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Should you add kiwifruit to your cereals? – An investigation of bioactive peptides produced with kiwifruit extract

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A dissertation

submitted in partial fulfilment

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By

Jessica Ellen Hampton

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Master of Science in Food Innovation

Should you add kiwifruit to your cereal? – An investigation of bioactive peptides produced with kiwifruit extract

by

Jessica Ellen Hampton

Oat is a popular cereal food which contains high amounts of beneficial compounds shown to exert positive effects on human health. It is notable for its relatively high protein content. Actinidin is a protease found in kiwifruit which has strong digestive properties. This study investigated if actinidin could be combined with oat to break down the endogenous oat proteins and generate peptides with bioactive properties, thus increasing their potential health effects. Kiwifruit extract was combined with oat at 37 °C for four hours to allow for maximum release of soluble protein into the samples. After the heat treatment step the samples were put through an *in vitro* digestion system. Changes in antioxidant activity were measured using total phenolic concentration (TPC), ferric reducing antioxidant power (FRAP), and angiotensin-converting-enzyme (ACE) inhibition assays. The Combined sample was not significantly different ($p > 0.05$) from the Kiwifruit Extract only sample for any compound analysed. However, the decrease in TPC, and increase in ACE was significantly greater ($p < 0.05$) in the Combined sample than the Rolled Oat only sample. Protein and peptide gels showed no difference between samples. Overall there was no evidence of significant changes to beneficial effects of the oat, as caused by the addition of actinidin. The addition of oat appeared to have a slight beneficial effect on the enzymatic activity and antioxidant content of kiwifruit. Future work investigating other bioactive compounds that are known be present in the oat is reccommended to gain a better understanding of the effect of actinidin on oat proteins.

Keywords: Actinidin, kiwifruit, oat, bioactive peptides, antioxidants, ACE inhibtion

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Introduction and Literature Review

1.1 Introduction

In 2019 the World Health Organisation reported that 74% of global deaths were attributed to non-communicable diseases such as heart disease, cancers, diabetes, and kidney disease (World Health Organisation, 2020). A large portion of these diseases are triggered or worsened by certain lifestyle factors and therefore the risk can be greatly reduced or managed with appropriate choices regarding diet, physical activity, sun exposure, and alcohol and tobacco use (Balwan & Kour, 2021). The pillar of diet reflects the need to consume fruit and vegetables, whole grains, and lean protein, and reduce consumption of saturated fat and salt. However, many do not consume enough nutrient-dense foods and have poor nutritional intakes, negatively affecting their health. In particular diabetes, and heart disease are linked with poor diet. Consumers are becoming more aware of this and are increasingly invested in what they are eating as they look to improve their health. This has contributed to the rise in functional foods which have health benefits above and beyond general nutrition factors (Henry, 2010). One way to produce functional food is to increase the bioavailability of what is inherently in the food product, contributing to a reduction in disease risk. This can be done through the production of the food itself, or by altering or treating the food with biological products (microbes or enzymes), heat, pH changes, or chemicals (Toldra et al., 2018). The effects of treatment can increase levels of bioactive compounds in the food products, leading to functional foods. Examples of this include the release of bioactive peptides from protein matrices, or the increasing the bioavailability of micronutrients in food products (Mazorra-Manzano et al., 2018; Tan & McClements, 2021). These types of food appeal to the consumer as they highlight the properties of the food itself and do not rely on supplementation or medication for the improvement of health. Generally, functional foods are well accepted, especially when the consumer is familiar with the 'regular' version of the product and the process in which it has been made 'functional' (Siro et al., 2008). This highlights the importance of developing well understood functional foods or finding ways to increase the benefits provided by already well-established foods.

1.2 Litrature review

1.2.1 Oats

Oats (*Avena sativa*) are a commonly consumed cereal around the world. They are part of the family Poaceae, which is divided into two tribes *Aveneae* (oats) and *Triticeae* (wheat, barley, and rye). Oats

have an inedible husk which is removed during processing, leaving the interior oat groat for consumption. Lightly steaming and pressing the groats into rolled oats is a common processing method as it retains the nutrition of a whole grain cereal but is easier and faster for the consumer to prepare (Decker et al., 2014). These rolled oats can be eaten raw, added to food as an ingredient, or further cooked into porridge or muesli. Oats are high in protein, fibre, and a wide variety of vitamins and minerals, but low in calories making them a nutritious option (Butt et al., 2008). Oats are very high in the soluble fibre β-glucan which has been associated with the reduction of risk of cardiovascular disease (CVD), diabetes, and cancer (Daou & Zhang, 2012). One bioactive compound of note is the avenanthramides-type antioxidants which are unique to cereals and touted as the main health advancing active compounds (Kim et al., 2021). Many beneficial mechanisms and pathways have been investigated and proposed but there has been little confirmation of them through human trials. As such, consumption of oats has been proposed and shown through *in vitro* study to influence many ailments such as high cholesterol and related cardiovascular issues, cancers, diabetes, as well as the effects of inflammation (Rasane et al., 2015). Oats differ from other cereals as their main storage proteins are globulins (11S, 12S) and avenins (3, E, F, A, and N9), not prolamin which is typical for cereals (Cavazos & de Mejia, 2013). Oats also have a protein content between 12-20% which is higher than many other cereals (Boukid, 2021). These key differences reflect a potential for increased bioactive peptides release from oats than what may be observed with other cereals. Many bioactive peptides have been identified in oats with angiotensin I-converting enzyme (ACE) and dipeptidyl peptidase-IV (DPP-IV) inhibition, antioxidant, anti-amnestic, and antithrombotic activity, stomach mucosal regulation, vasoactive stimulation, and activation of ubiquitin-mediated proteolysis mechanisms being key bioactive features (Bleakley et al., 2017; Cavazos & de Mejia, 2013). Some of these bioactive compounds have been isolated and some have been identified through *in silico* analysis. ACE and DPP-IV inhibition, and antioxidants tend to be found to have highest capacity for bioactivity in oats. The benefits of oats are already exceptionally good but there is potential to increase their bioactivity. Cheung *et al*. (2009) showed an increase in the ACE inhibition of oats that had been treated with thermolysin when compared to standard oat. Therefore, there are additional bioactive peptides in the food matrix of oats which are not able to be accessed by humans without additional processing or pre-digestion.

1.2.1.1 Bioactive peptides

Peptides are small sequences of amino acid joined by peptide bonds. Typically, they are 2-20 amino acids long and many multiple of these sequences connect to form protein molecules. *In vitro* and *in silico* analysis has shown that some specific individual peptide sequences have bioactive properties, the benefits of which has been seen in human trails, as reviewed by (Xu et al., 2019). These peptides are known as bioactive peptides and each type has slightly different functions and modes of action. Specific sequences act on specific systems in the body. The effects of the bioactive peptides are

dependent on the cleavage of the proteins in the right places from the food sources and the order and type of amino acids that from the chain (Toldra et al., 2018). The bioactive peptides must also survive digestion and reach their active site intact to have an effect. The functions of bioactive peptides have an array of benefits such as anti-diabetic, anti-cancer, immunomodulatory, gut health, heart health, and antioxidant properties, and there is potential for more benefits to be uncovered (Cavazos & de Mejia, 2013; Samtiya et al., 2021). These effects are not to be underestimated as the quantity of bioactive peptides found naturally in food can influence that same level of change that is seen through medicating but are much gentler on the body and do not come with side effects (Mazorra-Manzano et al., 2018). While there are bioactive peptides with high availability in their food form, additional are bound in the complex food protein matrix and unavailable. These additional compounds can be released by additional processing such as fermentation, pH, chemical, or heat treatment, and the action of enzymes (Toldra et al., 2018). Investigating the bioactive potential of foods through the release of bioactive peptides is an area of study which has enormous implications for the health of many. Increasing the bioavailability of bioactive peptides in foods will increase their health benefits. This is important to the public as 'functional foods' are gaining in popularity as food is increasingly seen as a way to effect positive change on wellbeing and not just as fuel.

1.2.1.2 Functions of bioactive peptides

Bioactive peptides are bioactive due to the physiological changes they can exert on humans. These bioactive properties can lead to a reduction in risk for certain diseases such as CVD, and diabetes. They can also provide antioxidant effects and associated benefits. Many of the benefits of bioactive peptides act to counter the effects of poor diet and inactivity related illness termed 'lifestyle diseases' (Yoshikawa et al., 2000). An overview of some of the benefits and undesirable effects of bioactive peptides are outlined in the following paragraphs.

1.2.1.3 Cardiovascular disease

Bioactive peptides have a variety of functions which contribute to lowering the risk for CVD. The main ones are antihypertensive properties through ACE inhibition and hypocholesterolemic properties through alterations to low density lipoprotein receptors (Erdmann et al., 2008). ACE is a part of the cardiovascular system of regulating blood pressure. However, when blood pressure is too high, ACE inhibitors must be used to counter the action of the ACE and lower the blood pressure. Some bioactive peptides can provide this function, resulting in lowered blood pressure (Wu et al., 2017).

1.2.1.4 Antidiabetic

Bioactive peptides can also have an effect on reducing the risk for diabetes. The main mechanism for this is inhibition of DPP-IV. DPP-IV is an enzyme which degrades gastric inhibitory polypeptides and glucagon-like peptides (Lambeir et al., 2003). Preventing this action is a key step in management of type-2 diabetes and many drugs have been developed to this effect. However natural sources of DPP-

IV inhibitors are preferred and have been identified in many biological sources with 222 food-derived sources described (Liu et al., 2019). These are very dependent on the use of enzymes for release from the food matrix so that they can be biologically available for use in the body.

1.2.1.5 Antioxidants

Antioxidants act against the damaging effects of free radicals by donating electrons to them resulting in their stabilisation. Having too many free radicals can cause damage to cells and increase the signs of aging and risk of disease (Liu et al., 2018). Some bioactive peptides have antioxidant activity which can reduce the presence of free radicals.

1.2.1.6 Hazardous effects

Using food processing techniques to release or form bioactive peptides is new and there are few studies regarding the safety or long-term health effects. This could be a concern as the processing could lead to the release of bioactive peptides new to humans. This includes the potential for release of toxic or allergenic peptides, negative changes to existing peptides, or unwanted interactions within the food, its environment, or during digestion (Liu et al., 2020).

1.2.2 Kiwifruit

Green kiwifruit (*Actinidia deliciousa*) are a small fruit intensively cultivated in New Zealand. They are typically consumed raw and are sweet but slightly sour when ripe. Nutritional analysis of green kiwifruit shows an extensive list of food components including a large variety of vitamins, minerals, and amino acids (Sivakumaran et al., 2018). As reviewed by Richardson *et al.* (2018), the considerable amounts of vitamins C, folate, potassium, and dietary fibre, are the most important features of kiwifruit and what sets them apart from other fruits. Notably kiwifruit contains high levels of soluble and insoluble fiber, totaling around 3% (Sivakumaran et al., 2018). Consumption of fiber has been linked to the maintenance of health through reduction of risk of CVD, diabetes, obesity, and improvement of bowel and digestive function (Lattimer & Haub, 2010). Kiwifruit also contain bioactive compounds with a high level of antioxidant activity. Antioxidant analysis of the common green 'Hayward' kiwifruit showed between 8.49µM TE/g and 23µM TE/g of antioxidant activity (Park et al., 2011). Four different antioxidant assays were used to measure slightly different aspects of their activity which account for the variation. This is reported to be higher than many other common fruits such as apples, pears, and grapes (Richardson et al., 2018). High intake of antioxidants is recommended for maintenance of health (Liu et al., 2018). Kiwifruit contains around 1-1.25% protein, and the protein present in the highest concentration is actinidin at around 40% (Boland, 2013). Actinidin has a molecular weight of 24 kda mark, with other dominant kiwifruit proteins, kiwellin and thaumatin-like protein, being smaller at 20 and 22 kda respectively (Maddumage et al., 2013). There are many benefits gained from these proteins, in particular actinidin as it is a protease. Its proteolytic activity has been

shown through its use as a meat tenderiser, and has also been proven in human trials to increase protein digestion through the gastrointestinal system (Park et al., 2020; Zhang et al., 2017).

1.2.2.1 Plant protease

Proteases are enzymes which facilitate the breakdown of proteins into peptides and amino acids by the hydrolysis of peptide bonds. They are found in plants, animals, bacteria, and fungi with each organism having a unique combination for their specific need. Proteases can be used to digest proteins completely or partially, as different proteases have different modes of action, levels of activity and ideal operation conditions which influences the outcome (Lopez-Otin & Bond, 2008). The addition of protease to food can lead to the release of bioactive peptides held in the food matrix to be released when this would not have been achieved by gastrointestinal digestion alone. Once again, the specific protease used can influence the quantity and quality of any peptides produced. Interest in plant proteases is increasing as animal consumption is decreasing. Increased uptake of vegan and vegetarianism is leading to the need for plant-based sources of products, including enzymes. Many plant proteases are in the cysteine family, such as bromelain in pineapple and papain in papaya. Plant peptides have been successfully used to facilitate the release of bioactive peptides from food matrix in plant and non-plant sources (Mazorra-Manzano et al., 2018).

1.2.2.2 Actinidin

A less known member of the cysteine protease family is actinidin, found in green kiwifruit. Actinidin has been shown to enhance the digestion of a variety of proteins, including proteins derived from cereals (Kaur et al., 2010). The release of bioactive peptides using actinidin has been described in hemp and meat protein substrates (Teh et al., 2016; Zhang et al., 2017). In both cases, increased ACE inhibition and antioxidant activity was observed in samples with actinidin compared to the non-treated samples. Based on the effects of the other plant cysteine proteins, actinidin should be an effective protease to use with the oats to produce bioactive peptides. Chalabi et al. (2014) found that actinidin had a protease activity between 7.8 - 28.8 U/g using a casein proteolytic assay. The variation was caused by different pH levels with the lowest pH, 3.0, producing the highest activity. When its proteolytic activity was directly compared to papain, actinidin was found to have a much more specific range (i.e., was effective on less types of proteins) but was as it was successful in digesting collagen, fibrinogen, and casein proteins (Chalabi et al., 2014). However, this study only investigated the digestion of animal proteins and further study of actinidins effects on plant-based proteins is needed. Plant proteases in general have not been extensively studied or used for production of bioactive peptides with one study discovering only 15% of cited work referencing plant proteases (Mazorra-Manzano et al., 2018). On top of this, around 75% of these references referred to bromelain and papain. Actinidin represents a small field of knowledge in this area but as a cysteine plant protease it is likely to have positive effects on the formation of bioactive peptides as previously shown in the

limited studies. Additionally, studies that have been completed use kiwifruit juice, or actinidin purified from kiwifruit, and not the kiwifruit flesh itself. Broadening the knowledge on the use of actinidin will lead to its increased use as a plant protease, especially in areas where the tropical pineapple and papaya are less accessible.

1.3 Aims and objectives

The aim of this study was to determine any effect of kiwifruit on oat proteins following their combination and processing through *in vitro* digestion and make a recommendation as to their potential for human health benefits. This was achieved through three main aims.

- **Aim 1:**

Quantify and visualise any changes after in vitro digestion in the combined sample as compared to kiwifruit alone and oat alone using protein quantification assays and gel electrophoresis.

- **Aim 2:**

Analyse changes in a variety of bioactive compounds after *in vitro* digestion in the combined sample as compared to kiwifruit alone and oat alone to get an overall picture of the bioactive potential for human consumption.

- **Aim 3:**

Analyse results in the context of human consumption to understand how the addition of kiwifruit may affect the quantity of bioactive compounds.

2

Materials and Methods

2.1 Raw sample preparation

2.1.1 Preparation of powders

2.1.1.1 Preparation of Kiwifruit extract

Proprietary freeze-dried kiwifruit powder 'Actazin' (Product Code FG01, Batch Number 10/36) was obtained from Massey University and stored frozen. 10 g of the Actazin powder was added to 40 ml of pH 7, 70 Mmol, sodium phosphate buffer and centrifuged at 3828 xg for 10 min at 4 °C. The supernatant was collected for use in the study and referred to as Kiwifruit extract.

2.1.1.2 Preparation of Oat flour

'Haraway's Traditional Rolled Oats' were purchased from a local supermarket for the study. A subsample of the oats was blended using a Nutribullet blender until a fine powder was formed. The resulting powder was kept frozen at -18 °C in an airtight bag and is referred to as Oat flour.

2.1.1.3 Soaking of Oats

Two samples were prepared to be run in the initial study to determine the effects of soaking the oats. For each sample, 1 g of oat flour was taken and suspended in sodium phosphate buffer (10 ml pH 7, 70 Mmol). Both were placed on a magnetic stirrer at 4 °C where one sample was left for 1 min and the second sample left for 24 h. These were centrifuged at 3828 xg for 10 min at 4 °C. The samples were referred to as Rolled oat only and Rolled oat only soaked, respectively.

2.1.2 Heat treatment study

The time and relative concentration of Oat and Kiwifuit were evalautated to determine the optimum combination. Kiwifruit extract and oat flour were combined to form samples at two concentrations as follows. Either 1 g or 0.5 of Oat flour was taken and added to 10 ml of Kiwifruit extract and left for 1 min. This sample was referred to as Combined. Combined, Rolled oat only (Section 2.1.1.3), and Kiwifruit extract (Seciton 2.1.1.1) samples were then subjected to incubation. Each sample was placed into a 37 °C water-bath with slight shaking enabled for 240 min. Subsamples were taken at 0, 30, 60, and 240 min. Samples were subsequently centrifiged at 3828 xg for 10 min at 4 °C. This was referred to as the 'heat treamtnet'.

2.2 *In vitro* digestion

Combined (Section 2.1.2), Rolled oat only (Section 2.1.1.3), and Kiwifruit extract (Section 2.1.1.1) samples that had undergone pre-treatment for 120 min were selected to undergo *in vitro* digestion. This meant that the effect of the kiwifruit was able to be determined and any existing bioactivity in the rolled oats or kiwifruit will be accounted for. Rolled oats were used as they are highly available in supermarkets, and the most frequently consumed type of oat. The whole oat was used which contrasts with other studies on cereals which use the protein concentrate.

The *in vitro* digestion system used in this study is based on the work of Sousa et al. (2020) with some adaptions. Schematic flow of the *in vitro* digestion process used is detailed in Figure 1. 2 ml of each sample, including a blank, was added to 2 ml of deionised water. Digestion occurred in three phases oral, gastric, and intestinal. In the oral phase, 300 U/ml of amylase was added to each sample. Samples were then incubated in an incubator at 37 °C for 2 min. Subsequently samples were transferred to a multi-stirrer hot plate and the temperature was maintained between 35-39 °C for the reminder of digestion. For the gastric phase, 30 ml of deionised water was added to each sample along with 0.8 ml of 1 M hydrochloric acid (HCL). A gastric juice was prepared containing 2.9 g pepsin and 10 ml 0.04 M HCL. Gastric juice was added to each sample to achieve 2000 U/ml of pepsin. After 120 min the pH was adjusted to 7 to stop gastric digestion. Intestinal digestion was started by adding 0.1 M phosphate buffer (pH 8) containing pancreatin to a final level of 100U/ml of trypsin activity/ml digesta. Intestinal digestion occurred for 120 min. Sub-samples were taken at the end of the oral phase and after 30, 60, and 120 min for each of the gastric and intestinal phases. Once collected all subsamples were immediately treated at 100 °C and cooled to 4 °C. Once cooled, all subs-samples were centrifuged at 5000 xg for 15 min at 4 °C. The supernatant from each sample was transferred to a fresh Eppendorf tube and frozen at -18 °C for subsequent analysis.

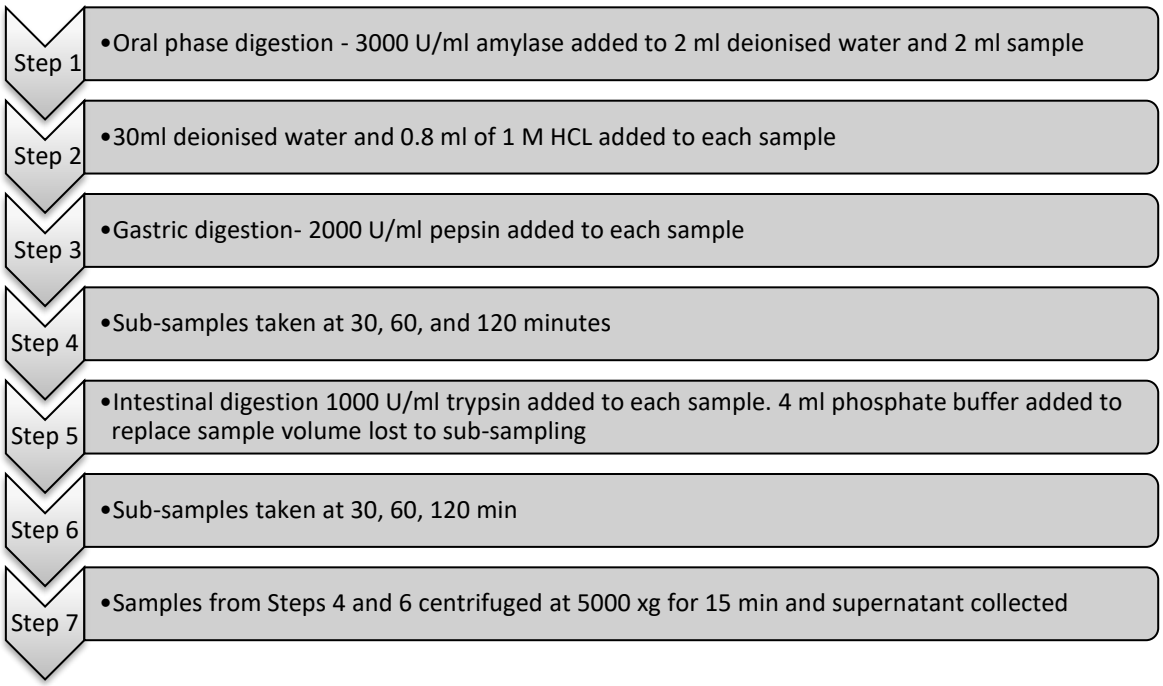

Figure 1 Schematic flow of *in vitro* digestion

2.3 Protein characterisation

2.3.1 Protein quantification assays

2.3.1.1 Biuret assay

The Biuret assay used was adapted from Sapan et al. (1999) and completed as indicated in Figure 2. A standard curve made from Bovine Serum Albumin (BSA), prepared in deionised water, was prepared at protein concentrations of 1, 2, 3, 4, and 5 mg/ml. A blank of 0 mg/ml protein was also prepared made using deionised water. 2 ml of each standard or each sample were added to test tubes with 3 ml of Biuret reagent. Samples were added to test tubes and incubated for at 37°C for 10 min. After incubation time each test tube was vortexed, and contents transferred to a 1 cm path length disposable cuvette to be read on a Spectrophotometer at 540 nm. Each sample and standard was completed in duplicate. Results were input into Excel to create a standard curve (Example provided in Appendix A.1) and protein of samples was determined. Results were expressed as mg protein per ml sample.

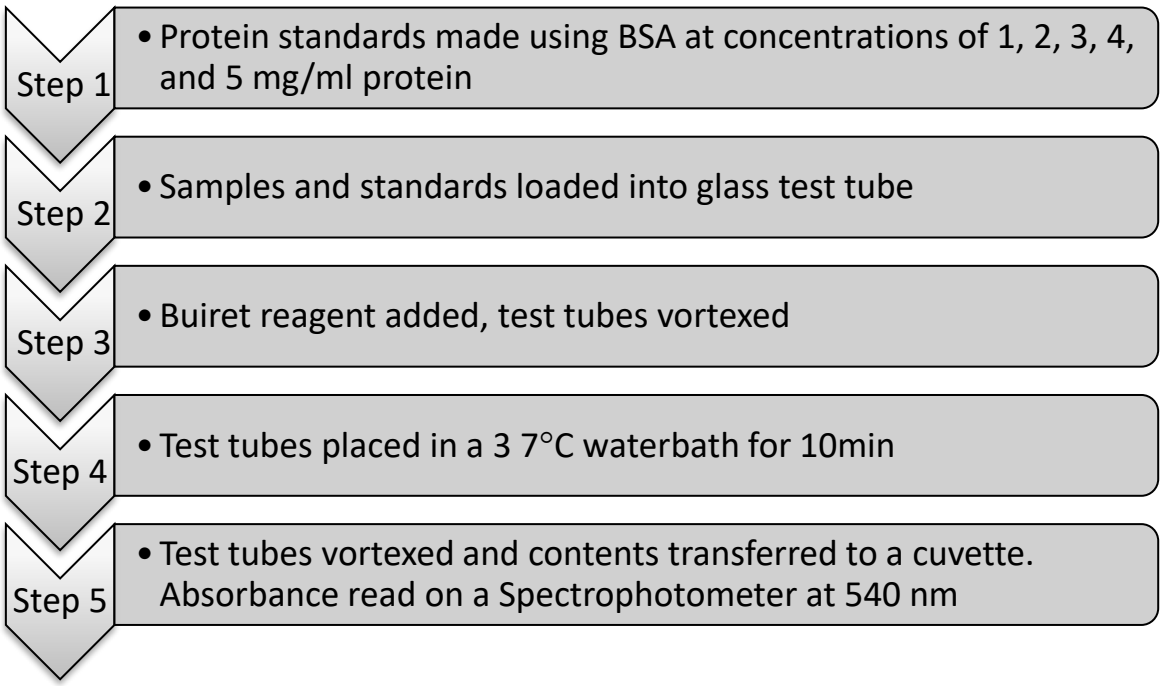

Figure 2 Schematic flow of Biuret assay

2.3.1.2 Bicinchoninic acid (BCA) aasay

The BCA assay was completed as described by Walker (2009) and as per Figure 3. A standard curve made from BSA and deionised water was prepared at protein concentrations of 0.065, 0.125, 0.25, 0.5, and 1 mg/ml. A blank with 0 mg/ml protein was made using deionised water. Samples used were Kiwifruit only and Rolled oat only (Section 2.1). 10 µL of each sample or standard was added in triplicate to a 96 well flat bottom microplate with 200 µL of BCA working reagent. BCA working reagent was made from a ratio of 50:1 of reagent A and B. The plate was covered with tinfoil and incubated at 37 °C for 30 min. After incubation, absorbance was read at 562 nm on a Fluorstar Omega microplate reader. Resulting data was input into Excel to create a standard curve (Example provided in Appendix A.2) which was used to determine protein content for samples. Results were expressed as mg protein per ml sample.

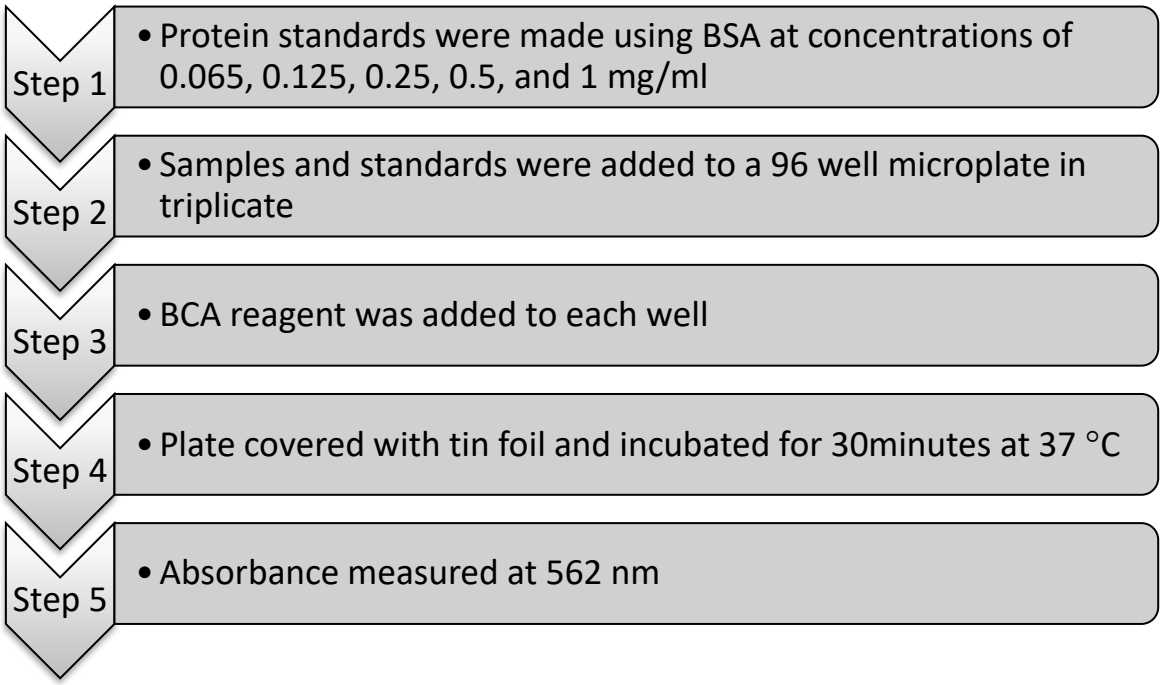

Figure 3 Schematic flow of BCA assay

2.3.1.3 Bradford assay

A Bradford assay adapted from Bradford (1976) was used as detailed in Figure 4. A standard curve made from BSA and deionised water was prepared at protein concentrations of 0.065, 0.125, 0.25, 0.5, and 1 mg/ml. A blank with 0 mg/ml protein was made using deionised water. Samples tested included Combined, Kiwifruit only, and Rolled oat only (Section 2.1), as well as the samples associated with the *in vitro* digestion (Section 0), diluted where appropriate. 10 μL of each standard and sample were added in triplicate to a 96 well flat bottom microplate. Bio-Rad reagent was diluted with 1 part reagent, 4 parts deionised water to make the working reagent. 200 μL of Bio-Rad working reagent was added to each well and the plate covered in tinfoil and incubated at room temperature for 5 min. After incubation, absorbance was measured on a Fluorstar Omega microplate reader at 595 nm. Data was analysed on Excel and resulting standard curve (Example provided in Appendix A.3) was used to determine protein content of samples. Results were expressed as mg of protein per ml of sample.

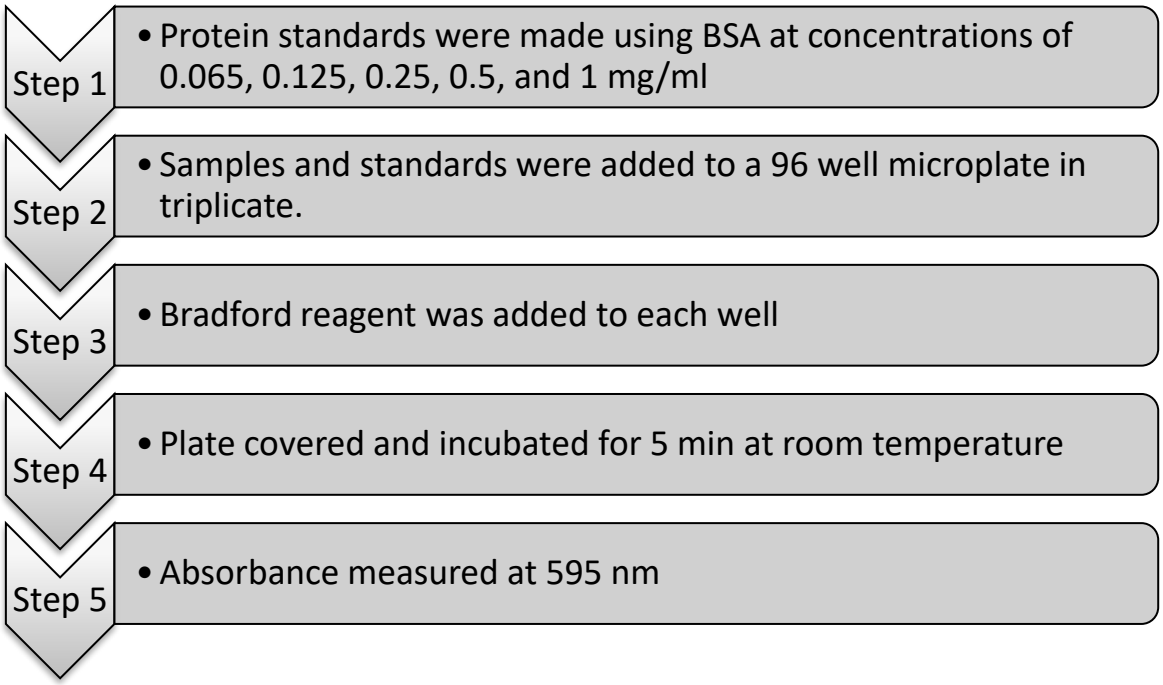

Figure 4 Schematic flow of Bradford assay

2.3.2 Protein quantification using gel electrophoresis

2.3.2.1 4-12% Bis-Tris Gel

Samples from the incubation study (Section 2.1.2) were used for this Gel. To increase protein concentration all samples from the incubation study were concentrated by placing in a SpeedVac set at 50 °C for 6 h. The resulting pellets were rehydrated by adding 25 µL of 4x lithium dodecyl sulphate (LDS) sample dye and placing in a 70 °C water bath for 15 min (except for Rolled at only samples which used 30 µL diluted 1x LDS dye and no heat treatment). A 4-12% Bis-Tris commercial gel was prepared in an electrophoresis tank and approximately 1.5 L of 2-(N-morpholino)ethanesulfonic acid (MES) running buffer added. 25 µL of sample were pipetted into the wells. 8 µL of 'Precision Plus Protein Standards All Blue' was added as a molecular marker. 200 V of current was applied for 30 min. After electrophoresis the gel was removed and placed in fixing solution (50% methanol, 7% acetic acid) on a rocker for 15 min at room temperature. Then the gel was rinsed by placing in deionised water on the rocker for 5 min. The rinsing step was repeated three times with deionised water replaced each time. Then water was replaced with 20 ml of GelCode Blue to stain the proteins. Gel was placed on the rocker for 1 h. After this the gel was transferred to a large container with deionised water and left to rock for 48 h before being scanned.

2.3.2.2 20% Peptide Gel

2.3.2.2.1 Gel preparation

A 20% sodium dodecly sulphate (SDS) gel was made to use for peptide analysis. This was made by combining 6800 µL of deionised water, 5200 µL of 1.5M Tris (pH 8.8), 200 µL of 10% SDS, and 8000 µL

of 30% acrylamides until completely combined. Then 100 µL of 10% ammonium persulfate (APS) and 10 µL of Tetramethylethylenediamine (TEMED) were added to complete the gel. This was poured between Mini Gel glass plates at 0.75 mm thick and held in place to allow to set. Isobutanol was spread along the top to prevent air exposure and drying. Once set, a 4% stacking gel was made. This followed the same method as the SDS gel but with the following modifications to materials: 4560 µL of deionised water, 1880 µL of 0.5M Tris (pH 6.8), 70 µL of 10% SDS, and 1000 µL of 30% acrylamides, 50 µL of 10% APS and 7.5 µL of TEMED. This was poured over the top of the SDS gels while still in between the glass panes, and a 10-well comb inserted. Once set, the glass plates were carefully removed from their holder and gels wrapped in a wet paper towel and then wrapped in Clingfilm. Gels were stored in the fridge until needed.

2.3.2.2.2 Gel running

Gels were run as described in Section 2.3.2.1. Samples run on this gel were from the *in vitro* digestion study as described in Section 0. 10 µL of Precision Plus Protein Dual Xtra Standards 250-2kda was used as a molecular marker when running these gels. Gels made using this method were stained using the Coomassie Blue staining method as follows. Gel was fixed for 1 h using fixing solution (50% methanol and 10% acetic acid). It was then rinsed using deionised water for 5 min. Rinse step was completed 3 times using fresh deionised water each time. Gel was stained using Coomassie Blue staining solution (0.1% Coomassie Brilliant Blue R-250, 50% methanol and 10% acetic acid) for 20 min. Gel was de-stained using de-staining solution (40% methanol and 10% acetic acid) for 2 h with the solution changed multiple times. The gel was placed into storage solution (5% acetic acid) to continue de-staining for a further 22 h. All fixing, staining, and de-staining steps were completed on a rocker with gentle agitation of the gels.

2.3.3 N-α-carbobenzoxy-L-lysine-p-nitrophenol (PNP) enzyme activity assay

Samples from the incubation study (Section 2.1.2) were used to evaluate the enzymatic activity of Actinidin using the 2.3.3N-α-carbobenzoxy-L-lysine-p-nitrophenol (PNP) enzyme activity as described by Boland and Hardman (1972) with modifications below. 1.386 ml of each sample was added to a new Eppendorf tube and 7 µL of each of 50 mM ethylnediaminetetraacetic acid (EDTA) and 500 mM dithiotreitol acid (DTT) were added. To perform the assay 900 µL of sodium phosphate buffer (pH 7, 70 Mmol) was added to a 1 cm path length disposable cuvette. 50 µL of sample (containing DTT and EDTA) was added and cuvette placed into spectrophotometer at 348 nm. Each sample was set to 0 before adding substrate. 50µL of 4 mM Z-Lys-pNP solution was added and absorbance recorded every 10 s for 90 s. The resulting data was input into Excel and the rate of change in the linear region used to determine the protease activity of each sample using Equations 1, 2, and 3.

Equation 1:

$$Activity = \frac{(sample\ rate - blank\ rate)}{\Delta \in} = M.min^{-1}\ \ \Delta E = 5400\ M^{-1}.cm^{-1}$$

Equation 2:

$$M.min^{-1}x\ 0.001L = mol.min^{-1}$$

Equation 3:

$$mol.min^{-1}\ x\ 10^{6} = \ \mu mol.min^{-1}$$

2.3.4 Bioactivity assays

2.3.4.1 Total Phenolic Concentration (TPC)

Total phenolic concentration was determined following protocol from Ainsworth and Gillespie (2007). Standard solutions were made using gallic acid and 70% methanol at concentrations of 0, 12.5, 25, 50, 75, 100, and 150 μg/ml. Samples from both the incubation study and the *in vitro* digestion (Sections 2.1.2 and 0) were tested. 20 μL of each sample and standard were pipetted into a 96 well flat-bottomed microplate. 100 μL of reagent was added. This was 2N Folin-Coicalteu reagent which had been diluted 10-fold to 0.2 N concentration using deionised water. Lastly, 80 μL of 7.5% sodium carbonate was added to each well. The plate was covered with tin foil, incubated at room temperature for 2 h and then read in a Fluorstar Omega plate at 760 nm. Results were input into Excel to determine the concentration of unknown samples (Example provided in Appendix A.4). Results were expressed as 'gallic acid equivalents per gram weight'.

2.3.4.2 Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power (FRAP) was determined following protocol from Huang et al. (2005) Iron (II) sulphate ($FeSO_4$) was used to make standard solutions of 1, 0.8, 0.6, 0.4, and 0.2 mM. Deionised water only was used as a blank. Samples from both the incubation study and the *in vitro* digestion (Sections 2.1.2 and 0) were tested. 20 μl of each sample and standard were added to wells of a 96 well flat bottom microplate. Working reagent was made using a 10:1:1 ratio of 300 mm acetate buffer, 10 mM 2,3,5-Triphenyltetrazolium chloride in 40 mM HCL, and 20 mM iron(lll) chloride. 200 μL of the working reagent was added to each well. The microplate was covered and incubated at 37 °C for 2 h and then read on Fluorstar Omega microplate reader at 593 nm. Resulting data was input into Excel to create a standard curve to calculate unknown concentrations (Example provided in Appendix A.5). Results were expressed as μmol Fe^{3+}/g sample.

2.3.4.3 Angiotensin-converting enzyme inhibition (ACE) assay

This assay was used to determine the angiotensin-converting enzyme inhibition (ACE) power of the samples. It is based on work by Ibrahim et al. (2017); Jimsheena and Gowda (2009). A sodium borate buffer (SBB) was made using 0.05 M boric acid, 0.3 M sodium chloride, and ultra-purified water. 10μL Angiotensin-converting-enzyme (ACE) prepared at 2 U/ml. ACE was then diluted to 1 U/ml per tube using of SBB and 35 5 μL aliquots were prepared. 31 μL of SBB was added to each aliquot of ACE, and then 10 μL of sample was added to its respective tube. Samples from the *in vitro* digestion (Section 2.2) were tested. Rolled oat only and Combined samples were performed in triplicate while Kiwifruit only samples were performed in duplicate. A blank from the end of digestion was included in triplicate, as well as a positive control (10 μM captopril in ultra-purified water), and negative control (SBB). After adding the samples, tubes were incubated at 37 °C for 10 min. After incubation, 13 μL of substrate was added to start the reaction and tubes were left to incubate at 37 °C for 1 h. Substrate was 0.005 M of and hippuryl-L-histidyl-L-leucine (HHL) in SBB. The reaction was stopped by adding 25 μL of 1 M HCL, with colour development obtained by adding 100 μL of pyridine and 50 μL of benzene sulfonyl chloride. Vortex machines were used to aid the mixing and colour formation in tubes. Tubes were briefly placed on ice to cool and then 200 μL of each sample was pipetted to a 96 well flat-bottomed microplate. Standards of 100, 200, 400, 600, 800, and 1000 μM of hippuric acid were made and added to the plate in triplicate. The plate was read on a Fluorstar Omega microplate reader at 410 nm. Data was transferred to Excel and Equation 4 was used to determine ACE inhibition power of the samples (Example standard curve provided in Appendix A.6).

Equation 4*:

$$ACE\ inhibition\ \% = 100 - \frac{THA}{CHA} X100$$

*THA was the average of hippuric acid in the sample test wells and CHA was the average concentration of hippuric acid in the negative control wells. This was calculated individually for each sample.

2.3.5 Statistical analysis

Statistical analysis was completed using Minitab software. Tests performed were either one way Analysis of Variance (ANOVA) followed by the Tukey post hoc test (if needed) or T-tests. Statistical significance was determined at $p < 0.05$. All experimentation and analysis was completed in at least duplicate. For the samples from the incubation study, samples were compared across time points. Data from samples derived from *in vitro* digestion were compared between preparation methods (Rolled oat only, Kiwifruit extract only, and Combined).

3

Results and discussion

3.1 Characterisation of kiwifruit and oat extracts

The purpose of characterisation was to determine the protein content of the raw samples, Oat flour and Actazin, using three protein determination assays.

The results from this section would be used to assist the designing of the pilot study. It would also aid with the experiments to ensures that the most appropriate methods would be adapted and applied.

3.1.1 Protein Quantification

The initial approach of re-suspending the raw dried powders in the sodium phosphate buffer (pH 7, 70 Mmol) was modified due to the difficulty in extracting adequate protein amount from the Oat flour and Actazin powders. The powders when prepared as described for the preparation of Kiwifruit extract and Soaking of Oats (Sections 2.1.1.1 and 2.1.1.3) without centrifugation absorbed the buffer solution and became gels or very thick pastes. To minimise this effect, the centrifugation step was included in the final protocol for the Kiwifruit extract. During centrifugation, many insoluble particles are removed from the sample. This study is interested in the actinidin in the sample. Actinidin is a soluble protein which is not removed by centrifugation and remains in the supernatant (Boland, 2013). Using the supernatant of the kiwifruit allows a concentrated enough sample whilst ensuring the compounds of interest are maintained.

Three different types of protein quantification assays were performed on the Kiwifruit extract, Rolled oat only and Rolled oat only soaked samples. First of all Biuret was used on the Kiwifruit extract, Rolled oat only samples (prepared without centrifugation steps) but had interference from the kiwifruit (formation of a copper precipitate) and did not adequately dissolve particulates. BCA was trialled next on the Kiwifruit extract and Rolled oat only samples (with centrifugation steps) but also had interference from the kiwifruit sugars, and unseen particulates in samples. Bradford was tried last on the Kiwifruit extract, Rolled oat only, and Rolled oat only soaked samples (with centrifugation steps) and was the most effective. There is a large amount of variation between the results obtained by the different assays as shown by Table 1.0 (Raw data in Appendix B.1).

Table 1 Quantification of protein (mg/g) in oat and kiwifruit powders using Biuret, BCA, and Bradford methods (mean ± standard deviation)

	Protein Assay		
Sample	**Biuret**	**BCA**	**Bradford**
Kiwifruit extract	NA	336 ± 2.48	3.26 ± 0.07
Rolled oat only	550 ± 12.0	150 ± 22.5	1.79 ± 0.09
Rolled oat only soaked	-	-	1.81 ± 0.01

The results presented in Table 1.0 all give very different values for the protein quantity of the samples. This is because each assay measures protein in a different way and therefore there will be variation in the end results. Also, there limitations and interferences which can occur with different assays, making some more suitable than others. The Biuret assay was the first choice of assay to use for protein quantification in this study as the protein content of the whole system was of interest. This is reflective of how the oat and kiwifruit would be consumed in a real-life environment. Therefore, the study wanted to reflect this by measuring the effect on the whole protein. The Biuret assay can remain accurate despite the presence of small particulates in the samples. Additionally, the incubation step helps to break these down even smaller.

During the Biuret assay, an orange precipitate was formed in the Kiwifruit extract samples. Upon further investigation, this was deemed to be an interaction between some of the amino acids contained in kiwifruit with the copper in the reagent. The Biuret assay cannot be reliably used to determine the protein concentration of a crude kiwifruit extract due to this interference. For the Rolled oat only sample the assay seemed okay but as kiwifruit would be used in combination with the oat flour for the remainder of the study another assay option was needed. There was also some concern about the size of the oat particulates in the sample which could interfere with the accuracy of the readings.

The next assay trialled was the BCA assay which can work with homogenates if they have enough clarity. There was large amounts of variation in the results from this assay making it difficult to determine the most appropriate concentrations. This may have been due to interference from compounds in the oat or kiwifruit, the presence of small particulates in the mixtures, or low protein content.

The Bradford assay was tried next with the samples. The Bradford assay has been shown to work with very low amount of proteins, to 0.006 mg/ml (Chutipongtanate et al., 2012). As this would only be looking at the soluble fraction of proteins in a low protein sample, a sensitive assay was needed to ensure accurate measurement. The Bradford assay was the final assay used to measure the protein content of the samples. The Kiwifruit extract, Rolled oat only, and Rolled oat only soaked samples were

analysed and the results were consistent and reliable with no interference of kiwifruit or oat compounds detected.

There are many types of protein quantification assays available, but they are not all suitable for all uses. There were many challenges in using the raw materials of oat and kiwifruit including low protein content, and interference of compounds found in them. Initially the whole sample homogenate was intended to be used but the particulates that this left in the samples interacted with the assays and interfered with the results. These reasons led to the choice of Bradford assay of soluble protein as the protein quantitative method for use in the study. Actinidin, the kiwifruit protein we are interested in, is a soluble protein so will be analysed by this method (Boland, 2013). The protein in the oats varies in solubility which could be a limitation in this study (Ma & Harwalkar, 1984). This is shown by the 24 hour soaking of oats producing a similar protein quantity as the non-soaked oats Table 1. However it is likely because of the cold temperate the sample was held at for the duration of the soaking. Also the use of an extraction buffer or solvent could yield an increase in the protein content of soaked oats. This could be explored for future studies as a way to increase the measurable protein content of the oats to get a better idea of any effect of the kiwifruit on them. For the purposes of this study, not undertaking the 24 h soaking step is good. This study aims to keep the samples as close to their real life use as possible and pre-soaking for 24 hours does not reflect this.

3.2 Optimisation of Kiwifruit and Oat heat treatment

Protein quantity and quality over the duration of treatment was investigated to determine the best combination to use in the main study. Assays were supported using gel electrophoresis to visualise any changes in the protein size distribution during the incubation.

3.2.1 Protein characterisation

3.2.1.1 Protein quantification

Bradford assays were used to determine the quantity of soluble protein in each sample at four time points during the heat treatment; 0, 30, 60, and 240 min. Kiwifruit extract protein content was significantly lower at the end (240 min) of the heat treatment compared to all earlier time points ($p < 0.05$) (Table 2). Rolled oat only protein content significantly increased at each time point of the heat treatment ($p < 0.05$) (Table 2). The soluble protein content for the Rolled oat only samples more than doubled over the 4 h heat treatment. The combined extracts protein content fluctuated over time, with the second (30 min) time points protein content being significantly higher than other time points, and the fourths (240 min) being significantly lower ($p < 0.05$) (Table 2). Subsamples at the first (0 minutes) and third (60 min) time points were not significantly different ($p < 0.05$) (Table 2). Raw data is provided in Appendix B.2.

Table 2 Quantification of protein (mg/g) in Kiwifruit and Oat extracts for the duration of the heat treatment

	Time (min)			
Sample	**0**	**30**	**60**	**240**
Kiwifruit extract	3.99 ± 0.02^{a}	3.78 ± 0.09^{a}	3.83 ± 0.06^{a}	2.95 ± 0.13^{b}
Rolled oat only	0.83 ± 0.05^{d}	1.23 ± 0.10^{c}	1.62 ± 0.13^{b}	1.93 ± 0.04^{a}
Combined	2.64 ± 0.05^{ab}	2.79 ± 0.22^{a}	2.65 ± 0.35^{ab}	2.18 ± 0.08^{b}

^mean ± standard deviation, superscript letters in each row indicate significant differences in protein content at time points for that sample (calculated using ANOVA ($p < 0.05$)).

The protein quantity for the oats increases significantly ($p < 0.05$) (Table 2) at each time point of the heat treatment. This is expected as the Bradford assay is measuring the soluble protein content. As the oat sits in the phosphate buffer over the course of the heat treatment, the heat aids in the release of bound proteins from the oat matrix, thus increasing the soluble protein content of the extract. The kiwifruit sample remains stable but then decreases significantly ($p < 0.05$) (Table 2) at the end point of the heat treatment. This is likely due to the high level of proteases in the sample, which degrade the proteins over time. The combined sample remains fairly stable throughout the heat treatment with some significant differences but this is likely due to natural fluctuations and sampling differences, eg the sample may not have been completely mixed and settling may have occurred, giving an inaccurate reading of the actual protein content.

3.2.1.2 Protein quality

Gel electrophoresis using a commercial Bis-Tris 4-12% gel was used to visualise protein in Kiwifruit extract at time 0, and Rolled oat only, and Combined samples extracts at all subsample points during the heat treatment. The main protein band in the combined sample is at 25 kda which is actinidin. This is not shown in the Kiwifruit extract sample which has no observable bands. The Rolled oat only samples have multiple observable bands, with the most intense at 50 kda and 10 kda. These are representative of oat proteins globulin, and glutelin. All samples containing kiwifruit (Kiwifruit extract and Combined) show very heavy areas and signs of interference in the gel, preventing observation of their protein bands.

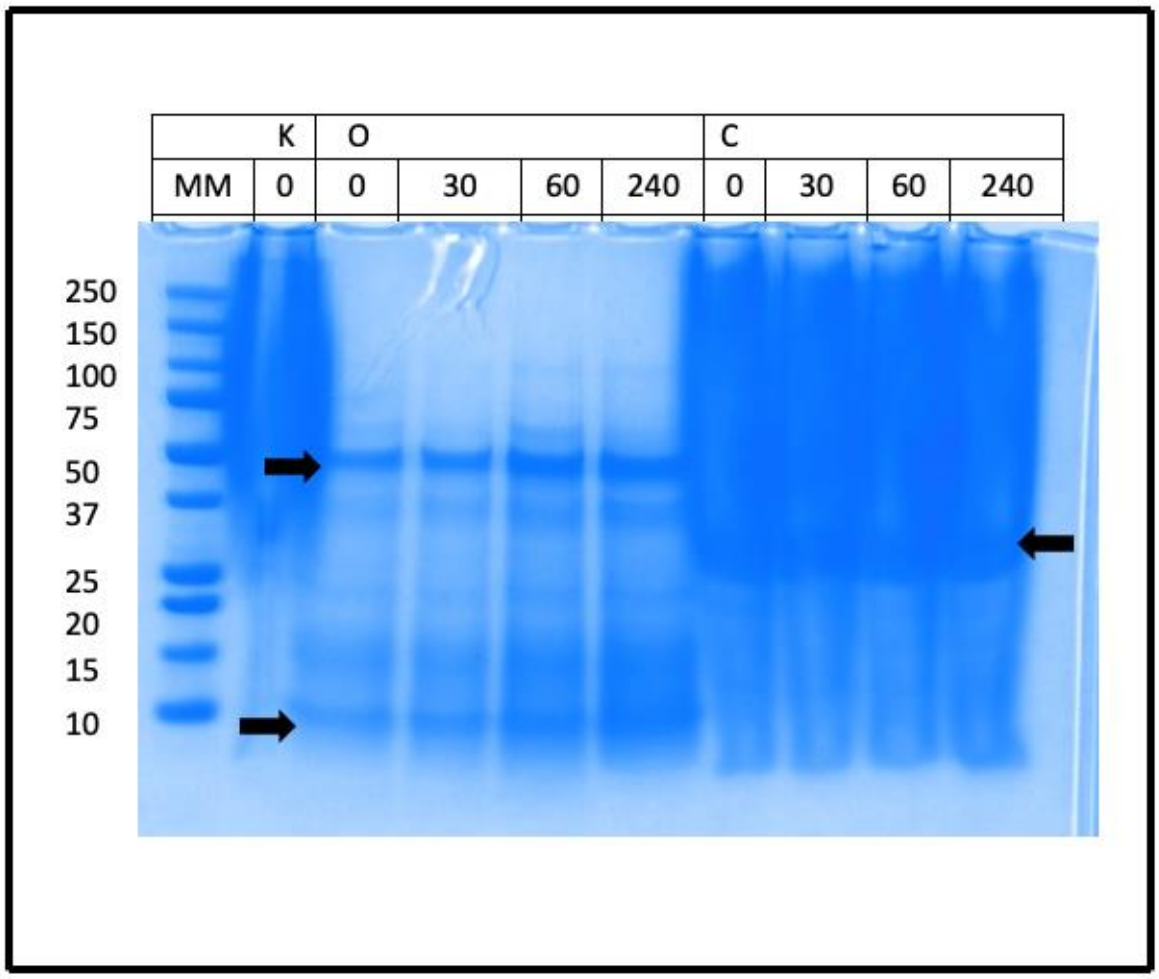

Figure 5 Gel scan of kiwifruit extract before heat treatment, and oat and combined extracts for the duration of the heat treatment (time in minutes). K = Kiwifruit extract , O = Rolled oat only, C =Combined, MM = molecular marker. Major bands at 50 kda identified as globulin, 25 kda as actinidin, and 9 kda as glutelin.

Most obvious on this gel is the interference in lanes which contain samples with kiwifruit. The protein content of these samples was low as per the Bradford assay results in Table 2. Because of this, all samples were run on a SpeedVac at 50 °C for approximately 5 h to remove water and increase the protein content to ensure visibility of protein on the gel. However, this high temperature may have negatively impacted the kiwifruit in the samples. The samples used in this gel were crude extracts of kiwifruit and oat and contained a complex mix of compounds other than protein. In particular, kiwifruit contains a large amount of sugar. The exposure to high heat during the SpeedVac processing may have caused a maillard reaction of the kiwifruit sugars, binding them together and creating large molecules unable to pass through the gel matrix. This is the potential cause of the smeared appearance of the kiwifruit containing samples. Further supporting this was an observation of a colour change of the samples post SpeedVac. Initially samples were a pale translucent yellow green colour and then after samples were an opaque golden orange, consistent with the browning seen as a result of the maillard

reaction. Benlloch-Tinoco et al*.,* (2012) also found heating caused a significant ($P < 0.05$) increase in viscosity and red-yellow hue of kiwifruit puree. This was using microwave heat and the authors attributed the changes to loss of pigment and alteration of pectin formation and not maillard browning (Benlloch-Tinoco et al., 2012). Despite the interference, there is an observable band at 28 kda in all of the combined samples. This is consistent with actinidin, the major kiwifruit protein. This band is not seen in the oat only samples so must be from the kiwifruit. However it is also not seen in the Kiwifruit extract sample, but the effect of the smearing is worse in this sample, which likely prevented this protein band from forming. There was no interference in any of the Rolled oat only samples and so there are multiple bands clearly visible on the gel. The strong band in the oat samples is a globulin (12S) protein. Globulins account for the majority of the protein in oat so it makes sense for this to be the darkest band. There are different types of globulins in oat but 12S occurs in the highest quantity. Additional globulins, the α-polypeptides, occurs around 37 kda where a lighter band is seen. Additionally, oat proteins prolamin and albumin occur around 15 kda where a final protein band is seen (Mäkinen et al., 2017). The Rolled oat only samples become darker as the time points progress. As samples were low in protein, they were loaded with concentrated versions of original samples and protein content not accounted for or adjusted to match. As shown in Table 2, the protein content of the oats increased over the course of the heat treatment, so this increase in band intensity is expected.

All samples indicate protein migration below the lowest molecular marker (10 kda) which could indicate the formation of lower molecular weight peptides in the samples.

A follow up gel on the pilot study using non-concentrated Kiwifruit extract and Combined samples was completed on Bis-Tris 4-12% commercial gel (Figure 6). This compared both sample extracts at all time points during the heat treatment. A major band was identified in all samples at around 22-27 kda. This is likely actinidin.

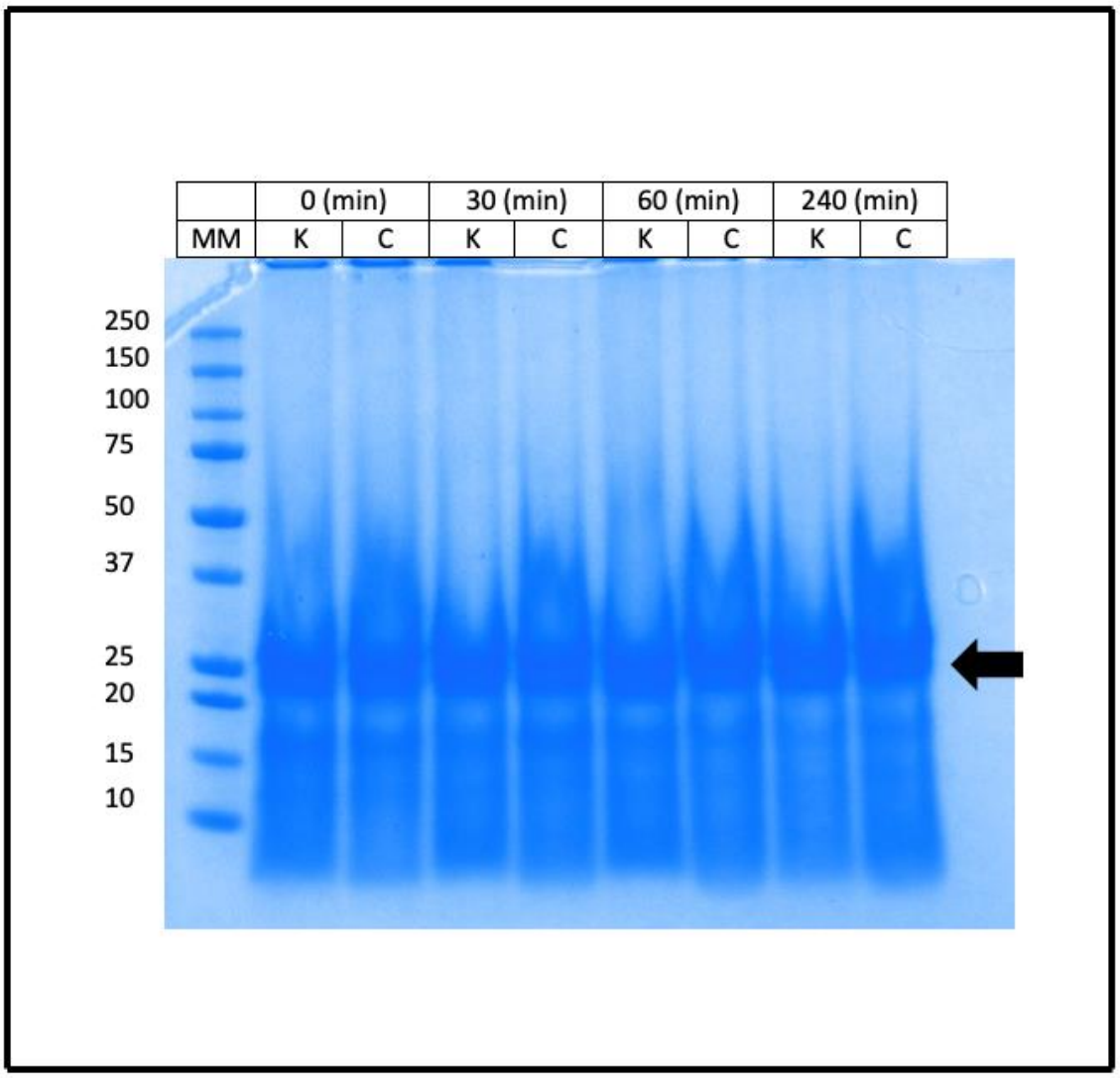

Figure 6 Gel scan of kiwifruit, and combined extracts over four hour heat treatment. K = Kiwifruit extract , C = Combined, MM = molecular marker. The major kiwifruit protein actinidin is identified at 25 kda.

The gel in Figure 6 was overloaded with too much protein so there are few identifiable bands present. Having too much protein meant that the bands have not separated and have joined together. The main band identified covers at around 22 to 27 kda. The majority of kiwifruit proteins are found in this region with the main kiwifruit protein, actinidin, having a molecular weight of 24 kda (Nieuwenhuizen et al., 2007). The other kiwifruit proteins, kiwellin and thaumatin-like protein also have similar molecular weights. Due to the large amount of kiwifruit in the sample and high protein loading volume, these proteins have been unable to separate into distinct bands. There is another band visible at 17 kda which could be oat albumins or prolamins. However, the major oat protein globulin is not able to be seen on the gel. This would be seen around the 50 kda mark but as these samples are un-concentrated, the oat protein quantity is too low to show up on the gel. The band seen at 17 kda is more likely to be from the kiwifruit.

3.2.2 Enzyme activity

The PNP assay was used to determine the enzymatic activity of the Kiwifruit extract, Rolled oat only, and Combined samples at time 0, and after the 4 h of heat treatment.

Our data showed that enzymatic activity of the kiwifruit significantly decreased ($P < 0.05$) (Table 3) over the course of the heat treatment. The enzymatic activity of oat sample measured before the heat treatment (0.16 ± 0.05) shows it contains similar enzymatic properties in approximately 20% capacity of the kiwifruit extract. Raw data is provided in Appendix B.3.

Despite a trend of losing enzymatic activity, there was no significant difference ($p > 0.05$, (Table 3)) in the combined sample with the 4-hour heat treatment.

Table 3 Enzymatic activity of kiwifruit and oat powders using PNP assay ($\mu mol.min^{-1}$) ^

Sample	Time (min)	
	0	**240**
Kiwifruit extract	0.75 ± 0.78^{a}	0.20 ± 0.04^{b}
Rolled oat only	0.16 ± 0.05	NA
Combined	0.62 ± 0.18	0.30 ± 0.12

^mean $\pm$ standard deviation, superscript letters in each horizontal row indicate significant difference in the enzymatic activity at the time points (calculated using T-test ($P<0.05$)).

Table 3 shows that the enzymatic activity of oats is low compared to kiwifruit. This was expected as kiwifruit contains the enzyme actinidin and has a known application of protein digestion (Kaur et al., 2010). Actinidin was still active after the four-hour heat treatment, albeit in a significantly lower ($p < 0.05$) (Table 3) amount. This indicates that the actinidin was able to be active on the proteins in the samples throughout the entire course of the heat treatment. The enzymatic activity of the combined sample decreased over the four hours but not significantly ($p > 0.05$) (Table 3). This indicates that there may be some effect from the oats at preserving the enzymatic activity over time, perhaps the introduction of a new complex system slowing autocatalysis of enzymes. There is no data obtained by this study to show the effect of the heat treatment on the enzymatic activity of the oats. However a study by (Skoglund et al., 2008) indicated that time soaking raw oat had no significant difference ($p > 0.05$) on the enzymatic activity of the oat. Additionally, they found a significant increase ($p < 0.05$) in the enzymatic activity as an effect of temperature. This study only investigated the effects on one specific enzyme, polyphenol oxidase, and not total enzymatic activity so these results may not reflect the context of this experiment. This gives an indication of the survivability of oat enzymes which could contribute to the non-significant change in the combined samples enzymatic activity. Although they are much smaller in quantity, they may be higher quality.

3.2.3 Bioactivty

Antioxidant activity and ACE inhibition were measured at all time points during the heat treatment for the samples.

3.2.3.1 Antioxidant activity

Total phenolic concentration of each sample extract was measured at four time points (0, 30, 60, 240 min) throughout the heat treatment as shown in Table 4. Kiwifruit extract was significantly lower ($p < 0.05$) (Table 4) at the second time point (60 min), but other than this was consistent throughout. Rolled oat only also remained consistent throughout the heat treatment until the end point (240 min) when it significantly increased ($p < 0.05$) (Table 4). The Combined sample experienced significant increases and decreases ($p < 0.05$) (Table 4) over the course of the heat treatment, with the final value significantly lower than the starting value. Raw data is provided in Appendix B.4

Table 4 Total phenolic concentration (gallic acid equivalents/g) in treated kiwifruit and oat extracts for the duration of the heat treatment

Sample	Time (min)			
	0	30	60	240
Kiwifruit extract	1045 ± 23.6^{a}	912 ± 13.0^{b}	1059 ± 14.7^{a}	1011 ± 38.2^{a}
Rolled oat only	45.1 ± 0.81^{b}	43.4 ± 0.94^{b}	43.3 ± 2.70^{b}	57.3 ± 2.30^{a}
Combined	1235 ± 40.3^{ab}	1178 ± 73.4b^{c}	1333 ± 17.6^{a}	1068 ± 25.4^{c}

^mean ± standard deviation, superscript letters in each row indicate significant differences in total phenolic concentration at time points for that sample (calculated using ANOVA ($P<0.05$)).

Total phenolic concentration increased for Rolled oat only, remained the same for Kiwifruit, and decreased for the combined sample. Even though the combined sample shows a significant decrease ($p < 0.05$) (Table 4) between start and end values it is unlikely a true representation of the antioxidant content. Each time point of the combined sample is different with the total phenolic concentration increasing and decreasing over time. Therefore, the end point measurement is unreliable. The fact that individually neither sample decreased over time is further indication that the statistics do not tell the entire story. Lastly, the variation for each combined sample, especially the first and second time points, is quite large which could alter the results enough to skew statistical significance. Total phenolic concentration is just one way of analysing antioxidant potential and so another assay to measure ferric reducing / antioxidant power was also used.

The results of the analysis over the heat treatment time points are shown in Table 5. The Kiwifruit extract sample was significantly higher ($p < 0.05$) (Table 5) at the start of the heat treatment. The decrease occurred within the first 30 minutes and then remained stable for the duration of the heat treatment. The Rolled oat only sample had no significant difference ($p < 0.05$) (Table 5) at any point during the heat treatment. The Combined sample had some variation in ferric reducing / antioxidant

power but overall had significantly decreased ($p < 0.05$) (Table 5) from start to end. Raw data is provided in Appendix B.5

Table 5 Ferric reducing / antioxidant power (mmol Fe^{3+}/g sample) of kiwifruit and oat extracts for the duration of heat treatment

	Time (min)			
Sample	**0**	**30**	**60**	**240**
Kiwifruit extract	21.1 ± 4.44^{a}	12.8 ± 0.54^{b}	14.0 ± 0.42^{b}	13.7 ± 1.97^{b}
Rolled oat only	0.31 ± 0.04	0.28 ± 0.07	0.29 ± 0.01	0.33 ± 0.03
Combined	19.7 ± 1.23^{a}	14.0 ± 0.48^{bc}	16.6 ± 1.30^{b}	11.8 ± 0.84^{c}

^mean ± standard deviation, superscript letters in each row indicate significant differences in ferric reducing / antioxidant power at time points for that sample (calculated using ANOVA ($P<0.05$).

Over the four-hour period the Kiwifruit extract and Combined samples both significantly decreased ($p < 0.05$) (Table 5) but the Rolled oat only did not. The Kiwifruit extract decreased in a greater amount than the Combined sample indicating some protective effect from the oat, preserving the ferric reducing antioxidant power in the sample.

3.2.4 Conclusion

The purpose of the heat treatment study was to determine the reaction conditions for the main study, in particular concentration or enzyme and substrate and if a pre-treatment (heat and soaking) was needed. Using the Bradford assay and only investigating the soluble protein of the samples meant that the full four-hour pre-treatment and heating step was necessary to ensure the maximum amount of protein in the sample. This is supported by Table 2 which shows the protein of the oat sample was highest at the 4 h mark.

3.3 *In vitro* digestion study

The *in vitro* digestion study will investigate any effect of the kiwifruit on oat. Using optimised methods as determined in the pilot study. The kiwifruit, and oat Combined sample, Kiwifruit extract sample and Rolled oat only sample that underwent Heat treatment were all treated to *in vitro* gastrointestinal digestion. Bradford assays were be used to determine protein concentration of samples and gel electrophoresis were be used to compare the fractionation proteins. Analysis was performed before and after *in vitro* digestion for all samples. Bioactivity assays were be undertaken to determine how the TPC, FRAP, and ACE inhibition changed after *in vitro* digestion.

3.3.1 Protein and peptide quantification

3.3.1.1 Protein quantification

A Bradford assay was used to measure the amount of soluble protein in the samples after *in vitro* digestion. This was compared to the protein content as measured after the conclusion of the heat treatment as shown in Table 6. The protein content of all samples decreased after *in vitro* digestion. The Rolled oat only sample had the lowest protein content after the heat treatment and the highest protein content after *in vitro* digestion. Raw data is provided in Appendix B.6.

Table 6 Protein quantity (mg/g) of kiwifruit and oat extracts after treatment and *in vitro* digestion

Sample	Heat treated	*In vitro* digestion
Kiwifruit extract	2.95 ± 0.13	0.005 ± 0.002
Rolled oat only	1.93 ± 0.04	0.040 ± 0.007
Combined	2.18 ± 0.08	0.010 ± 0.001

^ mean ± standard deviation.

The protein content should not change before and after the *in vitro* digestion as the protein cannot go anywhere. It is, however, broken down into peptides throughout the course of digestion. The Bradford assay used to quantify the protein is not suited to peptides and therefore their presence may not be represented in the final figure produced (Chutipongtanate et al., 2012). Therefore, the protein quantity has not changed, just their size which is not represented by the Bradford assay.

3.3.1.2 Protein and peptide quality

A homemade 4-20% SDS gel was used to investigate and analyse the formation of any peptides in the samples. The Kiwifruit extract, Rolled oat only and Combined samples were investigated over the course of *in vitro* digestion with subsamples from three phases of gastric digestion (30, 60, and 240 min) and three phase of intestinal digestion (30, 60, and 240 min) run through the gel. Samples were of original concentration and unadjusted for protein content. During the gastric phase a band was visible between 2 and 5 kda in the oat and combined samples (Figure 7). This faded over the course of gastric digestion. This band was not present during intestinal digestion for any of the samples (Figure 8).

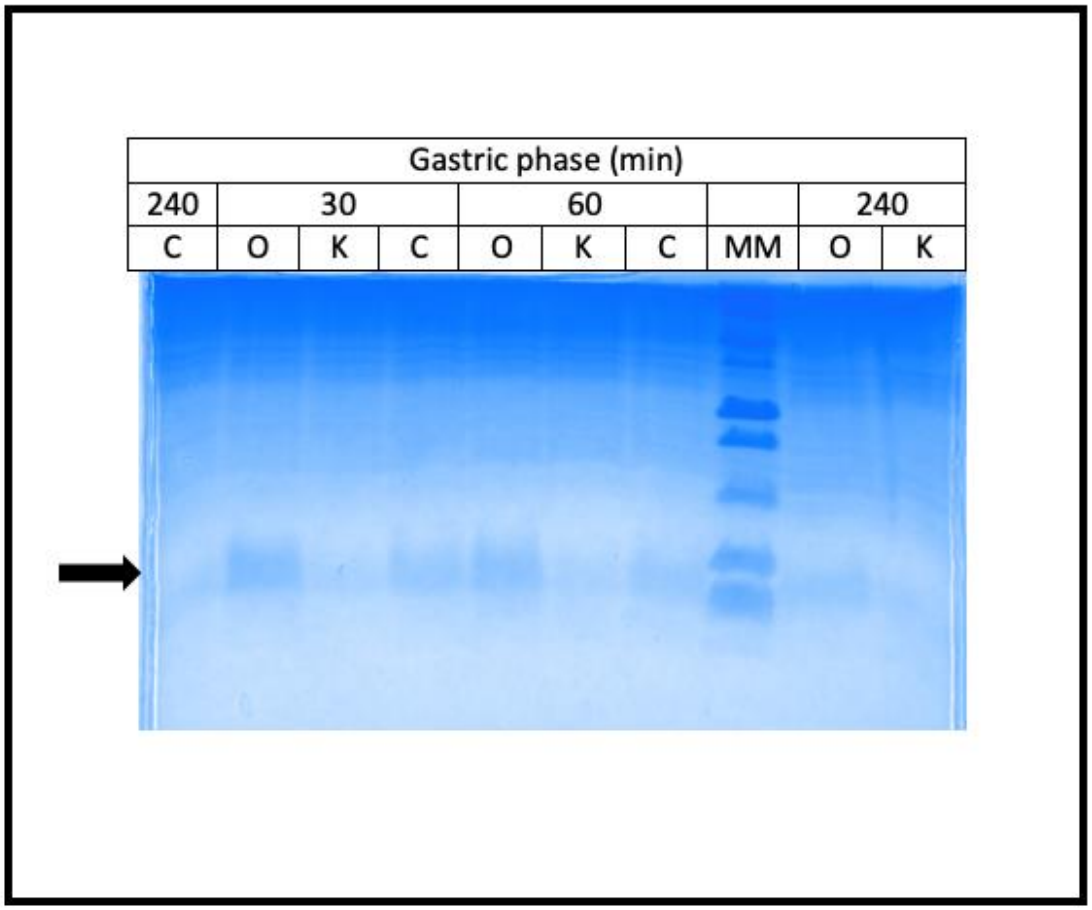

Figure 7 Gel scan of kiwifruit, oat, and combined samples at 30, 60, and 120 min of gastric digestion, K = Kiwifruit extract , O = Rolled oat only, C =Combined, MM = molecular marker,MM = molecular marker, the main band of interest is identified around the 2-5 kda mark, intermittently in columns.

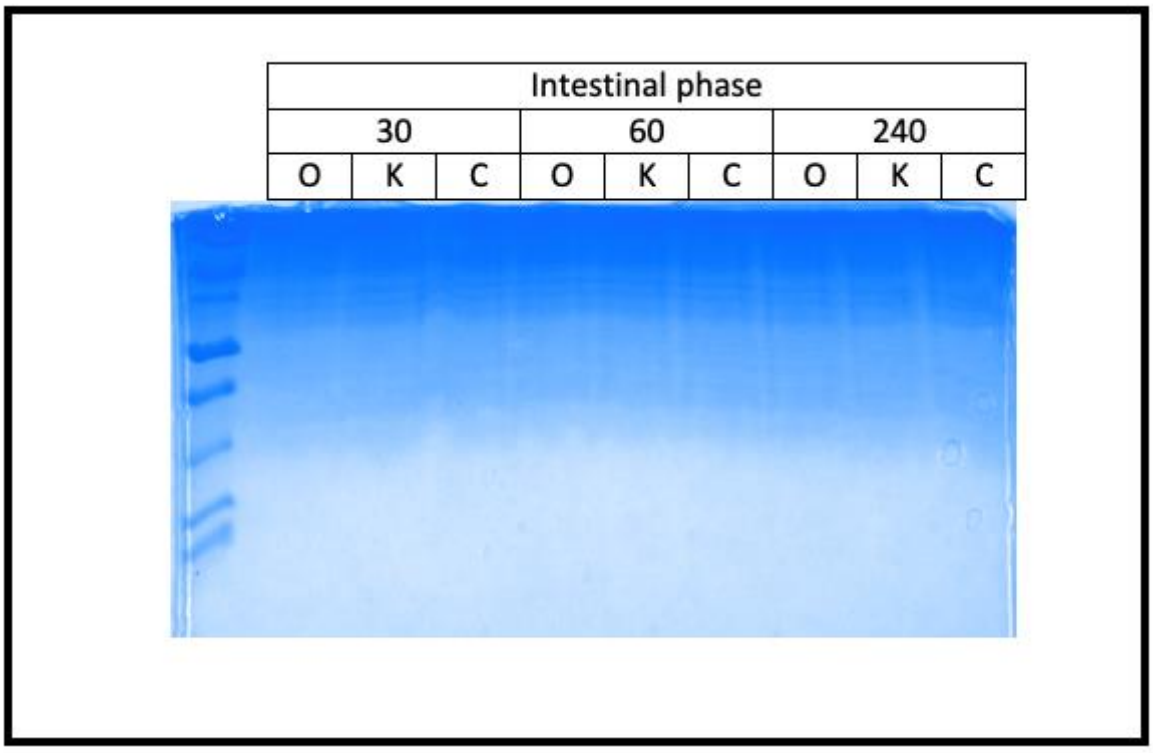

Figure 8 Gel scan of Kiwifruit extract (K), Rolled oat only (O) and Combined (C) samples at 30, 60, and 120 min of intestinal digestion, MM = molecular marker, no major bands are identified.

The reasoning behind running 20% peptide gels was to determine if any peptides with very low molecular weights were formed throughout the *in vitro* digestion. As shown by Figure 7 and Figure 8, there is little to no occurrence of peptides formed. The band at 2-5 kda as shown in Figure 7 is most prominent in the Rolled oat only sample, visible in the Combined sample, and very slightly seen in the Kiwifruit extract sample. It fades as the gastric phase progresses and is not seen by the start of the intestinal phase. This indicates that the peptides are fully broken down into free amino acids during the gastric phase. This result was not found by other studies which instead saw increases in the bioactivity and peptide formation of oats after *in vitro* digestion (Bleakley et al., 2017; Sánchez-Velázquez et al., 2021). As mentioned previously in 3.1.1 the low quantity of oat protein in the samples may contribute to this, in that there is simply not enough oat protein present to see on the gel.

3.3.2 Bioactivity

Bioactivity assays were used to investigate if the changes in the protein of the samples had also changed bioactive compounds in the samples. The change in bioactivity from after treatment to after *in vitro* digestion was calculated to give a percentage change for each sample. ACE inhibition used a change value not a percentage due to starting values of zero. Statistical analysis was performed on the overall change in activity to determine if any of the samples were different from another.

3.3.2.1 Antioxidant activity

All samples decreased in total phenolic concentration after *in vitro* digestion. The Kiwifruit extract and Combined samples decreased significantly more ($p < 0.05$) (Table 7) than the Rolled oat only sample. Raw data is provided in Appendix B.7.

Table 7 Total phenolic concentration (Gallic acid equivalents /g weight) of kiwifruit and oat extracts after treatment and *in vitro* digestion, blank corrected^

Sample	Heat treated	*In vitro* digestion	Percent decrease
Kiwifruit extract	1011 ± 38.2	60 ± 3.41	94.1 ± 0.37[a]
Rolled oat only	57.3 ± 2.30	46.9 ± 3.89	18.1 ± 6.97[b]
Combined	1067 ± 25.4	70.6 ± 2.70	93.3 ± 0.11[a]

^ mean ± standard deviation, superscript letters in each row indicate a significant difference in total phenolic concentration for the sample (measured using ANOVA ($p < 0.05$)).

While all samples decreased after *in vitro* digestion, they decreased at different rates. The Kiwifruit extract and Combined samples decreased at a much higher rate than the Rolled oat only. However, the Kiwifruit extract and Combined samples started with much higher phenolic concentrations with values in the thousands compared to the Rolled oat only was only at 57 Gallic acid equivalents/g . This indicates that most of the phenolic activity in the Combined sample is coming from the influence of

the kiwifruit and not the oat. However, the combined sample started and ended with a slightly higher phenolic concentration indicating there may be a preservation effect from the oats slowing or preventing some of the antioxidant degradation. This is supported by the significantly lower ($p < 0.05$) (Table 7) percentage decrease in total phenolic concentration seen by the oat sample, indicating that its antioxidant activity is mostly maintained through *in vitro* digestion.

Another antioxidant assay, FRAP, was conducted on the Kiwifruit extract, Rolled oat only, and Combined samples after heat treatment and *in vitro* digestion to determine the effect on antioxidants in a different context. The ferric reducing / antioxidant power of all samples decreased after *in vitro* digestion but not at significantly different levels ($p > 0.05$) (Table 8). Raw data is provided in Appendix B.8.

Table 8 Ferric reducing / antioxidant power (mmol Fe^{3+} /g weight) of kiwifruit and oat extract after treatment and *in vitro* digestion, blank corrected

Sample	Heat treated	*In vitro* digestion	Percent decrease
Kiwifruit extract	13.7 ± 1.97	0.93 ± 0.07	93.1 ± 0.54
Rolled oat only	0.33 ± 0.03	0.02 ± 0.01	93.8 ± 3.69
Combined	11.8 ± 0.84	0.97 ± 0.12	91.7 ± 1.02

^ mean ± standard deviation

The Kiwifruit extract had the highest FRAP value after the heat treatment and was similar to the Combined sample. The Rolled oat only sample had a much lower FRAP value. *In vitro* digestion caused the FRAP of all samples to decrease by more than 90%. The majority of the FRAP of the Combined sample comes from the addition of kiwifruit not oat. The apparent protective factor of the oat on the kiwifruit's antioxidant compounds does not seem to apply in this context.

3.3.2.2 Angiotension converting enzyme inhibition (ACE)

There was no ACE inhibition detected in the Kiwifruit extract and Combined samples after the heat treatment but before *in vitro* digestion. The Rolled oat only sample had a moderate level of ACE inhibition at this time point. All samples saw an increase in ACE inhibition after *in vitro* digestion. The increase for the Kiwifruit extract and Combined samples was significantly higher ($p < 0.05$) (Table 9) than the increase observed for the Rolled oat only samples. Raw data is provided in Appendix B.9.

Table 9 Angiotensin-converting-enzyme inhibition (%) of kiwifruit and oat extract after treatment and *in vitro* digestion

Sample	Heat treated	*In vitro* digestion	Increase in ACE inhibition
Kiwifruit extract	0	49.3 ± 2.07	49.3 ± 2.07^{a}
Rolled oat only	36.1 ± 16.5	59.8 ± 5.28	23.7 ± 6.14^{b}
Combined	0	54.2 ± 7.41	54.2 ± 7.41^{a}

^ mean ± standard deviation, superscript letters in each row indicate significant difference in the ACE inhibition of sample at time point (measured using ANOVA ($p < 0.05$)).

The increase of ACE inhibition for the Kiwifruit extract and Combined samples was large but not significantly different ($p < 0.05$) (Table 9) from each other. The Rolled oat only samples also increased in ACE inhibition, but to a lesser extent. Notably, the Kiwifruit extract and Combined samples started from no ACE inhibition after the heat treatment. This indicates that the *in vitro* digestion process has a positive effect on the proteins, enabling the release of bioactive peptides to facilitate this increase in ACE inhibition. The majority of these peptides come from the kiwifruit in the combined sample as the increase is mirrored by the Kiwifruit extract sample. The oat only sample also increased in ACE inhibition which would be reflected in the final inhibition capability of the combined sample, but makes up a smaller part.

3.3.3 General discussion

The bioactivity measurements of all the samples are lower than expected. For example, Bleakley et al. (2017) indicates oat should have close to 95% ACE inhibition whereas in this study it was found to be 59% at the highest. A possible explanation is that the former used *in silico* prediction of oat isolates while this present study used a crude rolled oat extract. Therefore, the results would not be as high due to natural variation of the sample and experimental factors. Additionally, a study on kiwifruit juice showed a decrease of approximately 30% of total phenolic concentration rather than the 94% in the present study (Ma et al., 2019). This could be down to different *in vitro* digestion systems used, notably the addition of bile salts, strict maintenance of pH, and the use of liquid nitrogen to stop digestion and preserve samples in the latter study.

In this study a small amount of oat was used in each sample as the materials were difficult to work with. The kiwifruit and oat flours were both powders which absorbed a large amount of liquid and to ensure they were fluid enough to work with ended up quite diluted. There are a number of background compounds coming from the kiwifruit which is evident in the gel runs. It is reasonable to assume that any effect of the kiwifruit on the oat is obscured by the kiwifruit itself in the samples.

Different measures of bioactivity will give a different picture of the effect as well. For example, TPC and FRAP are both measures of antioxidant activity, but TPC showed a decrease of 18% for Rolled oat only sample and FRAP showed a decrease of 93% for the same sample. Investigating and testing the right compounds will be very important in determine the actual effects of kiwifruit on the bioactivity of oat. A bioactive compound to investigate in future studies is avenanthramides which are unique to oats. They have been shown to have greater survivability through to the end of *in vitro* intestinal digestion so it may be easier to measure effects (Chen et al., 2019). One thing that this study did not

consider was the change in bioactive compounds throughout the different time points of the *in vitro* digestion. To have an effect on human health, bioactive compounds must be absorbed in adequate quantities in the intestine (therefore intestinal phase of digestion) (Dima et al., 2020). The intestinal phase of the *in vitro* digestion system used by this study was 120 min long and quantity of each bioactive compound was only measured at the end point. Theoretically, bioactive components can be absorbed by the body at any stage of intestinal digestion. In the *in vitro* system used, the release of bioactive components was dependant on the degradation of protein chains into their smaller bioactive peptide fragments. As the *in vitro* digestion continued, peptide fragments formed would have been further degraded into free amino acid or non-bioactive peptides and not absorbed as they would have been in a human model. Therefore, the end point measurement of each bioactive compound may not truly reflect the content produced by the samples over the course of *in vitro* digestion.

Lastly there is some indication that the complex food matrix can hinder the stability of the bioactive peptides, resulting in lower amounts or less desirable compounds (Udenigwe & Fogliano, 2017). Therefore, this study, which uses the oat and kiwifruit proteins in their complex natural matrix may have lower bioactivity results but be more accurate to reality.

4

Overall Conclusions

The benefit of adding kiwifruit extracts to oat protein was assessed in terms of its added effect towards producing oat bioactive peptides through the *in vitro* digest system. The study shows no evidence on the beneficial effect of adding kiwifruit to oat in bioactive peptide properties, such as total phenolic concentration, ferric reducing antioxidant power, and ACE inhibition. Additionally, there was no visible formation of peptides throughout the process of *in vitro* digestion.

There was one vital limitation to interpret the current results: the concentration of oat soluble protein treated with relatively high concentration of kiwifruit extracts were too low. The initial intention was to enhance the effect of actinidin from kiwifruit extract with the given oat, yet the current data has hindered the full understanding of any changes to the oat that may have occurred if concentration were adjusted. For future, additional studies using the purified oat total protein and actinidin extracts could be the next step to minimise the effects of the food matrix and focus entirely on the effects of the proteins.

An unexpected effect of this study is that the combined samples had decreases of a lesser extent for enzymatic activity and TPC as compared to the kiwifruit alone. This suggests the oat may have a beneficial effect on the kiwifruit by trapping it in the complex matrix, leading to the increased survivability of these compounds, instead of the other way around. This would make for an interesting avenue of future study. It is difficult to get accurate results due to the variable and complex nature of food products, but it is important to try as this is most reflective of the actual real world consumption.

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Appendix A

Standard curves

A.1 Example standard curve for Biuret assay

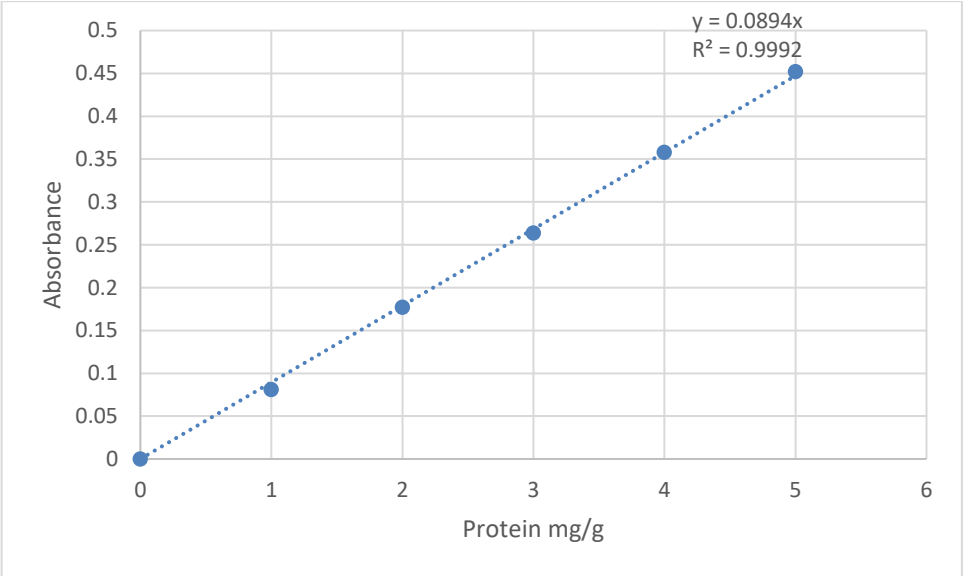

Figure 1: Biuret standard curve

A.2 Example standard curve for Bicinchoninic acid (BCA) aasay

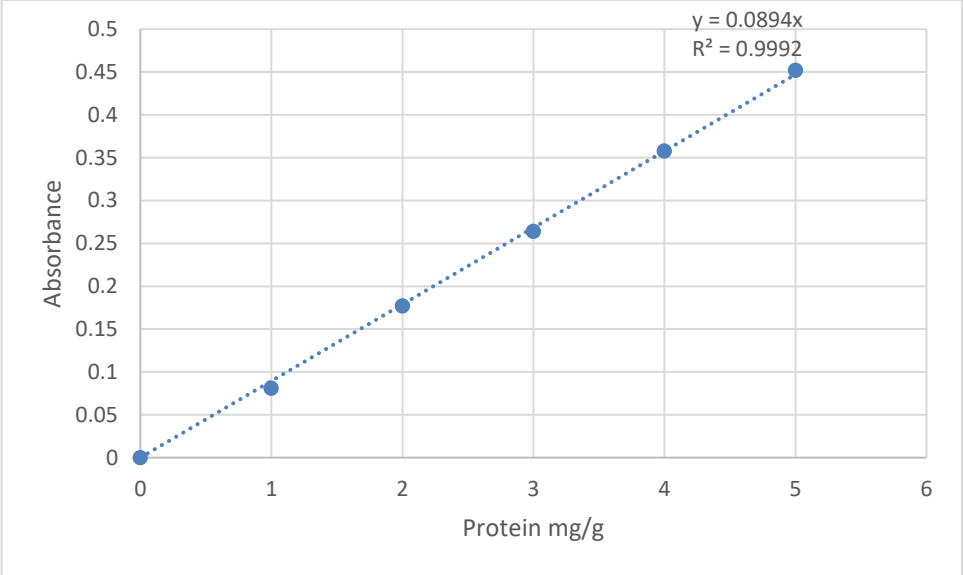

Figure 2: Bicinchoninic acid standard curve

A.3 Example standard curve for Bradford assay

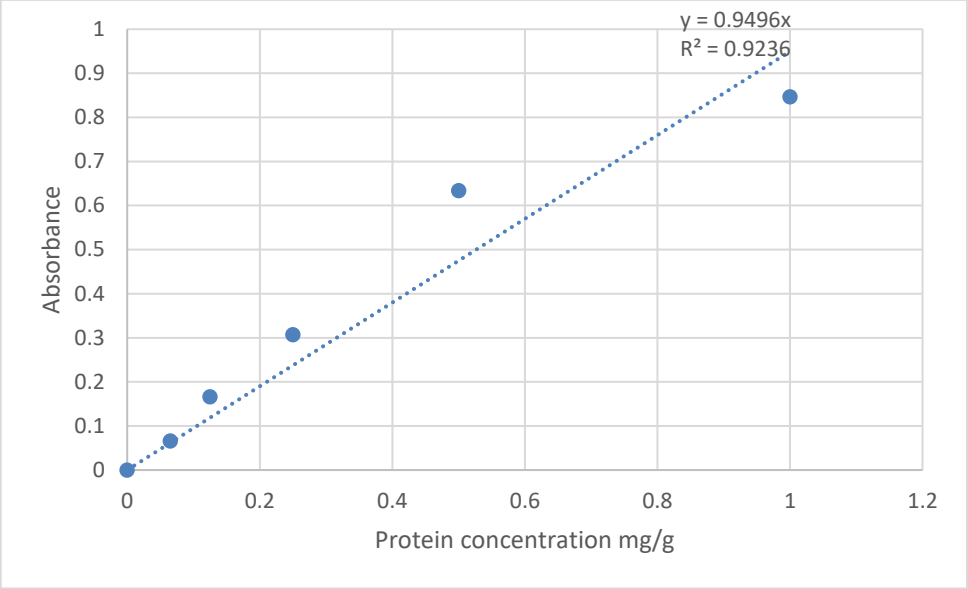

Figure 3: Bradford standard curve

A.4 Example standard curve for Total Phenolic Concentration assay

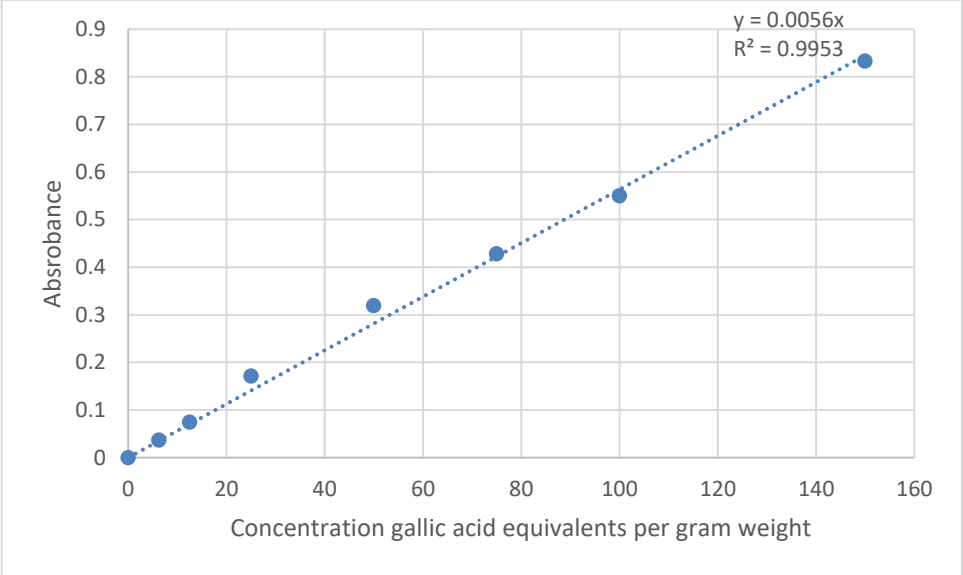

Figure 4: Total Phenolic Concentration standard curve

A.5 Example standard curve for Ferric Reducing Antioxidant Power assay

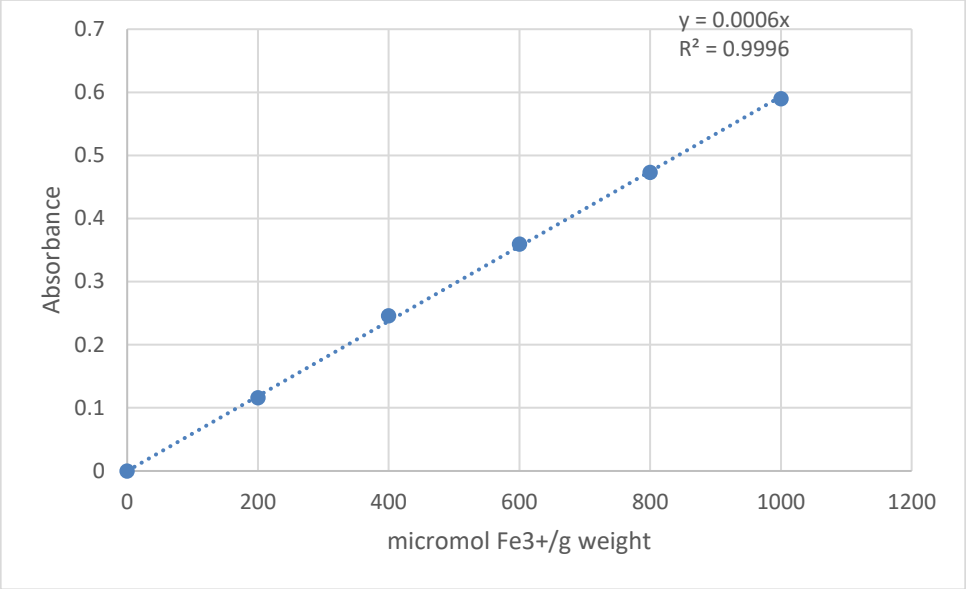

Figure 5: Ferric Reducing Antioxidant Power standard curve

A.6 Example standard curve for Angiotensin-converting enzyme inhibition (ACE) assay

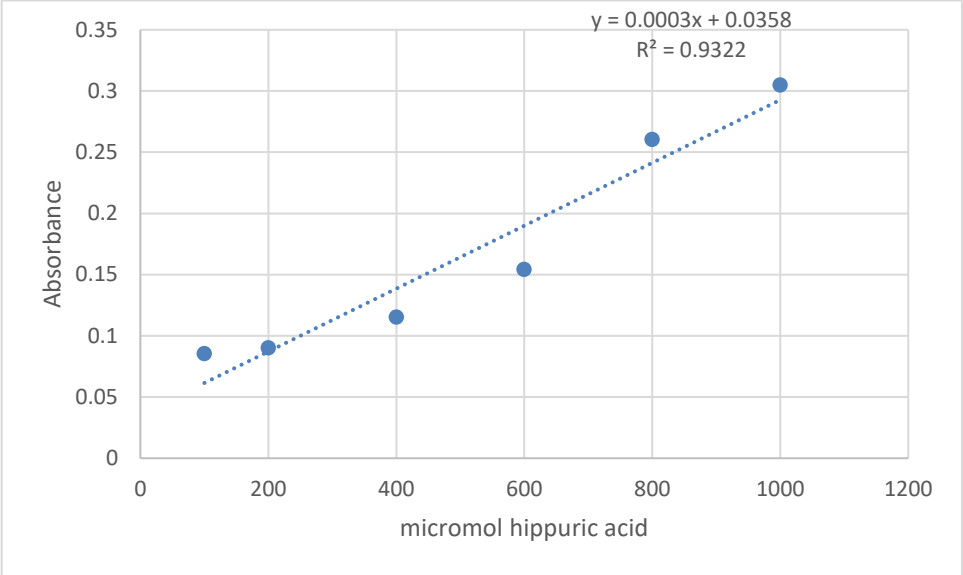

Figure 5: Angiotensin-converting enzyme inhibition standard curve

Appendix B

Raw data

B.1 Characterisation of kiwifruit and oat extracts protein quntifiucation

Table 1 Quantification of protein (mg/g) in kiwifruit and oat powders using biuret, BCA, and Bradford methods raw data

Assay	Sample	Protein concentration
Biuret	Rolled oat only	558.836689
Biuret	Rolled oat only	541.834452
BCA	Kiwifruit extract	333.8305984
BCA	Kiwifruit extract	333.8305984
BCA	Rolled oat only	165.920769
BCA	Rolled oat only	165.920769
Bradford	Rolled oat only	1.8096074
Bradford	Rolled oat only	1.864444
Bradford	Rolled oat only	1.864444
Bradford	Kiwifruit extract	3.17325228
Bradford	Kiwifruit extract	3.30699088
Bradford	Kiwifruit extract	3.29888552
Bradford	Rolled oat only soaked	1.6560649
Bradford	Rolled oat only soaked	1.831542
Bradford	Rolled oat only soaked	1.9302479

B.2 Optimisation of Kiwifruit and Oat heat treatment protein quantification

Table 2 Quantification of protein (mg/g) in kiwifruit and oat extracts for the duration of the heat treatment raw data

Sample	Time (min)	Protein content
Kiwifruit extract	0	3.999627942
Kiwifruit extract	0	3.992186773
Kiwifruit extract	0	3.969863269
Kiwifruit extract	30	3.701981211
Kiwifruit extract	30	3.873128081
Kiwifruit extract	30	3.776392894
Kiwifruit extract	60	3.768951725
Kiwifruit extract	60	3.821039903
Kiwifruit extract	60	3.895451586
Kiwifruit extract	240	2.809041019
Kiwifruit extract	240	2.991349642
Kiwifruit extract	240	3.050878988
Rolled oat only	0	0.89159068
Rolled oat only	0	0.80040527
Rolled oat only	0	0.81053698
Rolled oat only	30	1.32725431
Rolled oat only	30	1.22593718
Rolled oat only	30	1.12462006
Rolled oat only	60	1.4893617
Rolled oat only	60	1.62107396
Rolled oat only	60	1.74265451
Rolled oat only	240	1.92502533
Rolled oat only	240	1.96555218
Rolled oat only	240	1.88449848
Combined	0	2.583071342
Combined	0	2.67617896
Combined	0	2.649576784
Combined	30	2.870374849
Combined	30	2.952841596
Combined	30	2.532527207
Combined	60	2.702781137
Combined	60	2.27980653
Combined	60	2.98210399
Combined	240	2.271825877
Combined	240	2.162756953
Combined	240	2.117533253

B.3 Optimisation of Kiwifruit and Oat heat treatment Enzyme activity

Table 2 Enzymatic activity of kiwifruit and oat powders using PNP assay in ($\mu mol.min^{-1}$) raw data

Sample	Time (min)	Enzyme activity
Kiwifruit extract	0	1.592592593
Kiwifruit extract	0	1.5
Kiwifruit extract	0	0.203703704*
Kiwifruit extract	240	0.27037037
Kiwifruit extract	240	0.207407407
Rolled oat only	0	0.537037037
Rolled oat only	0	0.592592593
Rolled oat only	0	0.814814815
Combined	0	0.777777778
Combined	0	1
Combined	0	1.12962963
Combined	240	0.537037037
Combined	240	0.592592593
Combined	240	0.814814815

^samples indicated with * were considered outliers and not included in statistical analysis

B.4 Optimisation of Kiwifruit and Oat heat treatment Total phenolic content

Table 4 Total phenolic concentration (gallic acid equivalents/g) in treated kiwifruit and oat extracts for the duration of heat treatment raw data

Sample	Time (min)	Gallic acid equivalents/g
Kiwifruit extract	0	1062.5
Kiwifruit extract	0	1017.857143
Kiwifruit extract	0	1053.571429
Kiwifruit extract	30	898.3050847
Kiwifruit extract	30	915.2542373
Kiwifruit extract	30	923.7288136
Kiwifruit extract	60	1067.79661
Kiwifruit extract	60	1042.372881
Kiwifruit extract	60	1067.79661
Kiwifruit extract	240	974.5762712
Kiwifruit extract	240	1050.847458
Kiwifruit extract	240	1008.474576
Rolled oat only	0	45.89285714
Rolled oat only	0	44.28571429
Rolled oat only	0	45.17857143
Rolled oat only	30	42.37288136
Rolled oat only	30	44.23728814
Rolled oat only	30	43.55932203
Rolled oat only	60	40.16949153
Rolled oat only	60	45.08474576
Rolled oat only	60	44.57627119
Rolled oat only	240	57.62711864
Rolled oat only	240	59.49152542
Rolled oat only	240	54.91525424
Combined	0	1196.428571
Combined	0	1276.785714
Combined	0	1232.142857
Combined	30	21.86440678
Combined	30	24.40677966
Combined	30	24.40677966
Combined	60	26.27118644
Combined	60	26.77966102
Combined	60	26.94915254
Combined	240	20.84745763
Combined	240	21.86440678
Combined	240	21.3559322

B.5 Optimisation of Kiwifruit and Oat heat treatment Ferric reducing / antioxidant power

Table 5 Ferric reducing / antioxidant power (mmol Fe^{3+}/g sample) of kiwifruit and oat extracts for the duration of heat treatment raw data

Sample	Time (min)	mmol Fe^{3+}/g sample
Kiwifruit extract	0	20.229265
Kiwifruit extract	0	25.87660148
Kiwifruit extract	0	17.11058665
Kiwifruit extract	30	13.33002153
Kiwifruit extract	30	12.25368438
Kiwifruit extract	30	12.66766021
Kiwifruit extract	60	14.3235635
Kiwifruit extract	60	13.99238284
Kiwifruit extract	60	13.49561186
Kiwifruit extract	240	12.75045537
Kiwifruit extract	240	12.41927471
Kiwifruit extract	240	15.9794668
Rolled oat only	0	0.340525961
Rolled oat only	0	0.269723533
Rolled oat only	0	0.325354012
Rolled oat only	30	0.200364299
Rolled oat only	30	0.312965723
Rolled oat only	30	0.314621626
Rolled oat only	60	0.283159463
Rolled oat only	60	0.274879947
Rolled oat only	60	0.3013744
Rolled oat only	240	0.30634211
Rolled oat only	240	0.337804272
Rolled oat only	240	0.359331015
Combined	0	20.81928523
Combined	0	19.89211059
Combined	0	18.37491571
Combined	30	14.571949
Combined	30	13.74399735
Combined	30	13.74399735
Combined	60	15.48269581
Combined	60	16.39344262
Combined	60	18.04934592
Combined	240	12.50206988
Combined	240	11.92250373
Combined	240	10.84616658

B.6 *In vitro* digestion study protein quantification

Table 6 Protein quantity (mg/g) of kiwifruit and oat extracts after heat treatment and *in vitro* digestion raw data

Sample	Time	Protein quantity
Kiwifruit extract	After treatment	2.809041019
Kiwifruit extract	After treatment	2.991349642
Kiwifruit extract	After treatment	3.050878988
Kiwifruit extract	After *in vitro* digestion	0.056866049
Kiwifruit extract	After *in vitro* digestion	0.195871946
Kiwifruit extract	After *in vitro* digestion	0.009477675
Kiwifruit extract	After *in vitro* digestion	0.01053075
Rolled oat only	After treatment	1.92502533
Rolled oat only	After treatment	1.96555218
Rolled oat only	After treatment	1.88449848
Rolled oat only	After *in vitro* digestion	0.066343724
Rolled oat only	After *in vitro* digestion	0.053706824
Rolled oat only	After *in vitro* digestion	0.045282224
Rolled oat only	After *in vitro* digestion	0.054759899
Rolled oat only	After *in vitro* digestion	0.053706824
Rolled oat only	After *in vitro* digestion	0.048441449
Combined	After treatment	2.271825877
Combined	After treatment	2.162756953
Combined	After treatment	2.117533253
Combined	After *in vitro* digestion	0.058972199
Combined	After *in vitro* digestion	0.047388374
Combined	After *in vitro* digestion	0.060025274
Combined	After *in vitro* digestion	0.061078349
Combined	After *in vitro* digestion	0.049494524
Combined	After *in vitro* digestion	0.062131424

B.7 *In vitro* digestion study Total phenolic concentration

Table 7 Total phenolic concentration (gallic acid equivalents/g) in treated kiwifruit and oat extracts after heat treatment and *in vitro* digestion raw data

Sample	Time	gallic acid equivalents/g
Kiwifruit extract	After treatment	974.5762712
Kiwifruit extract	After treatment	1050.847458
Kiwifruit extract	After treatment	1008.474576
Kiwifruit extract	After *in vitro* digestion	974.5762712
Kiwifruit extract	After *in vitro* digestion	1050.847458
Kiwifruit extract	After *in vitro* digestion	1008.474576
Kiwifruit extract	After *in vitro* digestion	974.5762712
Rolled oat only	After treatment	57.62711864
Rolled oat only	After treatment	59.49152542
Rolled oat only	After treatment	54.91525424
Rolled oat only	After *in vitro* digestion	96.07142857
Rolled oat only	After *in vitro* digestion	92.85714286
Rolled oat only	After *in vitro* digestion	89.28571429
Rolled oat only	After *in vitro* digestion	85.71428571
Rolled oat only	After *in vitro* digestion	88.03571429
Rolled oat only	After *in vitro* digestion	87.14285714
Combined	After treatment	26.94915254
Combined	After treatment	20.84745763
Combined	After treatment	21.86440678
Combined	After *in vitro* digestion	109.2857143
Combined	After *in vitro* digestion	116.25
Combined	After *in vitro* digestion	113.75
Combined	After *in vitro* digestion	116.0714286
Combined	After *in vitro* digestion	114.4642857
Combined	After *in vitro* digestion	111.6071429

B.8 *In vitro* digestion study Ferric reducing / antioxidant power

Table 8 Ferric reducing / antioxidant power (mmol Fe^{3+}/g sample) of kiwifruit and oat extracts for the duration after heat treatment and *in vitro* digestion raw data

Sample	Time	mmol Fe^{3+}/g sample
Kiwifruit extract	After treatment	12.75045537
Kiwifruit extract	After treatment	12.41927471
Kiwifruit extract	After treatment	15.9794668
Kiwifruit extract	After *in vitro* digestion	1.013149022
Kiwifruit extract	After *in vitro* digestion	1.063722185
Kiwifruit extract	After *in vitro* digestion	1.134524612
Kiwifruit extract	After *in vitro* digestion	1.178354686
Rolled oat only	After treatment	0.30634211
Rolled oat only	After treatment	0.337804272
Rolled oat only	After treatment	0.359331015
Rolled oat only	After *in vitro* digestion	0.203978422
Rolled oat only	After *in vitro* digestion	0.195549562
Rolled oat only	After *in vitro* digestion	0.175320297
Rolled oat only	After *in vitro* digestion	0.173634525
Rolled oat only	After *in vitro* digestion	0.182063385
Rolled oat only	After *in vitro* digestion	0.178691841
Combined	After treatment	12.50206988
Combined	After treatment	11.92250373
Combined	After treatment	10.84616658
Combined	After *in vitro* digestion	1.134524612
Combined	After *in vitro* digestion	1.094066082
Combined	After *in vitro* digestion	1.016520566
Combined	After *in vitro* digestion	1.201955496
Combined	After *in vitro* digestion	1.328388402
Combined	After *in vitro* digestion	0.974376264

B.9 *In vitro* digestion study Angiotensin-converting-enzyme inhibition

Table 9 Angiotensin-converting-enzyme inhibition (%) of kiwifruit and oat extract after heat treatment and *in vitro* digestion raw data

Sample	Time	Inhibition %
Kiwifruit extract	After treatment	-16.60156535*
Kiwifruit extract	After treatment	-55.41992567*
Kiwifruit extract	After treatment	-22.46094049*
Kiwifruit extract	After *in vitro* digestion	50.7812488
Kiwifruit extract	After *in vitro* digestion	47.85156123
Rolled oat only	After treatment	52.97851448
Rolled oat only	After treatment	20.0195293
Rolled oat only	After treatment	35.40038905
Rolled oat only	After *in vitro* digestion	56.64062394
Rolled oat only	After *in vitro* digestion	55.90820205
Rolled oat only	After *in vitro* digestion	66.89453044
Combined	After treatment	-63.47656649*
Combined	After treatment	-12.93945588*
Combined	After treatment	-27.58789374*
Combined	After *in vitro* digestion	58.10546773
Combined	After *in vitro* digestion	45.65429555
Combined	After *in vitro* digestion	58.83788962

^negative values (indicated by *) are calculated as 0