Master thesis in Food Technology and Nutrition

# Evaluation of the Effect of Quinoa-containing bread varieties on acute blood glucose and insulin responses

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## Popular science summary

As I am sure that you have heard before, increasing obesity is a problem that is plaguing the 21st century. Diseases such as type 2 diabetes and cardiovascular disease are also on the rise. One option that has been suggested to help prevent all of this is low GI foods. GI, which is an abbreviation for glycemic index, is a way to compare how much your blood sugar changes after eating something. Almost everything that we eat will result in an increase of your blood sugar, also called blood glucose. This elevation can be smaller or larger depending on what you eat and, what gives most of a spike is carbohydrate rich foods.

Carbohydrates can be found in your average pasta, potatoes and bread amongst a number of other items. What happens is the carbohydrates you consume will be broken down in your intestines into smaller parts called glucose, galactose and fructose. These will then be transported into your bloodstream so that your body can store it and use them as energy wherever it is needed and the transportation of the glucose is what causes the spike in your blood sugar. A larger spike is often indicative of a high GI for that food. Insulin is a hormone that helps your body take up the sugars from your blood. Insulin can be likened to a key that unlocks small doors from your blood to the tissues in your body that the sugars can then go through. When you eat, the amount of insulin present in your blood is increased to make sure that all the sugars that your body needs for energy can be taken up from the blood.

Quinoa is a grain that has been around for a long time and it is traditionally most commonly used in South America and comes in a variety of colours. It has been shown that quinoa has a low GI and therefore this study will see if a bread made from the low GI quinoa can also have a lower spike than your regular wheat bread.

Three breads were made from three differently coloured quinoa. The quinoa used was a mix of 50% boiled whole quinoa grains and 50% quinoa flour made by milling raw grains. In total the quinoa bread contains 70% quinoa and 30% wheat flour, whilst the control bread was made using 100% wheat flour. Everyone participating in the study was given a portion of bread to consume and then had their finger pricked to collect capillary blood samples to measure the blood glucose and insulin. At the same time the participants were asked to fill out a form asking how hungry they felt, how much they wanted to eat and how full they felt on a scale of 1-100.

The control bread was known to be high GI and therefore caused a high rise in both blood sugar and insulin and when comparing the results for the quinoa breads with those of the control there was no difference to be seen in the blood sugar or insulin response. However, the quinoa breads all showed a decreased hunger and the desire to eat for the participants as well as an increase in how full the participants felt. One reason as to why there was no difference in blood glucose or insulin as originally thought could be because of how the breads were prepared and the effect could be avoided by boiling all the quinoa seeds beforehand.

# Abstract

Three quinoa-containing breads made from three different varieties of quinoa were presented as test products to be compared with a control of regular white wheat bread. The study was a randomised controlled single-blind, crossover in which blood glucose, insulin and subjective appetite sensations “Hunger”, “Satiety” and “Desire to eat” were measured in regular intervals during a three hours period after food consumption. Insulin levels and blood glucose response showed no significant difference compared to the control, however, all three measured subjective appetite sensations resulted in a significant improvement compared to the control for all three quinoa-containing breads. No significant differences could be seen between the three quinoa varieties for any of the test variables.

## Acknowledgments

I would like to thank my supervisors Anne Nilsson and Juscelino Tovar for their continuing support throughout this work. I would also like to thank Claudia Lazarte for being my examiner and the department of Food Technology, Engineering and Nutrition at Lund University for allowing me to perform all practical work at their facilities. Finally, I would like to say a special thanks to Rocio Morales Vargas for our continued work together throughout these months. It has been an honour and a privilege to work beside you on this project.

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# Introduction

Quinoa has been a staple in many households in South America for a long time. Since the 21st century the crop has started to be used more and more around the world and in 2013 the Food and Agricultural Organisation of the United Nations (FAO) declared it the year of the quinoa. Quinoa is not only a ‘super food’ in terms of its nutritional content and antioxidant properties, the extremely versatile conditions in which the crop can thrive makes it possible to grow almost anywhere in the world. (Vilcacundo & Hernández-Ledesma, 2017)

At the same time as quinoa is slowly trying to make a name for itself in our western world, a larger, more sinister trend is being put in the limelight. Chronic diseases such as diabetes and cardiovascular disease as well as their precursor metabolic syndrome are increasing in the world. These diseases can to a certain degree be known as lifestyle dependent illnesses that can be caused by a diet rich in calories, high GI foods in particular, along with a sedentary lifestyle (Brand-Miller, 2003). Obesity has now reached epidemic levels across the world and has been linked to numerous diseases and diseased states as the once mentioned before along with a list of others (Zyoud et. al, 2022).

Bread is a staple product in many households across the globe but unfortunately the one that seems to be the generally most common is the plain white wheat bread that has a relatively low nutritional value. Additionally, wheat bread is considered a high GI food (Borczak et. al, 2018), but bread in general has a great capacity to be fortified to add more nutritional value.

In this study the aim was to combine the popular food, bread, with the nutritional grain quinoa with the hypothesis of transferring some of the beneficial properties of quinoa into a product that is readily enjoyed by a large number of people.

# Background

## Quinoa

Quinoa (*Chenopodium quinoa*) belongs to the family of pseudo cereals and is most commonly grown in South American countries such as Bolivia, Peru and Argentina. The crop has a high stress tolerance and can grow under many varying conditions which is one of the reasons why it is now spreading more into Europe and other parts of the world. The grain can be cooked and eaten as is, fermented to make beer, or milled into a flour from which can be produced many different baked goods such as cookies or bread. Also, the leaves of the quinoa crop can be consumed in salads (Vilcacundo & Hernández-Ledesma, 2017). The nutritional benefits of quinoa are many, take protein for example, not only do quinoa grains have a higher overall protein content than for example rice, rye and barley, but also contain all essential amino acids in sufficient amount to cover the FAOs recommendations and is considered to have a complete amino acid profile (Nowak et. al, 2016). Quinoa also contains higher levels of fatty acids than most common cereals such as wheat, including relatively high amounts of linoleic and alpha-linolenic acid which are both essential fatty acids. The fatty acids in quinoa are accompanied by a high content of vitamin E which protects them from free radicals that cause oxidative rancidity of fat (Vilcacundo & Hernández-Ledesma, 2017). Additionally, quinoa also contains higher amounts of other vitamins such as riboflavin, folic acid and vitamin C as well as minerals such as iron, zinc and magnesium. Quinoa also contains a number of bioactive substances such as phytosterols and polyphenols which may have antioxidant, anti-inflammatory and anti-cancer effects (Alvarez-Jubete et. al, 2010). Finally quinoa has been shown to have a low glycemic index at around 35-53, depending on cooking method, when using pure glucose as reference (Gordillo-Bastidas et. al, 2016).

## Starch

Starch is the major component of the carbohydrates present in food. It consists of two types of polymeric glucose chains, amylose and amylopectin. From a nutritional viewpoint, starch can be divided into rapidly digested starch, slowly digested starch and resistant starch. The first two categories make up what is known as available starch. The available starch is hydrolysed in the small intestine by the enzymes amyloglucosidase and $\alpha$-pancreatic amylase into glucose residues that can be absorbed into the bloodstream. (McCleary et. al, 2019)

## Glycemic Index (GI)

The glycemic index refers to the glycemic response of a carbohydrate rich food during the first two hours after consumption compared to a reference value. Blood glucose is measured in intervals during two hours post food consumption of a portion containing 50g of available carbohydrates and a graph is plotted. From this, the incremental area under the graph is calculated and compared to a reference value that is usually obtained after consumption of an equivalent amount of carbohydrates from a reference product, such as wheat bread or sometimes pure glucose. The values are then classified into one of three categories based on the percentage compared to the reference. The scale goes from 0-100 and the three categories are: high GI (70 and above), moderate GI (56 to 69) or low GI (55 and below) (Kim, 2020). On the scale pure glucose is regarded as 100 and used as the reference. If white bread is being used at a reference all values should be multiplied with 0.71 to correspond to the values for when pure glucose is used as the reference (Romão et. al, 2021).

## Insulin

Insulin is a peptide hormone which is produced in the pancreas. The hormone is secreted into the bloodstream where it can bind to receptors on target cells throughout the body. One important binding site for insulin is activation of additional transport proteins called GLUT 4. GLUT 4 transports glucose from the extracellular matrix into the cell. This is the action by which insulin regulates the blood glucose levels after a meal. GLUT 4 is mostly present in adipose tissue and skeletal muscle where excess glucose can be stored as fat and glycogen respectively. Several other means of glucose uptake by the cells of the body exist, mainly glucose transporters GLUT 1 through 3 and 5, however these work independently from insulin. The secretion of insulin from the pancreas is triggered by increased concentrations of glucose in the blood (Watson & Pessin, 2001). Apart from this there are several other triggers for insulin secretion, such as hormones and neural stimuli. One important example is that stimuli from the vagus nerve will lead to insulin secretion when a person enters what is known as the cephalic phase of digestion which includes seeing, smelling and thinking of food (Teff, 2000). Hormonal stimuli can come from different places, incretin hormones originating from the gut such as glucagon/like peptide-1 (GLP-1) and glucose-dependant insulinotropic polypeptide (GIP) has a positive effect on insulin secretion from the pancreas whilst somatostatin has a negative effect. GLP-1 has also been shown to have long term effects on insulin levels due to increased cell mass of the $\beta$-cells of the pancreas which are responsible for insulin production (MacDonald et. al, 2002). Some individuals can develop something called insulin resistance. The mechanisms behind this remain unclear but the condition refers to an inadequate response to normal or heightened levels of insulin in the body. There are some clinical syndromes that have been shown to be associated with insulin resistance, the most prominent of these being diabetes and metabolic syndrome. (Wallace & Matthews, 2002)

## Obesity, Diabetes type 2 & Cardiovascular Disease

Obesity and overweight is plaguing the modern world and its prevalence is growing at an unnerving pace. With it comes a number of illnesses and one of the most strongly connected is diabetes type 2. It has even been shown that weight gain can increase your risk of diabetes type 2 of up to 9% per kg gained (Golay & Ybarra, 2005). Weight gain is a direct result of overconsumption of food and calories and the solution is to be in a calorie deficit over an extended period of time. It has been shown that a diet consisting of low GI foods may help to lower the body weight of people belonging to the obese category (Zafar et. al, 2019). Several studies have shown a connection between a number of chronic illnesses and a diet consisting of a high glycemic load (GL) and high GI foods. Glycemic load is a value for the GI of a food times the amount of carbohydrates in the consumed portion. A high GL diet has been connected to metabolic syndrome as well as a number of illnesses such as type 2 diabetes and cardiovascular disease (Brand-Miller, 2003). Zafar et. al (2019) also found that people suffering from type 2 diabetes or who are prediabetic can benefit from a low GI diet since it has the possibility to positively improve blood glucose control for the individual.

## Appetite sensations

Obesity and being overweight is caused by an overconsumption of calories over an extended period of time. This could be a result of low physical activity or a high intake of high calorie foods. The human body is striving to be in an energy balance where the energy consumed is approximately equal to the energy that is expanded. This is done by a variety of factors that impact how much energy we consume in the shape of food. Several hormones are released after acute food intake to signal a feeling of fullness. The same way there are hormones that after a longer period without any food intake signals the feeling of hunger (Suzuki et. al, 2012). How full or how hungry a person feels can directly impact how much food they consume and directly impacts the energy balance of their body and an increase in hunger sensations can eventually lead to an overconsumption of calories.

# Objectives

This study aims to evaluate the effects of quinoa/wheat composite bread on acute blood glucose and insulin as well as subjective appetite sensations. Breads made with different quinoa varieties were investigated and if they have the possibility to lower the blood glucose and insulin response following consumption compared to a standard bread made from wheat. The variables to be studied are blood glucose, insulin as well as a selection of subjective appetite sensations namely the subjects' sense of hunger, satiety and desire to eat. The appetite sensations enable an evaluation of the test person's subjective sensations in connection to each of the different test products.

# Materials and Methods

## Materials

The quinoa varieties which were of three different colours (Saltå Kvarn, Sweden), wheat flour (ICA, Sweden), salt (Falksalt, Sweden) and yeast (Kron Jäst, Sweden) were all purchased commercially from a supermarket in Lund, Sweden. Insulin analysis was performed using an ELISA insulin kit (Mercodia, Sweden). Blood glucose was analysed using Hemocue microcuvettes (Hemocue®, Sweden) and a Hemocue glucose reader (Hemocue®, Sweden). The starch analyses utilised the following chemicals and enzymes: $Na_3PO_4$ (Sigma-Aldrich, Germany), KOH (Merck, Germany), NaOH (VWR chemicals, Sweden), Amyloglucosidase (Roche Diagnostics, Germany), $\alpha$-amylase (Sigma-Aldrich, Germany), Ethanol (Solveco, Sweden), Termamyl (Sigma-Aldrich, Germany), pancreatin (Sigma-Aldrich, Germany), HCl (VWR international, Sweden), CaCl (Alfa Aesar, Germany), and $MgCl_2$ x $6H_2O$ (Merck, Germany).

## Bread preparations

Four different breads were developed prior to this study to be used as test products. Three breads were made using a specific variety of quinoa in a 70/30 % weight ratio of quinoa to wheat flour. As well as one control bread made from 100 % white wheat flour.

The quinoa-containing breads were made in a bread maker (Sage®, Heston Blumenthal®, United Kingdom). 185.5 g of uncooked quinoa (Saltå Kvarn) was boiled for 15 minutes in 371 g tap water with the lid closed. Once cooked the pot was placed in ice water for five minutes and then left to cool a further 25 minutes on the countertop with the lid on. The cooked quinoa was then added to the bread baker followed by 360 g of tap water and 185.5 g of ground quinoa flour, made by grounding raw quinoa seeds (Saltå Kvarn). 4.6 g of salt (Falksalt) was added followed by 159 g of commercially bought wheat flour (ICA). A small indentation was made in the wheat flour and 4.6 g of instant yeast (Kron Jäst) was added into the indentation. The bread was baked

using pre-programmed settings for whole grain rapid with the following alterations: Punch down times were all set to 5 seconds and the Rise 3 was set to 10 minutes. Once the bread entered the kneading mode on the machine the lid was opened and a spatula was used to manually mix the dough further to ensure no residue was stuck on the edges or at the bottom of the machine. Once the bead had finished baking it was left on the countertop for around 60 minutes wrapped in a damp towel until cold and subsequently placed in a plastic bag and stored in the fridge overnight. The next day the bread was placed on the countertop for approximately two hours to reach room temperature. To avoid product losses due to crumbs the breads were cut into approximate portion sizes, all slightly larger than the correct portion size, and the individual portions wrapped in aluminium foil, placed in a plastic bag and stored in the freezer until used for the trial. When used in the trial the bread portions were removed from the freezer and left at room temperature to thaw overnight before the trial. The weight of each portion was corrected to the amount containing 50 g of available starch on the day of the trial when plating the portions.

The wheat bread was prepared in a similar fashion to the quinoa bread. 360 g of tap water was added to the bread baker followed by 530 g of white wheat flour (ICA) and 4.6 g of salt (Falksalt). An indentation was made in the flour and 4.6 g of instant yeast (Korn Jäst) was added. The bread baker was set to the standard basic bread protocol. Once finished baking the breads were left wrapped in a damp towel for one hour at room temperature to cool and subsequently cut into appropriate portions, wrapped in aluminium foil and placed in a plastic bag and finally frozen. The same procedure preparing the bread for the trial day was used as for the quinoa breads.

The portion sizes all contained 50g available starch and were 123g, 209g, 218g and 228g for wheat bread, quinoa variety Z (QVZ), quinoa variety X (QVX) and quinoa variety Y (QVY) respectively.

## Total Starch Analysis

Frozen samples of bread were thawed and crumbled and subsequently left to air dry on a tray for 1-2 days. Once completely dry the samples were milled manually using a pestle. The milled bread samples were stored in a plastic container enclosed with parafilm to reduce the presence of additional moisture and oxygen. The total starch (TS) content of the samples was analysed after complete hydrolysis to glucose with a thermophilic amylase and amyloglucosidase, after pretreatment with KOH, according to the method described by Björck & Sijleström (1992).

## Resistant Starch Analysis

The resistant starch (RS) content of the samples was measured by the method described by Åkerberg et. al. (1998). Due to regulations currently in place at the facilities where the analysis took place the ethanol precipitation step was altered. The regulations prohibit use of heated

ethanol due to safety reasons and according to Åkerberg et. al. (1998) the sample should be left for one hour to precipitate in ethanol that has been preheated to 60 ℃. In order to adhere to the regulations, room temperature ethanol was used instead and the samples were left to precipitate overnight as described in the method by Sigma (2022).

## Available Starch

The amount of available starch (AS) in the sample breads was determined by the following equation:

$$AS = TS - RS \quad (1)$$

The amount of RS is subtracted from the TS content of the breads to yield the amount of AS.

## Moisture content

The moisture content of the breads were determined by overnight dehydration in the same way as used by Holm et, al (1986). A sample of fresh bread was weighed and subsequently placed in an oven (TS 8056, Termaks, Bergen, Norway) at 105 degrees centigrade overnight. Thereafter the sample was left to cool for 1 hour in a desiccator and weight again. The values were compared to gain the moisture content on percentage of the total weight of the bread.

## Description of Human Trial

The trial has been approved by an ethics committee with Swedish Ethical Review Authority diary number 2019-00980. Additionally, the study was conducted according to the guidelines laid down in the Declaration of Helsinki. Finally, the study follows a randomised controlled singel-blind, crossover design.

The trial consisted of four days, separated by approximately one week. Prior to a trial day the participants were asked to consume an individually standardised evening meal consisting of a chosen number of white bread slices with or without a chosen topping. All participants were asked to refrain from consuming any products containing high amounts of dietary fibre during the day before each trial day as well as avoid strenuous physical activity and alcohol consumption. The aforementioned factors can influence results in particular intake of high fibre food and strenuous exercise. The participants were also asked to keep the day before each trial day standardised to the best of their abilities and consume similar foods at similar times each day. The evening meal was to be consumed at 21:00 the night before and from that time the participants were to be fasting and refraining from all food and water until arriving at 7:30 on the trial day. The layout of each trial day was identical, independent of the test product. The

participants were weighed upon arrival and asked to sit down for 5-10 minutes before the initial (fasting) blood sample was taken. The participants were then presented with the test product accompanied by a 200- or 250-mL glass of water depending on the individual person's preference, and asked to consume everything at an even pace during twelve minutes. At the fifteen-minute mark the first post meal blood sample was taken. The following blood samples were taken at 30, 45, 60, 90, 120, 150 and 180 minutes after initiating consumption of the meal. During the three hours after the person consumed the test product no food or water was allowed to be consumed.

### Inclusion and exclusion criteria

The participants in the trial consisted of 18 apparently healthy adults that volunteered their time. The ratio between males and females was 7 to 11. To be included the participants were to be in the ranges of 18-40 years old and BMI between 20 and 28. No participant followed a vegan diet nor had any form of major food allergies or intolerances such as celiac disease or lactose intolerance. Additionally, no GI tract diseases or discomforts such as IBS were allowed in the participants as well as no metabolic diseases. Finally, all participants had to be non-consumers of any type of nicotine-containing products such as cigarettes or snus.

## Physiological Test Parameters

### Test Variables in blood

#### *Glucose*

Blood glucose was measured instantly at every time point. One drop of blood was collected in a cuvette that was immediately placed into a Hemocue glucose reader (Hemocue® Glucose 201+, Hemocue®, Ängelholm, Sweden) and the results were noted.

#### *Insulin*

Samples were collected in microtainer tubes and were left for 30-60 min to coagulate at room temperature and subsequently centrifuged for 10 min at 4000g (ST40R, Thermo Scientific, Massachausetts, USA). The separated serum was transferred from the microtainer to labelled eppendorf tubes and stored in a freezer for further analysis. Insulin was measured by using the Mercodia insulin ELISA kit (Mercodia, Uppsala, Sweden). All samples were left to thaw until room temperature for around 30 min before analysis. The samples were prepared in the 96 well plate according to the protocol by Mercodia (2021) and subsequently measured spectrophotometrically at 450 nm (Spectrostar Nano, BMG labtech, Ortenberg, Germany). The plates were washed using an automatic plate washer (Atlantis 2, LabVision, Romania).

### *Sampling procedure*

All samples were taken according to the following procedure: The tip of the finger was cleaned using chlorhexidine alcohol on an injection swab. The tip of the finger was held with slight pressure and a micro lancette was used to punch a hole in the skin. Two drops were gently pressed out and wiped away. The third drop was placed into a cuvette for blood glucose measurement. A few additional drops were placed in a labelled microtainer tube for subsequent insulin analysis.

### Subjective appetite sensations

All participants were given a form with the questions: How hungry do you feel? How satiated do you feel? and How strong is your desire to eat? Each question was to be answered by the participant marking on a 10 cm line going from not/none at all to extremely much/high following the method previously used by Hossain et al, (2021). The form was answered at the timepoints 0, 30, 60, 90, 120, 150 and 180 min post meal.

## Statistical analysis

All results were plotted against time and the positive integrals were calculated using GraphPad Prism (version 9.0, GraphPad Software, San Diego, CA, USA) and stored for statistical analysis in Minitab (MINITAB Statistical Software, version 21.4.0.0, Minitab, Minitab Inc., State College, PA, USA). General Linear Model ANOVA was used to analyse the data, followed by comparisons with Tukey's post hoc test if significant results were obtained, with a significance level of $P \leq 0.05$. The number of participants in this study was 18 however due to inadequate and/or incomplete samples or answers the number of participants for each analysis were as follows: 17 for blood glucose, 16 for subjective appetite sensations and 14 for insulin.

# Results

## Starch Analysis

Table 1 summarises the results from the analysis of total and resistant starch as well as the calculated value for available starch together with the results of the moisture content in the bread and resulting portion sizes for the four breads containing 50g available starch each.

**Table 1.** Values for starch content and portion sizes.

| Test product | Total starch in dry matter [g/100g] | Resistant starch in dry matter [g/100g] | Moisture content in the breads [%] | Available starch in dry matter [g/100g] | Available starch in fresh sample [g/100g] | Portion size [g] |
|---|---|---|---|---|---|---|
| QVX | 60.18 | 3.59 | 40.57 | 56.58 | 22.96 | 218 |
| QVY | 59.02 | 3.30 | 39.37 | 55.72 | 21.94 | 228 |
| QVZ | 64.11 | 3.53 | 39.41 | 60.58 | 23.87 | 209 |
| Control | 77.90 | 1.90 | 53.28 | 76.0 | 40.49 | 123 |

*Results from analysis of total and resistant starch (TS and RS) as well as moisture content in the test and reference products, and available starch (AS) calculated by subtracting RS from TS. As well as portion sizes based on 50g of AS for control products (bread made from wheat flour) as well as three test products (bread containing different quinoa varieties ) QVX, QVY and QVZ.

## Blood glucose

The mean peak in blood glucose occurs around the 30 min after the start of the breakfast for all four products. Figure 2 below depicts the average glucose response curves for all four products, control, QVX, QVY and QVZ against time during 120 min post meal consumption. As can be seen in figure 2 all four curves follow a similar appearance and peak height. The incremental and absolute graphs mimic each other, and the absolute graph shows that all fasting values were similar for all test products. Statistical analysis showed that no significant differences in postprandial blood glucose responses (iAUC, 0-120 min) between the quinoa varieties or in comparison with the control product.

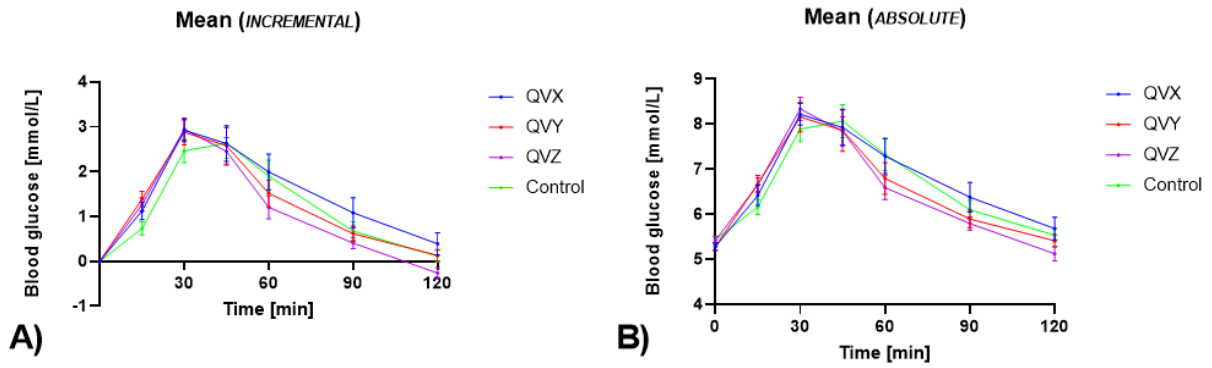


**Figure 2.** Average blood glucose responses of the four products, control, QVX, QVY and QVZ plotted against time during 120 min post meal. A shows incremental values and B shows absolute values with N=17.

## Insulin

Figure 3 shows the average insulin levels peak at around 30 min post meal consumption. The curve appears visually to have a slightly lower peak for the control product than the three quinoa products which appear to all have a similar peak height. Figure 3A shows the incremental values of the insulin levels and figure 3B shows the absolute values. All average fasting values appear to be similar and independent of the test product according to figure 3B. No significant differences in insulin levels (iAUC, 0-120 min) could be found between the varieties or in comparison with the control.

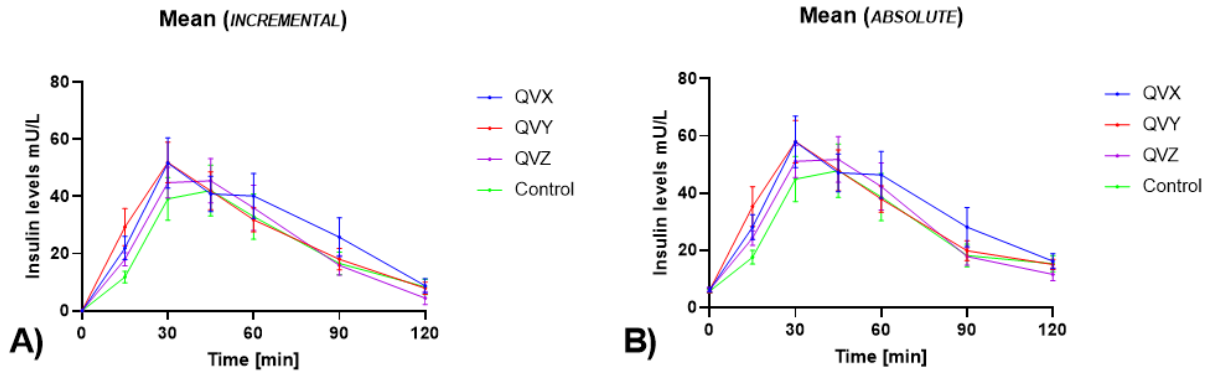


**Figure 3.** Average insulin levels in mU/L plotted against time for the four test products, control, QVX, QVY and QVZ. A shows incremental mean values and B shows absolute mean values with N=14.

## Subjective appetite sensations

All three subjective appetite sensations measured, hunger, satiety and desire to eat, share the same trends as shown in figure 4A, 4B and 4C respectively. The control product tended to result in lower satiety respective higher hunger and desire to eat responses than the remaining test products and it continues to have this appearance through the entire test period.

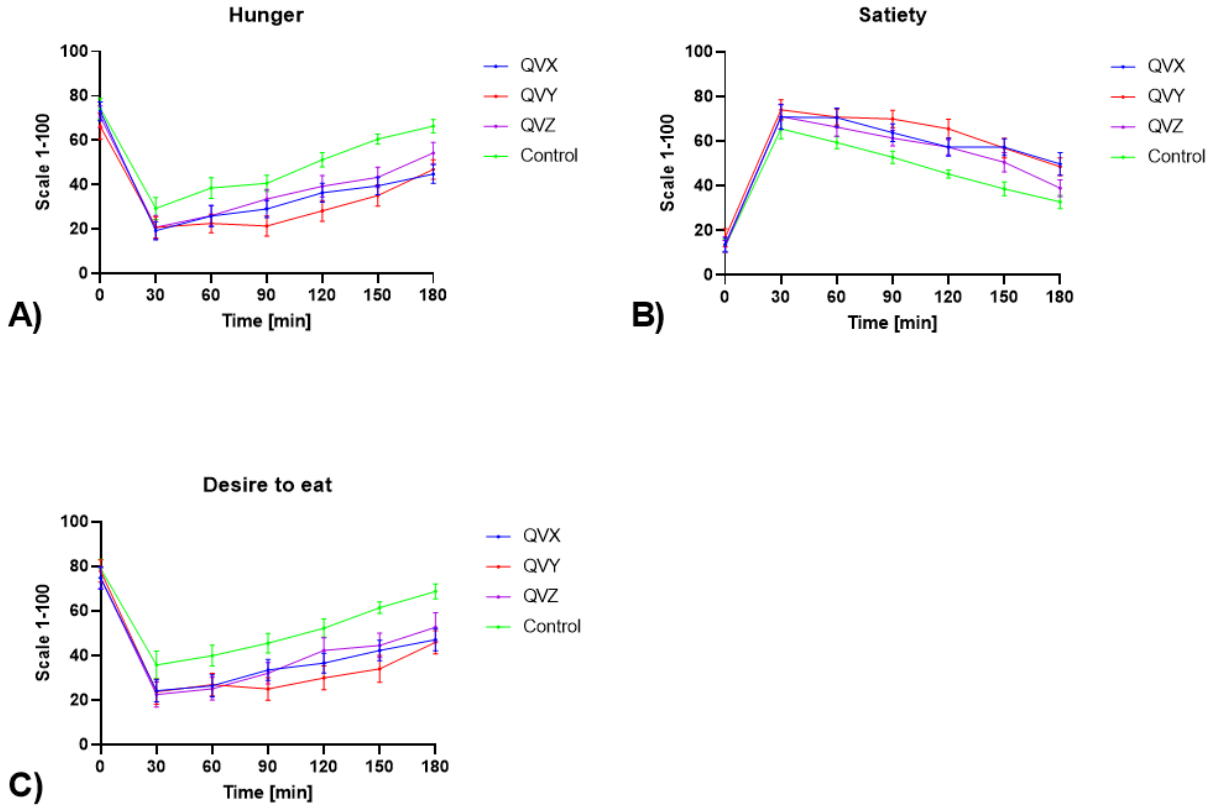


**Figure 4.** Average answers to the questions A) "How hungry do you feel?" B) "How satiated do you feel?" and C) "What is your desire to eat?" on a scale of 1-100 for the three test products made from quinoa, QVX, QVZ and QVY and the control product, plotted against time with N=16.

As it can be seen in table 2 all three quinoa varieties showed a significant difference compared to the control for all three appetite sensations. QVY showed the most prominent significant difference with $P \leq 0.001$ for all three sensations. QVX also showed a significant difference at $P \leq 0.001$ for hunger but only $P \leq 0.01$ for satiety and desire to eat. Finally, QVZ showed a significant difference at $P \leq 0.01$ for desire to eat and $P \leq 0.05$ for hunger and satiety. When looking at only the endpoints (180 minutes) statistical analysis showed a significant difference for QVX and QVY compared to the control for all parameters. QVZ also showed significant differences to the control for hunger and desire to eat however not for satiety. There were no significant differences between the three quinoa varieties themselves.

**Table 2.** Mean subjective appetite sensations (Hunger, satiety and desire to eat) after consuming test and control products for breakfast. *

| | **Control** | | **QVX** | | **QVY** | | **QVZ** | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| *Subjective appetite sensations* | | | | | | | | |
| Hunger AUC (0-180 min) [mm*min] | $8718^{A}$ | 468 | $6268^{B}$ | 569 | $5538^{B}$ | 679 | $6758^{B}$ | 693 |
| Satiety AUC (0-180 min) [mm*min] | $8530^{A}$ | 311 | $10545^{B}$ | 597 | $11091^{B}$ | 596 | $9973^{B}$ | 557 |
| Desire to eat AUC (0-180 min) [mm*min] | $9266^{A}$ | 584 | $6717^{B}$ | 676 | $6052^{B}$ | 833 | $6896^{B}$ | 812 |
| Hunger endpoint (180 min) [mm*min] | $66.38^{A}$ | 2.98 | $44.75^{B}$ | 4.19 | $46.88^{B}$ | 4.40 | $54.31^{B}$ | 4.78 |
| Satiety endpoint (180 min) [mm*min] | $32.75^{A}$ | 3.01 | $49.75^{B}$ | 5.04 | $48.56^{B}$ | 3.91 | $38.94^{AB}$ | 3.79 |
| Desire to eat endpoint (180 min) [mm*min] | $68.81^{A}$ | 3.32 | $47.13^{B}$ | 5.04 | $45.94^{B}$ | 5.20 | $52.69^{B}$ | 6.49 |

*Mean values for all five test variables for control (Wheat bread), and the three different quinoa containing breads QVX, QVY and QVZ as well as the mean value of the endpoint at 180 min for all three subjective appetite sensations. Significant difference between the values at $P \leq 0.05$ in each row is denoted by differing superscript letters. AUC denoting Area Under the Curve, and SEM standard error of mean.

# Discussion

The results show that all breads containing quinoa varieties promote a glucose response similar to that of regular white wheat flour bread (WB). Previous studies have shown that quinoa is a grain that has a low GI value as can be seen in a review by Gordillo-Bastidas et. al, (2016). Depending on preparation and cooking methods quinoa has a GI value of between 35-53. WB has repeatedly been described as a high GI food that is measuring above 70 on the GI scale using pure glucose as the reference (Borczak et. al, 2018). Additionally, quinoa has been reported to have higher amounts of polyphenols than wheat (Gordillo-Bastidas et. al, 2016) which has been shown to have a lowering effect on blood glucose that could account for the low GI previously reported by the grain (Kim et. al, 2016). According to the results displayed in Figure 2 the test products, independent of variety, result in a similar postprandial blood glucose response as WB, and hence, could also be suggested to have a high GI value. The results from this study therefore contradicts the hypothesis that the low GI of the quinoa grains would also be conferred to the quinoa bread. One reason for this could be that the amount of free glucose is higher in the quinoa products than for the control. The quinoa-containing breads were made using a 1:1 ratio of boiled quinoa kernels and quinoa flour milled from raw untreated quinoa kernels. Some participants described a sweet taste in some of the test products and further testing of the amounts of free glucose in the test products is recommended. Supporting this hypothesis is a study by Lorenz & Nyanzi (1989) which show quinoa flour has a high $\alpha$-amylase activity. The measurements of amylase activity were made using an amylograph and displayed in Brabender units (BU). Quinoa measured 120 BU compared to wheat which was estimated to measure at around 2000 BU under the same conditions. Further supporting this is a study by Azizi et. al (2021) which showed that quinoa shows a significantly higher $\alpha$-amylase activity compared to wheat. This indicates that quinoa has a much higher $\alpha$-amylase activity and therefore the potential to degrade starch into free glucose and $\alpha$-oligoglucans. A higher amount of free glucose in the quinoa bread products compared to intact quinoa grains could explain the elevated blood glucose response despite quinoa containing several blood glucose response-decreasing factors. Amylase activity is lost when boiling due to denaturation and inactivation of the enzyme (Singh et. al, 2015) henceforth replacing the quinoa flour used in this study with one that has been previously treated, for example by boiling of the intact quinoa grains and subsequently drying the kernels before milling, could be a promising option for reducing the GI.

Additionally heat treatment of starch will lead to an unwinding of the structure of the amylose and amylopectin in the starch granule and even complete disruption of the granule itself which increases the starch digestibility (Xie et. al, 2020). 50% of the quinoa used was boiled quinoa seeds which therefore are heat treated in two subsequent steps, first the boiling followed by the baking. This could indicate that the increase in digestibility of the starch in the quinoa could lead to a higher uptake of glucose than for quinoa which has only been through one heat treatment step and therefore be a possible explanation for the conflicting result.

It follows that no significant difference could be seen in regards to insulin between any of the test products. Insulin secretion is highly dependent on blood glucose levels and therefore it follows that the results for the two would mimic each other to a certain extent, even though there are a number of exceptions to this rule. What can be seen however when viewing figure 3 is that the insulin concentrations after the control appears to be marginally (no significance obtained) lower than the three quinoa varieties in the early period. This could perhaps be attributed to the rather large difference in portion sizes for the test products where the portions of all three quinoa breads are almost twice the size of the control bread. The larger portions could possibly lead to an increase in insulin secretion due to stimuli from the vagus nerve, which responds to the cephalic phase of digestion, where both the view of the food as well as the act of eating are included (Geary, 2004). When viewing the test products, the portions of the quinoa containing breads are visibly larger, and when consuming the food the intensity will inevitably differ due to the different portion sizes whilst still adhering to the same time restraint.

The test variables where significant differences could be seen between test and control products were the three subjective appetite sensations, hunger, satiety and desire to eat. As presented in table 2 all quinoa breads showed significant differences compared to the WB for all three sensations. When viewing figures 4A, 4B and 4C it can be seen visually that the control resulted in overall inferior appetite sensations than all three quinoa bread varieties. One factor that could play a part is what can be seen in the insulin curve in figure 3. Insulin responses appears visually to be slightly lower for the control than for the quinoa varieties and insulin is a hormone that can have a decreasing effect on appetite and is also suggested to be able to further increase the appetite suppressing actions of CCK (cholecystokinin) (Geary, 2004).

Apart from the possible connections to insulin levels there are also a number of other possibilities. The difference in the portion sizes is most likely the main reason for the statistical difference. More food leaves the test subjects feeling more satiated and less hungry. Even if this is the only reason, the different portions all contain the same amount of available starch which is one of the main contributors to the total amount of calories in a portion of bread. Though not possible in hindsight to calculate the exact caloric content of the portion sizes used, it is possible to speculate some based on the nutritional information provided on the packages of the quinoa kernels and wheat flour used (can be seen in appendix 1). Calculated from the nutritional labels the one loaf of the wheat bread contained around 1800 kcal and the three quinoa-containing breads around 1900 kcal, with some variation depending on the variety, per loaf. However what should be noted is that whilst the wheat bread was light and airy in texture the quinoa-containing breads were a lot more dense and moist making the weight of the quinoa-containing loves higher. This resulted in that even if the portion sizes were widely different in terms of the weight the number of portions per loaf was approximately the same for all four breads. This would then indicate that the extra calories in the portions of quinoa-containing bread might not be enough to be the sole reason for the significant difference that could be seen compared to the control.

However, since the exact weight of the finished loves were not noted this is all speculation and more investigations into the caloric content of the quinoa-containing breads should be done before any conclusions can be drawn.

As stated on the nutritional labels of the wheat flour and quinoa kernels used, which can be seen in the appendix 1, quinoa contains higher amounts of protein and dietary fibre than the wheat flour per 100g. Intake of high amounts of protein and fibre respectively has been shown to have an appetite suppressing role (Sharafi et. al, 2018). Additionally, the gut hormone GLP-1 has been shown to have an effect on appetite as well as insulin secretion. The hormone is released after a meal and has been shown to increase glucose dependent insulin secretion (Wang et, al. 2022). In a previous study by Wang et. al, (2022), quinoa has been shown to increase GLP-1 secretion in obese mice. This effect, if transferable to humans, could be another possible explanation to the differences seen in appetite sensations compared to the wheat bread control. An increase in GLP-1 could also be another possible explanation for the marginal (no significance) differences seen in figure 3 between the control and the three quinoa-containing breads.

Additionally, if the endpoints at 180 minutes are studied in the figures 4A, 4B, and 4C, the quinoa breads all appear to give a higher sensation of satiety along with lower sensation of hunger and desire to eat. This is further substantiated by the statistical analysis, seen in table 2, which showed a significant difference for all three quinoa breads compared to the WB for hunger and desire to eat and showed a significant difference for QVX and QVY compared to the WB for satiety. At this point in time the person perhaps sits down to have a second meal. The less hungry person will most likely unconsciously eat less than a person who is more hungry. These are all especially important aspects when it comes to aiding people trying to reduce their caloric intake. For example, people who belong to the obese category and are trying to lose weight in order to improve their health. It can also be helpful in the prevention of obesity which is caused by an extended calorie surplus over time.

When comparing the three products with different quinoa varieties, the QVY can be seen to result in the most pronounced difference compared to the control on all three appetite sensations, followed by QVX and finally QVZ. One reason for the difference (not significant) between the three could be due to the difference in polyphenol content of the three quinoa varieties. According to Ballester-Sánchez et. al, (2019) amongst the three varieties used QVY was most abundant in polyphenols, closely followed by QVX, and finally QVZ that contained the lowest amount. Polyphenols have been suggested as a reason for increased secretion of GLP-1 by quinoa (Wang et. al, 2022). Potentially the differences in the amounts of polyphenols between quinoa products in this study could explain the discrepancy between the products with respect to effects on appetite sensations Thus, QVY with the highest amount of polyphenols, also resulted in the most advantageous appetite scores.

### Limitations

The major limitations of this study is the limited number of participants as well as the varying number of participants for each of the analyses. Additionally, regarding the subjective appetite sensations, the study design is lacking due to the large variation in portion sizes of the four products. For more accurate and reliable results on appetite sensations a study comparing equal portion sizes on either a weight or calorie basis should be performed.

## Conclusion

Quinoa shows a great deal of potential to be incorporated into products that can help prevent obesity and its many accompanying illnesses. The formulation of the specific test product used in this study showed a significant improvement compared to regular white wheat bread in subjective appetite sensations for all three varieties included in the study, with the most prominent being QVY. No significant differences could be seen in the postprandial blood glucose and insulin responses depending on test products. The results somewhat contradict previous articles depicting quinoa as a low GI food. However, this is hypothesised to be due to a higher content of free glucose in the quinoa-containing breads due to unintentional increase in $\alpha$-amylase activity. To avoid this the quinoa flour used for bread baking in this study is suggested to be pre-treated to inactivate any potential $\alpha$-amylase.

To further investigate the effects on quinoa containing breads some suggestions for further work includes pretreatment of quinoa to inactivate and potential any potential $\alpha$-amylase. Additionally more objective measures of hunger and appetite should be investigated for example by measuring hormone levels in the blood post meal consumption. Finally a more thorough investigation into the appetite sensations is required utilising the same portions for all four products in order to get a better representation of the effects of the quinoa in the bread.

# References


Alvarez-Jubete, L., Wijngaard, H., Arendt, E.K. & Gallagher, E. (2010) Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chemistry, 119,* 770-778. https://doi.org/10.1016/j.foodchem.2009.07.032

Azizi, S., Azizi, M.H. & Rajaei, P. (2021) Investigating and comparing the enzymatic activity of wheat, Quinoa and Amaranth. *J food safe & hyg, 7(2), 110-120.*

Ballester-Sánchez, J., Gil, J.V., Haros, C.M. & Fernández-Espinar, M.T. (2019) Effect of Incorporating White, Red or Black Quinoa Flours on Free and Bound Polyphenol Content, Antioxidant Activity and Colour of Bread. *Plant Foods Hum Nutr,* 74, 185–191. https://doi.org/10.1007/s11130-019-00718-w

Björck, I. & Siljeström, M. (1992) In-vivo and in-vitro digestibility of starch in autoclaved pea- and potato products. *J Sci Food Agric 58,* 541-553. https://doi.org/10.1002/jsfa.2740580414

Borczak, B., Sikora, M., Sikora, E., Dobosz, A. & Kapusta-Duch, J. (2018) Glycaemic index of wheat bread. *Starch, 70.* https://doi.org/10.1002/star.201700022

Brand-Miller, JC. (2003) Glycemic load and chronic disease. *Nutrition Reviews*, 61(5), 49–55. doi:10.1301/nr.2003.may.S49-S55.

Geary, N. (2004) *Hunger and Satiation*. Encyclopedia of Endocrine Diseases, Elsevier. pp 459-468. https://doi.org/10.1016/B0-12-475570-4/01166-5.

Golay, A. & Ybarra, J. (2005) Link between obesity and type 2 diabetes. *Best Practice & Research Clinical Endocrinology & Metabolism, 19(4),* 649-663. https://doi.org/10.1016/j.beem.2005.07.010.

Gordillo-Bastidas, E., Díaz-Rizzolo, D.A., Roura, E., Massanés, T. & Gomis, R. (2016) Quinoa (Chenopodium quinoa Willd), from Nutritional Value to Potential Health Benefits: An Integrative Review. *Journal of Nutrition & Food Sciences, 6(3).* doi: 10.4172/2155-9600.1000497

Hossain, M. M., Tovar, J., Cloetens, L., Florido, M. T. S., Petersson, K., Prothon, F., & Nilsson, A. (2021). Oat Polar Lipids Improve Cardiometabolic-Related Markers after Breakfast and a Subsequent Standardized Lunch: A Randomized Crossover Study in Healthy Young Adults. *Nutrients*, *13*(3), 988. https://doi.org/10.3390/nu13030988

Kim, D. (2020) *Chapter 14 - Glycemic Index*. Obesity Global Impact and Epidemiology. pp 183-189. https://doi.org/10.1016/B978-0-12-818839-2.00014-4

Kim, Y., Keogh, J.B. & Clifton, P.M. (2016) Polyphenols and Glycemic Control. *Nutrients, 5;8(1),* 17. doi: 10.3390/nu8010017

Lorenz, K. & Nyanzi, F. (1989) Enzyme activities in quinoa (Chenopodium quinoa). *International Journal of Food Science and Technology, 24,* 543-551. https://ifst.onlinelibrary.wiley.com/doi/pdfdirect/10.1111/j.1365-2621.1989.tb00678.x?casa_token=RxTZWfAHiJQAAAAA:DOyI-h6l-kzYB73_q7Tzf-usCBTS-ksTcG9UYAP_mZgtsdU1SH4rYCx41F828hka3RO27tPPF5kS_nk [Accessed: 2023-05-12]

MacDonald, P.E,, El-Kholy, W., Riedel, M.J., Salapatek, A.M., Light, P.E. & Wheeler, M.B. (2002) The multiple actions of GLP-1 on the process of glucose-stimulated insulin secretion. Diabetes. *American Diabetes Association, 51(3), 34-42*. doi: 10.2337/diabetes.51.2007.s434

McCleary, B.V., McLoughlin, C., Charmier, L.M.J. & McGeough, P. (2019) Measurement of available carbohydrates, digestible, and resistant starch in food ingredients and products. *Cereal Chemistry, 97,* 114-137. https://doi.org/10.1002/cche.10208

Mercodia AB. (2021) *Mercodia Equine Insulin ELISA: Directions for Use*. (Version 8.0) https://www.mercodia.com/app/uploads/2022/11/Directions-for-Use-10-1205-01-Version-8.0-Lot.nr-30096-31916-32442.pdf

Nowak, V., Du, J.U. & Charrondière, R. (2016) Assessment of the nutritional composition of quinoa (Chenopodium quinoa Willd.). *Food Chemistry, 193, 47-54.* https://doi.org/10.1016/j.foodchem.2015.02.111.

Romão, B., Falcomer, AL., Palos, G., Cavalcante, S., Botelho, RBA., Nakano, EY., Raposo, A., Shakeel, F., Alshehri, S., Mahdi, WA., Zandonadi, RP. (2021) Glycemic Index of Gluten-Free Bread and Their Main Ingredients: A Systematic Review and Meta-Analysis. *Foods*, 10(3), 506. doi: 10.3390/foods10030506

Sharafi, M., Alamdari, N., Wilson, M., Leidy, HJ. & Glynn, EL. (2018) Effect of a High-Protein, High-Fiber Beverage Preload on Subjective Appetite Ratings and Subsequent Ad Libitum Energy Intake in Overweight Men and Women: A Randomized, Double-Blind Placebo-Controlled, Crossover Study. *Curr Dev Nutr, 23*. doi: 10.1093/cdn/nzy022.

Singh, K., Shandilya, M., Kundu, S. & Kayastha, A.M. (2015) Heat, Acid and Chemically Induced Unfolding Pathways, Conformational Stability and Structure-Function Relationship in Wheat α-Amylase. *PLoS One, 10(6).* doi: 10.1371/journal.pone.0129203

Suzuki, K., Jayasena, C.N. & Bloom, S.R. (2012) Obesity and Appetite Control. *Experimental Diabetes Research.* https://doi.org/10.1155/2012/824305

Teff, K. (2000) Nutritional implications of the cephalic-phase reflexes: endocrine responses, *Appetite, 34(2), 206-213*. https://doi.org/10.1006/appe.1999.0282.

Vilcacundo, R. & Hernández-Ledesma, B. (2017) Nutritional and biological value of quinoa (Chenopodium quinoa Willd.). *Current Opinion in Food Science, 14,* 1-6. https://doi.org/10.1016/j.cofs.2016.11.007.

Wallace, T.E. & Matthews, D.R. (2002) The assessment of insulin resistance in man, *Diabetes medicine, 19(7), 527-534.* https://doi.org/10.1046/j.1464-5491.2002.00745.x

Wang, T.Y., Tao, S.Y., Wu, Y.X., An, T., Lv, B.H., Liu, J.X., Liu Y.T. & Jiang, G.J. (2022) Quinoa Reduces High-Fat Diet-Induced Obesity in Mice via Potential Microbiota-Gut-Brain-Liver Interaction Mechanisms. *Microbiol Spectr, 10(3)*. doi: 10.1128/spectrum.00329-22

Watson, R.T & Pessin J.E. (2001) Intracellular organization of insulin signalling and GLUT4 translocation. Recent progress in hormone research, 56(1), 175-194. https://www.endocrine.org/~/media/endosociety/files/ep/rphr/56/rphr_vol_56_ch_09_intracellular_organization.pdf

Wilcox, G. (2005) Insulin and Insulin Resistance. *Clin Biochem Rev, 26*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1204764/pdf/cbr26_2pg019.pdf

Xie, X.,Qi, L.,Xu, C., Shen, Y., Wang, H. & Zhang, H. (2020) Understanding how the cooking methods affected structures and digestibility of native and heat-moisture treated rice starches. *Journal of Cereal Science, 95*. https://doi.org/10.1016/j.jcs.2020.103085.

Zafar, M. I., Mills, K. E., Zheng, J., Peng, M. M., Ye, X., & Chen, L. L. (2019) Low glycaemic index diets as an intervention for obesity: a systematic review and meta-analysis. *Obesity Reviews, 20,* 290– 315. https://doi.org/10.1111/obr.12791.

Zyoud, S.H., Shakhshir, M., Abushanab, A.S., Koni, A., Shahwan, M., Jairoun, A.A. & Al-Jabi, S.W. (2022) Global research trends on the links between insulin resistance and obesity: a visualization analysis. *transl med commun, 7, 18*. https://doi.org/10.1186/s41231-022-00124-6

Åkerberg, A.K.E., Liljeberg, H.G.M., Granfeldt, Y.E., Drews, A.W. & Björck, I.M.E. (1998) An In Vitro Method, Based on Chewing, To Predict Resistant Starch Content in Foods Allows Parallel Determination of Potentially Available Starch and Dietary Fibre. *American Society for Nutritional Sciences, 98*. doi: 10.1093/jn/128.3.651

# Appendix 1

Nutritional labels for the wheat flour and the quinoa kernels used for the preparations of the breads used in this study.


**Figure 5.** Nutritional labels from the A) wheat flour, B) QVX (quinoa variety X), C) QVY (quinoa variety Y) and D) QVZ (quinoa variety Z) used to prepare the breads used in this study.