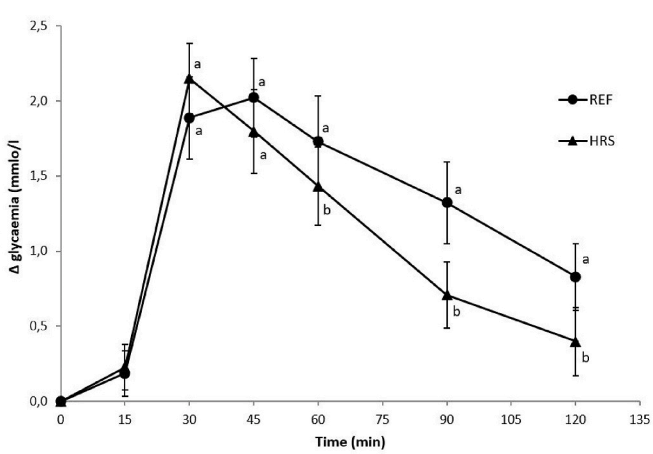

Fig. 1. Mean incremental postprandial glycaemia in all subjects after ingestion of REF (reference bread) and HRS (bread with a high amount of resistant starch), n = 37; for each time, different letters mean values significantly different (P ≤ 0.05).

Table 2
Insulin data (mUI/l) at fasting, 30 min after ingestion and increase up to 30 min by group for the two tested breads.

Time	REF N group	REF O group	HRS N group	HRS O group
0	11.64 ± 5.41	13.20 ± 6.19	11.80 ± 6.53	15.37 ± 6.19
30	52.85 ± 20.03	47.24 ± 30.48	50.07 ± 28.58	65.41 ± 28.37
Δ insulin	41.21 ± 17.65	35.78 ± 24.77	38.27 ± 27.84	49.74 ± 24.08

Data presented mean ± SD. REF – reference bread; HRS – bread with a high amount of resistant starch. N group – subjects with normal weight (BMI<25.0 kg/m^2); O group – subjects with overweight (BMI≥25.0 kg/m^2).

3.3. Visual analogue scales

The comparison of data between the two groups did not differ significantly and, for that reason, VAS scores were analyzed together. Fig. 2 represents the mean curves that express the perception of satiety to VAS responses. The adjustment to the respective baseline was made. Generally, the comparison of means between the two treatments in each moment demonstrated scores significantly higher for HRS bread than REF bread showing greater satiety to HRS bread. Only for the question "How hungry do you feel?", the scores were very similar. In respect to question "How satisfied do you feel?", scores were significantly higher at all moments: 15 (P = 0.008), 30 (P = 0.008), 45 (P = 0.014), 60 (P = 0.018), 90 (P = 0.005) and 120 min (P = 0.017). For the responses to "How full do you feel?" HRS bread revealed significantly higher scores at 90 (P = 0.038) and 120 min (P = 0.05) and relatively to responses to question, "How much do you think you can eat?" we registered significantly higher scores at 45 min (P = 0.031) and 90 min (P = 0.05).

Table 3 represents the mean iAUC for each question and treatment. All responses revealed increased satiety associated with HRS bread. Statistical analysis of the data showed significant differences for "How satisfied do you feel?" (P = 0.003) and "How much do you think you can eat?" (P = 0.005). For "How hungry do you feel?" and "How full do you feel?", *P-value* was, respectively, 0.055 and 0.073. Each treatment influenced satiety-related feelings differently.

To evaluate how long each feeling remains, we compared the mean scores at each moment (15, 30, 45, 60, 90 and 120) with the fasted value – Fig. 3. For REF bread the scores to "How satisfied do you feel?" at 120 min showed no significant differences to the fasting score. Similarly, to "How hungry do you feel?", no significant differences were observed between fasting score and 90 and 120 min scores, for the same bread. For HRS bread we observed significant differences from fasting to 120 min scores for all except for the question "How hungry do you feel?".

3.4. Glycaemic index

Generally, foods are classified as low (≤55), medium (56–69) or high (≥70) GI. According to the described methodology REF bread showed a GI of 82 while HRS bread presented a value of 60. According to this classification, REF bread presents a high GI food and HRS bread as a medium GI food.

4. Discussion

4.1. Glycaemia

These data shows that HRS bread causes a significantly lower postprandial glucose response than REF bread. The test portions of both breads were equivalent (50 g of available carbohydrates) whereby the RS present in the HRS bread have influenced the glucose response, namely the digestibility of the available starch fraction, especially from 45 min after consumption of the meal. These results are consistent with those observed by Hallstrom et al. (2011) where the authors have compared the glycaemic response to equivalent portions of bread (50 g of carbohydrates) with different RS content/portion (unknown category

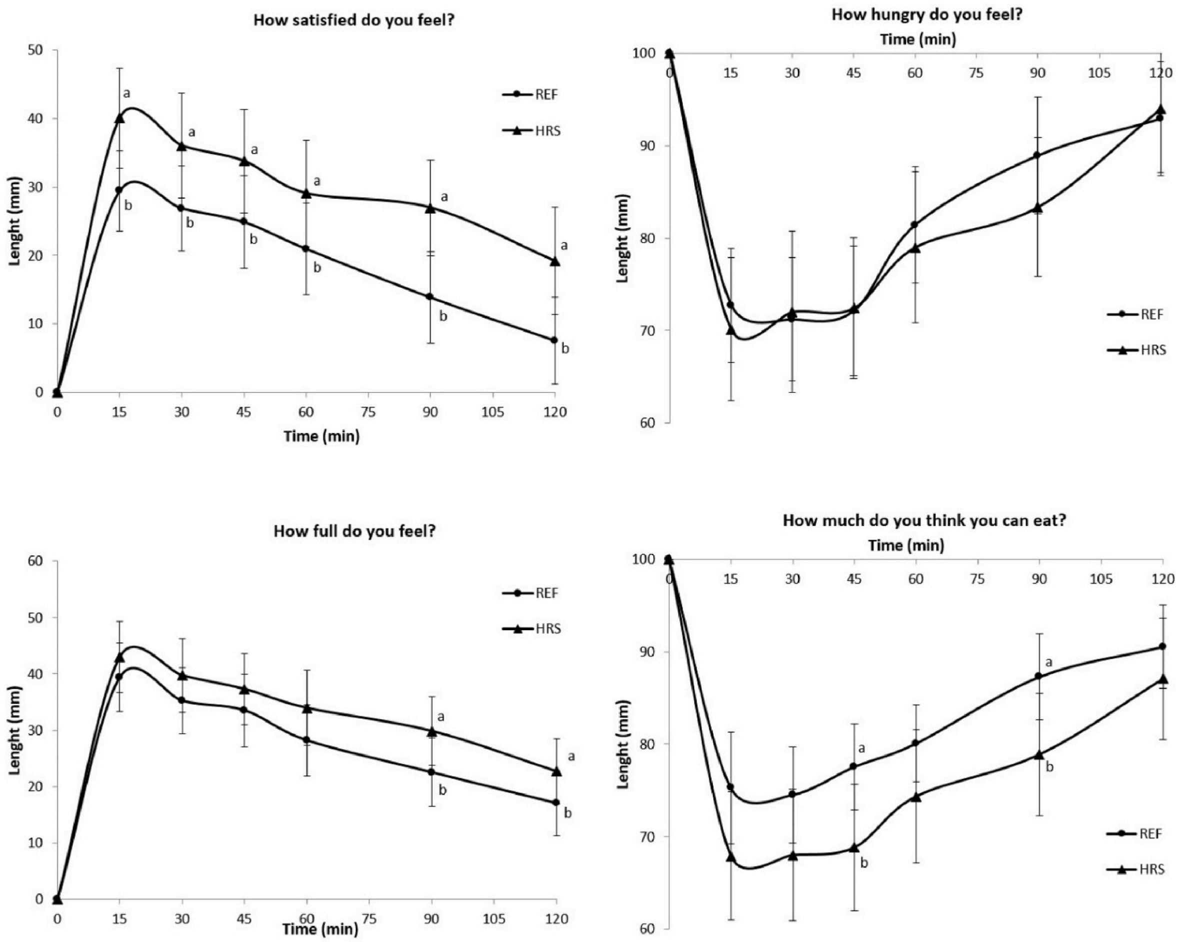

Fig. 2. Satiety related VAS questions, expressed as change from baseline to tested samples (HRS bread and REF bread); n = 37; for each moment, different letters means significantly different values (P ≤ 0.05).

Table 3
Mean iAUC (mm*min) to responses of VAS questions after ingestion of HRS bread and REF bread.

Questions	REF	HRS
How satisfied do you feel?	2544.6 ± 2130.6[a]	3680.6 ± 2632.5[b]
How hungry do you feel?	2501.6 ± 2202.6[a]	3398.2 ± 3051.2[a]
How full do you feel?	3328.4 ± 2292.4[a]	3902.1 ± 2367.1[a]
How much do you think you can eat?	2288.9 ± 1444.6[a]	3724.3 ± 3143.4[b]

REF – control bread; HRS – bread with a high amount of RS. Data presented as mean ± DP, n = 37. For each line, different letters mean significantly different values (P ≤ 0.05).

of RS): 1.5 g (control), 4.8 g and 7.7 g. This study found a significantly lower iAUC (0–120min) of the sample with 7.7 g of RS (13.0% total starch) compared to control. In our study, 2.5 g of RS/portion has demonstrated a significantly lowered glucose response than 0.7 g of RS while the glucose response to 4.8 g/portion at Hallstrom et al. investigation did not show significant differences to control. It is important to highlight that this sample (4.8 g/portion) was prepared with whole wheat flour which contains other fibre components that can influence differently the glycaemic response (Higgins, 2004).

Our research uses a bread-making technology to obtain the HRS bread that promotes the formation of retrograde starch, so our results express the effects of RS3. A new form of RS (RS5) could be formed in bread by complexing amylose with lipids. RS5 appears in breads that include some type of fat in their ingredients. The bread making process in our study followed the traditional Portuguese bread making process in which no fat is added. Therefore, as can be seen in Table 1, the lipid content is so low that the formation of RS5 was not considered.

Other studies investigated the effect of RS included in meals. Akerberg et al. (1998) have compared breads with different amounts of RS: 0.2% vs 3.5% vs 10.3% of total starch, included in a meal with foodstuffs rich in fat and protein. Only the glycaemic response of bread with 10.3% showed significant differences compared to the reference bread. Klosterbuer et al. (2012) investigated the effect of RS3 addition to a meal, keeping constant the remaining nutrients and report a significant reduction in iAUC (0–180) in a meal supplemented with 25 g of RS. Despite the differences between the sample test compositions, types of RS tested and the quantification methodology of RS (only at the last study is the same at the present investigation), the results of the referred studies agree with the present research.

4.2. *Insulin response*

Glucose response curves, up to 30 min, to both treatments were similar therefore, no differences were observed at insulin response. It should not be ignored that insulin response up to 120 min may display significant differences between the two treatments. These findings are also in agreement with Akerberg et al. (1998) and Hallstrom et al. (2011) who found no significant differences on insulin response (0–120 min) even when the glucose response was significantly different. Klosterbuer et al. (2012) also observed no significant differences on insulin response for a breakfast supplemented with 25 g of RS but the same breakfast with 20 g of RS and 5 g of pullulan showed significant differences on insulin response, in comparison to a low-fiber control

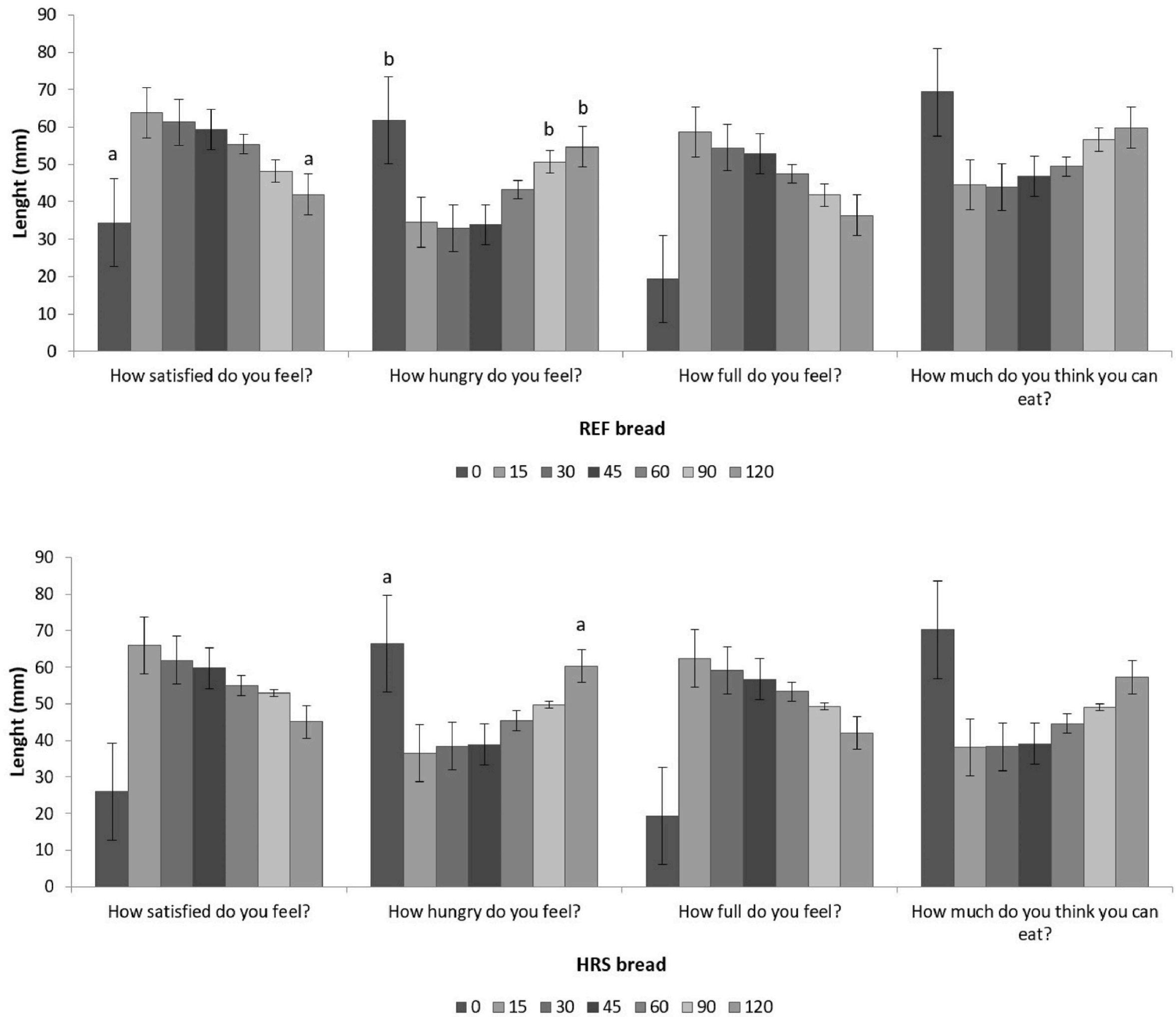

Fig. 3. Comparison of mean scores for each VAS responses at fasting and each evaluation moment to HRS bread and REF bread; n = 37; equal letters means not significantly different scores.

breakfast. Pullulan is a non-available polysaccharide produced by the fermentation of dextrin by *Aureobasidium pullulans*.

4.3. Appetite VAS scores

Satiation is defined as the sensation of fullness that develops during the progress of a meal and contributes to meal cessation, whereas satiety is defined as the sensation of fullness between one meal and the next (Sorensen et al., 2003). Both sensations are regulated by a multitude of environmental, central and peripheral signals. The ability of a particular food or meal to induce satiation and satiety has crucial importance to the ingested dose and the gap time between meals. These two variables can significantly influence food intake and determine energy balance. Satiety cannot be assessed with a simple question, therefore, VAS are commonly used.

For this study, we evaluated satiety based on VAS questions about satisfaction, hunger, fullness and prospective food consumption. VAS questions about "satisfaction" and "fullness" exhibited similar behaviour: low scores on fasting state followed by higher scores 15 min after intake the meal that, gradually, decreased up to 120 min. For the others two questions ("hunger" and "prospective food consumption") we observed the opposite behaviour: high scores on fasting state followed by lowest scores at 15 min that, progressively, increased up to 120 min. Our findings shows that HRS bread with 2.4% of RS was consistently more satiating than REF bread (0.8% of RS). HRS bread reveals higher satiety scores. This difference is very clear to "satisfaction" item in which the comparison of mean scores was significantly higher for all the evaluation moments (15, 30, 45, 60, 90 and 120 min). On VAS issues about "fullness" and "prospective food consumption", the significant differences at 90 and 120 min and at 45 and 90 min, respectively, are consistent with greater satiety of HRS bread. Likewise, the feeling of satiety expressed as iAUC revealed significantly higher values for "satisfaction" (3680.6 ± 2632.5 mm*min *vs* 2544.6 ± 2130.6 mm*min) and "prospective food consumption" (3724.3 ± 3143.4 mm*min *vs* 2288.9 ± 1444.6 mm*min) after HRS bread intake than REF bread intake. Also for "hungry" and "fullness", the iAUC values for HRS bread were higher but without statistical significance. Data obtained to understand how long the feeling of satiety lasts confirmed the capacity of HRS bread to induce higher satiety than REF bread. HRS bread showed no statistical differences between fasting and 120 min mean scores in just one question (hunger). REF bread showed no significant differences to "satisfaction" item between fasting and 120 min and to "hunger" item between fasting and 90 min and fasting and 120 min. HRS bread presents a longer duration of satiety than REF bread.

Few studies have investigated the effect of RS on satiety. The methodology used and the study designs do not allow the comparison of results. The satiety evaluation methods, itself a very subjective measure, were not the same, beyond the different composition of the meal wherein RS was included (the fat content is a very important factor in satiety evaluation as well as the presence of other fibre sources). Other differences between studies are the duration (period) of the study and the RS type tested (Higgins, 2004; Luhovyy et al., 2014). However, our study agrees with Willis et al. (2009) where those authors observed that a muffin supplemented with 8 g of RS induced greater satiety than a placebo. Al-Mana and Robertson (2018) demonstrated that RS acutely and significantly reduces food intake at an *ad libitum* meal in overweight/obese men compared with placebo but this effect was lost when the whole 24 h intake was evaluated. These data are contradictory to other studies developed with different methodologies but using the same satiety evaluation tool (VAS questions by Flint et al. (2000)). In a study by Bodinham et al. (2010), the consumption of 48 g of RS2 divided by breakfast and lunch, did not influence the feeling of appetite but reduced the energy intake at dinner meal. Klosterbuer et al. (2012) did not find significant differences in scores of satiety after eating a breakfast supplemented with 25 g of RS3 and there were no significant differences in the amount of *ad libitum* energy intake at the lunch buffet and for the remainder of the day. Willis et al. studies suggest that not all fibres influence satiety equally. These researchers demonstrate that RS is one of the fibre components with more influence on short-term satiety (Willis et al., 2009). The mechanisms to explain why fibre rich foods are more satiating seems to be related to the delay of gastric emptying and absorption of macronutrients. The increasing of gastrointestinal hormones production that acts as inducers of satiety, such as cholecystokinin, GLP-1 and tyrosine-tyrosine peptide, may also explain this effect after ingestion of fibre rich foods (Slavin, 2005). As well, Nilsson et al. (2008) found that RS may mediate satiety by altering colonic fermentation due to their prebiotic effects. Sorensen et al. (2003) in a review paper, reports that palatability of food or a meal inconsistently influences appetite and satiety. Highly palatable foods may increase or decrease satiety scores and the effects may not be predictable. In a recent review, Guo et al. (2021) also refer the potential effect of RS on reducing energy intake and inducing satiety supported by numerous studies despite the heterogeneity of the experimental designs.

Despite the subjectivity in the assessment of satiety, our results suggest the higher perception of satiety after ingestion HRS bread in comparison to REF bread. We may speculate that at an *ad libitum* condition will be ingested a lower HRS bread portion than REF bread. In this case, the glycaemic response and the energy intake will be lower, which could be important on a weight loss situation, weight maintenance or on diabetes. Further research is needed to understand the mechanisms of how RS influence satiety, namely monitoring the gut hormones and colonic fermentation.

4.4. Glycaemic index

GI index is a physiological concept used to classify carbohydrate containing foods. Foods with a high GI value release glucose rapidly into the bloodstream, while foods with a low GI value release glucose slower. In general, high-fibre foods are assigned a lower GI value (Nugent, 2005). Since resistant starch is one of the fibre components, its presence will influence the glycaemic index. In fact, some authors showed that increased RS content *per* test portion was correlated to a reduced GI (Hallstrom et al., 2011). In our study, the low GI of HRS bread (GI = 60) *vs* REF bread (GI = 82) was a consequence of slow glycaemic response of this bread. Given that both breads differ essentially by the RS content, we can assume that RS was the major contributor and the responsible to lower GI. According to EFSA, the beneficial effects of RS are verified when this component replaces the digestible starch. Our study confirms previous studies that RS can influence the rate of digestion of available starch (Granfeldt et al., 1995; Hallstrom et al., 2011; Klosterbuer et al., 2012) whereby their benefits seem to be present even when RS does not replace digestible starch. Further research is needed to clarify this effect and, if appropriate, propose a new health claim to EFSA. In our study, HRS bread was produced exclusively with white wheat and corn flours and presented an amount of 2.4% of RS (5% total starch). No studies are known that have tested bread samples with lower RS content and without the presence of other fibre sources. Nevertheless, this amount of RS showed a positive effect on glycaemic response. It will be important to investigate the beneficial effects of HRS bread in the context of a meal, along with other foodstuffs.

5. Conclusions

Bread with 2.4% of RS3, produced with white wheat and corn flour, presented a GI of 60 (medium GI). The higher amount of RS causes a significantly lower postprandial glucose response in comparison to a control (GI of 82). These results shows that RS3 influenced the digestibility of the available starch fraction. No differences were observed at insulin response. RS induces higher satiety scores and consequently the food intake. More research is needed to investigate these benefits in the context of a meal and the long-term effects.

Ethical approval

The study was approved by the Centro Hospitalar Lisboa Norte/ Faculdade de Medicina Ethics Commission. Written informed consent was obtained from all subjects before the start of the study.

CRediT authorship contribution statement

Olga Amaral: Conceptualization, Methodology, Formal analysis, Writing. **Catarina Guerreiro:** Conceptualization, Formal analysis, Co-supervised the whole experiment. **Ana Almeida:** Methodology, Revision of the manuscript. **Marília Cravo:** Conceptualization, Formal analysis, Co-supervised the whole experiment.

Declaration of competing interest

The authors declare that they have no conflict of interest.

The founders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

Acknowledgements

The authors would like to thank Profs. Antonieta Medeiros and Miquelina Pena for assistance in blood sampling. We are also grateful to the Beja city Hospital José Joaquim Fernandes (Baixo Alentejo Health Unit), namely Dra. Isabel Ramôa of the Diabetes Service in the clinical trial, including the provision of equipment and disposables for measuring capillary blood glucose and measurement of haemoglobin A1c to all study volunteers and Dra. Graça Cristina of the Clinical Pathology Service in the treatment of blood samples for the quantification of insulin. This research was funded by Polytechnic Institute of Beja, Portugal and Fundação para a Ciência e Tecnologia / GeoBioTec, grant number UIDP/04035/2020.

References

Akerberg, A., Liljeberg, H., & Bjorck, I. (1998). Effects of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. *Journal of Cereal Science, 28*(1), 71–80. https://doi.org/10.1006/jcrs.1997.0173

Al-Mana, N. M., & Robertson, M. D. (2018). Acute effect of resistant starch on food intake, appetite and satiety in overweight/obese males. *Nutrients, 10*(12). https://doi.org/10.3390/nu10121993. Article 1993.

Amaral, O., Guerreiro, C., Gomes, A., & Cravo, M. (2016). Resistant starch production in wheat bread: Effect of ingredients, baking conditions and storage. *European Food Research and Technology, 242*(10). https://doi.org/10.1007/s00217-016-2674-4, 1747-1747-1753.

Amini, S., Mansoori, A., & Maghsumi-Norouzabad, L. (2021). The effect of acute consumption of resistant starch on appetite in healthy adults; a systematic review and meta-analysis of the controlled clinical trials. *Clinical Nutrition ESPEN, 41*. https://doi.org/10.1016/j.clnesp.2020.12.006, 42-42-48.

Bodinham, C. L., Frost, G. S., & Robertson, M. D. (2010). Acute ingestion of resistant starch reduces food intake in healthy adults. *British Journal of Nutrition, 103*(6), 917–922. https://doi.org/10.1017/s0007114509992534

Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., & Wolever, T. M. S. (2005). Glycaemic index methodology. *Nutrition Research Reviews, 18*(1), 145–171. https://doi.org/10.1079/nrr2005100

CodexAlimentarius, C. (2009). Report on the 30th session of the codex committee on nutrition and foods for special dietary uses. In *ALINORM 09/32/26* (p. 46). Appendix II Rome.

EFSA Panel on Dietetic Products, N. A. (2011). Scientific Opinion on the substantiation of health claims related to resistant starch and reduction of post-prandial glycaemic responses (ID 681), "digestive health benefits" (ID 682) and "favours a normal colon metabolism" (ID 783) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA Journal, 9*(4). Retrieved 4 de Dez 2012, from http://www.efsa.europa.eu/en/efsajournal/doc/2024.pdf.

Englyst, H. N., Kingman, S. M., Hudson, G. J., & Cummings, J. H. (1996). Measurement of resistant starch in vitro and in vivo. *British Journal of Nutrition, 75*(5), 749–755. https://doi.org/10.1079/bjn19960178

European Commission. (2008). Commission Directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. https://www.legislation.gov.uk/eudr/2008/100/contents.

FAO/WHO. (1998). Carbohydrates in human nutrition. Report of a joint FAO/WHO expert consultation. *FAO Food & Nutrition Paper, 66*, 1–140. Retrieved 1998, from https://www.fao.org/3/w8079e/w8079e00.htm.

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scares in assessment of appetite sensations in single test meal studies. *International Journal of Obesity, 24*(1), 38–48. https://doi.org/10.1038/sj.ijo.0801083

Granfeldt, Y., Drews, A., & Björck, I. (1995). Arepas made from high amylose corn flour produce favorably low glucose and insulin responses in healthy humans. *Journal of Nutrition, 125*(3), 459–465. https://doi.org/10.1093/jn/125.3.459

Guo, J., Tan, L., & Kong, L. (2021). Impact of dietary intake of resistant starch on obesity and associated metabolic profiles in human: A systematic review of the literature. *Critical Reviews in Food Science and Nutrition, 61*(6), 889-889-905.

Hallstrom, E., Sestili, F., Lafiandra, D., Bjorck, I., & Ostman, E. (2011). A novel wheat variety with elevated content of amylose increases resistant starch formation and may beneficially influence glycaemia in healthy subjects. *Food & Nutrition Research, 55*. https://doi.org/10.3402/fnr.v55i0.7074. Article 7074.

Haralampu, S. G. (2000). Resistant starch - a review of the physical properties and biological impact of RS3. *Carbohydrate Polymers, 41*(3), 285–292. https://doi.org/10.1016/s0144-8617(99)00147-2

Haub, M. D., Hubach, K. L., Al-Tamimi, E. K., Ornelas, S., & Seib, P. A. (2010). Different types of resistant starch elicit different glucose reponses in humans. *Journal of Nutrition and Metabolism*. https://doi.org/10.1155/2010/230501, 2010.

Higgins, J. A. (2004). Resistant starch: Metabolic effects and potential health benefits. *Journal of AOAC International, 87*(3), 761–768. https://doi.org/10.1093/jaoac/87.3.761

Higgins, J. A., & Brown, I. L. (2013). Resistant starch: A promising dietary agent for the prevention/treatment of inflammatory bowel disease and bowel cancer. *Current Opinion in Gastroenterology, 29*(2), 190–194. https://doi.org/10.1097/MOG.0b013e32835b9aa3

Klosterbuer, A. S., Thomas, W., & Slavin, J. L. (2012). Resistant starch and pullulan reduce postprandial glucose, insulin, and GLP-1, but have No effect on satiety in healthy humans. *Journal of Agricultural and Food Chemistry, 60*(48), 11928–11934. https://doi.org/10.1021/jf303083r

Luhovyy, B. L., Mollard, R. C., Yurchenko, S., Nunez, M. F., Berengut, S., Liu, T. T., … Anderson, G. H. (2014). The effects of whole grain high-amylose maize flour as a source of resistant starch on blood glucose, satiety, and food intake in young men. *Journal of Food Science, 79*(12), H2550–H2556. https://doi.org/10.1111/1750-3841.12690

McCleary, B. V., McNally, M., Rossiter, P., Aman, P., Amrein, T., Arnouts, S., … Zheng, T. (2002). Measurement of resistant starch by enzymatic digestion in starch and selected plant materials: Collaborative study. *Journal of Aoac International, 85*(5), 1103–1111. https://doi.org/10.1093/jaoac/85.5.1103

Nilsson, A. C., Ostman, E. M., Holst, J. J., & Björck, I. M. (2008). Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast. *Journal of Nutrition, 138*(4), 732–739. https://doi.org/10.1093/jn/138.4.732

Nugent, A. P. (2005). Health properties of resistant starch. *Nutrition Bulletin, 30*(1), 27–54. https://doi.org/10.1111/j.1467-3010.2005.00481.x, 10.1111/j.1467-3010.2005.00481.x.

Robertson, M. D. (2012). Dietary-resistant starch and glucose metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care, 15*(4), 362–367. https://doi.org/10.1097/MCO.0b013e3283536931

Slavin, J. L. (2005). Dietary fiber and body weight. *Nutrition, 21*(3), 411–418. https://doi.org/10.1016/j.nut.2004.08.018

Sorensen, L. B., Moller, P., Flint, A., Martens, M., & Raben, A. (2003). Effect of sensory perception of foods on appetite and food intake: A review of studies on humans. *International Journal of Obesity, 27*(10), 1152–1166. https://doi.org/10.1038/sj.ijo.0802391

Willis, H. J., Eldridge, A. L., Beiselgel, J., Thomas, W., & Slavin, J. L. (2009). Greater satiety response with resistant starch and corn bran in human subjects. *Nutrition Research, 29*(2), 100–105. https://doi.org/10.1016/j.nutres.2009.01.004

Wolever, T. M. S. (2013). Is glycaemic index (GI) a valid measure of carbohydrate quality? *European Journal of Clinical Nutrition, 67*(5), 522–531. https://doi.org/10.1038/ejcn.2013.27

Wolever, T. M. S., Brand-Miller, J. C., Abernethy, J., Astrup, A., Atkinson, F., Axelsen, M., … Zhang, J. (2008). Measuring the glycemic index of foods: Interlaboratory study. *American Journal of Clinical Nutrition, 87*(1), 247S–257S. https://doi.org/10.1093/ajcn/87.1.247S. PMID: 18175765.

Xiong, K., Wang, J., Kang, T., Xu, F., & Ma, A. (2021). Effects of resistant starch on glycaemic control: A systematic review and meta-analysis. *British Journal of Nutrition, 125*(11), 1260–1269. https://doi.org/10.1017/S0007114520003700