Investigating the effects of Oat beta-glucan on *in-vitro* starch and lipid digestion

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Introduction

According to the World Health Organization, cardiovascular disease (CVD) is one of the leading causes of mortality and morbidity worldwide [1]. While behavioural and environmental factors play a role, lifestyle and diet are key in determining CVD risk. Diets rich in fruits, vegetables, legumes, and whole grains, which are high in dietary fibre, have been shown to reduce CVD risk by helping manage obesity and hypertension [2].

A common example of a widely consumed grain are oats (*Avena sativa L*) known for their nutritional benefits and versatility in various food products. A key component of oats contributing to their health promoting properties is beta-glucan, a type of non-starch polysaccharide primarily found in the oat endosperm cell walls [3]. Beta-glucans are composed of D-glucopyranosyl residues linked by beta 1,3 and beta 1,4 glycosidic bonds [4]. These unique linkages give beta-glucans their ability to impact physiological processes.

Research have shown beta-glucans from oats can lower post-prandial blood glucose and reduce low-density lipoprotein (LDL) cholesterol concentrations [5], recognized by the Food and Drug Administration and the European Food Safety Authority. These effects are due to the increased viscosity in the intestinal lumen, slowing digestion and carbohydrate absorption, influenced by the source, molecular weight, and food matrix [4]. Beta-glucans also reduce free fatty acid release during digestion by encapsulating lipids, though the precise mechanisms are still being studied [3].

Aims of the project

Previous studies have extensively explored the physiological effects of oat beta-glucans, including their role in promoting bile excretion and cholesterol removal, though the mechanisms are still under investigation [6]. This project aims to investigate the fundamental principles of oat beta-glucan in in-vitro starch and lipid digestion, focusing on:

1. Investigating the effects of oat beta-glucans on the concentration of reducing sugars (maltose equivalents) release.
2. Investigating the effects of oat beta-glucans on the amount of free fatty acids released (Palmitic Acid, Linoleic Acid, Oleic Acid, and Stearic Acid).
3. Investigating the structural differences among seven types of biscuits made from oat flake, oat flour, extracted oat beta-glucan BG28 (28% beta-glucan) and BG90 (90% beta-glucan), compared to wheat flour, wheat flake controls and another non-starch polysaccharide, guar gum flour

Methods

In-vitro digestion (INFOGEST)

In-vitro digestions of seven types of biscuits (wheat flour, wheat flake, oat flake, oat flour, BG28, BG90, and guar gum flour) were performed and repeated three times. The experiment used the three steps (oral, gastric, duodenal) standardised by the INFOGEST Cost Action [7]. 2.5g of each sample was placed into a 50ml tube and was added simulated salivary fluid (1:1 wt/wt) with alpha amylase (12mg/ml) and placed in a rotating incubator at 37°C for 5 minutes. The oral bolus is then diluted with simulated gastric fluid (1:1 vol/vol) containing gastric lipase and pepsin, incubated at pH 3 for 1 hour. The gastric chyme is diluted with simulated intestinal fluid (1:1 vol/vol), bile salts, and pancreatin, incubated at pH 7 for 2 hours. Two separate aliquots samples (50uL) were taken at 5, 35, 60, 65, 80, 95, 125, 155, 180 minutes. One aliquot was added Orlistat (1mM) to stop lipase activity at intestinal phase, and another aliquot was added Sodium Carbonate (0.3M) to stop amylase activity.

Microscopy

Biscuit samples were stained with Nile Red, Fast Green FCF, Calcofluor, Fluorescein and incubated for 24 hours at 37°C. Samples were washed 4 times with 1xPBS solution and mounted on slides using SecureSeal imaging spacers and imaged on a Nikon AX Confocal microscope. Further image analysis was conducted using ImageJ to subtract background signals and produce 3D images from Z-projections.

Digested biscuit samples treated with Sodium Carbonate were centrifuged 3 times at max speed (13000rpm) for 10 minutes, and supernatant was removed. Samples were visualised under polarised light with birefringent starch granules representing un-gelatinised starch, resistant to digestion.

Reducing sugar analysis (PAHBAH assay)

Firstly, a set of maltose standards (0-1000 µM) were prepared. Secondly, 10µL digested biscuit samples treated with Sodium Carbonate were diluted 1:200 with distilled water. 100µL of each standard and diluted samples were transferred into 1.5mL Safe-lock tubes. 1ml of PAHBAH solution was added to each tube and place in a floating rack in a boiling water bath for 5 minutes. Once the samples were cooled after 10 minutes, they are placed in the vortex for 1 minute. Lastly, 200µL of each samples were pipetted onto a clear, plastic flat base 96-well plate and absorbance level of each sample were measured using a spectrophotometry at 402nM. A standard curve was plotted and each concentration was calculated.

Fatty acid extraction and derivatisation

Firstly, a set of standards (0-50 mM) were prepared. Secondly, 10µL of digested biscuit samples treated with Orlistat and standards were homogenized with 90µL distilled water, 90µL Methanol, and 200µL Chloroform. The mixture was placed on a vortex for 1 minute and centrifuged from 10 mins at maximum speed. 150µL of the Chloroform layer was collected and transferred into 1.5mL glass vials. These vials were placed in a rotary evaporator for 30 minutes and 50µL of MFSTA (*N*-Trimethylsilyl-*N*-methyl trifluoroacetamide) was added. The mixture was incubated at room temperature for 12 hours. Lastly, 50µL of Heptane was added and sealed. Each sample was placed in a gas chromatography / mass spectrophotometry (GC/MS) to measure the content of fatty acid in lipid samples.

Statistics

The data were analysed using Prism Version 10.2.3. The significance level was set at $P > 0.05$ (2 tailed) for all data sets. The data were expressed as means of triplicates $\pm$ SEM. The differences between the types of biscuits, duration of digestion, and maltose release or fatty acid release were evaluated by a two-way analysis of variance.

Results and Discussion

The final results showed the percentage of maltose released relative to total starch content, with guar gum biscuits exhibiting the lowest maltose release (19.44 ± 2.66) and wheat flake biscuits the highest (33.67 ± 3.97). However, a two-way ANOVA test found no significant differences in maltose release between biscuit types during the duodenal phase. The overall low maltose release can be attributed to the limited starch digestion, as seen in Figure 2 and 3. These images reveal a significant amount of undigested starch granules, visible as starch birefringence. The refracted light from non-gelatinized starch granules in the polarized light images (Figure 3) supports the observation of incomplete starch digestion, explaining the low maltose release. This may be due to the encapsulation effect, where the viscous polysaccharide network forms a gel layer around starch granules, preventing enzyme access. The structural integrity of the flours can also be a factor since wheat and oat flakes released higher maltose in comparison to their flour version due to the hydrothermal treatment causing endosperms cells to be severely ruptured and gelatines, thus being susceptible to digestion. Additionally, processing factors like baking temperature and moisture content may have influenced gelatinisation levels [3].

For lipid digestion, BG90 biscuits exhibited the highest fatty acid release as seen in Figure 4, particularly for palmitic (56.0 ± 4.81) and stearic acids (32.68 ± 2.76), while guar gum biscuits had the lowest release for all fatty acids release (1.36 ± 0.96, 0.66 ± 0.47, 2.11 ± 1.49, 3.25 ± 2.30 for palmitic, linoleic, oleic, and stearic acid, respectively). This difference can be explained by the viscosity and gel-forming properties of guar gum, which restrict enzyme access to lipids, consistent with findings by Moschakis et al. (2014). In contrast higher molecular weight β-glucan like BG90 may promote lipid digestion by enhancing emulsification in comparison to BG28 biscuits. Biscuits made from oat flour and flakes also showed reduced fatty acid release, likely due to the formation of a gel layer by leaching β-glucans or the incomplete breakdown of the oat matrix. Additionally, other components present in oats, such as starch and proteins, may interfere with lipid digestion, as they can form complex structures that hinder enzyme access, similar to the mechanisms.

Overall, these results highlight the importance of the structural integrity of dietary fibres in regulating both starch and lipid digestion, with the balance between concentration, molecular weight, and food matrix integrity being crucial for optimizing digestion outcomes.

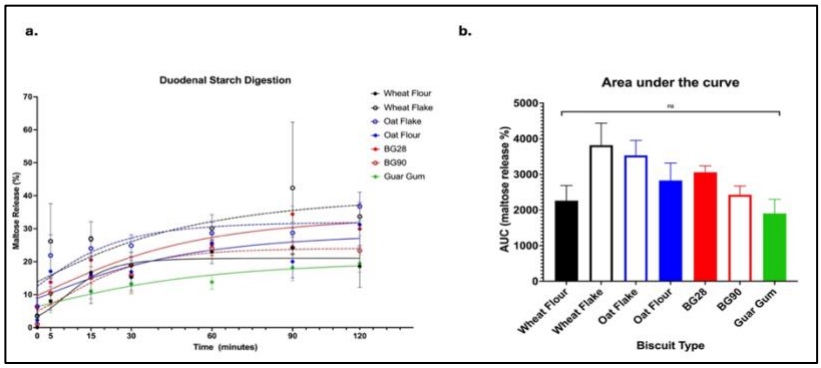

Figure 1: Maltose Release (%) during Duodenal Starch Digestion. **a**. Curve demonstrating the increase of maltose release (% w/w) in the duodenal stage of in-vitro digestion of seven types of biscuits as seen in the legend. Biscuit made from wheat flake released highest amount of maltose while biscuits made from guar gum released lowest amount of maltose. **b.** bar graph representing the area under curve of each maltose release curve (%). All the data shown are mean values of triplicates and Standard Error Mean. All sets of data was analysed using a Two-way ANOVA test.

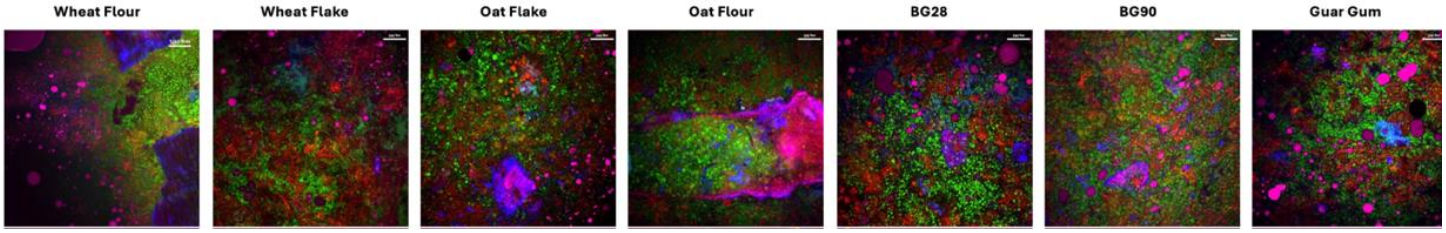

Figure 2: Fluorescence Confocal Microscopy comparing seven types of pre-digested biscuits. Images were captured using Nikon AX Confocal microscope. Samples were visualised using laser excitations (emissions) of 405nm (410-480nm), 488nm (500-550nm), and 640nm (645-670nm) for Calcofluor, Nile Red and Fast Green FCF respectively.

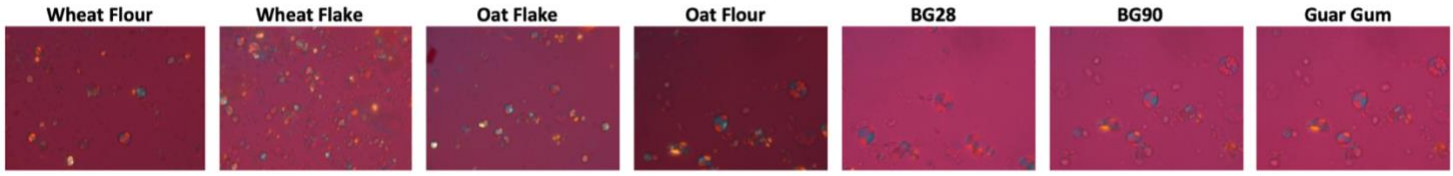

Figure 3: Polarised light images comparing seven types of post-digested biscuits. The birefringent starch granules shown demonstrate limited gelatinisation during biscuit baking, which contributes to the low rates of digestion observed.

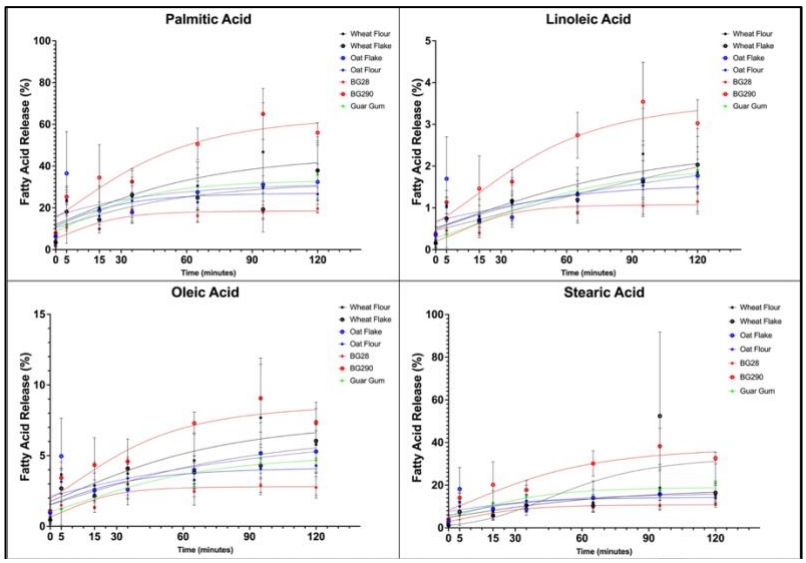

Figure 4: Fatty Acid Release (%) during Duodenal Digestion. Four curves demonstrating the release of Palmitic acid (16:0), Linoleic acid (18:2), Oleic acid (18:1), and Stearic acid (18:0) in the duodenal stage of in-vitro digestion of seven types of biscuits (as seen in the legend). Biscuits made from BG90 showed an overall high fatty acid release, while biscuits from BG20 showed a low fatty acid release. All the data shown are mean values of triplicates and Standard Error Mean. All sets of data was analysed using a Two-way ANOVA test.

Future Directors:

The results obtained so far provides a solid foundation for the broader aims of the project. However, since the project is still ongoing, further replication of the fatty acid extraction is necessary to reduce the variability in the results. Additionally, improving the imaging of the oat biscuits by optimizing the stain concentrations could enhance the visual data. As the project progresses, it may also be valuable to investigate the effects of oat beta-glucans on bile excretion.

Departures from original proposal

The original project plan was to use bread as the main food product, however based on availability issues, we have decided on using biscuit samples instead.

Value of studentship

Student

This studentship has been an incredible learning experience, providing me with hands-on experience in the lab and the opportunity to work with food products, an area I'm passionate about and will pursue in the future. I've developed new experimental techniques and strengthened my basic laboratory skills. I was also introduced to applications like Fiji and Prism, which have helped me better understand the process of data analysis and how to interpret large datasets. I am particularly grateful for the guidance and support of Balazs, Peter, and Xirui throughout the summer.

Supervisor

The work conducted as part of this project contributes to an ongoing study assessing the mechanisms of beta-glucan action in the gut, and the importance of structure and processing on enzymic digestion. It is hoped that these data will help to inform future in vivo studies.

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