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Structuring white rice with gellan gum reduces the glycemic response in healthy humans

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ABSTRACT

White rice has a high glycemic index and its consumption has been linked to an increased risk of developing type-2 diabetes mellitus, increased diabetes associated complications and obesity. In recent *in vitro* studies we have shown that addition of food hydrocolloids, such as low acyl gellan gum (LAGG), when cooking white rice potentially modifies starch digestion kinetics. The impact *in vivo* remains to be investigated. We aimed to determine the effect of adding LAGG to white rice on postprandial glycemic, gastrointestinal and appetitive responses in humans. Following LAGG *in vitro* characterisation, 12 healthy adults participated in a randomised, controlled, crossover study. They consumed isoenergetic meals of jasmine white rice (232 kcal) cooked with (Rice + LAGG) and without (Rice control) 3 % w/dry rice w LAGG. Blood glucose, intragastric meal appearance, meal volume and appetite were assessed serially for 2 h. The incremental area under the curve over two hours (iAUC2h) for blood glucose for the Rice + LAGG meal (93 ± 16 mmol/L·min) was significantly lower than that for the Rice control meal (160 ± 18 mmol/L·min), P=0.0007. Blood glucose rose postprandially to a peak at T=30 min, with the Rice control meal peak (7.3 ± 0.2 mmol/L) significantly higher than that for the Rice + LAGG meal (6.5 ± 0.2 mmol/L), $P < 0.01$. MRI images showed that for Rice + LAGG there were multiple rice boluses persisting intragastrically throughout the digestion time. There were no significant differences in appetite between meals. The addition of LAGG to the cooking process was effective in reducing postprandial blood glucose responses in healthy humans. If confirmed, this could potentially provide a simple and relatively inexpensive intervention to reduce the post prandial glycemic response to white rice.

1. Introduction

Elevated blood glucose is a recognised major risk factor for the development of negative health consequences, such as cardiovascular dysfunction (Mapanga & Essop, 2016) and more severe complications in those with poorly controlled diabetes (Edwards, Vincent, Cheng, &

Abbreviations: AUC, area under the curve; iAUC, incremental area under the curve; BMI, Body Mass Index; CR, compression ration; EGI, estimated glycemic index; FOV, field of view; GI, glycemic index; GLCM, grey level cooccurrence matrix; HASTE, Half-Fourier Single-shot Turbo spin-Echo; HI, hydrolysis index; LAGG, low acyl gellan gum; MRI, magnetic resonance imaging; ROI, region of interest; SBWC, small bowel water content; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SSF, simulated salivary fluid; TE, echo time; TR, repetition time; TPA, texture profile analysis; T2, transverse relaxation time; VAS, visual analogue scale.

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Feldman, 2008; Stratton et al., 2000). Controlling blood glucose levels is therefore important in reducing these risks. Diet plays a key role in blood glucose control. For individuals with impaired glucose metabolism, such as those living with pre-diabetes or diabetes, diets with low glycemic responses have consistently demonstrated beneficial effects for blood glucose management in both short-term and long-term studies (Howlett & Ashwell, 2008; Ojo, Ojo, Adebowale, & Wang, 2018; Riccardi, Rivellese, & Giacco, 2008; Zafar et al., 2019). Furthermore, habitually consuming foods high in rapidly absorbed carbohydrates has been linked with increased risk for type-2 diabetes (Salmeron et al., 1997; Schulze et al., 2004) and consumption of foods with low glycemic response can be beneficial for weight control (Brand-Miller, Holt, Pawlak, & McMillan, 2002; Ludwig et al., 1999).

Rice is a staple food for a large part of the world's population (Fukagawa & Ziska, 2019) and provides about 20 % of the global energy intake (van Dam, 2020). Preference is shown for highly refined white rice, which is low in dietary fibre and has a high glycemic index (GI), reflected in the high postprandial blood glucose response (Fukagawa & Ziska, 2019; van Dam, 2020). High consumption of white rice will not only elevate blood glucose postprandially in those diagnosed with type 2 diabetes, increasing the risk of complications, but also has been associated with increased risk for developing type-2 diabetes. This has been attributed to the high glycemic response of the rice (Bhavadharini et al., 2020; Hu, Pan, Malik, & Sun, 2012). This problem is particularly prominent in Asian populations where the daily consumption of white rice can be significantly higher than in the Western population; the average intake being three to four servings per day compared with two per week in Western populations (Hu et al., 2012).

Extensive dietary change to improve health outcomes is challenging, particularly in low- and middle-income countries (Blake et al., 2021). An attractive alternative could be to find simple modifications to food processing that could help create 'healthier' versions of commonly consumed foods.

Hydrocolloids are hydrophilic molecules of high molecular weight, such as polysaccharides, with uses in foods including viscosity modifiers and/or gelling agents (Alshammari et al., 2021). They can be used to reduce the glycemic response of high GI foods both *in vitro* and *in vivo* (Alshammari et al., 2021; Boers, Seijen-ten Hoorn, & Mela, 2015; Gouseti et al., 2014), although how this benefit can be exploited is yet to be fully understood. Lowering the GI of rice by using different combinations of xanthan gum (a hydrocolloid) and Japanese 'Koshiibuki' polished rice during food processing decreased postprandial blood glucose levels (Fuwa, Nakanishi, & Moritaka, 2016). The greatest effect was observed when the xanthan gum was added during the cooking process of the rice.

We have recently carried out *in-vitro* experiments to investigate the use of hydrocolloids to modify complex food matrices under gastrointestinal conditions, with the aim to modify starch digestibility (Muttakin et al., 2023). We have shown that low acyl gellan gum (LAGG), a hydrocolloid of bacterial origin (Alshammari et al., 2021), had high potential to reduce the Estimated Glycemic Index (EGI) of white jasmine rice *in-vitro*. LAGG appeared to create a gel-like layer on the surface of the rice and to slow down breakdown of simulated rice food boluses during *in vitro* intestinal digestion. The potential for LAGG to reduce the glycemic response to white rice *in-vivo* has not been investigated.

Building on that initial *in-vitro* work, the first aim of this study was to investigate the effect of adding LAGG to jasmine white rice cooking process using *in-vitro* methods and establish the most appropriate dose of LAGG for the subsequent *in vivo* study. The second aim was to test the hypothesis that the addition of LAGG to the cooking process of jasmine white rice will decrease the postprandial blood glucose response (primary outcome measure) compared with white rice cooked identically but without adding LAGG. We also hypothesised that this would have an effect on appetite, assessed using subjective appetite ratings and *ab libitum* intake measurement, and gastrointestinal responses, assessed using gastrointestinal magnetic resonance imaging (MRI) techniques to measure intragastric meal and gas volumes and small bowel water volumes.

2. Material and methods

2.1. In vitro characterization

2.1.1. Material

Low acyl gellan gum (LAGG) KELCOGEL® F was obtained from CP Kelco (Atalanta, GA). Thai Hom Mali fragrant jasmine rice (Green Dragon, Thailand, imported by Westmill Foods, Enfield, UK) was purchased from a local supermarket (Medina Foodstore, Nottingham, UK). Salivary alpha-amylase, pancreatic alpha-amylase, pancreatin, bile salt, pepsin enzyme, assay 4-hydroxybenzhydrazide (PAHBAH), maltose standard, stop solution (Na_2CO_3) and all other reagents were of analytical grade and purchased from Sigma–Aldrich (Merck Life Science, Gillingham, UK).

2.1.2. Sample preparation

Initial work to determine the LAGG dose range for the *in vivo* study considered 1 %, 2 %, 3 % and 4 % LAGG (w/dry rice weight). However, it was shown that at LAGG concentrations above 3 % (w/dry rice weight) the water phase became too thick for the subsequent cooking of the rice. Therefore, the range 0 % − 3 % LAGG was chosen for this *in vitro* initial screening.

Ten grams of raw jasmine rice were cooked in 15 g of water at 95 °C for 20 min with the addition of different amounts of LAGG, expressed as a percentage of LAGG weight over raw dry rice weight. Samples were initially cooked with a range of LAGG amounts to determine the sensible working range before the water phase became too thick to undertake the necessary subsequent processing. Sample A was the control cooked with no added LAGG. Samples B, C and D were cooked with 1 %, 2 % and 3 % added LAGG (w/w of dry rice), respectively. After the cooking was completed, the rice was mixed gently with a spoon. Samples were prepared fresh just before studying their digestion *in vitro*, and the cooking and digestion procedures were repeated in triplicates for each sample.

2.1.3. In vitro digestion and estimated glycemic index

For the *in vitro* digestion, the static INFOGEST protocol was followed as detailed in Supplementary Materials (Brodkorb et al., 2019; Minekus et al., 2014). For the oral phase no mastication step was used. For the intestinal phase, simulated intestinal fluid (SIF) containing pancreatic a-amylase was used before incubation at 37 °C for 120 min at pH 6.

At times 2.5, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min of intestinal digestion, 200 μL aliquots were extracted and α-amylase was inactivated as detailed in Supplementary Materials. Intestinal digestion started at time 0 with the addition of SIF containing pancreatic enzymes. Starch hydrolysis was measured as released reducing sugars using a 4-hydroxybenzhydrazide (PAHBAH) assay (Blakeney & Mutton, 1980). The rate of starch digestion was then expressed as the percentage of total starch hydrolysed at different times based on the method of Goni *et al.* (Goni, GarciaAlonso, & SauraCalixto, 1997). The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each rice sample by the corresponding area of the reference sample (white bread). The estimated glycemic index (EGI) was then calculated using the formula EGI=39.71 + (0.549 HI) (Goni et al., 1997).

2.1.4. Texture analysis

Texture analysis of the rice grains was performed using a Texture Analyser (TA.XTplus, Stable Micro Systems, Surrey, UK) equipped with a 35 mm diameter flat punch cylindrical probe as detailed in Supplementary Materials. Cooked the rice grains were transferred into a Petri dish and submerged in excess of water or of hydrochloric acid solution (0.01 mol/L), and kept soaked at room temperature (~20 °C) for a period of 1 h, 2 h, and 3 h before testing (Supplementary Fig. 1A). The compression experiment was then performed using a modified texture

profile analysis protocol (TPA) (Bourne, 1966; Friedman, Whitney, & Szczesniak, 1963; Rosenthal, 2010), with two sequential compressions, separated by the recovery time of 10 s. Three repeats for each condition were measured and averaged. The analysis was performed using an empirical approach, by evaluating the values of maximum force, slope of the force versus compression curve to determine effective stiffness and maximum pull of force on retract as well as compression ratio and stiffness.

2.1.5. Fluorescent microscopy

LAGG was labelled using 5-(4,6-dichlorotriazinyl) aminofluorescein (5-DTAF). 5-DTAF is a reactive dye usually used to stain proteins and has an absorption/emission maxima of 492/516 nm. The 5-DTAF label can bind strongly to the LAGG and therefore provide a unique fluorescent probe to demonstrate if the rice grain is coated by LAGG during cooking and/or if LAGG is able to penetrate below the grain surface. Briefly: a stock solution of 40 mg of 5-DTAF in 4.038 mL of DMSO was prepared. 0.2 mM of 5-DTAF was added to 10 mg/mL of LAGG sample at room temperature. Ten mM Na_2SO_4 was slowly added to this. The pH was then raised to 10 using NaOH at room temperature and after two hours the reaction was quenched using 1:2 ratio ethanol/sodium acetate buffer mixture. After this the unbound 5-DTAF was washed with ethanol:sodium acetate solution using a Buchner funnel with a 0.45 μm PTFE membrane under vacuum until the filtrate appeared clear. The fluorescently labelled LAGG was then dehydrated using ethanol and dried under vacuum overnight, ready to use. After this, the same cooking protocol described above was followed using jasmine rice with 3 % weight/dried rice weight unlabelled LAGG (control) or labelled LAGG. Cross sections of the cooked rice grains were then cut and underwent fluorescent microscopy.

2.2. In vivo study

2.2.1. Ethical approval, study participants and screening

The study was approved by the University of Nottingham Faculty of Medical and Health Sciences Research Ethics Committee (Ethics approval number 470–2001) and it was registered on clinicaltrials.gov with identifier NCT05080400. Informed written consent was obtained from all participants prior to the commencement of the study. Twelve healthy adult participants were recruited. Details of recruitment, screening and inclusion and exclusion criteria are provided in Supplementary Materials and in Supplementary Fig. 3).

2.2.2. Study design

This study was a randomised, controlled, cross-over trial. Twelve participants completed the human study, seven females and five males (Supplementary Fig. 3). They were 26 ± 2 years old and with a BMI of 23 ± 1 kg/m2. A schematic diagram of the timings of the study day's protocol is shown in Fig. 1. The participants arrived at 09:00 h after having fasted since 22:00 h the previous evening. Before the rice test meal, termed as time T=0 throughout, a baseline fasting finger prick blood test was carried out. The participants then filled the baseline visual analogue scale (VAS) appetite questionnaire described below and underwent an MRI scan of the abdomen. This provided baseline values for all endpoints and a further check that the stomach was not containing food before the study started.

The participants were then asked to sit at a table in a quiet room and they were provided with the rice meal immediately after it had been cooked. They were allocated the Rice + LAGG or rice control meal first according to a Latin square block design. They were asked to consume the rice test meal within 15 min. Both rice meals were presented in exactly the same way. The rice was provided with 330 mL of still water, at room temperature, and the participants were asked to consume all of the water with their meal.

Postprandially, fingerpick blood glucose sampling was then carried out 15 min after the meal start and every 15 min for 2 h, for a total of 9 sampling points including the fasting baseline. MRI images of the abdomen were also taken after feeding (T=15 min) and subsequently at 30-minute intervals for 2 h. The finger-prick, followed by the VAS for appetite were carried out immediately before the MRI scan. The MRI procedures took only a few minutes and the participants spent most of the time sitting up in a quiet room adjacent to the MRI scanner.

When this was all completed, an *ad libitum* pasta lunch meal was provided approximately 15 min after the final MRI scan, followed by one last VAS questionnaire. The participants were then instructed to keep food diaries which they were provided with for the rest of the day, to calculate the total energy intake for the day.

An identical study day was repeated with the other rice meal intervention following a washout period of approximately 7 days. At the end of their last visit, the participants were asked 3 yes/no standard questions: if the study was acceptable, if the meals were acceptable and if they perceived a difference between the two meals.

Care was taken to minimise confounding effects by carefully controlling the conditions of the preparation, presentation and consumption of the two intervention meals. The participants were asked to eat the same meal (of their choice) for dinner before each of the two study days to reduce variability and compliance with this was checked verbally at the study day. Although the rest of the diet on the day before the glucose study was not controlled participants were asked to avoid strenuous exercise and alcohol for 24 h prior to the study. The gum was tasteless under the investigated conditions.

2.2.3. Rice test meals

The study comprised two rice test meals based on jasmine rice cooked in water. The 'Rice control' meal was prepared using 185 g of raw jasmine rice placed with 365 mL of still bottled water (ASDA, UK) in a 1.5 L rice cooker (Cookworks™, Argos, UK). The rice cooker took 20 min to cook the rice before switching off automatically, at which point all the cooking water had been absorbed by the rice. After this 180 g of the cooked rice was weighed and served in a disposable dish with a disposable spoon and a 330 mL bottle of still water (Highland Spring, UK).

For the 'Rice + LAGG' meal preparation, firstly 5.5 g of LAGG (equivalent to a 3 % w/w of dried rice) were dispersed in 365 mL water,

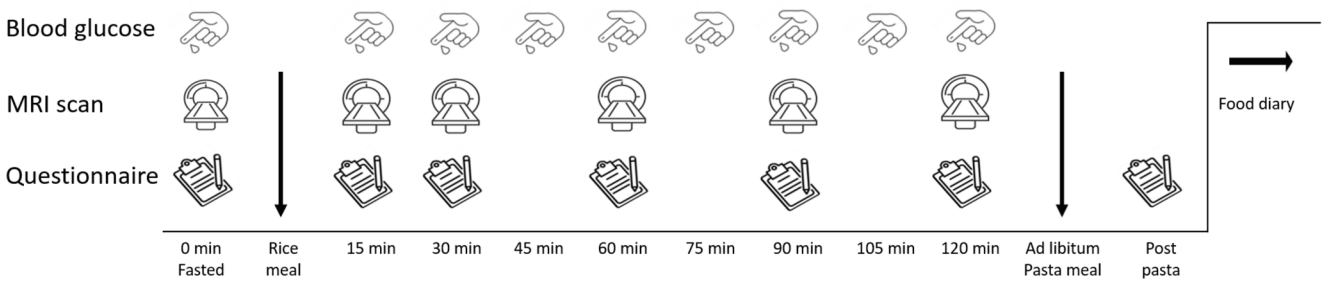

Fig. 1. Schematic diagram of the procedures carried out on a study day.

with the rice cooker turned on, for 10 min. The water/LAGG mixture was then stirred and an additional 50 mL of water was added to replace water lost as previously determined by a test of water weight loss on cooking. The aliquot of 185 g of raw rice was then added and cooked in the same way as above and fed immediately after cooking.

The cooked meal portion corresponded to 50 g of available carbohydrates (Wolever, Jenkins, Jenkins, & Josse, 1991), which is the standard used to test the GI of foods, and the meals provided 232 kcal each (isoenergetic) with the energy content of the LAGG considered to be zero kcal/g as recommended (Wolever et al., 1991).

2.2.4. Glycemic responses

Finger prick blood glucose measurements were carried out after careful hand washing, using warm soppy water that was carefully rinsed off and the hands dried. A single-use Unistik 3 lancet (Owen Mumford, Woodstock, Oxfordshire) and an Accu-Chek Performa hand-held blood glucose meter with Accu-Chek Performa test strips (both from Roche Diabetes Care, Inc, Indianapolis, Indiana) were used. The 2 h finger prick blood sampling duration was chosen as the incremental AUC for blood glucose over 2 h (iAUC 2 h) which represents the standard glucose test for assessing the glucose responses to a given glucose intake (Wolever et al., 1991).

2.2.5. Subjective appetite responses

Paper-based 100 mm VAS (Blundell et al., 2010; Flint, Raben, Blundell, & Astrup, 2000) were used to measure the subjective feeling of hunger, satiety, fullness, desire to eat and prospective food consumption. These were used as previously described (Alhussain, Macdonald, & Taylor, 2016; Alyami et al., 2019), with each VAS scale being anchored to extreme sensation, e.g. for hunger the lower anchor was 'Not hungry at all' and the higher anchor was 'I have never been more hungry'. Participants were asked to make a vertical line on each of the five questions on the scale that matched how they felt at that time point. A new VAS sheet was presented each time and removed after completion, to avoid bias from previous scores. The average or composite appetite score was then calculated for each participant at each time point and for each meal using the formula:

$$\text{Composite satiety score} = [\text{hunger} + (100 - \text{satisfaction}) + (100 - \text{fullness}) + \text{desire to eat} + \text{prospective consumption}]/5$$

The resulting composite appetite score had values between 0 and 100. Lower values in this context indicated more 'benefit' towards lower hunger, higher fullness and less desire to eat, whilst higher values indicated the opposite (Alhussain et al., 2016; Alyami et al., 2019).

2.2.6. Ad libitum meal

An *ad libitum* meal was served 15 min after all the glucose testing and MRI procedures were completed, as a lunch test, to assess *ad libitum* food intake. It consisted of a chilled, pre-prepared tomato and mozzarella pasta bake purchased from a supermarket (Tesco, UK). Three packs (450 g each) of pasta bake were heated in a 900 W microwave at full power for 10 min and stirred well at the end. The nutritional value of the pasta bake per 100 g was 109 kcal, comprising 16.4 g of carbohydrates, 4.9 g of protein and 2.3 g of fat. Percentage of total energy from carbohydrate was 60 %, from protein 18 % and from fat 19 %. The participants were given a large bowl with a single weighed portion of approximately 1300 g and a 330 mL bottle of still water. The participants were seated again in the same quiet room as for the rice meals and they were told that this portion was deliberately much larger than that normally consumed, and to eat from the bowl until satisfied, as used previously (Alyami et al., 2019; Hussein et al., 2015). They were instructed to drink all the water in the bottle with the pasta meal as they wanted. The amount of pasta left over was removed and weighed. The type of meal was selected as it would allow each spoonful to provide homogenous composition of ingredients, having been carefully stirred. The energy intake was calculated from the amount consumed as an objective measure of food consumption. The speed of the *ad libitum* meal consumption was measured by dividing the amount of pasta intake in g on the time taken to eat it in minutes (Alhussain et al., 2016).

2.2.7. Food diaries and total energy intake

Food diaries were given to the participants to keep a detailed record of food and beverages consumed over the study day, to calculate daily energy intake. The participants were instructed to report information on the size of the meal using household measures or by weighing the food using their own scale. They were also asked to provide information about the cooking methods and to provide brand names if appropriate. If the participants prepared a meal using several ingredients, they were asked to provide a recipe, the number of servings produced, and the actual amount consumed. The energy and nutrient content of the food and drink consumed was subsequently analysed using Nutritics software (Nutritics Ltd, Dublin, Ireland). The nutritional composition of non-available food items in the software were added manually using data from food labels. Where an identical food was not available, a best fit was chosen.

Total energy intake for the day included the intake of rice, the *ad libitum* pasta meal and the food reported in the food diary as having been consumed over the remainder of the day.

2.2.8. Magnetic resonance imaging

MRI imaging was carried out using a 1.5 T HDxt MRI scanner (General Electric Medical Systems, Milwakee, Wisconsin). At each time point, breath-hold, axial, multi-slice Half-Fourier Single-shot Turbo spin-Echo (HASTE) MRI scans were acquired to visualise the intragastric appearance of the rice meals with time and to measure the volumes of the stomach contents by manually tracing around each two-dimensional image of the meal in the stomach, as described in Supplementary Materials. This sequence collected 46 slices with echo time TE 60 ms and image resolution 1.56 mm × 1.67 mm with a slice thickness of 5.0 mm. This moderately T2 weighted sequence provided good contrast between liquids (appearing white), tissues or hydrated foods (appearing grey), and more solid/less hydrated food components (appearing black). The gastric volume time curves were fitted to calculate the time to empty half of the gastric meal contents (T50%) and average meal emptying rate (mL/min). A breath-hold, coronal, multi-slice, single-shot, fast spin echo (FSE) sequence was also acquired to assess the small bowel water content (SBWC). This acquired 32 slices with TE 326 ms and image resolution 1.56 mm × 3.13 mm with a slice thickness of 7.0 mm. More technical details of the MRI acquisition and data processing are available in Supplementary Materials.

The MRI scans were also reviewed qualitatively to explore what the appearance of the rice meals was in the stomach and if any characteristics could be noted, particularly the presence or absence of food boluses. Subsequently, the MRI images were also analyzed to assess quantitatively the solid and liquid components of the meals with time and also changes in meal image texture (Mayar, Smeets, van Duynhoven, & Terenzi, 2023; Milan et al., 2024; Otsu, 1979; van Eijnatten et al., 2024) in terms of homogeneity, contrast, local homogeneity, entropy and correlation (Arvis, Debain, Berducat, & Benassi, 2011; Conners & Harlow, 1980; Haralick, Shanmugam, & Dinstein, 1973; Vrbik et al., 2019). More details about image texture analysis are available in Supplementary Materials.

2.3. Statistical analysis

2.3.1. Sample size calculation

We did not have data on glucose responses to similar rice meals to carry out a formal power calculation. We based instead our estimated sample size of $n = 12$ on the paper by Fuwa *et al* (Fuwa et al., 2016) which used a reasonably similar set up. They showed a statistically significant reduction in postprandial blood glucose responses studying *n*

= 11 healthy humans who were fed a different white rice variety than this study (Japonica Koshiibuki rice) cooked adding a different gum (xanthan), and they compared it with consumption of the rice without the added gum.

2.3.2. Data analysis

Descriptive data are presented as means with the standard error of the mean (SEM) unless otherwise stated. For the *in vitro* study non-parametric Kruskal-Wallis test followed by Dunn's post hoc multiple comparisons test was used to analyze estimated GI values. The *in vivo* study had a paired study design. The iAUC 2 h was calculated for blood glucose for each participant and arm of the study. The iAUC2h is commonly used for glycemic response data calculation and it ignores the area of the curve beneath the fasting concentration (Brouns et al., 2005). For all the other data, the total AUC was calculated from the respective time curves for each participant and arm of the study. The Shapiro-Wilk normality was used to test for normal distributions. This confirmed a paired *t*-test was appropriate to compare AUCs for most data sets, apart from the stomach gas volume data for which a Wilcoxon match-paired signed rank test was used. The data were expressed as means with SEM. Two-way repeated measures ANOVA were used to compare the data from the time courses. The data was analysed using GraphPad Prism for windows version 9.2.0 (GraphPad Software, San Diego, California). Statistical significance of differences was assumed with a P value less than 0.05.

3. Results and discussion

3.1. In vitro estimated glycemic index

Addition of LAGG to the cooking water significantly reduced *in vitro* starch hydrolysis in the static digestion model in a dose-dependent manner. Two-way ANOVA analysis showed a significant main effect of percentage LAGG (P=0.0133) and a significant main effect of time (P=0.0126), with no significant interaction (P=0.2389). The addition of LAGG significantly affected the EGI of the cooked rice, Kruskal-Wallis test P=0.0014 (Fig. 2). LAGG significantly reduced the median EGI value by 27 %, from 94 for the control 0 % LAGG, to 69 for the 3 % LAGG samples ($P < 0.05$ post-hoc Dunn's multiple comparisons test difference from control sample 0 % LAGG).

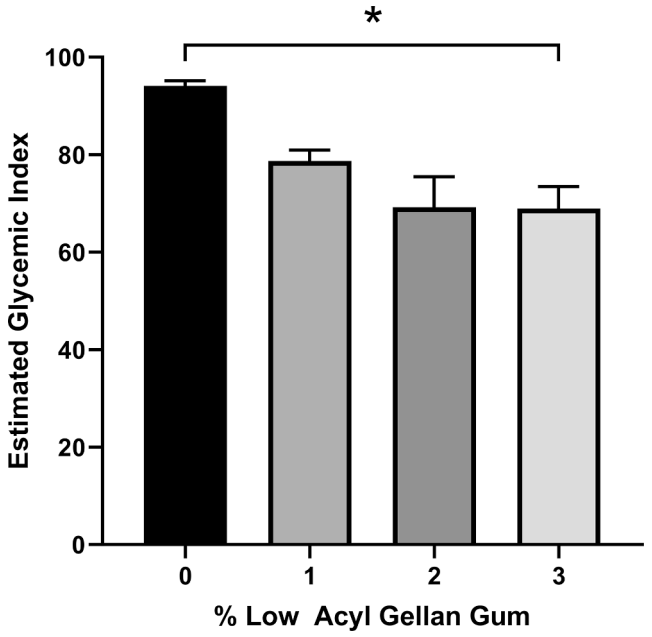

Fig. 2. Estimated Glycemic Index (EGI) of jasmine rice cooked with increasing amounts of low acyl gellan gum (LAGG), as determined by the *in vitro* static digestion model. The data are shown as medians and IQR of the triplicate samples. The medians varied significantly with % LAGG, Kruskal-Wallis test P=0.0014. *$P < 0.05$ post-hoc Dunn's multiple comparisons test difference from control sample 0 % LAGG.

These results were in agreement with our previous study carried out to investigate the effect of adding 1 % w/dry rice w LAGG to jasmine rice during cooking (Muttakin et al., 2023). White jasmine rice is a popular variety known to have a high GI (Ranawana, Henry, Lightowler, & Wang, 2009; Truong, Yuet, & Hall, 2014). Increasing LAGG concentration appeared to have a stronger effect in reducing EGI but adding more than 3 % w/dry rice w made the cooking water too thick before addition of the rice. For these reasons 3 % was determined as the optimal dose for the *in vivo* study. The INFOGEST *in vitro* digestion model used here had some limitations (Zhou, Tan, & McClements, 2023). The model was based on a static method, and as such it does not account for dynamic changes that would occur during normal *in vivo* digestion, including pH changes and feedback mechanisms.

Gellan gum is considered safe for dietary consumption. The United States Food and Drug Administration (FDA) has approved its use as a food additive. It was also approved by the European Community as a food additive with code E-418. Moreover, gellan gum is gluten free and is broadly utilised in gluten free foods to improve the texture and taste of pasta, biscuits, sweets and dairy products. It is harmless for people diagnosed with coeliac disease and is suitable for vegetarians, kosher and Halal diets, which means it is suitable for consumption across different populations. This study was carried out on a jasmine white rice, and its applicability to different varieties of rice remains to be investigated. Starch digestion depends on many factors including the ratio of amylose to amylopectin and the level of resistant starch present. The effect of hydrocolloids on the properties and digestibility of other varieties of rice would also depend on many factors, including the surface properties of the grains. One can hypothesise that for highly refined white rice grains the effect shown here would be relatively similar and transferable irrespectively of the rice variety.

3.2. Texture analysis

Rice grains exhibited marked differences in mechanical behaviour upon soaking time when cooked in water with or without LAGG. Fig. 3A and Fig. 3B show a marked reduction in the maximum force of the second compression (F_{MAX2}) for the Rice control, indicating that LAGG promotes a 'springier' texture of the rice grain. The magnified plot of the second compression peak shown in Fig. 3B also highlights the reduction in slope of the force gradient, which is also consistent with LAGG treatment resulting in a stiffer texture of the rice grains. We note that for an ideally elastic material the heights of the peaks on the first and second compressions should be identical. The 12 texture parameters evaluated from the compression curves are reported in Supplementary Table 1. Additional compression curves and results are shown in Supplementary Fig. 1B-D and Supplementary Fig. 2.

Fig. 3C and Fig. 3D show the values of F_{MAX2} and Stiffness at the 2nd compression as a function of soaking time. The graphs demonstrate a clear increase in stiffness following LAGG treatment. Additionally, it can be seen that the Rice control samples became softer with increasing soaking time, whilst treatment with LAGG appeared to protect the grains from softening over time.

The Texture Analizer results were consistent with the hypothesis that cooking the rice in LAGG has a profound effect on the stiffness and mechanical resistance of the rice grains, promoting the integrity of cooked rice grains. By contrast, the rice grains cooked in plain water tended to fracture and disintegrate more with prolonged soaking time. The observations derived from the compression test are in keeping with our initial *in vitro* studies (Muttakin et al., 2023) and with previous observations that gellan gum can modify the textural properties of rice (Chung, Liu, & Lim, 2007; Fang, Wang, Xu, & Zuo, 2018). We did not utilise fractured rice grains in these experiments, which potentially may

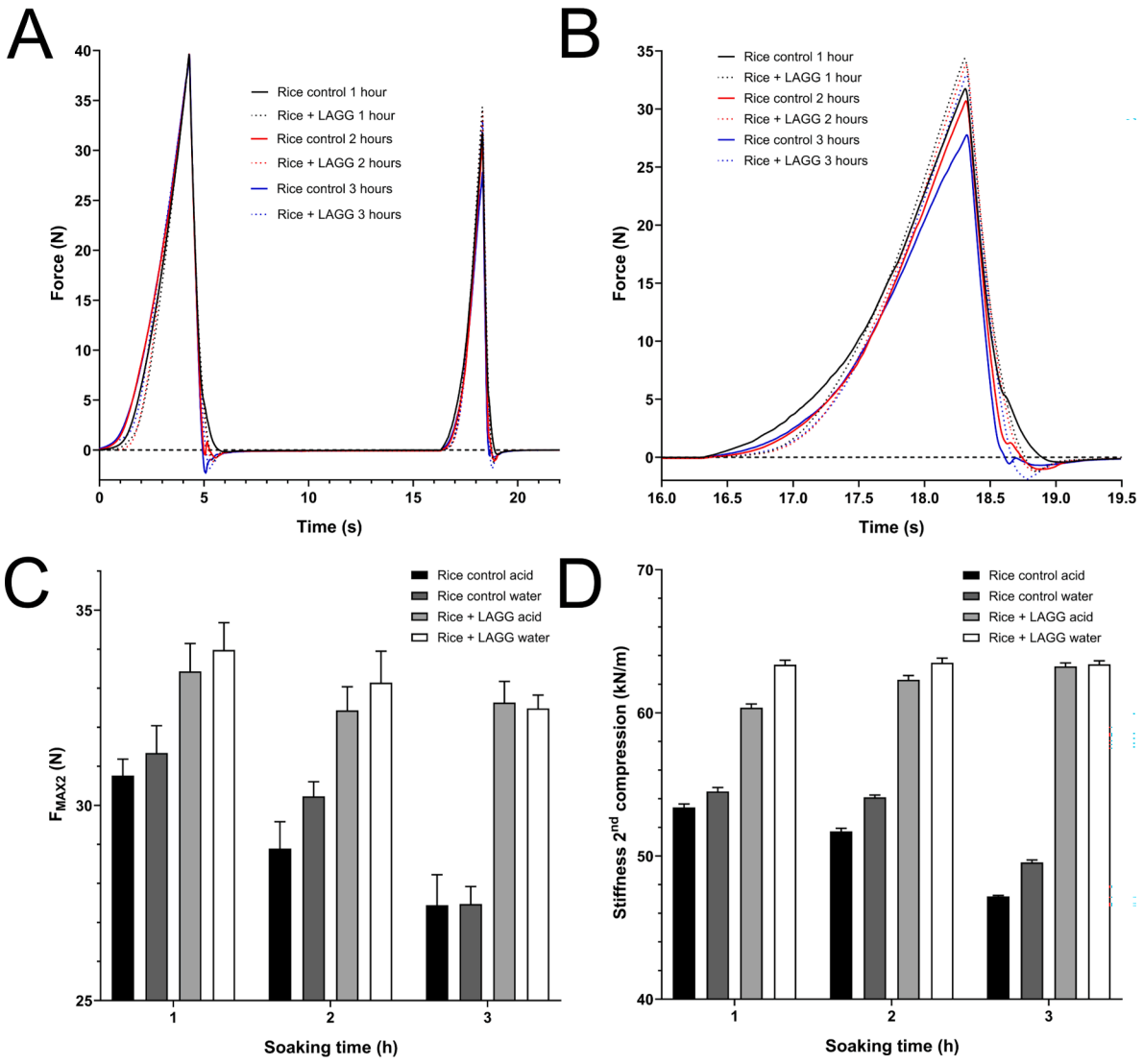

Fig. 3. Analysis of mechanical properties of rice grains, cooked with and without 3 % weight/dried rice weight LAGG, as a function of LAGG treatment, soaking time, and solution pH. (a) Shows comparison of mechanical behaviour of Rice + LAGG grains versus Rice control in a double-compression experiment. (b) Shows the area of the second compression, where differences between LAGG treatment and controls are most striking. The maximum force and the slope of the compression curve, which is proportional to stiffness of the material is markedly higher for the Rice + LAGG grains as compared to Rice control. (c) Shows values of the maximum force on second compression (F_{MAX2}) as a function of soaking time, the values reflect the loss of stiffness as well as the irreversible, plastic deformation of rice grains. (d) Shows stiffness determined by fitting the 2nd segment of the force versus compression curves as a function of soaking time for different treatments and acidity levels.

have introduced some bias towards more resilient grains. Considering that the rate of hydrolysis depends also on particle size, the stronger physical integrity of the rice cooked in LAGG solution can be expected to retain its physical integrity for longer, thus allowing a lower rate of enzymatic hydrolysis, suggesting that the LAGG treatment has the potential to influence the behaviour of the rice grains in the gastrointestinal tract.

3.3. Fluorescent microscopy

Example results of fluorescent microscopy images of cross sections of jasmine rice grains cooked with and without the 5-DTAF labelled LAGG are shown in Fig. 4. The green fluorescence signal that is clearly seen in Fig. 4A arises from the labelled LAGG. The fluorescence signal is distributed all around the outer surface of the rice grain confirming that during the cooking process the LAGG coated the grain. In Fig. 4A fluorescence green signal can also be seen inside the grain section, towards the middle of the grain, suggesting that during cooking the LAGG had not only coated the surface but had also penetrated below the surface of the grain.. In the control study the rice was cooked using unlabelled LAGG (Fig. 4B) and no fluorescent signal was visible as expected.

Having established that the addition of LAGG during cooking coated and penetrated the grains, reduced the EGI of jasmine rice and promoted rice grain integrity, it is worth considering possible explanations of the mechanisms for these effects, as research regarding the effect of LAGG on starch hydrolysis and GI is poorly explored in the literature compared with other food hydrocolloids. LAGG coating of the rice grain may act as an outside barrier and also as an inside barrier for enzymatic action in the rice grain, and it may also restrict the leakage of amylose during gelatinisation of the starch granule. It had been suggested that LAGG can resist well an acidic environment such as found in the stomach (Chung et al., 2007; Norton, Cox, & Spyropoulos, 2011). Other mechanisms at play could possibly involve competitive binding of amylase to the LAGG. These mechanisms could also potentially be amplified by further gelation and increase in gel strength of the LAGG in the acidic stomach environment. LAGG may also possibly strengthen aggregation of the individual rice grains during bolus formation after swallowing, further reducing surface exposure to enzymes during digestion.

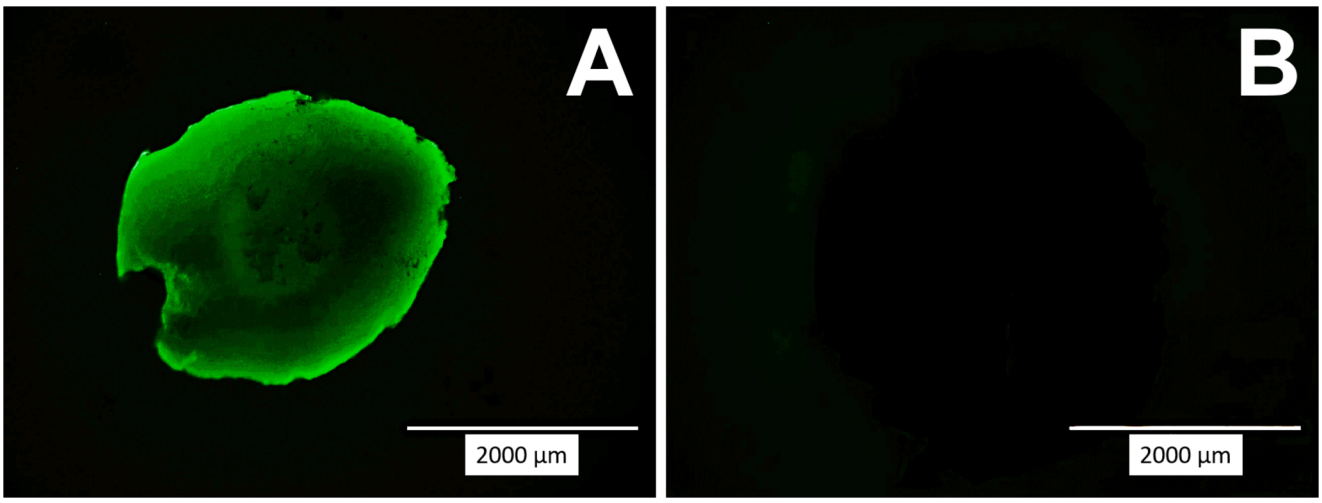

Fig. 4. Fluorescence microscopy images of: (A) Example of a fluorescence microscopy image of a cross section of a grain of jasmine rice cooked with 3% (w/dried rice weight) LAGG that was labelled with fluorescent dye 5-(4,6-dichlorotriazinyl) aminofluorescein (5- DTAF). (B) Example of a fluorescence microscopy image of a cross section of a grain of jasmine rice cooked with 3% (w/dried rice weight) unlabelled low acyl gellan gum (LAGG) as control.

3.4. In vivo study

The study procedures were tolerated well. All participants consumed the allocated two rice meals within the time required. There were no adverse events reported during the study. Twelve complete data sets were available for analysis for all the study outcomes save for the food diaries, which had one fewer data set since one participant failed to return a questionnaire.

3.5. Blood glucose

The blood glucose time courses are shown in Fig. 5. The baseline values were within the fasting healthy normal range (between 3.9 and 5.4 mmol/L). The mean fasting baseline values were comparable for the two arms of the study with 5.1 ± 0.1 mmol/L for the Rice control meal and 5.1 ± 0.1 mmol/L for the Rice + LAGG meal (P>0.9999).

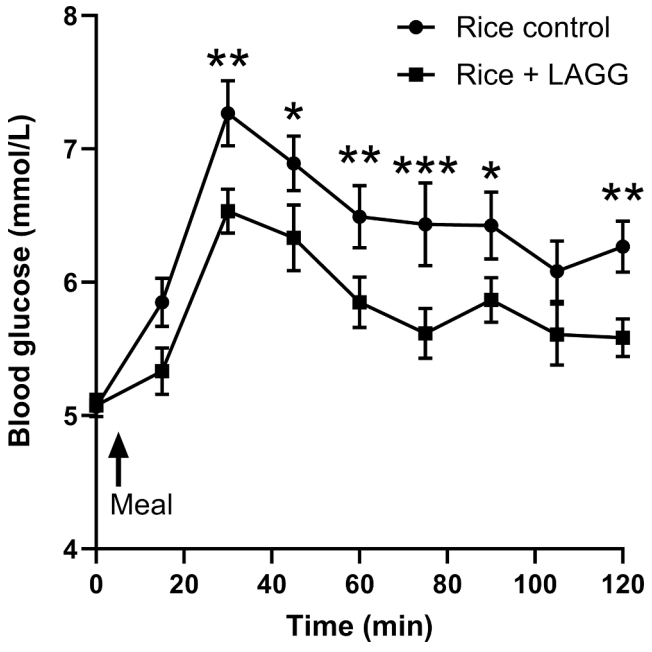

Fig. 5. Blood glucose time courses from T=0 to T=2 h for n = 12 participants who consumed the test meal with and without the addition of 3 % w/dry rice w low acyl gellan gum (LAGG). Two-way repeated measure ANOVA analysis showed a significant main effect of rice meal type (P < 0.0001) and a significant effect of time (P < 0.0001) with no interaction of rice meal type × time (P=0.1210). Data points are mean ± SEM. * P < 0.05, ** P < 0.01, *** P < 0.001.

Postprandially, the blood glucose values rose up to T=30 min and then gradually decreased. At the end of the sampling period at T=2 h the blood glucose values had not yet returned to baseline values. Numerically, the values for the Rice + LAGG meal remained consistently lower than those for the Rice control meal. Across the whole study the individual blood glucose values were more than 1 mml/L lower than control in 28 instances reaching up to 2.5 mmol/L lower than control. The largest mean difference in glucose values from control was observed at T=75 with a value of 0.8 ± 0.2 mmol/L.

The iAUC 2 h for finger prick blood glucose was the principal outcome of the study. The iAUC 2 h for blood glucose for the Rice + LAGG was 93 ± 16 mmol/L·min whilst that for the Rice control was 160 ± 18 mmol/L·min. This corresponds to a percentage difference of 42 % of Rice + LAGG compared to Rice control. The difference in iAUC 2 h was highly significant (Table 1). The individual blood glucose levels rarely went below baseline and therefore the significant differences revealed by the iAUC 2 h calculation (baseline corrected and ignoring negative areas below baseline) are unchanged when using a simple AUC 2 h trapezoidal integral (total AUC with no baseline correction) as shown in Table 1. Two-way repeated measure ANOVA analysis showed a significant main effect of rice meal type (P < 0.0001) and a significant effect of time (P < 0.0001) with no interaction of rice meal type × time (P=0.1210). Following this, exploratory post-hoc analysis of blood glucose means for each time point corrected for multiple comparisons showed significant postprandial differences at all time points apart from T=15 min and T=105 min (Fig. 5). Blood glucose rose postprandially to a peak at T=30 min, with the Rice control peak 7.3 ± 0.2 mmol/L significantly higher than that for the Rice + LAGG meal 6.5 ± 0.2 mmol/L (P < 0.01).

The blood glucose reduction observed here was compatible with that found when xanthan gum was added to a different variety of rice (Fuwa et al., 2016). The level of postprandial glucose peaks may be an important predictor of increased risk of cardiovascular disease in those living with diabetes (Ceriello, 2005). The size of the peak blood reduction following the Rice + LAGG meal observed here was of potential clinical importance.

The participants here were all healthy and mostly young, so it would be beneficial to know if the LAGG had similar (or even greater) effects on participants if they were people living with diabetes or from an older

Table 1
Summary results table for $n = 12$ participants who consumed the test meal with and without the addition of low acyl gellan gum (LAGG). Data are shown as mean ± standard error of the mean.

	Rice control	Rice + LAGG	*P value*
Glucose iAUC 2 h (mmol/L·min)	160 ± 18	93 ± 16	0.0007
Glucose AUC 2 h (mmol/L·min)	767 ± 20	697 ± 17	<0.0001
Gastric meal volume AUC 2 h (mL·min)	32340 ± 1831	30914 ± 1077	0.3959
Gastric meal volume half emptying time T50% (min)	107 ± 12	101 ± 6	>0.99
Gastric meal volume emptying rate (mL/min)	2.1 ± 0.2	2.0 ± 0.1	0.8598
Gastric gas volume AUC 2 h (mL·min)	2772 ± 507	1851 ± 237	0.1099
Total gastric meal volume AUC 2 h (mL·min)	35113 ± 1862	32765 ± 1196	0.1892
Gastric total volume half emptying time T50% (min)	109 ± 11	106 ± 7	0.8240
Gastric total volume emptying rate (mL/min)	2.1 ± 0.2	2.1 ± 0.2	0.6009
Small bowel water content AUC 2 h (mL·min)	2939 ± 454	2020 ± 260	0.0253
Composite appetite score AUC 2 h (mm·min)	6076 ± 584	5729 ± 493	0.2666
Hunger AUC 2 h (mm·min)	5186 ± 658	5080 ± 565	0.7398
Satisfaction AUC 2 h (mm·min)	5950 ± 541	6139 ± 578	0.5762
Fullness AUC 2 h (mm·min)	5683 ± 564	6140 ± 548	0.2945
Desire to eat AUC 2 h (mm·min)	5901 ± 806	5552 ± 627	0.4587
Prospective consumption AUC 2 h (mm·min)	6923 ± 565	6289 ± 652	0.1738
Amount of *ad libitum* lunch meal eaten (g)	577 ± 65	579 ± 74	0.9680
Energy intake from *ad libitum* lunch meal (kcal)	629 ± 70	631 ± 81	0.9680
Time taken to eat *ad libitum* lunch meal (min)	10 ± 1	10 ± 1	0.9292
Speed of eating *ad libitum* lunch meal (g/min)	57 ± 6	59 ± 8	0.6480
Self-reported energy intake for the reminder of the day (kcal) ($n = 11$)	1435 ± 166	1176 ± 210	0.1601
Self-reported carbohydrate's intake for the reminder of the day (g) ($n = 11$)	151 ± 20	139 ± 22	0.7249
Self-reported fat intake for the reminder of the day (g) ($n = 11$)	55 ± 10	45 ± 10	0.1445
Self-reported protein intake for the reminder of the day (g) ($n = 11$)	80 ± 14	55 ± 12	0.0068
Total energy intake for the whole day (kcal) ($n = 11$)	2329 ± 258	2064 ± 206	0.1606

The self-reported intakes are reported for $n = 11$ as 1 food record questionnaire was not returned. Abbreviations: AUC 2 h: area under the curve for 2 h.

cohort. The participants were also predominantly Caucasian, whilst it has been shown that glycemic responses can be higher in Chinese participants compared with Europeans after exposure to rice or glucose (Kataoka et al., 2013). Use of the fingerpick blood tests measured capillary blood glucose levels. It is known that there is a small difference between blood glucose levels taken from capillary compared with venous sources (Boyd, Leigh, & Stuart, 2005). Venous blood is more likely to be influenced by local uptake by tissue/muscle hence it may give a less accurate reflection of whole body glucose metabolism.

3.6. Appetite responses and food intake

The time courses for the composite appetite VAS score are shown in Fig. 6. The difference of the AUC overall composite score was not statically significant for the two meals being 6076 ± 584 for Rice control and 5729 ± 493 for Rice + LAGG (P=0.2666). Two-way repeated measure ANOVA analysis showed no interaction of rice meal type × time

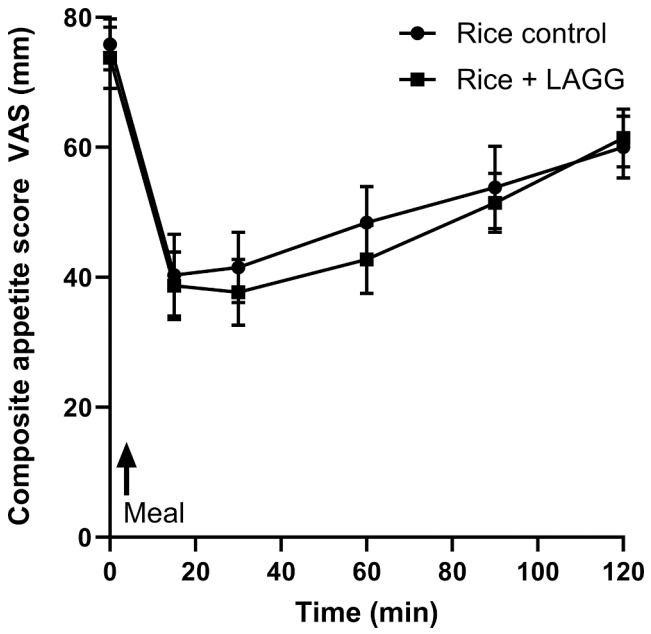

Fig. 6. Composite appetite score visual analogue scale (VAS) time courses from $n = 12$ healthy participants who consumed the rice meal with and without the addition of 3 % w/dry rice w low acyl gellan gum (LAGG) during the cooking process. Lower values in this context indicate lower hunger, higher fullness and less desire to eat, whilst higher values indicated the opposite. Two-way repeated measure ANOVA analysis showed no interaction of rice meal type × time (P=0.7123), no significant effect of rice meal type (P=0.2804) and a significant effect of time ($P < 0.0001$). Values shown are mean ± SEM.

(P=0.7123), no significant effect of rice meal type (P=0.2804) and a significant effect of time ($P < 0.0001$). The individual time courses for the hunger, satisfaction, fullness, desire to eat and prospective food consumption VAS are shown in Supplementary Fig. 4.

The amount consumed from the *ad libitum* meal (Table 1) was not significantly different between rice meal groups (P=0.9680). The time taken to consume the *ad libitum* meal for both the groups was 10 ± 1 min. The mean rate of eating of the *ad libitum* lunch meal (g/min) was 57 ± 6 g/min for the Rice control group and 59 ± 8 g/min for the Rice + LAGG group (P=0.6480). The final composite appetite VAS score after the *ad libitum* meal was 9 ± 2 for Rice control and 10 ± 1 for the Rice + LAGG group and the difference between groups was not significant (P=0.8254). The mean energy intake from the self-reported dietary intake records following the consumption of the Rice control meal was 2329 ± 258 kcal/day and the Rice + LAGG meal was 2064 ± 206 kcal/day. However, this difference was not statistically significant (P=0.1606).

In assessing the total energy intake for the day, use of a food diary may have introduced under or over reporting (Poppitt, Swann, Black, & Prentice, 1998). This was minimised by training the participants in the use of the diary and would be less likely to occur in this participant group, than in those who are living with obesity (Poppitt et al., 1998). No significant difference in the mean composite appetite score or total energy intake for the whole day was found between the Rice + LAGG meal and the Rice control meal. At the end of their last visit, all 12 participants answered 'yes' to the question if the study was acceptable and 'yes' to the question if the meals were acceptable. Only 4 out of 12 participants answered 'yes' to the question if they could perceive a difference between the two rice meals.

3.7. Stomach volumes and small bowel water content

On the moderately T2 weighted scans the rice meals inside the stomach provided good signal (Fig. 7). The good contrast between meal,

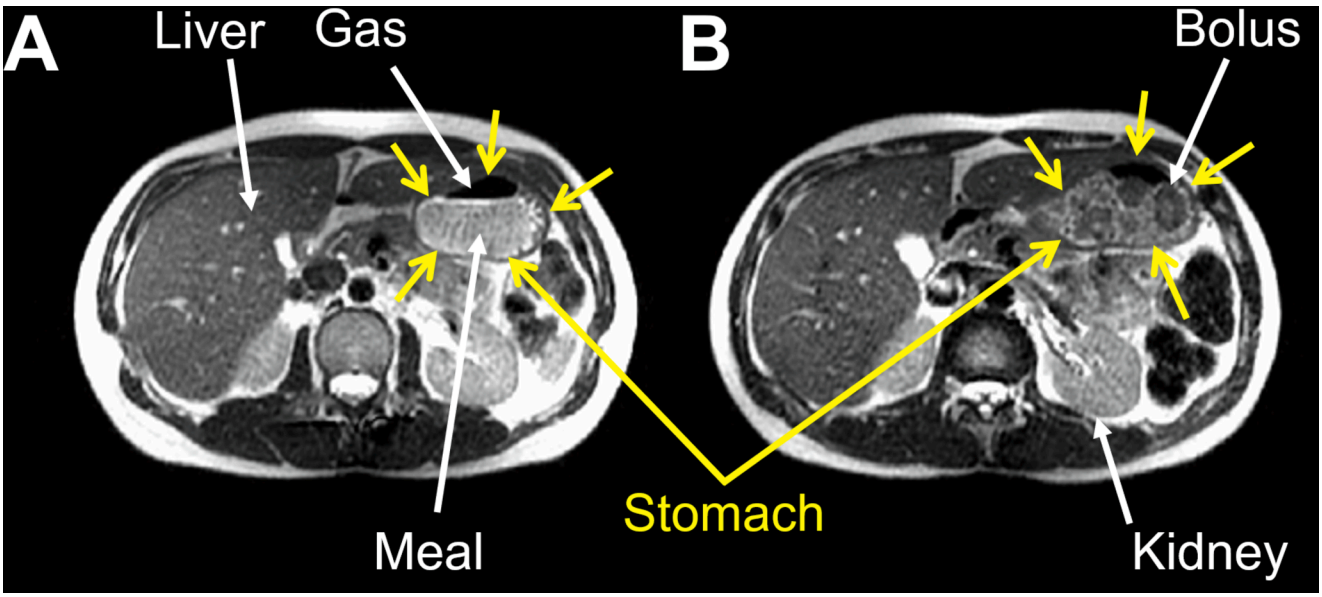

Fig. 7. (A) MRI image acquired axially through the stomach of a study participant at T=90 min after feeding the Rice control meal. (B) Corresponding MRI image from the same participant taken at the same time point but after consuming the rice + 3 % w/dry rice w low acyl gellan gum (Rice + LAGG) meal. In (B) darker, round boluses are visible inside the stomach. Anatomical landmarks such as the liver and kidneys are also indicated by white arrows for ease of orientation and the stomach boundaries are indicated by the yellow arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

organs and gas facilitated the tracing of the regions of interest (ROIs) for the rice meal in the stomach and separately for the stomach gas on each image where they were visible. The MRI baseline images allowed a check that all participants had come in fasted for their study day, which was confirmed. The stomach at baseline contained only a small volume of resting gastric juices (Supplementary Fig. 5), averaging 52 ± 13 mL for the Rice control meal, and 40 ± 6 mL for the Rice + LAGG meal, with no significant difference (P=0.9259). The average time courses for gastric meal volume (excluding gas, as presented in the remainder of this section) are shown in Fig. 8.

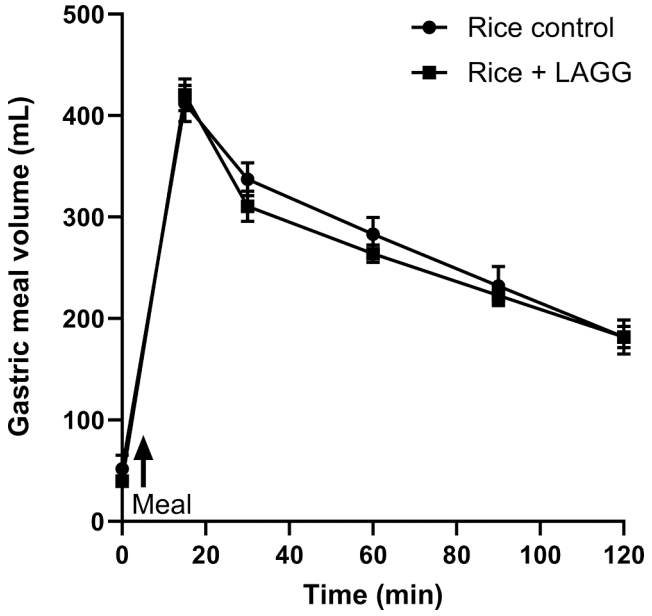

Fig. 8. Gastric meal volume time courses from $n = 12$ healthy participants who consumed the rice meal with and without the addition of 3 % w/dry rice w low acyl gellan gum (LAGG) during the cooking process. Two-way repeated measure ANOVA analysis showed no interaction of rice meal type × time (P=0.4287), no significant effect of rice meal type (P=0.4337) and a significant effect of time (P < 0.0001). Data points are mean ± SEM.

Gastric meal volumes rose postprandially at T=15 min to approximately the same average volume, 412 ± 17 mL for Rice control and 420 ± 16 mL for Rice + LAGG with no significant difference (P=0.9852). Subsequently, gastric meal volume declined. The postprandial volumes for Rice + LAGG were lower than for Rice control, but the difference was modest and not significant. By T=2 h the gastric meal volumes had not yet returned to the fasting baseline volumes. The AUC 2 h for the gastric meal volume for white rice with and without LAGG are shown in Table 1. There was a modest difference between these two values and that was not significant. From the curve fitting, one can estimate that GE would be complete in around 180 – 210 min in total.

For the gastric meal volumes, the two-way repeated measure ANOVA analysis showed no interaction of rice meal type × time (P=0.4287), no significant effect of rice meal type (P=0.4337) and a significant effect of time (P < 0.0001). The gastric volume emptying curves were primarily exponential (83 % of the emptying curves fitted best for exponential curve) and the fits were overall good, with average R2 = 0.92 ± 0.02. The half GE time T50% for the meal (excluding gas volume) was not significantly different between Rice control 107 ± 12 min and Rice + LAGG 101 ± 6 min (paired Wilcoxon's P>0.99). The meal volume emptying rate was also not different between Rice control 2.1 ± 0.2 mL/min and Rice + LAGG 2.0 ± 0.1 mL/min (paired *t* test P=0.8598).

The time courses for gastric gas volume and total gastric volume are shown respectively in Supplementary Fig. 6. Gastric gas volume did not show a recognisable pattern with time, oscillating within an average band of between 15 mL and 30 mL of intragastric gas and no significant differences between meals. Given the small amount of intragastric gas present, when the individual gas volumes were added to the individual meal volumes at each time point to calculate total gastric volume (meal plus gas), the increase in volume was only modest and the results for GE time and rate did not change markedly. Total gastric volume T50% was 109 ± 11 min for the Rice control meal not different from the total gastric volume T50% for the Rice + LAGG meal which was 106 ± 7 min (paired *t* test P=0.8240). Total gastric volume emptying rate was 2.1 ± 0.2 mL/min for the Rice control meal again not different from that for the Rice + LAGG meal 2.1 ± 0.2 mL/min (paired *t* test P=0.6009). The total gastric volume AUC for the Rice + LAGG meal was 32765 ± 1196

mL·min compared to that for the Rice control meal 35113 ± 1862 mL·min with no significant difference (P=0.1892).

Gastric meal volume T50% and gastric meal volume emptying rate were not significantly different between meals indicating that the delivery of chyme to the duodenum from both meals was similar, and that therefore overall GE rate was not part of the mechanism underlying the differences in blood glucose levels. Duodenal energy feedback may also have played a role as the Rice + LAGG is likely to have delivered energy to the duodenum slower than the Rice control. While lower energy delivery would speed gastric emptying, rice boluses aggregation in the stomach would tend to slow emptying, so it is possible that these two factors could have opposed each other's effect.

The time courses for SBWC are shown in Supplementary Fig. 6C. Small bowel water decreased after feeding and there was a modest but significant difference between the two meals with the Rice control values being higher than those for Rice + LAGG, possibly driven by baseline differences.

SBWC is a key parameter that allows insights into small intestinal function and its response to foods. SBWC depends on small bowel motility, GE of nutrients from the stomach, and the balance of secretion and absorption of fluids in response to the feeding intervention (Dellschaft, Hoad, Marciani, Gowland, & Spiller, 2022). The reduction in SBWC observed here for the Rice + LAGG meal compared to Rice control was modest and possibly influenced by baseline differences.

For the MRI part of the study, a limitation was that the participants were imaged in the supine position. However, the participants were only lying in the machine for approximately 8 min at each scanning time, so this is unlikely to have had a strong effect, and the positioning of the participants was the same for both arms of the study.

3.8. Intragastric rice meals appearance and texture

The rice meal had a characteristic grainy appearance on the MRI images as shown in the example in Fig. 7. In this type of MRI sequence, intragastric fluids (e.g. secretion, water from the drink) appeared brighter than the rice and surrounding organs and formed a fluid layer on top of the rice. A small amount of black intragastric gas was often present on top of the water layer. During the course of digestion, the Rice control appeared to become brighter and to lose the grainy appearance, becoming more homogeneous chyme with time than immediately after ingestion. Within the Rice control meal some boluses could be identified after ingestion in 9 of the participants but these boluses were only visible at later time points in 2 of the 12 participants (Supplementary Table 2).

Conversely the Rice + LAGG remained consistently darker, and some

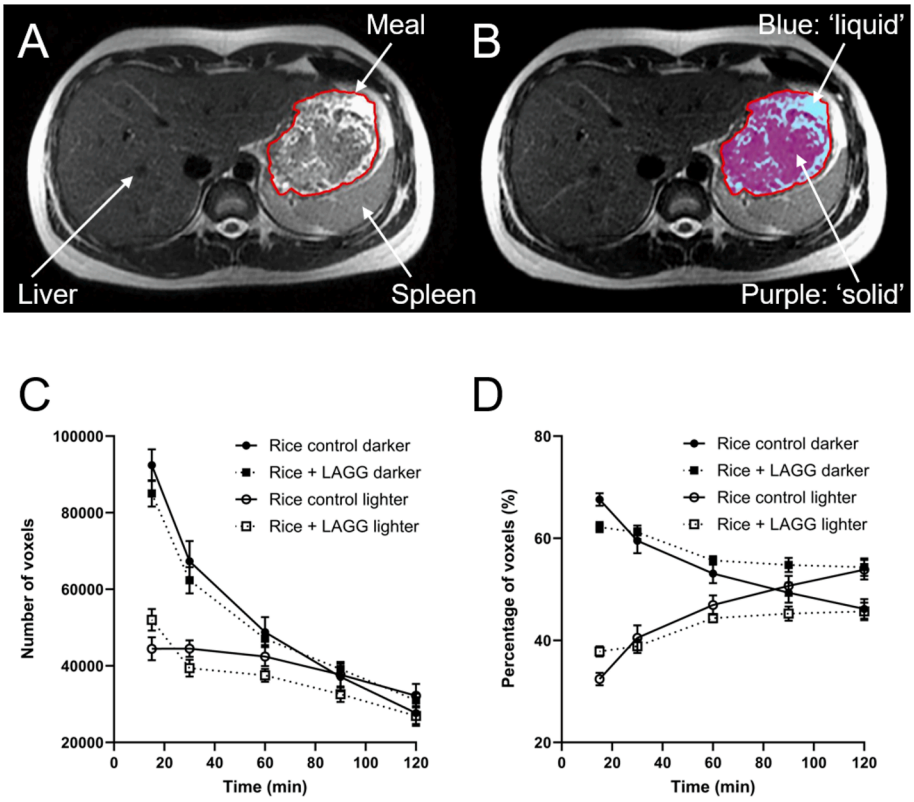

Fig. 9. Time courses of the solid and liquid components of the rice meals quantified using the method of Otsu. (A) Example of a T2-weighted axial MRI image of the stomach of a participant with a region of interest drawn in red around the meal in the stomach and (B) the same image but with the darker image pixels (corresponding to the more 'solid' component of the meal in the stomach) colour coded in purple and the lighter image pixels (corresponding to the more 'liquid' component of the meal in the stomach) colour coded in blue. (C) Time course of number of darker (more solid) and of lighter (more liquid) pixels of the meal in the stomach for both the Rice control and Rice + (3 % w/dry rice w) LAGG meals. For the lighter (more liquid) number of pixels data, two-way repeated measure ANOVA analysis showed a significant interaction of rice meal type × time (P=0.0041), no significant effect of rice meal type (P=0.1554) and a significant effect of time (P < 0.0001). For the darker (more solid) number of pixels data, two-way repeated measure ANOVA analysis showed a significant interaction of rice meal type × time (P=0.0370), no significant effect of rice meal type (P=0.6254) and a significant effect of time (P < 0.0001). (D) Time course of the percentage of darker (more solid) and of lighter (more liquid) pixels of the meal in the stomach for both the Rice control and Rice + LAGG meals. For the lighter (more liquid) percentage of pixels data, two-way repeated measure ANOVA analysis showed a significant interaction of rice meal type × time (P=0.0004), no significant effect of rice meal type (P=0.1102) and a significant effect of time (P < 0.0001). For the darker (more solid) percentage of pixels data, two-way repeated measure ANOVA analysis showed a significant interaction of rice meal type × time (P=0.0004), no significant effect of rice meal type (P=0.1102) and a significant effect of time (P < 0.0001). Values shown are mean ± SEM from $n = 12$ participants for who consumed the test meal with and without the addition of 3 % w/dry rice w LAGG. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

grainy, textured appearance persisted throughout the period after ingestion. Clearly defined, darker, multiple rice boluses (Fig. 7B) could be seen in all 12 participants after ingestion and were still visible at later time points (T=90–120 min) in 11 participants (Supplementary Table 2). The darker MRI appearance of the rice boluses can be interpreted as chyme that is less hydrated and/or comprising water molecules that are less mobile. This is in turn consistent with LAGG facilitating structuring of the boluses and making them more resistant to breakdown.

The time courses of the solid and liquid components of the rice meals quantified from the MRI images using the method of Otsu (Mayar et al., 2023; Milan et al., 2024; Otsu, 1979; van Eijnatten et al., 2024) are shown in Fig. 9. The plots show a rapid drop in the number of image pixels attributed to the liquid component of the meal for the Rice + LAGG meal up to T=30 min. After that the liquid component is consistently lower for the Rice + LAGG meal compared to Rice control indicating increased fluid sieving for the Rice + LAGG meal. Two-way repeated measure ANOVA analysis showed a significant interaction of rice meal type × time (P=0.0041) for the lighter (more liquid) component of the meal.

Example Haralick texture analysis feature maps are shown in Fig. 10, with the corresponding time courses shown in Supplementary Fig. 7. The curves show significant differences in all 5 Haralick texture parameters between the two rice meals. Compared to the Rice control meal, for the Rice + LAGG meal Homogeneity was lower (consistent with more tones of grey); Contrast was higher (consistent with more disparate neighbouring pixel intensities); Local homogeneity was lower (consistent with lower similarity of pixels); Entropy was higher (consistent with larger image randomness and complexity) and Correlation was higher (consistent with increased correlation of pixels to their neighbours).

4. Conclusions

This study showed that the addition of LAGG to the cooking process of white jasmine rice coated and penetrated the jasmine rice grains, decreasing the EGI and increasing their stiffness, promoting the grains' integrity, as determined in the *in vitro* studies. *In vivo* the Rice + LAGG significantly decreased postprandial blood glucose levels and peak blood glucose compared with untreated white rice, after an acute dose in a healthy adult population. This is an exciting finding but whether the effect would be sustained after a repeated (chronic) exposure remains to be determined. The study set up and funding did not allow for collecting blood and measuring hormone peptides. Without data from key hormones regulating glucose metabolism such as insulin, glucagon, GIP and GLP-1, it is difficult to speculate on physiological mechanisms for the observed glycemic responses. The postprandial duration of this study was 2 h. This was chosen as the standard time frame for measuring the GI of foods. Looking at the time curves from this study the blood glucose and stomach volumes had not yet gone back to fasting baseline values at the 2 h time point. The curves can be extrapolated, showing that a longer time window to 180–210 min would be more appropriate to capture the return to baseline of the study endpoints. This can inform future studies.

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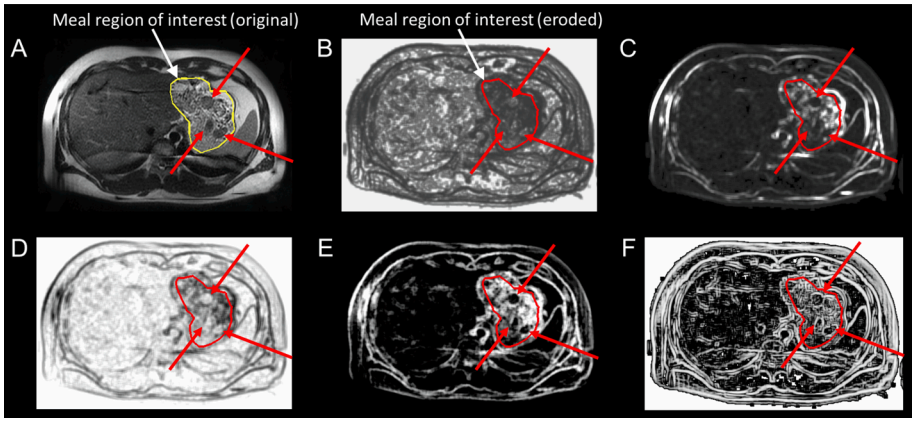

Fig. 10. (A) Example of a T2-weighted axial MRI image of the stomach of a participant with a region of interest drawn in yellow around the meal in the stomach. The following 5 Fig. panels are the Haralick image texture maps derived from the original image (A) featuring the texture characteristics: (B) Homogeneity, (C) Contrast, (D) Local homogeneity, (E) Entropy and (F) Correlation. The panels B-F also show in red the eroded region of interest used to sample the mean Haralick feature values. Food boluses in the stomach are indicated by the red arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.115090.

References

Alhussain, M. H., Macdonald, I. A., & Taylor, M. A. (2016). Irregular meal-pattern effects on energy expenditure, metabolism, and appetite regulation: A randomized controlled trial in healthy normal-weight women. *American Journal of Clinical Nutrition, 104*(1), 21–32. https://doi.org/10.3945/ajcn.115.125401

Alshammari, N. A., Taylor, M. A., Stevenson, R., Gouseti, O., Alyami, J., Muttakin, S., Bakalis, S., Lovegrove, A., Aithal, G. P., & Marciani, L. (2021). Effect of intake of food hydrocolloids of bacterial origin on the glycemic response in humans: Systematic review and narrative synthesis. *Nutrients, 13*(7), 2407. https://doi.org/10.3390/nu13072407

Alyami, J., Whitehouse, E., Yakubov, G. E., Pritchard, S. E., Hoad, C. L., Blackshaw, E., Heissam, K., Cordon, S. M., Bligh, H. F. J., Spiller, R. C., Macdonald, I. A., Aithal, G. P., Gowland, P. A., Taylor, M. A., & Marciani, L. (2019). Glycaemic, gastrointestinal, hormonal and appetitive responses to pearl millet or oats porridge breakfasts: A randomised, crossover trial in healthy humans. *British Journal of Nutrition, 122*(10), 1142–1154. https://doi.org/10.1017/s0007114519001880

Arvis, V., Debain, C., Berducat, M., & Benassi, A. (2011). Generalization of the cooccurrence matrix for colour images: Application to colour texture classification. *Image Analysis and Stereology, 23*(1), 63–72. https://doi.org/10.5566/ias.v23.p63-72

Bhavadharini, B., Mohan, V., Dehghan, M., Rangarajan, S., Swaminathan, S., Rosengren, A., Wielgosz, A., Avezum, A., Lopez-Jaramillo, P., Lanas, F., Dans, A. L., Yeates, K., Poirier, P., Chifamba, J., Alhabib, K. F., Mohammadifard, N., Zatonska, K., Khatib, R., Keskinler, M. V., Wei, L., Wang, C. S., Liu, X. Y., Iqbal, R., Yusuf, R., Wentzel-Viljoen, E., Yusufali, A., Diaz, R., Keat, N. K., Lakshmi, P. V. M., Ismail, N., Gupta, R., Palileo-Villanueva, L. M., Sheridan, P., Mente, A., & Yusuf, S. (2020). White rice intake and incident diabetes: A study of 132,373 participants in 21 countries. *Diabetes Care, 43*(11), 2643–2650. https://doi.org/10.2337/dc19-2335

Blake, C. E., Frongillo, E. A., Warren, A. M., Constantinides, S. V., Rampalli, K. K., & Bhandari, S. (2021). Elaborating the science of food choice for rapidly changing food systems in low-and middle-income countries. *Global Food Security, 28*. https://doi.org/10.1016/j.gfs.2021.100503

Blakeney, A. B., & Mutton, L. L. (1980). A simple colorimetric method for the determination of sugars in fruit and vegetables. *Journal of the Science of Food and Agriculture, 31*(9), 889–897. https://doi.org/10.1002/jsfa.2740310905

Blundell, J., de Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah, S., Schuring, E., van der Knaap, H., & Westerterp, M. (2010). Appetite control: Methodological aspects of the evaluation of foods. *Obesity reviews, 11*(3), 251–270. https://doi.org/10.1111/j.1467-789X.2010.00714.x

Boers, H. M., Seijen-ten Hoorn, J., & Mela, D. J. (2015). Effect of hydrocolloids on lowerign blood glucose. In *In 18th Gums and Stabilisers for the Food Industry Conference* (pp. 193–208). Wrexham, Wales: Glyndwr Univ.

Bourne, M. C. (1966). Measure of shear and compression components of puncture tests. *Journal of Food Science, 31*(2), 282–291. https://doi.org/10.1111/j.1365-2621.1966.tb00494.x

Boyd, R., Leigh, B., & Stuart, P. (2005). Capillary versus venous bedside blood glucose estimations. *Emergency Medicine Journal, 22*(3), 177–179. https://doi.org/10.1136/emj.2003.011619

Brand-Miller, J. C., Holt, S. H. A., Pawlak, D. B., & McMillan, J. (2002). Glycemic index and obesity. *American Journal of Clinical Nutrition, 76*(1), 281S–S285. https://doi.org/10.1093/ajcn/76.1.281S

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assuncao, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A. R., Martins, C., Marze, S., McClements, D. J., Ménard, O., Minekus, M., Portmann, R., Santos, C. N., Souchon, I., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., & Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

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

Ceriello, A. (2005). Postprandial hyperglycemia and diabetes complications - Is it time to treat? *Diabetes, 54*(1), 1–7. https://doi.org/10.2337/diabetes.54.1.1

Chung, H. J., Liu, Q., & Lim, S. T. (2007). Texture and in vitro digestibility of white rice cooked with hydrocolloids. *Cereal Chemistry, 84*(3), 246–249. https://doi.org/10.1094/cchem-84-3-0246

Conners, R. W., & Harlow, C. A. (1980). A theoretical comparison of texture algorithms. *IEEE Transactions on Pattern Analysis and Machine Intelligence, 2*(3), 204–222. https://doi.org/10.1109/tpami.1980.4767008

Dellschaft, N., Hoad, C., Marciani, L., Gowland, P., & Spiller, R. (2022). Small bowel water content assessed by MRI in health and disease: A collation of single-centre studies. *Alimentary Pharmacology and Therapeutics, 55*(3), 327–338. https://doi.org/10.1111/apt.16673

Edwards, J. L., Vincent, A. M., Cheng, H. T., & Feldman, E. L. (2008). Diabetic neuropathy: Mechanisms to management. *Pharmacology and Therapeutics, 120*(1), 1–34. https://doi.org/10.1016/j.pharmthera.2008.05.005

Fang, S., Wang, J., Xu, X. J., & Zuo, X. B. (2018). Influence of low acyl and high acyl gellan gums on pasting and rheological properties of rice starch gel. *Food Biophysics, 13*(2), 116–123. https://doi.org/10.1007/s11483-018-9517-8

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

Friedman, H., Whitney, J., & Szczesniak, A. (1963). The texturometer - a new instrument for object texture measurement. *Journal of Food Science, 28*, 390–396. https://doi.org/10.1111/j.1365-2621.1963.tb00216.x

Fukagawa, N. K., & Ziska, L. H. (2019). Rice: Importance for global nutrition. *Journal of Nutritional Science and Vitaminology, 65*, S2–S3. https://doi.org/10.3177/jnsv.65.S2

Fuwa, M., Nakanishi, Y., & Moritaka, H. (2016). Effect of xanthan gum on blood sugar level after cooked rice consumption. *Food Science and Technology Research, 22*(1), 117–126. https://doi.org/10.3136/fstr.22.117

Goni, I., GarciaAlonso, A., & SauraCalixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427–437. https://doi.org/10.1016/s0271-5317(97)00010-9

Gouseti, O., Jaime-Fonseca, M. R., Fryer, P. J., Mills, C., Wickham, M. S. J., & Bakalis, S. (2014). Hydrocolloids in human digestion: dDynamic in-vitro assessment of the effect of food formulation on mass transfer. *Food Hydrocolloids, 42*, 378–385. https://doi.org/10.1016/j.foodhyd.2014.06.004

Haralick, R. M., Shanmugam, K., & Dinstein, I. (1973). Textural features for image classification. *IEEE Transactions on Systems Man and Cybernetics, SMC3 (6)*, 610–621. https://doi.org/10.1109/tsmc.1973.4309314

Howlett, J., & Ashwell, M. (2008). Glycemic response and health: Summary of a workshop. *American Journal of Clinical Nutrition, 87*(1), 212S–S216. https://doi.org/10.1093/ajcn/87.1.212S

Hu, E. A., Pan, A., Malik, V., & Sun, Q. (2012). White rice consumption and risk of type 2 diabetes: Meta-analysis and systematic review. *Bmj, 344*, e1454.

Hussein, M. O., Hoad, C. L., Wright, J., Singh, G., Stephenson, M. C., Cox, E. F., Placidi, E., Pritchard, S. E., Costigan, C., Ribeiro, H., Ciampi, E., Nandi, A., Hedges, N., Sanderson, P., Peters, H. P. F., Rayment, P., Spiller, R. C., Gowland, P. A., & Marciani, L. (2015). Fat emulsion intragastric stability and droplet size modulate gastrointestinal responses and subsequent food intake in young adults. *Journal of Nutrition, 145*(6), 1170–1177. https://doi.org/10.3945/jn.114.204339

Kataoka, M., Venn, B. J., Williams, S. M., Te Morenga, L. A., Heemels, I. M., & Mann, J. I. (2013). Glycaemic responses to glucose and rice in people of Chinese and European ethnicity. *Diabetic Medicine, 30*(3), E101–E107. https://doi.org/10.1111/dme.12080

Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., & Roberts, S. B. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics, 103* (3). https://doi.org/10.1542/peds.103.3.e26.

Mapanga, R. F., & Essop, M. F. (2016). Damaging effects of hyperglycemia on cardiovascular function: Spotlight on glucose metabolic pathways. *American Journal of Physiology-Heart and Circulatory Physiology, 310*(2), H153–H173. https://doi.org/10.1152/ajpheart.00206.2015

Mayar, M., Smeets, P., van Duynhoven, J., & Terenzi, C. (2023). In vitro 1H MT and CEST MRI mapping of gastrointestinal milk protein breakdown. *Food Structure, 36*, Article 100314. https://doi.org/10.1016/j.foostr.2023.100314

Milan, A. M., Barnett, M. P. G., NMcNabb, W. C., Roy, N. C., Coutinho, S., Hoad, C. L., Marciani, L., Nivins, S., Sharif, H., Calder, S., Du, P., Gharibans, A. A., O'Grady, G., Fraser, K., Brernsein, D., Rosanowski, S. M., Sharma, P., Shrestha, A., & Mithen, R. F. (2024). The impact of heat treatment of bovine milk on gastric emptying and nutrient appearance in peripheral circulation in healthy females: a randomized controlled trial comparing pasteurized and ultra-high temperature milk. *American Journal of Clinical Nutrition*, in press. https://doi.org/10.1016/j.ajcnut.2024.03.002.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., & Brodkorb, A. (2014).

A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function, 5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Muttakin, S., Bakalis, S., Fryer, P. J., Alshammari, N. A., Marciani, L., & Gouseti, O. (2023). Reducing starch digestibility of white rice by structuring with hydrocolloids. *Food Research International, 174*, Article 113490. https://doi.org/10.1016/j.foodres.2023.113490

Norton, A. B., Cox, P. W., & Spyropoulos, F. (2011). Acid gelation of low acyl gellan gum relevant to self-structuring in the human stomach. *Food Hydrocolloids, 25*(5), 1105–1111. https://doi.org/10.1016/j.foodhyd.2010.10.007

Ojo, O., Ojo, O. O., Adebowale, F., & Wang, X. H. (2018). The effect of dietary glycaemic index on glycaemia in patients with type 2 diabetes: a systematic review and meta-analysis of randomized controlled trials. *Nutrients, 10* (3). https://doi.org/10.3390/nu10030373.

Otsu, N. (1979). A threshold selection method from gray-level histograms. *IEEE Transactions on Systems, Man and Cybernetics, 9*, 62–66. https://doi.org/10.1109/TSMC.1979.4310076

Poppitt, S. D., Swann, D., Black, A. E., & Prentice, A. M. (1998). Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *International Journal of Obesity, 22*(4), 303–311. https://doi.org/10.1038/sj.ijo.0800584

Ranawana, D. V., Henry, C. J. K., Lightowler, H. J., & Wang, D. (2009). Glycaemic index of some commercially available rice and rice products in Great Britain. *International Journal of Food Sciences and Nutrition, 60*, 99–110. https://doi.org/10.1080/09637480802516191

Riccardi, G., Rivellese, A. A., & Giacco, R. (2008). Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes. *American Journal of Clinical Nutrition, 87*(1), 269S–S274. https://doi.org/10.1093/ajcn/87.1.269S

Rosenthal, A. J. (2010). Texture profile analysis -how important are the parameters? *Journal of Texture Studies, 41*(5), 672–684. https://doi.org/10.1111/j.1745-4603.2010.00248.x

Salmeron, J., Ascherio, A., Rimm, E. B., Colditz, G. A., Spiegelman, D., Jenkins, D. J., Stampfer, M. J., Wing, A. L., & Willett, W. C. (1997). Dietary fiber, glycemic load, and risk of NIDDM in men. *Diabetes Care, 20*(4), 545–550. https://doi.org/10.2337/diacare.20.4.545

Schulze, M. B., Liu, S. M., Rimm, E. B., Manson, J. E., Willett, W. C., & Hu, F. B. (2004). Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. *American Journal of Clinical Nutrition, 80*(2), 348–356. https://doi.org/10.1093/ajcn/80.2.348

Stratton, I. M., Adler, A. I., Neil, H. A. W., Matthews, D. R., Manley, S. E., Cull, C. A., Hadden, D., Turner, R. C., & Holman, R. R. (2000). Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): Prospective observational study. *BMJ, 321*(7258), 405–412. https://doi.org/10.1136/bmj.321.7258.405

Truong, T. H., Yuet, W. C., & Hall, M. D. (2014). Glycemic index of American-grown jasmine rice classified as high. *International Journal of Food Sciences and Nutrition, 65*(4), 436–439. https://doi.org/10.3109/09637486.2013.869797

van Dam, R. M. (2020). A global perspective on white rice consumption and risk of type 2 diabetes. *Diabetes Care, 43*(11), 2625–2627. https://doi.org/10.2337/dci20-0042

van Eijnatten, E. J. M., Camps, G., Guerville, M., Fogliano, V., Hettinga, K., & Smeets, P. A. M. (2024). Milk coagulation and gastric emptying in women experiencing gastrointestinal symptoms after ingestion of cow's milk. *Neurogastroenterology and Motility, 36* (1). https://doi.org/10.1111/nmo.14696.

Vrbik, I., Van Nest, S. J., Meksiarun, P., Loeppky, J., Brolo, A., Lum, J. J., & Jirasek, A. (2019). Haralick texture feature analysis for quantifying radiation response heterogeneity in murine models observed using Raman spectroscopic mapping. *PLOS One, 14* (2). https://doi.org/10.1371/journal.pone.0212225.

Wolever, T. M. S., Jenkins, D. J. A., Jenkins, A. L., & Josse, R. G. (1991). The glycemic index: Methodology and clinical implications. *American Journal of Clinical Nutrition, 54*(5), 846–854. https://doi.org/10.1093/ajcn/54.5.846

Zafar, M. I., Mills, K. E., Zheng, J., Regmi, A., Hu, S. Q., Gou, L. N., & Chen, L. L. (2019). Low-glycemic index diets as an intervention for diabetes: A systematic review and meta-analysis. *American Journal of Clinical Nutrition, 110*(4), 891–902. https://doi.org/10.1093/ajcn/nqz149

Zhou, H. L., Tan, Y. B., & McClements, D. J. (2023). Applications of the INFOGEST in vitro digestion model to foods: A review. *Annual Review of Food Science and Technology, 14*, 135–156. https://doi.org/10.1146/annurev-food-060721-012235

Supplementary Materials

Reducing the glycemic response to white rice by structuring with gellan gum: a randomized controlled trial in healthy humans

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1. Supplementary Materials and Methods

1.1 In-vitro digestion

For the *in vitro* digestion, the static INFOGEST protocol was followed with some modifications (Brodkorb et al., 2019; Minekus et al., 2014). Enzyme activities were assumed as stated by the supplier, and the same batches were used in all experiments. Electrolyte compositions of the simulated digestive fluids were as in (Brodkorb et al., 2019). Briefly, 5 g of the cooked rice was transferred into 50 mL conical tubes. For the oral phase no mastication step was used. Five mL of simulated salivary fluid (SSF) containing 75 u/mL human salivary amylase (Sigma–Aldrich) were added to each tube, vortex mixed (Fisher Brand, UK), and left to stand for 2 minutes. The gastric phase was then initiated by adding 10 mL of simulated gastric fluid (SGF) containing 288 mg of pepsin (Sigma–Aldrich, 2000U/mL) to each tube. The samples were vortex mixed and incubated at 37°C for 30 min at pH 1.5. For the intestinal phase, 20 mL of simulated intestinal fluid (SIF) containing 4 mL of pancreatic a-amylase (Sigma–Aldrich, 100U of trypsin activity/mL) were added to each tube, and the tubes were vortex mixed and incubated at 37°C for 120 min at pH 6.

1.2. Determination of estimated glycemic index

At times 2.5, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min of intestinal digestion, a 200 µL aliquot was transferred from the digestion tube to a 1.5 mL test tube containing 200 µL of stop solution (0.3 M Na_2CO_3 to stop enzyme activity by raising the pH above the activity range, as in (Muttakin et al., 2023) and vortexed to inactivate the α-amylase. Intestinal digestion started at time 0 with the addition of SIF containing pancreatic enzymes. There was necessarily a delay from adding the intestinal digestive fluids to taking the first sample (i.e., time required to mix the sample with the fluids and measure the pH) and therefore the first sampling time was standardised to 2.5 min for all samples, for consistency. Also, care was taken to avoid taking large particles from the sample in the aliquot. Starch hydrolysis was measured as released reducing sugars using a 4-hydroxybenzhydrazide (PAHBAH) assay (Blakeney & Mutton, 1980). The samples were first centrifuged for 5 minutes at 12,500 rpm using a mini centrifuge (Thermo Fisher Scientific, Waltham, MA), then 20 µl of the supernatant was transferred to a new tube and diluted in water (50:1). Next, 100 µL of the diluted supernatant was transferred into a new tube, 1 mL of freshly prepared PAHBAH solution was added (250 mg PAHBAH dissolved in 4.75 mL of 0.5M HCl, then mixed with 45 mL of 0.5M NaOH) and the tube was placed into a 95°C water bath for 5 min. The samples were then allowed to cool to room temperature before transferring to cuvettes. Absorbance at a wavelength of 405 nm measured using a spectrophotometer (Genesys 10 Vis, Thermo Fisher Scientific, Waltham, MA) was then compared with the maltose standard curve. The rate of starch digestion was expressed as the percentage of total starch hydrolysed at different times with 100% indicating hydrolysis of the total starch content of the investigated rice portion. The percentage of starch hydrolysis of the study samples was calculated based on the method of Goni *et al.* (Goni, GarciaAlonso, & SauraCalixto, 1997). A similar digestion experiment was carried out using white bread as a control sample. The

hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each rice sample by the corresponding area of the reference sample (white bread). The EGI was then calculated as suggested by Goni *et al.* (Goni et al., 1997) using the formula EGI = 39.71 + (0.549 HI).

1.3 Texture analysis

Texture analysis of the rice grains was performed using a Texture Analyser (TA.XTplus, Stable Micro Systems, Surrey, UK) equipped with a 35 mm diameter flat punch cylindrical probe. The force was detected using a 5 kg loading cell. The rice was cooked with and without 3% w/dry rice w LAGG as described below in the *in vivo* study section. Straight after cooking, the rice grains were transferred into a Petri dish and submerged in excess of water or of hydrochloric acid solution (0.01 mol/L), and kept soaked at room temperature (~20°C) for a period of 1h, 2h, and 3h.

For the texture measurements, Soaking in excess liquid allowed to avoid clamping of the grains and also to evaluate the mechanics of the grains with time under acid conditions, as a model of what would be the case in the stomach, 15 rice grains were positioned together on the flat substrate, with the grains' major axis parallel to the surface (Supplementary Fig. 1A). The 15 grains were positioned avoiding contact between the grains and excess fluid was blotted using a delicate lens cleaning tissue. The compression experiment was then performed using a modified texture profile analysis protocol (TPA) (Bourne, 1966; Friedman, Whitney, & Szczesniak, 1963; Rosenthal, 2010), with two sequential compressions, separated by the recovery time of 10 s. Before placing the rice grains into the machine, the gap between the surfaces was zeroed at 20 N compression force, which then enabled calculating the absolute contact distance. These values of the contact distance enabled in turn to calculate the effective height of rice grains, and, correspondingly, the compression ratio, $CR\% =$

$\frac{h_1-h_2}{h_1}100\%$, where h_1 and h_2 are contact heights on the first and second compressions. Due to the general uniformity of rice grains and relatively small variability of the grain diameter, the error in determining the CR% was found to be less than 5%. The use of a bed of 15 grains had a positive effect on reducing the measurement errors as compared to a single grain compression approach (Yu et al., 2019).

During measurements, the upper probe (flat punch) descended at the speed of 0.5 mm/s and the trigger force was set at 0.01 N. At this point, the compression distance, h_1, was recorded. The probe continued downward motion until reaching the set maximum force of 40 N. At this point, the maximum compression distance, h_{1MAX}, was recorded. The probe then withdrew to a distance of 0.5 mm above the contact point and a dwell of 10 s was imposed. The second compression cycle was performed by compressing rice grains to the same distance as in the first compression cycle, h_{1MAX}. Then, the probe withdrew to complete the test. Three repeats for each condition were measured and averaged. The analysis was performed using an empirical approach, by evaluating the values of maximum force, slope of the force versus compression curve to determine effective stiffness and maximum pull of force on retract as well as compression ratio. The compression ratio was calculated using the expression: $CR\% = \frac{h_1-h_2}{h_1}100\%$, where h_1 and h_2 are contact heights on the first and second compressions. Stiffness was determined using linear fitting of the Force vs Distance curve in the range of forces between 8 and 36 N, i.e. 20% and 90% of maximum compression, respectively. A similar procedure was applied for the determination of stiffness during second compression, with the fitting ranges adjusted accordingly to account for the reduction in the values of the maximum force. Stiffness was evaluated for both the approach and the retract segments of the curve. The pull-off force was determined by taking the minimum value of the force upon the retraction of the probe and presented with a negative sign to indicate the opposite direction of the force. The stiffening ratio was determined as Stiffens2/Stiffness1.

The F_{MAX} ratio was determined as $F_{MAX}1/F_{MAX}2$. The adhesion ratio was determined as Pull-off1/Pull-off2. The Plasticity parameter was determined as the ratio of the stiffness values obtained from the retract and approach segments of the curve.

1.4 Glucose meter calibration

The accuracy of the AccuCheck blood glucose meter was checked against standard laboratory Yellowsprings glucose analyser 2300 (using the glucose oxidase method) in the David Greenfield Human Physiology laboratory at the University of Nottingham. Fifteen blood samples were measured with the AccuCheck in triplicate and the mean of each blood sample measurement was compared against the corresponding measurement on the Yellowsprings meter. The test showed that the AccuCheck used for this study matched well the standard laboratory equipment readings with linear regression R^2=0.9937 and $P < 0.0001$ over the whole range of values of interest for this study.

1.5 In vivo study

Healthy adult participants were recruited via posters placed around the University of Nottingham campus. The inclusion criteria were: aged between 18 and 65 years, with a Body Mass Index (BMI) between 18.5 kg/m² and 24.9 kg/m² and with no medical conditions or previous gastrointestinal surgery which could affect study measurements. Exclusion criteria included: having a fasting blood glucose greater than 5.4 mmol/L, use of medication which could interfere with study measurements, for example acid suppressants or anti-spasmodics, dislike of or intolerance to the products served in the study, being not suitable for MRI scanning (e.g., presence of metal implants in the body), and pregnancy.

Participants received information materials and if interested were invited to attend a short appointment (approximately 30 minutes) when informed written consent to participate

in the study was obtained. They were then screened, having fasted for at least 11 hours prior, to determine if they were eligible for the study. Screening included a finger prick blood sample to test for fasting blood glucose level (method described below). Demographic data were also collected, including weight and height to calculate the BMI. Twelve participants completed the study (see study flow diagram in Supplementary Fig. 3).

The initial study design was single-blind with the participants kept blind to the rice intervention cooking process. However, after data collection the data sets were further blinded to the operator by a member of staff not involved with the analysis and the blinding code was broken only after the analysis was completed.

The participants arrived at 09:00 hours after having fasted since 22:00 hours the previous evening and avoiding alcohol, strenuous exercise, and caffeine for the 24 hours prior. Participants attended the two study days approximately 7 days apart. They were allowed to consume their habitual diet between each study visit but they were asked to eat the same evening meal of their own choice before each study day.

1.6 Magnetic resonance imaging

MRI imaging was carried out using a 1.5T XDxt MRI scanner (General Electric Medical Systems, Milwakee, Wisconsin). At each time point the participants were placed in the scanner supine, feet first, with a 12-element body receiver wrapped around the abdomen. A 3-plane localizer scan was performed first to locate the organs in the body, followed by a calibration scan. This procedure took approximately 30 seconds. After this, the stomach was imaged first using an axial Half-Fourier Single-shot Turbo spin-Echo (HASTE) sequence. This acquired 46 contiguous slices through the abdomen in a single breath-hold of 26 seconds, with a field of view (FOV) of 400 mm × 320 mm (right-left and anterior-posterior respectively). The echo time TE was 60 ms and the repetition time TR was 549.7 ms. The

acquired image resolution was 1.56 mm × 1.67 mm with a slice thickness of 5.0 mm. This sequence was moderately T2 weighted therefore providing good contrast between liquids (appearing bright), tissues or hydrated foods (appearing grey), and more solid / less hydrated food components (appearing black). Lastly, small bowel water content (SBWC) was imaged using a coronal single-shot fast spin echo (FSE) sequence. This acquired 32 contiguous slices through the abdomen in two separate breath-holds of 24 seconds, with a FOV of 400 mm × 400 mm (right-left and head-feet respectively). The TE was 326 ms and the TR was 1511.7 ms. The acquired image resolution was 1.56 mm × 3.13 mm with a slice thickness of 7.0 mm. This sequence was heavily T2 weighted therefore showing liquids appearing very bright and other tissues and food components appearing black.

1.7 Analysis of MRI images

For the analysis of MRI, at each time point the stomach meal volume and stomach gas volume were calculated by manually drawing corresponding regions of interest (ROIs) on the stomach images using MIPAV (Medical Image Processing, Analysis, and Visualization software, US National Institute for Health) (McAuliffe et al., 2001) and summing the volumes across all slices. Each gastric volume time curve was fitted to calculate the time to empty half of the gastric meal contents (T50%). The average meal emptying rate (mL/min) was also calculated from the fit. Total stomach volume was then calculated as the sum of meal plus gas volume at each time point.

SBWC was measured using in-house developed software as validated and used previously (Dellschaft, Hoad, Marciani, Gowland, & Spiller, 2022; Hoad et al., 2007), Briefly, the SBWC assessment assumes that in the heavily T2 weighted images any bright intensity pixel with a signal intensity at and above a threshold level set from the cerebrospinal fluid within that particular image stack (i.e. as an internal normalisation) represents freely mobile/liquid

water. Based on this principle, regions of interest were manually drawn on all visible small bowel water signal and their total volume summed up by the software, yielding the freely mobile SBWC in mL at that time point.

The MRI scans were also reviewed qualitatively to explore what the appearance of the rice meals was in the stomach and if any characteristics could be noted, particularly the presence or absence of food boluses. This task was undertaken with the operator blind to the meal type. Subsequently, the MRI images were also analyzed to assess quantitatively the solid and liquid components of the meals with time and also changes in meal image texture with time as follows.

The method of Otsu (Otsu, 1979) was used to assess the solid and liquid components of the meals. This is a histogram based method to segment images into different regions depending on their pixel intensities. It has been utilized recently by others to appraise intragastric proportions of milk coagulum and liquid *in vitro* and *in vivo* (Mayar, Smeets, van Duynhoven, & Terenzi, 2023; Milan et al., 2024; van Eijnatten et al., 2024). Briefly, the intragastric meal ROIs drawn for the gastric volume measurements were converted to binary masks using MIPAV software. The masks were loaded on Matlab® (version R2018a The MathWorks Inc, USA) together with the original images. Otsu's method was then applied to calculate the number of darker (corresponding to the more solid component of the meal in the stomach) and lighter (corresponding to the more liquid component of the meal in the stomach) voxels. The voxels in the segmented image (image x mask) were divided into 3 separate regions using 2 thresholds from the multithresh Matlab function. The upper threshold was then used to split the image into 'liquid' (above the higher threshold) and 'solid' (below this higher threshold) and the number of pixels for each region calculated.

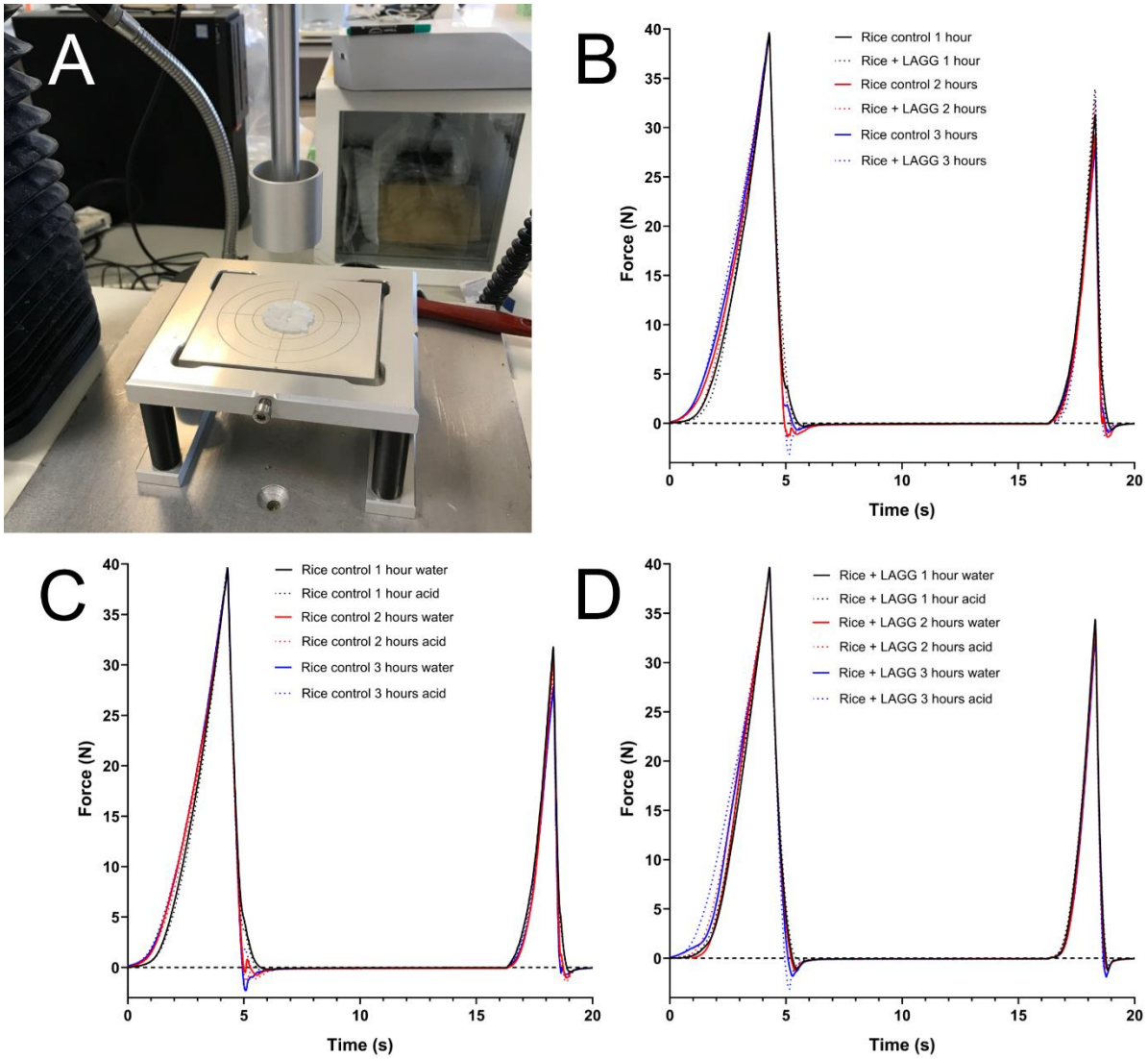
Image texture analysis has also been used recently to quantify intragastric meal image characteristics (van Eijnatten et al., 2024). There are 14 Haralick textural features for image

classification described in the original paper (Haralick, Shanmugam, & Dinstein, 1973). However, some are highly correlated and it has been proposed (Arvis, Debain, Berducat, & Benassi, 2011; Conners & Harlow, 1980; Vrbik et al., 2019) that 5 Haralick features based on a grey level cooccurrence matrix (GLCM) are most useful: homogeneity, contrast, local homogeneity, entropy and correlation. Here, these 5 Haralick image texture operators were calculated using the MIPAV software. The intragastric meal ROIs drawn for the gastric volume measurements were first eroded to avoid artificial areas of high signal change at the edges of meal volume. The eroded ROIs were then applied to each of the Haralick feature maps and the mean feature values calculated.

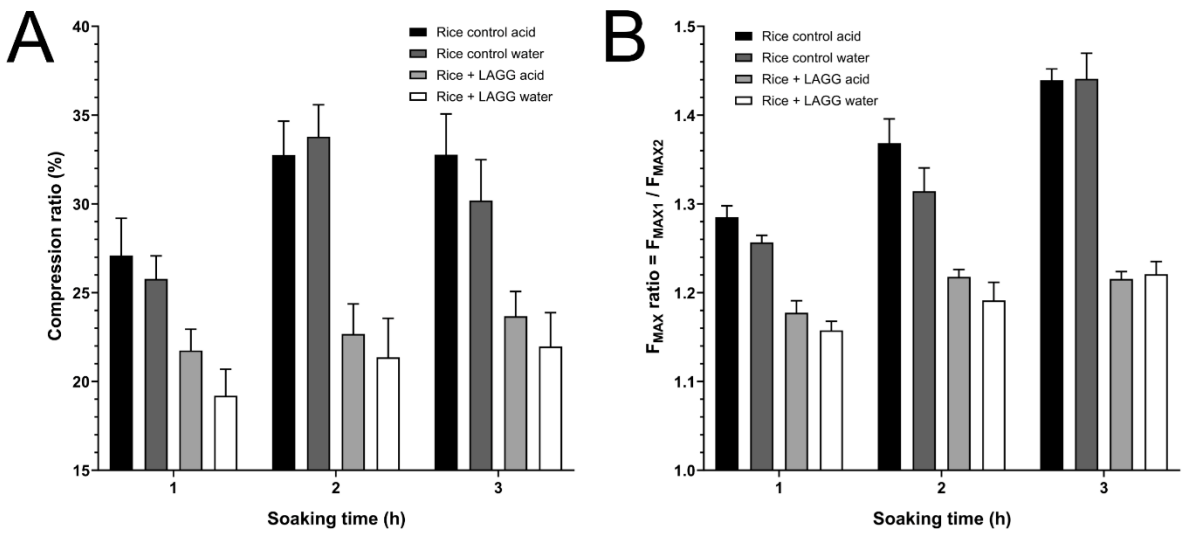
Supplementary Table 1

Summary results of the twelve parameters extracted from the force-versus-compression texture analyzer curves using a 'two-bite', sequential compressions protocol. The parameters extracted during the first and second compression cycles have indices '1' and '2', respectively. Parameters directly extracted from the force curves include: compression ratio (%), which reflects irreversible compression of rice grains induced by the first compression cycle, effective stiffness defined as a slope of the linear part of the force-indentation curve recorded during compression, F_{MAX} is the maximum force, whereby F_{MAX1} reflects the set point of 40 N, and F_{MAX2} reflects the reduction in the force due to rice grain compression, and thus provides additional parametrisation of the degree of the irreversible decompression of rice grains, pull-off force is extracted from the minimum force point during probe's retraction from the bed of the rice grains. Based on the primary parameters, several derived parameters were extracted to reflect relative changes of specific attributes. Stiffening Ratio, F_{MAX} Ratio, and Adhesion Ratio describe, respectively, the ratio of stiffness, F_{MAX} and Pull-off Force values recorded on the 1st and 2nd compression cycle ('bite'). The Plasticity parameter describes the ratio of stiffness recorded during decompression and compression arms of the force curve (rows 2 and 3 of the table) for the 1st and the 2nd 'bite' respectively. The larger values of the plasticity parameter reflect stronger extent of irreversible deformation (e.g. plastic deformation, cracking). The data are given as average values of triplicate measurements with standard deviations (SD). Values with the standard deviation that can be determined to the certainty of the last digit only are given in parentheses.

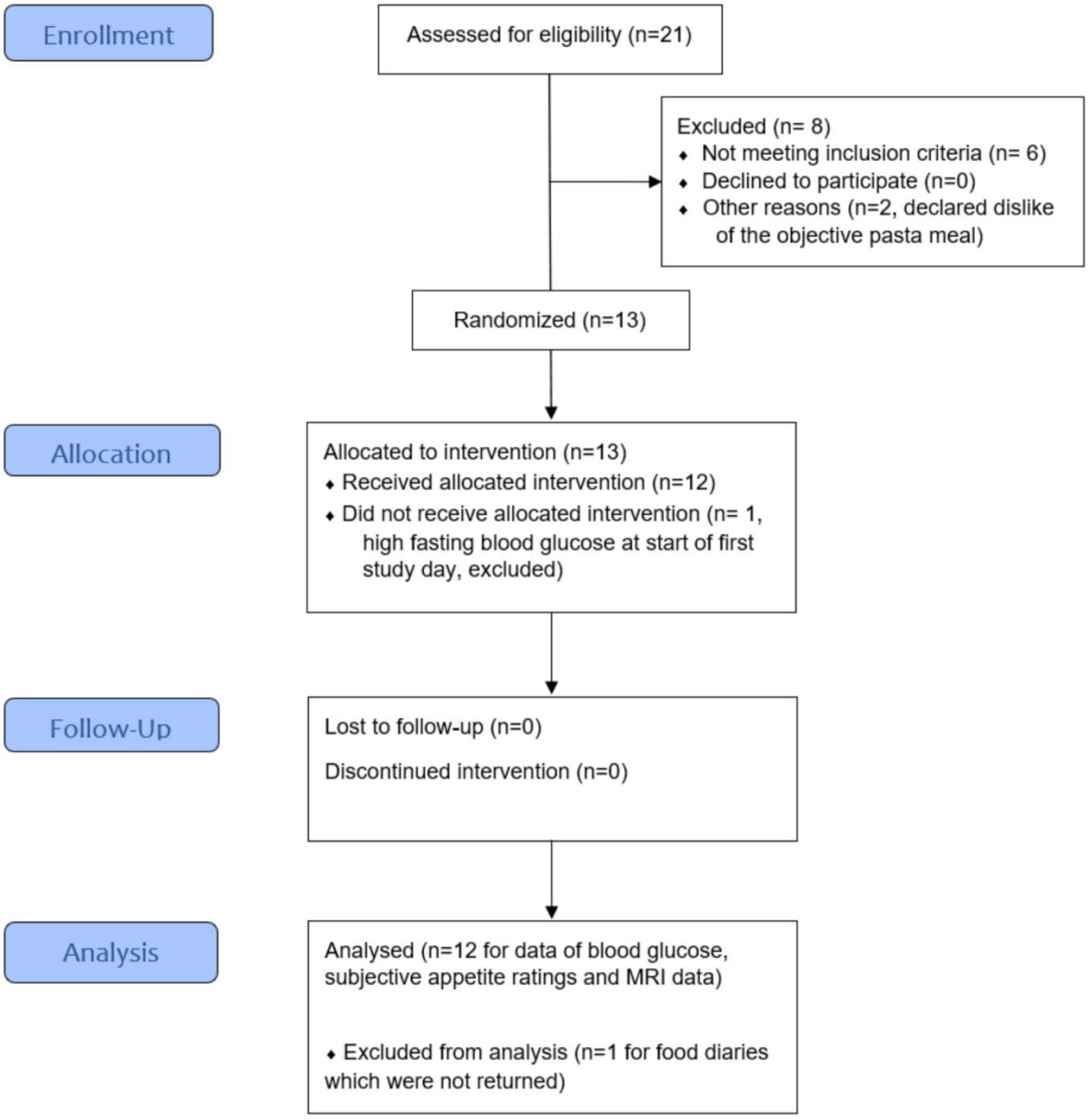
	Rice + LAGG in water 1h	**Rice + LAGG in acid 1h**	**Rice control in water 1h**	**Rice control in acid 1h**	**Rice + LAGG in water 2h**	**Rice + LAGG in acid 2h**	**Rice control in water 2h**	**Rice control in acid 2h**	**Rice + LAGG in water 3h**	**Rice + LAGG in acid 3h**	**Rice control in water 3h**	**Rice control in acid 3h**
Compression Ratio (%)	19.2 ± 1.5	21.7 ± 1.2	25.8 ± 1.3	27.1 ± 2.1	21.4 ± 2.2	22.7 ± 1.7	33.8 ± 1.8	32.8 ± 1.9	22.0 ± 1.9	23.7 ± 1.4	30.2 ± 2.3	32.8 ± 2.3
Stiffness 1 (N/m)	33994.5 ± 67.9	32411.4 ± 21.5	30194.9 ± 98.2	30852.9 ± 133.5	32995.2 ± 56.0	29130.4 ± 31.3	25607.9 ± 96.4	27025.6 ± 103.4	29038.4 ± 18.1	28958.4 ± 28.9	27752.0 ± 89.0	29239.5 ± 94.1
Stiffness 2 (N/m)	63358.7 ± 318.8	60354.1 ± 264.0	54508.3 ± 275.7	53389.4 ± 244.8	63498.3 ± 324.0	62308.9 ± 299.8	54101.3 ± 159.9	51718.4 ± 218.1	63386.1 ± 245.3	63244.4 ± 243.7	49550.5 ± 172.2	47177.3 ± 72.8
Stiffening Ratio (-)	1.86(4)	1.86(2)	1.80(5)	1.73(0)	1.92(4)	2.13(9)	2.11(3)	1.91(4)	2.18(3)	2.18(4)	1.78(5)	1.61(3)
F_{MAX} 1 (N)	39.33 ± 0.24	39.36 ± 0.19	39.38 ± 0.22	39.53 ± 0.25	39.48 ± 0.13	39.49 ± 0.15	39.73 ± 0.23	39.53 ± 0.28	39.65 ± 0.28	39.66 ± 0.1	39.58 ± 0.25	39.49 ± 0.11
F_{MAX} 2 (N)	33.98 ± 0.70	33.43 ± 0.71	31.34 ± 0.70	30.76 ± 0.42	33.14 ± 0.81	32.43 ± 0.61	30.23 ± 0.37	28.89 ± 0.69	32.48 ± 0.35	32.63 ± 0.54	27.47 ± 0.45	27.44 ± 0.78
F_{MAX} Ratio (-)	1.157 ± 0.010	1.177 ± 0.014	1.257 ± 0.008	1.285 ± 0.013	1.191 ± 0.020	1.218 ± 0.008	1.314 ± 0.026	1.368 ± 0.027	1.221 ± 0.014	1.215 ± 0.008	1.441 ± 0.029	1.439 ± 0.013
Pull-off F 1 (N)	-1,15(5)	-0.18(1)	-0.05(2)	-0.20(1)	-1.24(6)	-0.80(2)	-0.50(4)	-1.38(8)	-1.83(4)	-3.13(2)	-2.29(7)	-0.67(0)
Pull-off F 2 (N)	-1.25(8)	-0.27(6)	-0.42(0)	-0.65(1)	-1.16(7)	-1.13(5)	-1.03(1)	-1.39(2)	-1.89(8)	-1.38(6)	-0.55(5)	-0.95(3)
Adhesion Ratio (-)	0.9(7)	2.2(7)	4.1(6)	0.7(1)	1.0(7)	0.7(1)	0.4(9)	0.9(9)	0.9(7)	2.2(7)	4.1(6)	0.7(1)
Plasticity 1 (-)	3.4(2)	3.4(5)	4.2(2)	4.1(8)	3.5(2)	4.3(4)	5.1(9)	5.0(0)	4.5(6)	4.5(7)	5.1(0)	4.8(2)
Plasticity 2 (-)	1.6(9)	1.7(2)	2.0(9)	2.1(9)	1.8(4)	1.7(7)	2.1(8)	2.5(5)	1.8(6)	2.0(4)	2.6(4)	2.5(4)



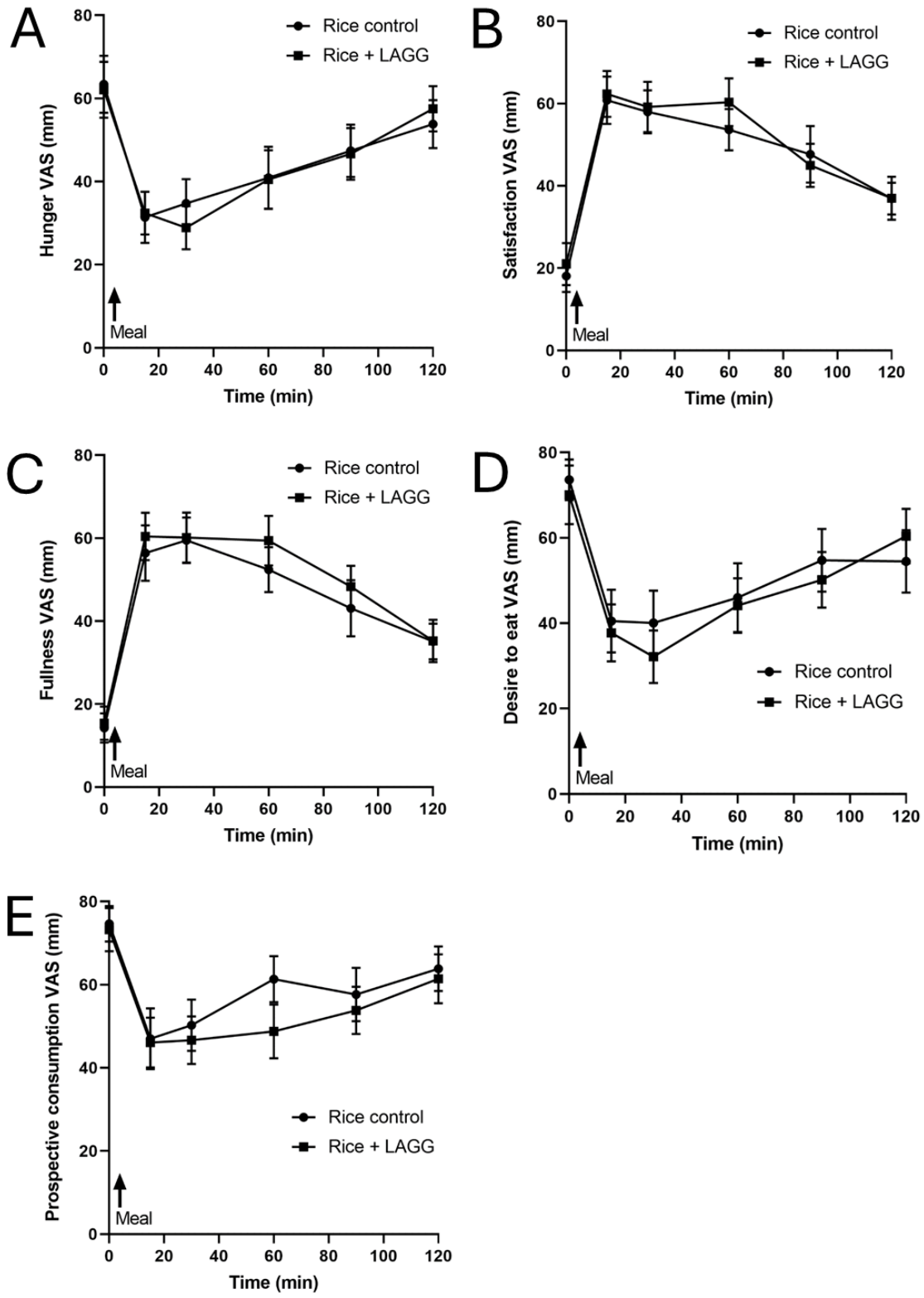
Supplementary Fig. 1. (A) Image showing the TA.XTplus Texture Analyzer set up for the sequential compression experiments. For each run, 15 rice grains, cooked with or without 3% w/dry rice w low acyl gellan gum (LAGG) and then soaked in water or acid for 1h, 2h and 3h were positioned together on the flat substrate, with the grains' major axis parallel to the surface and tested using a flat punch probe. The following three graphs show the analysis of mechanical properties of the rice grains as a function of LAGG treatment, soaking time, and solution pH. (B) Comparison of mechanical behaviour of LAGG treated rice grains versus control soaked in acid solution for 1h, 2h and 3h. (C) Comparison of mechanical behaviour of control rice grains soaked either in water or acid solution for 1h, 2h and 3h. (D) Comparison of mechanical behaviour of LAGG treated rice grains soaked either in water or acid solution for 1h, 2h and 3h.



Supplementary Fig. 2. Additional results from the TA.XTplus Texture Analyzer experiments whereby for each run, 15 rice grains, cooked with or without 3% w/dry rice w low acyl gellan gum (LAGG) and soaked in water or acid for 1h, 2h and 3h were tested using a flat punch probe. (A) Values of the compression ratio as a function of soaking time, the values are proportional to the degree of irreversible, plastic deformation of rice grains. (B) Values of FMAX Ratio as a function of soaking time, the values are proportional to the fragility of rice grains following initial compression. For purely elastic, non-fragile material the values of FMAX Ratio = 1. The data are shown as average of triplicate measurements ± SD.

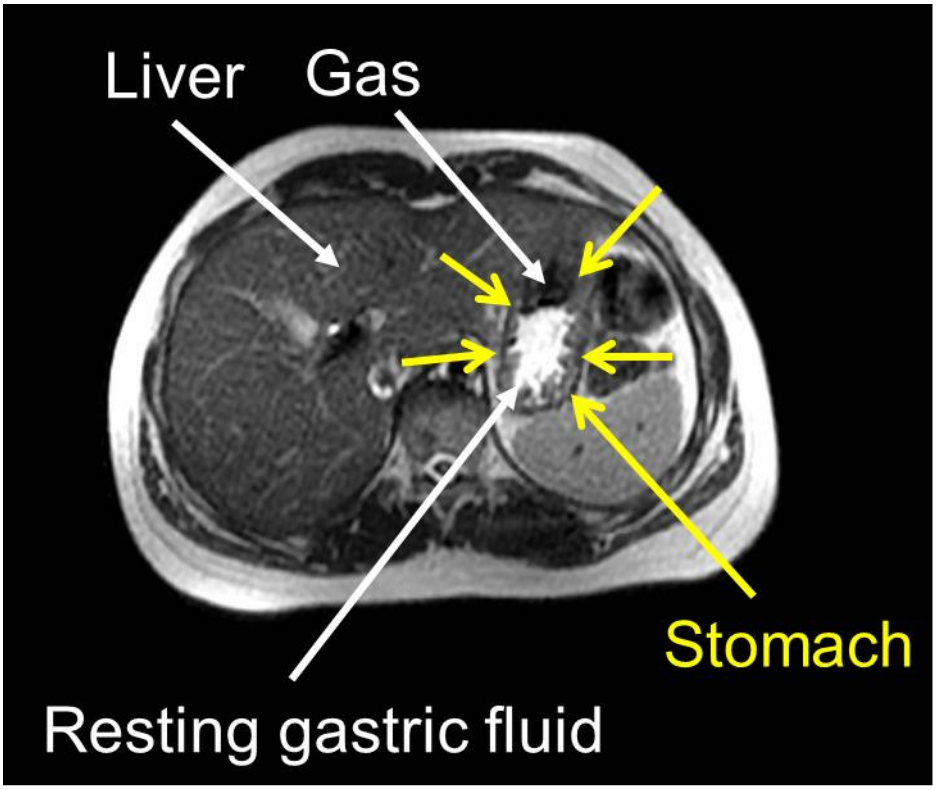


Supplementary Fig. 3. CONSORT 2010 flow diagram of the *in vivo* study(Schulz, Altman, & Moher, 2010).

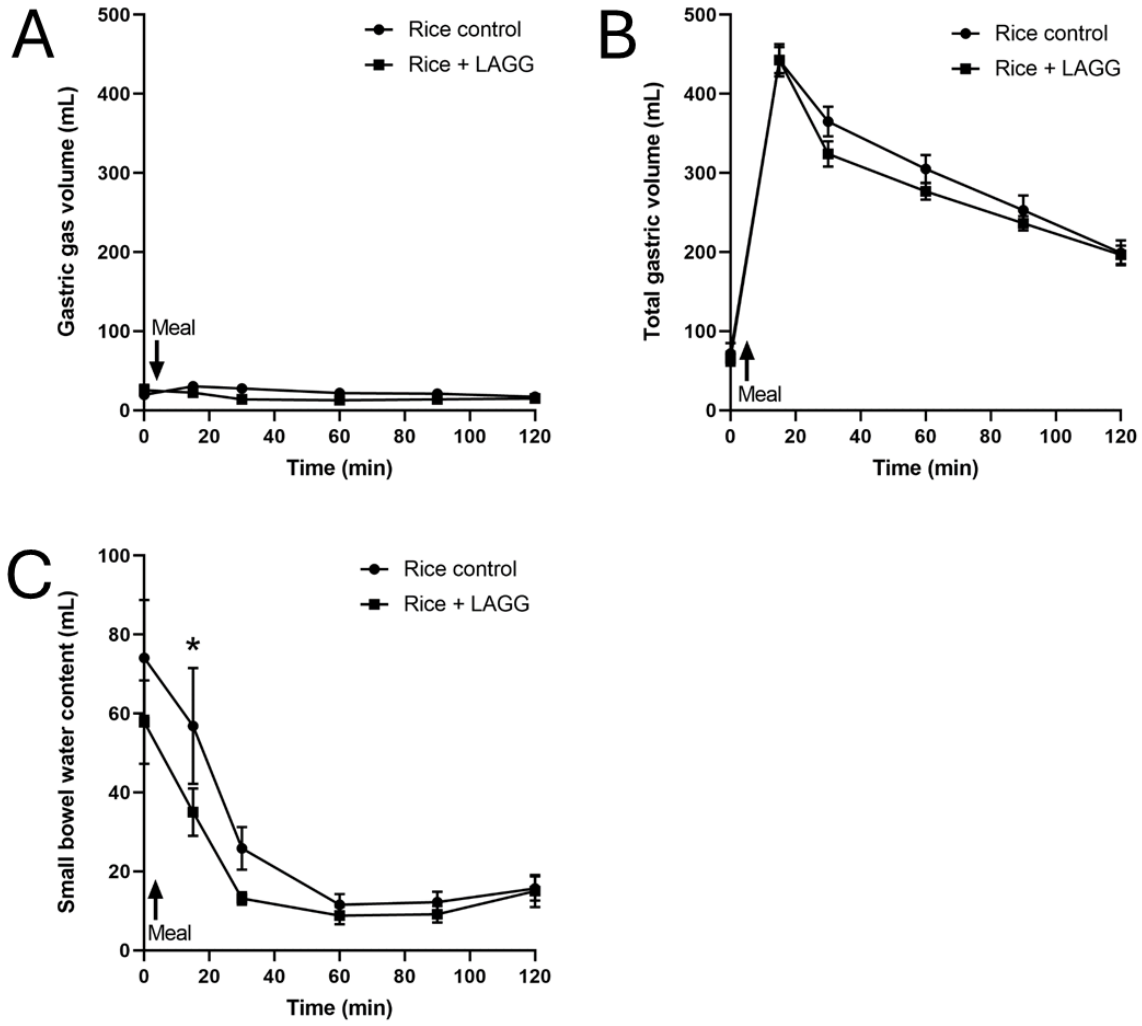


Supplementary Fig. 4. Visual analogue scale (VAS) time courses from *n* = 12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. (A) Hunger. Two-way repeated measure ANOVA

analysis for hunger VAS scores showed no significant interaction of rice meal type × time ($P = 0.7830$), no significant main effect of rice meal type ($P = 0.8242$) and a significant main effect of time ($P < 0.0001$). (B) Satisfaction. Two-way repeated measure ANOVA analysis for desire to eat VAS scores showed no significant interaction of rice meal type × time ($P = 0.3237$), no significant main effect of rice meal type ($P = 0.5236$) and a significant main effect of time ($P < 0.0001$). (C) Fullness. Two-way repeated measure ANOVA analysis for fullness VAS scores showed no interaction of rice meal type × time ($P = 0.7184$), no significant effect of rice meal type ($P = 0.2827$) and a significant effect of time ($P < 0.0001$). (D) Desire to eat. Two-way repeated measure ANOVA analysis for desire to eat VAS scores showed no significant interaction of rice meal type × time ($P = 0.6442$), no significant effect of rice meal type ($P = 0.5173$) and a significant effect of time ($P < 0.0001$). (E) Prospective consumption. Two-way repeated measure ANOVA analysis for prospective consumption VAS scores showed no significant interaction of rice meal type × time ($P = 0.4604$), no significant effect of rice meal type ($P = 0.1894$) and a significant effect of time ($P < 0.0001$). Data points are mean ± SEM.



Supplementary Fig. 5. MRI image acquired axially through the stomach of a study participant at fasted baseline, before a rice feeding intervention. Resting gastric fluid appearing white in this moderately T2-weighted sequence can be seen as well as intragastric gas appearing black, both indicated by white arrows. The liver is also indicated by a white arrow as an anatomical landmark for ease of orientation. The stomach boundaries are indicated by the yellow arrows



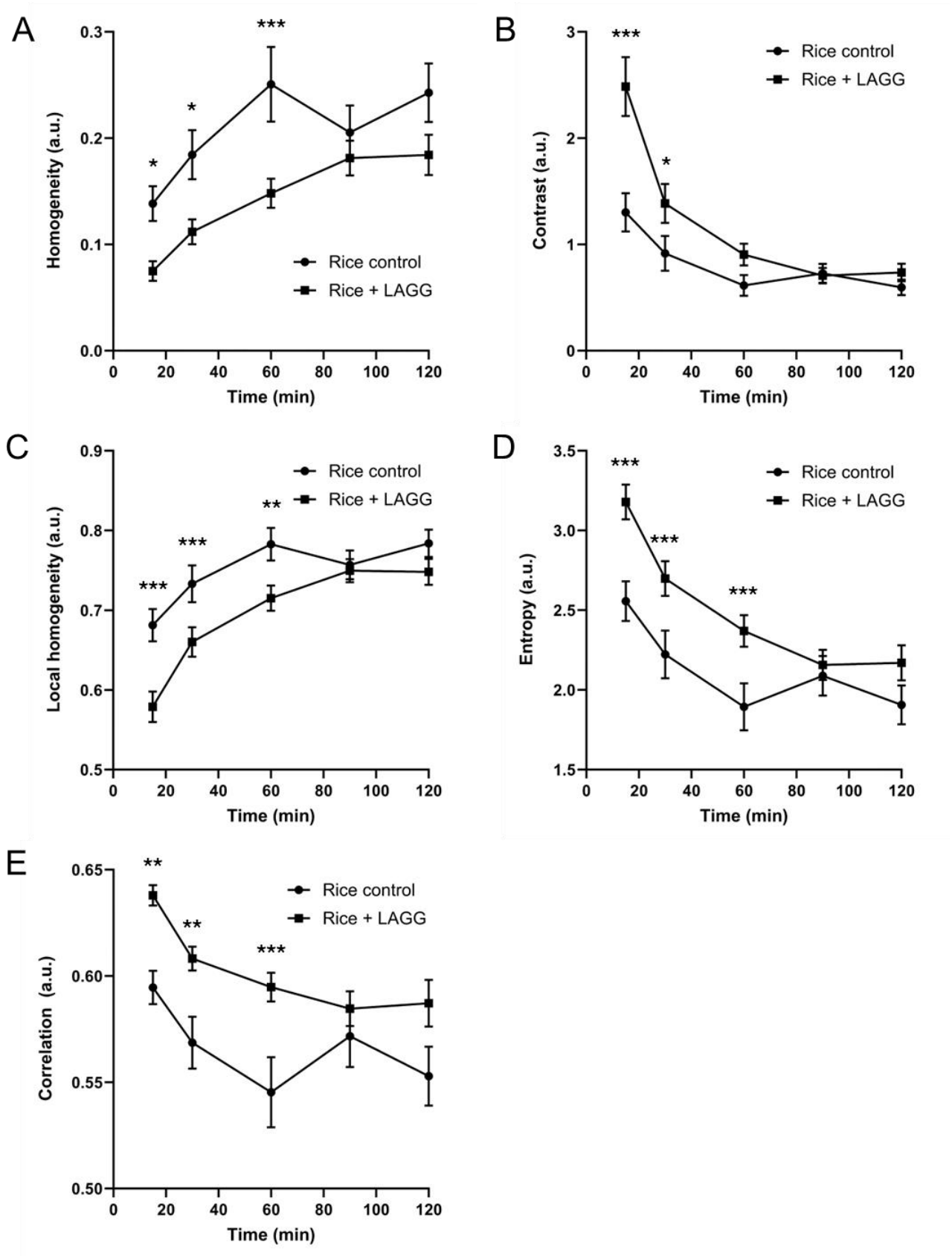
Supplementary Fig. 6. Additional results from the MRI imaging study. (A) Gastric gas volume time courses. Two-way repeated measure ANOVA analysis showed no significant interaction of rice meal type × time (P = 0.0991), no significant effect of rice meal type (P = 0.1128) and no significant effect of time (P = 0.0536). (B) Total gastric volume (meal volume plus gas volume) time courses. Two-way repeated measure ANOVA analysis showed no significant interaction of rice meal type × time (P = 0.2668), no significant effect of rice meal type (P = 0.2313) and a significant effect of time (P < 0.0001). (C) Small bowel water content time courses. Two-way repeated measure ANOVA analysis showed no significant interaction of rice meal type × time (P = 0.2859), a significant effect of rice meal type (P = 0.0337) and a significant effect of time (P < 0.0001). The data are from *n* = 12 healthy participants who consumed the rice meal with and without the addition of 3% w/dry rice w low acyl gellan gum (LAGG) during the cooking process. Values shown are mean ± SEM

from *n* = 12 participants for who consumed the test meal with and without the addition of 3% w/dry rice w LAGG. * $P < 0.05$.

Supplementary Table 2

Summary table indicating whether the operator could distinguish (green tick mark) or not distinguish (red cross mark) rice boluses in the individual MRI images of the stomach of each of the 12 healthy participants immediately after feeding and at later time points T=90-120 min after feeding.

	Rice control		**Rice + Gellan**	
Participant	Boluses visible after feeding	Boluses visible at later time points	Boluses visible after feeding	Boluses visible at later time points
1	✓	✗	✓	✗
2	✓	✗	✓	✓
3	✓	✓	✓	✓
4	✗	✗	✓	✓
5	✓	✗	✓	✓
6	✗	✗	✓	✓
7	✓	✗	✓	✓
8	✗	✗	✓	✓
9	✓	✗	✓	✓
10	✓	✓	✓	✓
11	✓	✗	✓	✓
12	✓	✗	✓	✓



Supplementary Fig. 7. Time courses of the five Haralick image texture features calculated for both the rice control and rice + LAGG meals. The graphs show respectively the Haralick

texture characteristics for: (A) Homogeneity. Two-way repeated measure ANOVA analysis showed no significant interaction of rice meal type × time (P = 0.2360), a significant effect of rice meal type (P = 0.0041) and a significant effect of time (P < 0.0001). (B) Contrast. Two-way repeated measure ANOVA analysis showed a significant interaction of rice meal type × time (P < 0.0001), a significant effect of rice meal type (P = 0.0052) and a significant effect of time (P < 0.0001). (C) Local homogeneity. Two-way repeated measure ANOVA analysis showed a significant interaction of rice meal type × time (P = 0.0028), a significant effect of rice meal type (P = 0.0040) and a significant effect of time (P < 0.0001). (D) Entropy. Two-way repeated measure ANOVA analysis showed a significant interaction of rice meal type × time (P = 0.0111), a significant effect of rice meal type (P = 0.0032) and a significant effect of time (P < 0.0001). (E) Correlation. Two-way repeated measure ANOVA analysis showed no significant interaction of rice meal type × time (P = 0.2533), a significant effect of rice meal type (P = 0.0023) and a significant effect of time (P < 0.0001). Values shown are mean ± SEM from *n* = 12 participants for who consumed the test meal with and without the addition of 3% w/dry rice w LAGG. * P < 0.05, ** P < 0.01, *** P < 0.001.

Supplementary References

Arvis, V., Debain, C., Berducat, M., & Benassi, A. (2011). Generalization of the cooccurrence matrix for colour images: application to colour texture classification. *Image Analysis and Stereology, 23* (1), 63-72. https://doi.org/10.5566/ias.v23.p63-72.

Blakeney, A. B., & Mutton, L. L. (1980). A simple colorimetric method for the determination of sugars in fruit and vegetables. *Journal of the Science of Food and Agriculture, 31* (9), 889-897. https://doi.org/10.1002/jsfa.2740310905.

Bourne, M. C. (1966). Measure of shear and compression components of puncture tests. *Journal of Food Science, 31* (2), 282-291. https://doi.org/10.1111/j.1365-2621.1966.tb00494.x.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assuncao, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A. R., Martins, C., Marze, S., McClements, D. J., Ménard, O., Minekus, M., Portmann, R., Santos, C. N., Souchon, I., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., & Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14* (4), 991-1014. https://doi.org/10.1038/s41596-018-0119-1.

Conners, R. W., & Harlow, C. A. (1980). A theoretical comparison of texture algorithms. *Ieee Transactions on Pattern Analysis and Machine Intelligence, 2* (3), 204-222. https://doi.org/10.1109/tpami.1980.4767008.

Dellschaft, N., Hoad, C., Marciani, L., Gowland, P., & Spiller, R. (2022). Small bowel water content assessed by MRI in health and disease: a collation of single-centre studies. *Alimentary Pharmacology and Therapeutics, 55* (3), 327-338. https://doi.org/10.1111/apt.16673.

Friedman, H., Whitney, J., & Szczesniak, A. (1963). The texturometer - a new instrument for object texture measurement. *Journal of Food Science, 28*, 390-396. https://doi.org/10.1111/j.1365-2621.1963.tb00216.x.

Goni, I., GarciaAlonso, A., & SauraCalixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17* (3), 427-437. https://doi.org/10.1016/s0271-5317(97)00010-9.

Haralick, R. M., Shanmugam, K., & Dinstein, I. (1973). Textural features for image classification. *Ieee Transactions on Systems Man and Cybernetics, SMC3* (6), 610-621. https://doi.org/10.1109/tsmc.1973.4309314.

Hoad, C. L., Marciani, L., Foley, S., Totman, J. J., Wright, J., Bush, D., Cox, E. F., Campbell, E., Spiller, R. C., & Gowland, P. A. (2007). Non-invasive quantification of small bowel water content by MRI: a validation study. *Physics in Medicine and Biology, 52* (23), 6909-6922. https://doi.org/10.1088/0031-9155/52/23/009|issn 0031-9155.

Mayar, M., Smeets, P., van Duynhoven, J., & Terenzi, C. (2023). In vitro 1H MT and CEST MRI mapping of gastrointestinal milk protein breakdown. *Food Structure, 36*, 100314. https://doi.org/10.1016/j.foostr.2023.100314.

McAuliffe, M. J., Lalonde, F. M., McGarry, D., Gandler, W., Csaky, K., & Trus, B. L. (2001). Medical image processing, analysis and visualization in clinical research. In *14th IEEE Symposium on Computer-Based Medical Systems* (pp. 381-386). Bethesda, Md.

Milan, A. M., Barnett, M. P. G., NMcNabb, W. C., Roy, N. C., Coutinho, S., Hoad, C. L., Marciani, L., Nivins, S., Sharif, H., Calder, S., Du, P., Gharibans, A. A., O'Grady, G., Fraser, K., Brernsein, D., Rosanowski, S. M., Sharma, P., Shrestha, A., & Mithen, R. F. (2024). The impact of heat treatment of bovine milk on gastric emptying and nutrient appearance in peripheral circulation in healthy females: a randomized controlled trial comparing pasteurized and ultra-high temperature milk. *American Journal of Clinical Nutrition*, in press. https://doi.org/10.1016/j.ajcnut.2024.03.002.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D. J., Ménard, O., Recio, I., Santos, C. N., Singh, R. P., Vegarud, G. E., Wickham, M. S. J., Weitschies, W., & Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function, 5* (6), 1113-1124. https://doi.org/10.1039/c3fo60702j.

Muttakin, S., Bakalis, S., Fryer, P. J., Alshammari, N. A., Marciani, L., & Gouseti, O. (2023). Reducing starch digestibility of white rice by structuring with hydrocolloids. *Food Research International, 174*, 113490. https://doi.org/10.1016/j.foodres.2023.113490.

Otsu, N. (1979). A threshold selection method from gray-level histograms. *IEEE Transactions on Systems, Man and Cybernetics, 9*, 62-66. https://doi.org/10.1109/TSMC.1979.4310076.

Rosenthal, A. J. (2010). Texture profile analysis -how important are the parameters? *Journal of Texture Studies, 41* (5), 672-684. https://doi.org/10.1111/j.1745-4603.2010.00248.x.

Schulz, K. F., Altman, D. G., & Moher, D. (2010). CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ, 340*. https://doi.org/10.1136/bmj.c332.

van Eijnatten, E. J. M., Camps, G., Guerville, M., Fogliano, V., Hettinga, K., & Smeets, P. A. M. (2024). Milk coagulation and gastric emptying in women experiencing gastrointestinal symptoms after ingestion of cow's milk. *Neurogastroenterology and Motility, 36* (1). https://doi.org/10.1111/nmo.14696.

Vrbik, I., Van Nest, S. J., Meksiarun, P., Loeppky, J., Brolo, A., Lum, J. J., & Jirasek, A. (2019). Haralick texture feature analysis for quantifying radiation response heterogeneity in murine models observed using Raman spectroscopic mapping. *Plos One, 14* (2). https://doi.org/10.1371/journal.pone.0212225.

Yu, L., Witt, T., Bonilla, M. R., Tumer, M. S., Fitzgerald, M., & Stokes, J. R. (2019). New insights into cooked rice quality by measuring modulus, adhesion and cohesion at the level of an individual rice grain. *Journal of Food Engineering, 240*, 21-28. https://doi.org/10.1016/j.jfoodeng.2018.07.010.