# NUTRITIONAL COMPOSITION, PROTEIN DIGESTIBILITY AND MINERAL BIOACCESSIBILITY OF PLANT-BASED BURGERS COMPARED WITH A BEEF BURGER.

Marta Maria Gamez Fernandez

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Division of Nutritional Sciences
School of Biosciences
Sutton Bonington Campus
Loughborough
Leicestershire
LE12 5RD

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*Abstract*

Background. Food production is the largest cause of global environmental change with animal protein being the dietary group with the strongest impact, especially ruminant meat. In contrast, plant-based foods cause fewer adverse environmental effects, but they typically contain anti-nutritional factors that reduce protein digestibility and mineral bioaccesibility. Food processing in general, and cooking in particular, can reduce anti-nutritional factors' activity, and this could improve the nutritional adequacy of plant-based meat alternatives (PBMAs). Hence, whether plant-based meat alternatives are a meat-equivalent source of these nutrients remains unknown.

Aims. The first objective was to examine the nutritional composition and the effect of cooking on it, in comparison with a beef burger. The second objective was to analyse the content of antinutritional factors (ANFs) such as phenolics and phytic acid in beef and plant-based burgers. Finally, the third objective was to investigate the bioaccessibility of iron and zinc and the protein digestibility in beef and plant-based burgers.

Methods. A total of 8 plant-based burgers (Vivera 1 and 2, The Meatless Farm, Quorn and 4 Sainsbury's burgers (mushroom and jackfruit, mixed vegetable, onion and parsley, and quinoa) along with a beef burger (Birds Eye Beef) were acquired. Both cooked and raw burgers were subjected to energy, protein and mineral content determination. Cooked burgers were subjected to analysis of phytic acid and total phenolics as well as to determination of the bioaccesibility of iron and zinc and the digestibility of proteins.

Results. Plant-based burgers composed of highly concentrated plant proteins (Vivera 1 and 2, The Meatless Farm, Quorn, and Sainsbury's onion and parsley) had slightly less energy and similar or even more protein compared to the beef burger. In addition, plant-based burgers were richer in minerals than the beef burger, but they also had higher levels of sodium. Cooking tended to increase the concentration of minerals, protein and energy. Plant-based burgers had higher levels of total phenolics and phytic acid than the beef burger, for which protein digestibility was greater. Iron solubility was similar between the beef burger and the plant-based burgers containing plant protein concentrates. However, zinc solubility was greater for the beef burger than for the plant-based burgers. A burger fortified with iron and zinc (The Meatless Farm) had higher soluble iron and similar soluble zinc than the beef burger, but it also had higher amounts of non-soluble iron and zinc, as fortified minerals might be bounded by ANFs. In conclusion, PBMAs are not nutritionally identical replacements of meat products, with the concentration of ANFs along with the solubility of iron and zinc being key differences. Also, the impact of ANFs on mineral absorption may depend on other factors such as food processing, interactions with microbiota, and mineral status and hence their impact on PBMAs nutritional adequacy needs further investigation.

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# 1 Introduction.

*Food, sustainability and health*.

In 2017 global population reached 7.5 billion and the United Nations (UN) predict this will rise to 8.5 billion by 2030 and almost 10 billion by 2050 (Aiking and de Boer, 2018). The Food and Agriculture Organisation (FAO) projects a 60% food demand increase by 2050 (Aiking and de Boer, 2018). In September 2015, UN presented the 17 Sustainable Development Goals (SDGs) as an incentive to prioritise and integrate issues such as food security, food sustainability, climate change and the broader aim of staying within planetary boundaries (Aiking and de Boer, 2018). Each of the nine planetary boundaries represent a system or a process that is important for regulating and maintaining stability of the planet. They define global biophysical limits that humanity should operate within to ensure a stable and resilient Earth system, i.e. conditions that are necessary to foster prosperity for future generations (Willett et al., 2019). These are climate change, ocean acidification, stratospheric ozone depletion, atmospheric aerosol loading, nitrogen and phosphorus flows to the biosphere and oceans, global freshwater use, land system change, rate of biodiversity loss and chemical pollution (Rockström, 2009). Food production is the largest cause of global environmental change (Willett et al., 2019) and many of these planetary boundaries are strongly interlinked by protein production, with nitrogen cycle acceleration in a pivotal role (Aiking and de Boer, 2018).

Although global food production of calories has kept pace with population growth, more than 820 million people have insufficient food and many more consume low-quality diets that cause micronutrient deficiencies and diet-related obesity as well as diet-related non communicable diseases. Unhealthy diets are the largest global burden of disease and pose a greater risk to morbidity and mortality than does unsafe sex, alcohol, drug, and tobacco use combined. In addition, the global shift to unhealthy diets is also contributing to environmental degradation (Willett et al., 2019).

*Diet transition*

Currently, there is a dual diet transition in progress. Booming economies are increasing their consumption of meat (China) and dairy (India) like Western Europe did half a century ago. In Europe, a reverse transition away from animal products is about to break through (Aiking and de Boer, 2018). However, in global terms, the intake of meat is above recommendations for a healthy diet (Willett et al., 2019).

From the environmental perspective, animal foods are the dietary group with the strongest impact, especially ruminant meat. However, plant-based foods cause fewer adverse environmental effects

(Figure **1**). In fact, most studies assessing the environmental effect of diets find decreasing effects with increased replacement of animal source foods with plant-based foods. Importantly, diets that replaced ruminants with other alternatives, such as fish, poultry, and pork, also show reduced environmental effects, but to a smaller extent than vegan and vegetarian diets (Willett et al., 2019).

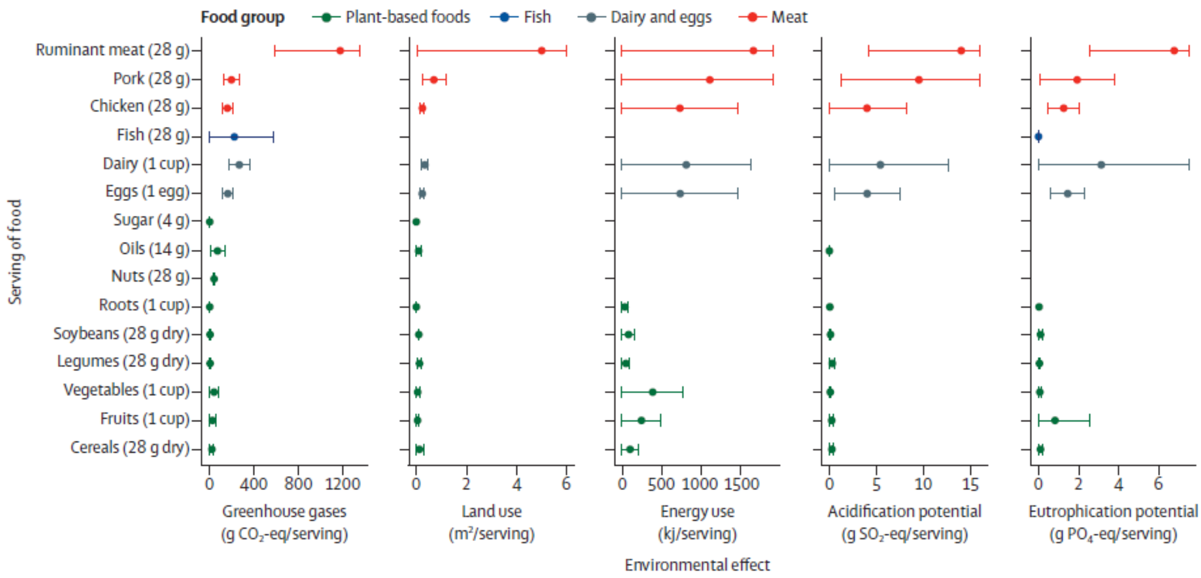



*Figure 1 Environmental effects per serving of food produced. Bars are mean (SD). $CO_2$=carbon dioxide. Eq=equivalent. $PO_4$=phosphate. $SO_2$=sulphur dioxide. From the EAT-Lancet Commission on healthy diets from sustainable food systems (Willett et al., 2019)*

Nevertheless, just following health dietary guidelines would reduce GHG emissions significantly, in addition to being much healthier than current dietary habits. Even cutting the climate change impact in half is feasible by adopting a culturally acceptable and cheap diet (Aiking and de Boer, 2018). The EAT-Lancet commission on healthy diets from sustainable food systems proposed a reference healthy diet which involves a global reduction in the intake of red meat, starchy vegetables and eggs and a global increase in plant-based foods such as legumes, whole grains, nuts, fruits and vegetables (Figure **2**).

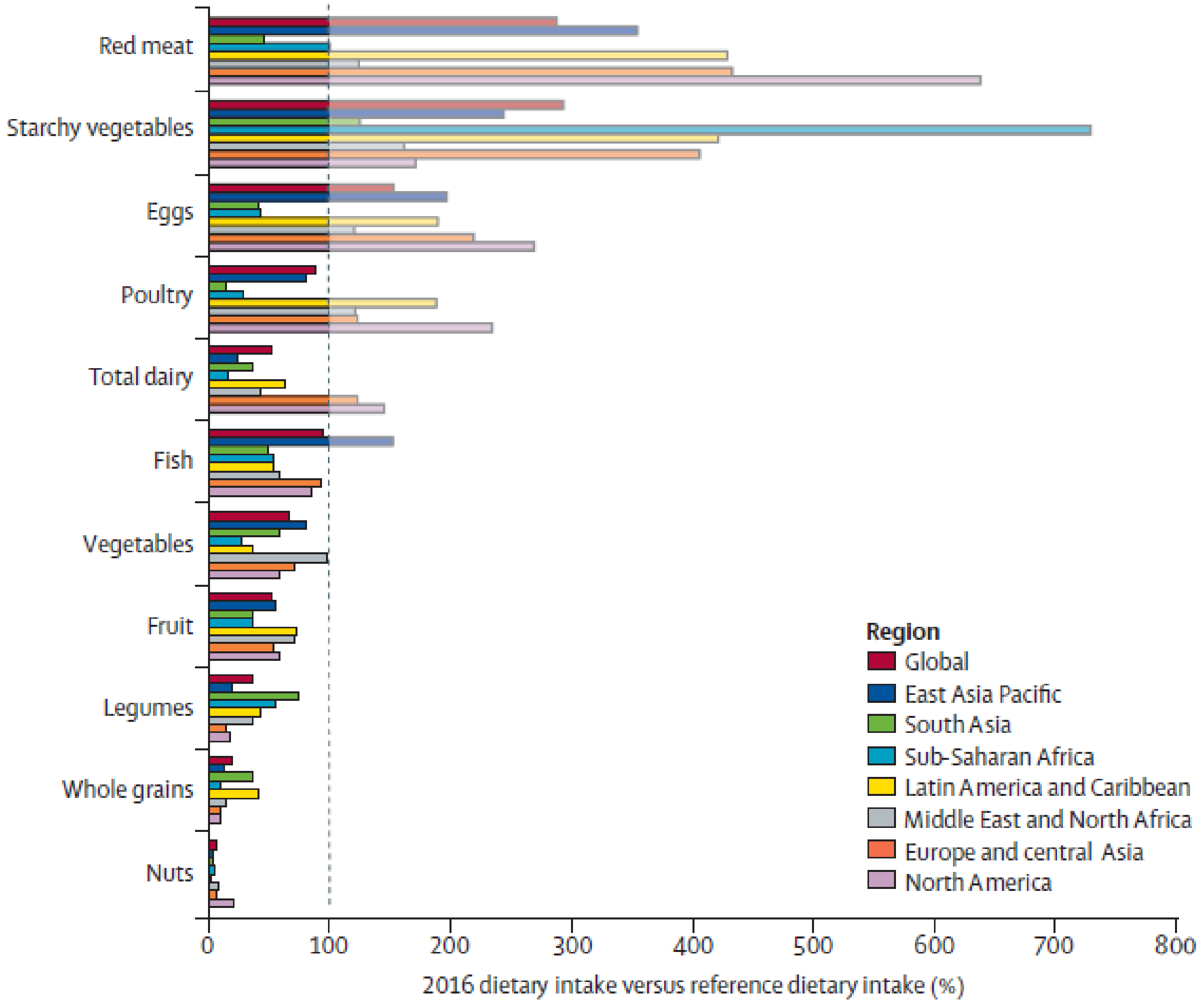



*Figure 2 Diet gap between dietary patterns in 2016 and reference diet intakes of food. The dotted line represents intakes in the reference diet by EAT–Lancet Commission on healthy diets from sustainable food systems (Willett et al., 2019).*

Also, this commission stated that dietary changes from current diets to healthy diets are likely to substantially benefit human health, avoiding about 10.8 - 11.6 million deaths per year, a reduction of 19 - 23.6% (Willett et al., 2019).

Addressing the environmental and animal welfare issues caused by intensive animal protein production involve various trade-offs. A better animal welfare and a reduction in the use of antibiotics would reduce feed efficiency. Another barrier in the protein transition comes from the consumers perspective, with many resistant to the idea of eating less meat (Van der Weele et al., 2019) and switching from meat-centred meals to meals based on pulses, vegetables, nuts, mushrooms, algae, seaweed or insects (Aiking and de Boer, 2018). This has led to a search for alternatives, including plant-based meat alternatives (PBMAs).

*Meat alternatives overview.*

PBMAs designed to be meat analogues approximate the aesthetic qualities (primarily texture, flavour, and appearance) and/or chemical characteristics of specific types of meat. Some vegetarian meat analogues are based on centuries-old recipes for wheat gluten, rice, mushrooms, legumes, tempeh, or pressed-tofu, with flavouring added to make the finished product taste like chicken, beef, lamb, ham, sausage, seafood, etc. Analogues simulating coarse ground-meat products such as burgers may contain textured proteins (such as textured soy flour and concentrates). In addition, mycoprotein from a filamentous fungus (*Fusarium venenatum*) is another protein used to create a variety of plant-based products (Malav et al., 2015).

A recent publication by Van der Weele et al. performs an integrative comparison of meat alternatives by considering the nutritional implications, potential sustainability gains and required technological and social-institutional change of five meat alternatives (i.e., cultured meat, algae, insects, plant-based meat alternatives (PBMAs) and pulses). Pulses, existing PBMAs and whole insects offer the greatest environmental gain along with the lowest required technological innovation. However, novel PBMAs with highly refined ingredients need moderate technological innovation, but this will still contribute to the environmental gain as much as existing PBMAs. Cheese/dairy and egg-based meat alternatives also require low technological innovation, but they have low environmental gain. Finally, moderate and high technological innovations are needed to develop meat alternatives based on protein extracted from insects, cultured meat and algae. Furthermore, the potential impact on environmental gain of these alternatives is still uncertain (see figure **3**).

| Sustainability gains | Required technological innovation | | |
|---|---|---|---|
| | Low | Moderate | High |
| High | Pulses | | |
| Moderate | Existing PBMA<br>Whole insects | Novel PBMA with highly refined ingredients | |
| Low | Cheese/ Dairy<br>Eggs | | |
| Uncertain | | Protein extracted from insects | Cultured Meat<br>Algae |

*Figure 3 Sustainability gains and required technological innovation of meat alternatives (Van der Weele et al., 2019).*

Within the meat alternatives with high and moderate environmental gain, PBMAs need the lowest social-institutional change, followed by beans and ultimately by insects (Van der Weele et al., 2019) (See figure **4**).

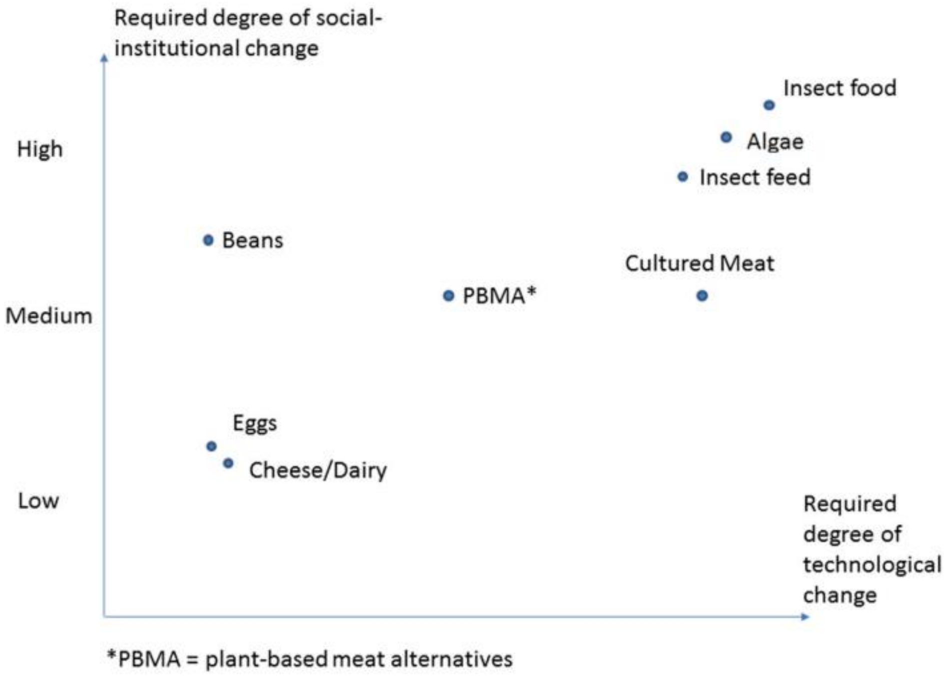



*Figure 4 Required degree of social-institutional and technological change of meat alternatives (Van der Weele et al., 2019).*

From the nutritional perspective and in the context of global protein overconsumption (see figure **2**), animal-sourced food intake in developed countries can be reduced by at least one third without any replacement and this would not result in protein deficiency (Van der Weele et al., 2019). However, the quality of protein consumed is also important. While meat is recognised as a good source of essential amino acids among other nutrients, plant foods (and hence PBMAs) have an inferior amino acid composition (Van der Weele et al., 2019). Compared to animal foods, cereals, nuts and seeds contain reduced amounts of lysine but have similar content of sulphur amino acids (cysteine and methionine). In contrast, legumes tend to have higher concentrations of lysine than other plant-foods and reduced content of sulphur amino acids (Yu, 2009). For this reason, a diet that includes a variety of pulses will contribute to the necessary intake of the essential amino acids (Messina, 2014, Melina et al., 2016). However, the bioavailability of proteins varies across food sources and this has been linked to the presence of anti-nutritional factors (ANFs) in plant foods (FAO, 2013). ANFs are substances that impair nutrient absorption and are naturally occurring secondary metabolites that protect the plant against some biological stresses such as pest attacks. These metabolites include tannins and phytic acid (PA) (Avilés-Gaxiola et al., 2018) and are present in higher concentrations in the outer layers of the grain (Filho et al., 2017). These compounds have the ability to form insoluble complexes with proteins and minerals, which reduce their bioavailability (Sant' Ana et al., 2019). Also, the digestibility of plant proteins is affected by vegetal cell walls and seed coats as they reduce digestive enzyme accessibility (Sa et al., 2019). Nevertheless, this generalised reduced protein digestibility might not be the case of PBMAs as they are usually based on pulses and grains that have been processed and are incorporated into the product as isolates, concentrates or extrudates (Kumar et al., 2017, Malav et al., 2015). Processing usually applied to these foods can be very effective to improve the protein quality and digestibility. These methods are physical processes and dry or wet fractioning to produce flours (20 – 30% protein content), enriched flours (30 – 50%), concentrates (40-80%) and isolates (>90%). Also, thermal processing and extrusion usually increase protein digestibility (Sa et al., 2019). In fact, isolated soy protein (the most common source of protein in PBMAs) has a Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1, the highest possible rating and equivalent to animal protein (Kumar et al., 2017, Hughes et al., 2011). Although PDCAAS has been used for years to assess protein digestibility, the use of a new method called Digestible Indispensable Amino Acid Score was proposed and recommended in 2014 by FAO (FAO, 2013, Mathai et al., 2017). Isolated soy protein has been reported to have a DIAAS of 98 % (with sulphur amino acids (SAA) as the first limiting amino acids). This is lower than the values reported for whey protein isolate (125%, histidine as limiting amino acid), but it is also higher than the DIAAS for other plant proteins such as pea protein concentrate (73%, SAA limiting SAA) (Mathai et al., 2017).

Reducing meat intake may have other consequences for human health. For example it has been reported that a 47% reduction of red and processed meat led to a fall in white cell count (Simpson et al., 2019). Meat is a good source of minerals such as iron and zinc. According to FAO data, approximately 2 billion people in the world suffer from micronutrient deficiencies. Specifically, 30% of global population is anaemic, many due to iron deficiency, and an estimated 17.3% is at risk of inadequate zinc intake. Thus, both zinc and iron deficiencies constitute a significant public health problem (Dahdouh et al., 2019).

Dietary iron is often classified as haem iron and non-haem iron, present in animal and plant food sources and generally in the ferric form ($Fe^{3+}$). Non-haem iron is obtained from plant sources, while meat is a primary source of haem iron, although small amounts are present in some plants and fungi. Iron from animal foods is better absorbed than from plant foods (EFSA, 2015). Even though meat is a good source of iron and is overconsumed, iron deficiency is a worldwide public health issue attributed to more than 60% of anaemia cases (Tiekou Lorinczova et al., 2020), affecting both industrialised and non-industrialised countries (WHO&UN, 1993). In 2014, the World Health Organisation set a target of 50% reduction of anaemia in women of reproductive age by 2025 (WHO, 2014). However, the 0.2 - 0.3% per year improvement in anaemia in the past two decades (Mason et al., 2013) is insufficient to meet the WHO targets. As Western diet is high in saturated fat and low in fibre, current recommendation to switch Western society to a more plant-based diet will benefit sustainability and human health (Willett et al., 2019, Aiking and de Boer, 2018). However, this necessary dietary change might compromise iron status, and hence this risk needs to be assessed. Muscle and liver tissues contain partially digested cysteine-containing peptides which are the main enhancers of iron absorption. In contrast, other proteins block iron absorption. This is the case of the conglycinin fraction of soy protein (EFSA, 2015), commonly used as the main ingredient of PBMAs.

ANFs such as phytic acid and iron-binding phenolic compounds (tannins) are also a known inhibitors of iron absorption that reduce the bioavailability of iron from plant foods (WHO, 2004).

Zinc is a component of more than 300 enzymes involved in multiple metabolic processes including gene expression and metabolism of other micronutrients. Zinc also has a central role in cellular and humoral immunity. Both animal (lean red meat) and plant foods (whole-grain cereals, pulses, and legumes) are good sources of zinc, but again ANFs impair zinc absorption as well, making plant foods poorer sources of zinc. Again, dietary proteins in a meal also influence zinc absorption. It has been shown that animal protein (chicken, beef) increases zinc absorption. Remarkably, zinc absorption from legume-based diets (e.g. white bean and lupin protein) is comparable with that from animal protein-based diets despite a higher ANFs content (WHO, 2004).

PA or Phytate is myo-inositol hexaphosphate, which is made up of an inositol ring with six phosphate groups (figure **5**) and is the plant storage form of phosphorus, so is found in high concentrations in seeds, cereals, and pulses . The antinutritional effects of PA are due to its high cation binding capacity, explained by its double charged phosphate groups and the inability of human digestive enzymes to degrade it. PA is also important to consider when assessing iron and zinc bioavailability (Dahdouh et al., 2019). Its cation binding capacity is a function of the number of phosphate groups on the inositol ring. There are six inositol phosphate forms, each of which is named according to the number of phosphate groups attached to the inositol ring, i.e. IP1 to IP6. Inositol hexaphosphate (IP6) contains 6 phosphate groups, is the most abundant form in mature unprocessed cereals, legumes and oleaginous seeds which have not been stored, and the strongest in terms of mineral binding capacity. It can be degraded by simple processing methods, such as soaking, germination, and fermentation (Gabaza et al., 2018, FAO/IZiNCG, 2018), converting IP6 to lower IPs, which interfere less with the bioavailability of zinc and iron (FAO/IZiNCG, 2018). PA is relatively heat stable during normal household boiling temperatures of 100ºC, but in industrial processing such as canning or extrusion cooking when higher temperatures are used there will be some loss(FAO/IZiNCG, 2018). Mechanical processing such as milling unrefined cereals and dehulling legumes can also lead to significant reductions in phytate, as well as minerals  (FAO/IZiNCG, 2018).

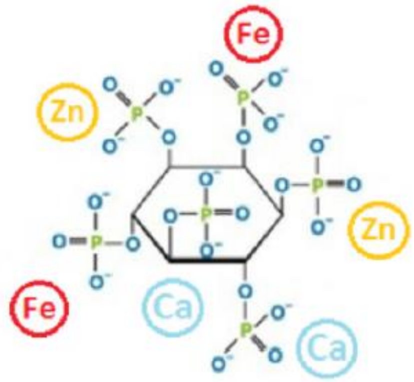



*Figure 5 IP6 structure and mineral binding capacity, from FAO/IZiNCG, Rome, 2018 (FAO/IZiNCG, 2018)*

In processed foods (i.e. fermented, boiled, soaked, etc.), significant amounts of IP6 are degraded to lower IPs, and therefore the relative amounts of IP5, IP4 and IP3 increase. In processed foods, a range between 3 and 84% of IPs (in cereals, legumes and pulses) are from lower IPs values compared to IP6. Often, the values are around 30 - 40% of lower IPs to IP6 (Figure **6**) (Dahdouh et al., 2019). Based on the evidence above it might be predicted that PBMAs originally rich in IP6 may have improved iron and zinc availability due to the impact of industrial processing on PA cation binding capacity.

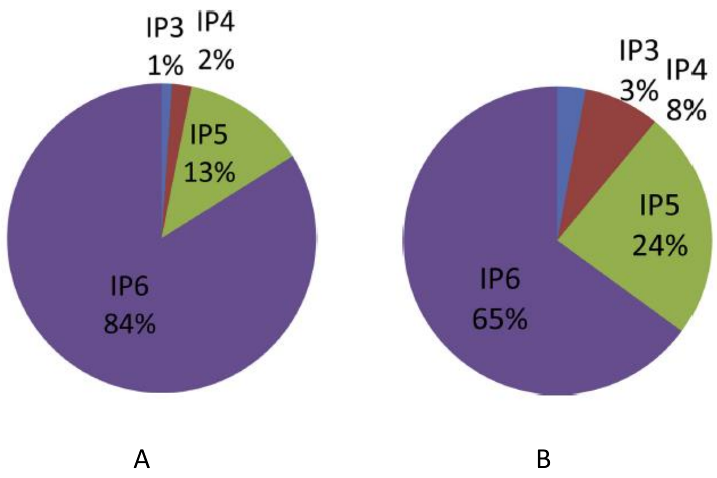



*Figure 6 Amount of IPs in raw (A) and processed (B) foods from PhyFoodComp by Dandouh et al (Dahdouh et al., 2019)*

Because the negative effects of phytate on zinc and iron absorption are dose dependent, the use of phytate:iron molar ratio as well as phytate:zinc molar ratio is recommended to estimate the impact of phytate on zinc and iron bioavailability (FAO/IZiNCG, 2018).

The other main ANF are tannins (figure **7**), which are polyphenols present in plants. They are generally classified into hydrolysable and condensed tannins. The hydrolysable forms are quickly degraded and are present in small amounts in foods. Condensed tannins are polymerised products of flavan-3-ol (cathechin) and flavan-3,4-diol (or mixtures of these). They are also referred to as flavolans or procyanidins and are the main polyphenols in foods. In general tannins are resistant to heat, and the industrial processes that can reduce their content in foods (dehulling, soaking, germination and addition of chemicals with a high affinity for tannins) are laborious and/or expensive (Sarwar Gilani et al., 2012). Tannins have the ability to form insoluble precipitates with proteins (reducing their absorption), and to prevent iron from being absorbed through the formation of insoluble complexes with the mineral (Delimont et al., 2017).

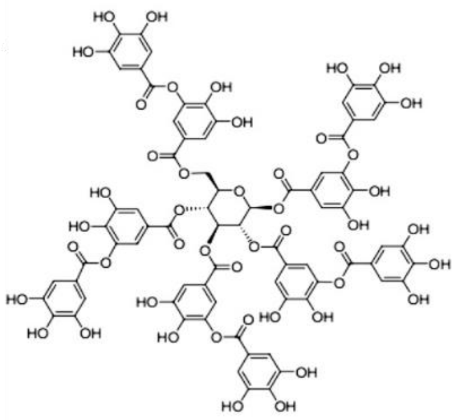


*Figure 7 Tannic acid from Delimont et al.(Delimont et al., 2017)*

*Effect of cooking on nutritional profile*

Cooking foods has been shown to impact nutrient composition, mineral bioaccessibility and protein digestibility (Liu et al., 2019, Sa et al., 2019, Margier et al., 2018). Mineral content reduces with cooking (Margier et al., 2018, Meiners et al., 1976, Kimura and Itokawa, 1990), especially when boiling (Kimura and Itokawa, 1990). Cooking can positively affect protein digestibility as it causes protein denaturation, reduced resistance to enzymes attack and leaching out of unfavourable components. It has been shown that protein digestibility of peas increases with cooking due to a reduction in PA and tannin content, among other reasons. However, overheating may depress protein digestibility owing to an increased hydrophobicity, non-enzymatic browning, thermal cross-linking and the formation of toxic compounds and complexes between proteins and tannins/phytates (Sa et al., 2019).

# 2 Aims of study.

This thesis is focused on examining the nutritional composition and digestibility of PBMAs. In addition, another goal of the project was to investigate whether the process of cooking influenced the nutritional composition of the foods. To address this question, the following approaches were taken:

1. Proximate analysis of cooked and raw PBMA. This included determination of the energy, protein, lipid, fibre, and ash contents, as well as the mineral, amino acid and fatty acid compositions.
2. Analysis of ANF such as phytic acid and total phenolics (tannins).
3. Digestibility of proteins and bioaccessibility of minerals (Zn and Fe).

# 3 Methods.

## 3.1 Description of samples and cooking.

Samples were different plant-based burgers and a beef burger, which was used for comparison purposes. All burgers were commercially available in supermarkets in the UK. Most of the burgers were presented in packs of two units, although some of them were in packs of 4 and 8 units. As burgers arrived at the laboratory, they were stored in the freezer at -20 °C until they were used for relevant analysis.

*Set 1 of burgers*. This comprised of a group of six different burgers, one being a beef burger (see Table 1). One burger from each pack was cooked and the rest were left raw. Both cooked and raw samples were then used for proximate analysis. A full description of their ingredients and nutritional facts can be found in appendices 1a and 1b.

*Table 1. List of burgers in set 1.*

| BRAND | PRODUCT NAME AND DESCRIPTION | CODE |
|---|---|---|
| Vivera | Seasoned vegetarian burgers made with rehydrated textured soya protein and red onion. | VIV 1 |
| Sainsbury | The Smoky Jack Quarter Pounder Smoky Mushroom & Jackfruit vegan burgers. | SMJ |
| The Meatless Farm | Meat free burgers made with soya, pea and rice proteins. | TMF |
| Quorn | Meat free savoury burger, made with mycoprotein. | QUO |
| Birds Eye | Beef quarter pounders with chopped onion and seasoning. | BEB |
| Sainsbury | Mixed vegetable burgers with chilli and coriander. | SMX |

*Set 2 of burgers*. A second set of burgers was obtained to perform mineral bioaccessibility, protein digestibility assays and determination of the ANFs present. As burgers are intended to be consumed cooked prior to consumption, all burgers were cooked for these analyses to get a more realistic insight. We aimed to acquire the same six burgers used in set 1, but only three of those burgers were available, therefore three new burgers were purchased as well as the 3 originals (Table 2). Once again, a full description of their ingredients and nutritional facts can be found in appendices 2a and 2b.

*Table 2 List of burgers in set 2. *Burgers different from set 1.*

| BRAND | PRODUCT NAME AND DESCRIPTION | CODE |
|---|---|---|
| Vivera | Plant-based seasoned burger made from rehydrated soya and wheat protein (with added iron and vitamin B12). * | VIV 2 |
| Sainsbury | Seasoned vegetarian burgers with onion and parsley * | SOP |
| The Meatless Farm | Meat free burgers made with soya, pea and rice proteins. | TMF |
| Quorn | Meat free savoury burger, made with mycoprotein. | QUO |
| Birds Eye | Beef quarter pounders with chopped onion and seasoning. | BEB |
| Sainsbury | Quinoa, sweet potato & lentils burgers seasoned with garlic puree, chipotle chilli and garam masala * | SQU |

**Cooking.** Even though the packaging on each burger  suggested a different cooking method, we performed a standard cooking method for all samples, cooking in a fan oven (Lamona double oven LAM4405, Howdens, London, UK) at 210°C for 15 minutes. The temperature of each burger was confirmed to be over 70°C as they were removed from the oven. Then, burgers were cooled for 5 minutes and refrozen. All samples were weighed before and after cooking to calculate weight losses that can be seen in Appendix 3.

Afterwards, both cooked and raw burgers from set 1 were first freeze-dried and ground with a hand blender to reduce burgers to smaller pieces and then with a coffee grinder to eventually transform burgers into powder, ready for subsequent analysis. Set 2 of burgers was divided into three aliquots after cooking: one for in vitro digestion to study iron and zinc bioaccesibility, one for in vitro digestion to assess protein digestibility and another one for analysis of ANFs. Aliquots intended for in vitro digestion did not need freeze-drying, whereas those intended for determination of ANFs did. Details on the moisture and dry weight of samples can be found in Appendix 4.

## 3.2 Bomb calorimetry

To determine total energy content, freeze-dried cooked and raw burgers from set 1 were used. The bomb calorimeter was a Parr 6400 model from Parr Instrumental Company (Illinois, USA). Operational work consisted of loading 1 g of sample placed in a crucible in the bomb head. The ignition string was attached so that it touched the sample and the ignition wire. Once the calorimeter bomb lid was shut the apparatus was programmed and the sample was run in determination mode.

## 3.3 Protein determination.

50 mg of freeze-dried, cooked and raw burgers were weighed into tin foil capsules and crimped. Aspartic acid (CE instruments; 10.52% N) was used as a standard. Both samples and standards were placed in the autosampler and run on the EA1112 organic elemental analyser (Thermo Fisher) in N/Protein configuration. A calibration curve was created with a bypass (50mg of aspartic acid), blanks and standards. Then this curve was used to determine the nitrogen content of each sample. Finally, nitrogen was converted to protein using a conversion factor of 6.25.

## 3.4 Total mineral content.

Total mineral content was analysed on a triple quadrupole ICP-MS (inductively coupled plasma-mass spectrometry)(iCAP TQ, Thermo-Fisher Scientific, Bremen, Germany) on freeze-dried cooked and raw burgers from set 1. Samples were pre-treated with microwave acid digestion. This was performed by digesting 0.2g of each sample with 6 ml of $NHO_3$ (Primar Plus™) in the microwave (Anton Paar GmbH, St. Albans, U.K.). After digestion, samples were diluted with 14 ml of milliQ water. Prior to ICP-MS analysis, 1 ml of each digested sample was diluted with 9 ml of milliQ water. The concentrations of 32 minerals was obtained: lithium, beryllium, boron, sodium, magnesium, aluminium, phosphorus, sulphur, potassium, calcium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, germanium, arsenic, selenium, rubidium, strontium, molybdenum, silver, cadmium, cesium, barium, thallium, lead and uranium.

## 3.5 Total phenolics determination (tannic acid equivalent).

This assay was performed on cooked and freeze-dried burgers from set 2 following the Folin Ciocalteu method described by FAO/IAEA (FAO/IAEA, 2000). 0.1g of samples were diluted in 4 ml of 0.1M NaOH (Sigma Aldrich) and centrifuged for 10 minutes at 10000 rpm. 25 µl of the supernatants were dispensed into a 96 well plate (Sarstedt Ag & Co. KG, Germany) along with previously prepared tannic acid standards (0, 0.2, 0.4, 0.6, 0.8 and 1mg/ml). 200 µl of water, 25 µl of $Na_2CO_3$ and 25 µl of Folin Ciocalteu reagent (both from Sigma Aldrich) were added to all wells. After 1 hour of incubation the plate was placed in a plate reader (Bio-Rad Laboratories, Inc. Model 680 XR) and absorbance read at 655nm.

## 3.6 Phytic acid assay and calculation of phytate:mineral ratios.

Cooked and freeze-dried burgers from set 2 were used for phytic acid determination. For this purpose, an enzymatic kit from Megazyme International Ireland was used and the procedure was conducted following the manufacturer protocol. Reagents not provided in the kit were procured from Sigma Aldrich. The method principle is the release of total phosphorus from food by the action of phytase and alkaline phosphatase. Then, total released phosphorus is measured by colorimetric method. Prior to enzymatic treatment, burgers were subjected to acid extraction of inositol phosphates. Phytase provided in the kit, which is specific for phytic acid ($IP_6$) and the lower myo-inositol phosphate forms (i.e. $IP_2$, $IP_3$, $IP_4$ and $IP_5$), releases their phosphates. Hence, alkaline phosphatase is used to release the final phosphate from $IP_1$. Afterwards, the total phosphate released reacts with ammonium molybdate in acidic conditions to form molybdenum blue. After incubation for 1 hour in a water bath at 40 °C, absorbance was read in a spectrophotometer at 655 nm. The standard curve was used to calculate concentration of phytic acid in the burgers

Calculation of the phytate:mineral molar ratios was done as described by Dahdouh et al. (Dahdouh et al., 2019)

$$\frac{\frac{Phytate\ (mg)}{660\ (MW)}}{\frac{Zn\ (mg)}{65.38\ (AtW)}}$$

*Equation 1 PA:Zn molar ratio*

$$\frac{\frac{Phytate\ (mg)}{660\ (MW)}}{\frac{Fe\ (mg)}{55.845\ (AtW)}}$$

*Equation 2 PA:Fe molar ratio*

Where:

- Atw: Atomic weight

- MW: Molar weight

Because total iron and zinc was determined on burgers from set 1, and phytic acid was analysed on burger from set 2, phytate-mineral molar ratios were calculated for the three burgers that were present in both sets, i.e. The Meatless Farm, Quorn and Birds Eye Beef.

### 3.7 In vitro digestion with Infogest method.

Digestion of burgers was performed using the Infogest method described by Brodkorb et al (Brodkorb et al., 2019) and Minekus et al (Minekus et al., 2014). It is a static in vitro simulation of gastrointestinal food digestion and a standardized and international consensus method (Figure **8** shows an overview of the method).

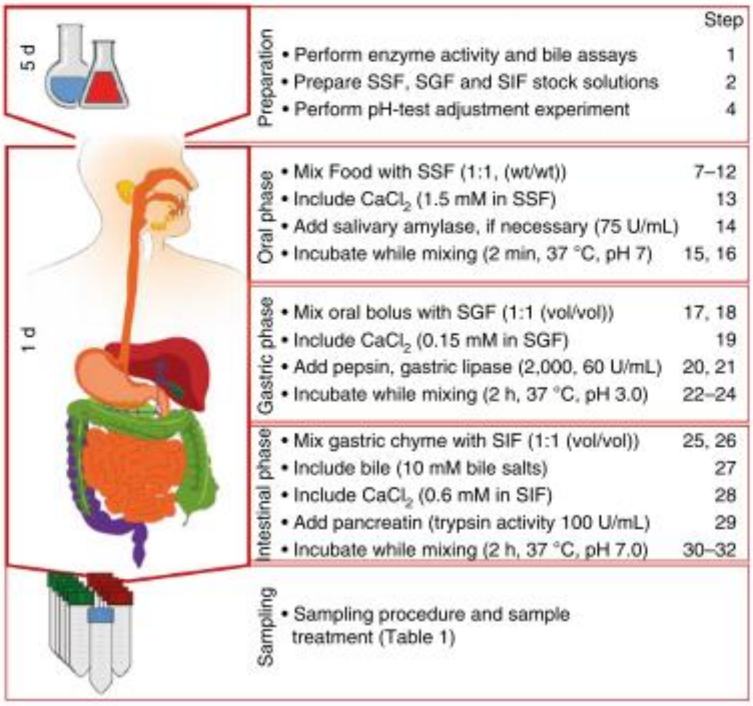



*Figure 8 Flow diagram of the INFOGEST 2.0 digestion method from Brodkorb et al (34).*

This technique was used to determine protein digestibility and mineral bioaccessibility, with some modifications of the procedure implemented for the latter and described in the section called determination of iron and zinc bioaccessibility.

The protocol can be divided into three parts: preparation, digestion procedure and sample treatment for subsequent analysis. Prior to the day of the in vitro digestion, enzymes and electrolyte solutions simulating digestion fluids (i.e. Simulated salivary fluid (SSF), simulated gastric solution (SGS) and simulated intestinal fluid (SIF)) were prepared. A description of the simulated digestion fluids can be seen in table 3. The digestion procedure consisted of three phases (oral, gastric and intestinal) to which samples were exposed sequentially. Each phase was initiated by mixing samples with the corresponding simulated digestion fluid (1:1) along with enzymes (i.e. amylase in the oral phase, pepsin in the gastric phase and pancreatin and bile in the intestinal phase). A specific pH value needs to be achieved in each phase so 0.01 M NaOH or 6 M HCl were used to bring the pH to 7 (oral and intestinal phases) or 3 (gastric phase), then samples were incubated with added reagents at 37 °C in a shaking water bath. The timing of the incubation was 2 minutes in the oral phase, and 2 hours in the gastric and another 2 hours in the intestinal phases.

*Table 3. Volumes of electrolyte stock solutions of digestion fluids for a volume of 400 mL diluted with water (1.25× concentrations). Simulated digestion fluids for the oral (SSF), gastric (SGF) and intestinal (SIF) digestion phases are mixed at a 1.25× concentration using the electrolyte stock solutions and water according (Brodkorb et al., 2019).*

| CONSTITUENT | STOCK CONCENTRATION (g/L) | SSF (mL) | SGF (mL) | SIF (mL) |
|---|---|---|---|---|
| KCl | 37.3 | 15.1 | 6.9 | 6.8 |
| $KH_2PO_4$ | 68 | 3.7 | 0.9 | 0.8 |
| $NaHCO_3$ | 84 | 6.8 | 12.5 | 42.5 |
| NaCL | 117 | 0 | 11.8 | 9.6 |
| $MgCl_2(H_2O)_6$ | 30.5 | 0.5 | 0.4 | 1.1 |
| $NH_4CO_3$ | 48 | 0.06 | 0.5 | 0 |
| $CaCl_2(H_2O)_2$ | 0.3M | 0.025 | 0.005 | 0.04 |
| HCl (adjust accordingly) | 6 M | pH 7 | pH 3 | pH 7 |

Materials for in vitro digestion were as follows: NaOH, HCl, KCl, $KH_2PO_4$, $NaHCO_3$, NaCl, $MgCl_2(H_2O)_6$, $(NH_4)_2CO_3$ and $CaCl_2$ $(H_2O)_2$ were provided by Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). α-amylase from Bacillus sp. (≥ 400 units/mg protein), pancreatin from porcine pancreas (8xUSP),

bovine bile, 1,4-piperazinediethanesulfonic acid disodium salt (PIPES) and dialysis tubing (high retention seamless cellulose tubing, average flat width 23 mm, molecular weight cut-off 12,400 kDa) were all from Sigma Aldrich (Dorset, UK) products. Stable isotopes ($^{57}Fe$ and $^{70}Zn$) (95% enrichment) were from Isoflex, USA. Pefabloc was from Fisher Scientific, Loughborough, UK.

### 3.7.1 Determination of protein digestibility.

After digestion, the tubes were centrifuged at 4,500 × *g* for 30 minutes and the supernatant was separated from the pellet and both fractions were collected and stored for subsequent analysis. Pellets were subjected to total protein determination to measure insoluble protein content in burgers. To that end, the procedure was the same as described for protein determination of cooked and raw burgers in the section above. Digestibility of proteins was calculated using equation below.

$$Protein\ digestibility\ \% = \frac{P_{tot} - P_{ins}}{P_{tot}} * 100$$

Where $P_{tot}$ is the total protein (mg/100g) in cooked burger and $P_{ins}$ is the insoluble protein in pellets after in vitro digestion (mg/100g).

### 3.7.2 Determination of iron and zinc bioaccesibility.

Iron and zinc bioaccessibility was determined after in vitro digestion following the INFOGEST method (Brodkorb et al., 2019, Minekus et al., 2014) with some modifications; (i) pancreatin was added in order to achieve a final enzyme activity of 100 U/mL protease activity in the final digestion mixture and bile was added to achieve a concentration of 2 mM in the final digestion mixture. The reduction in pancreatin and bile acid was in order to reduce their iron and zinc concentration in the digesta and these concentrations have previously been shown to be sufficient in the determination of iron and zinc bioaccessibility (Glahn et al., 2015). (ii) Isotopic labelling of reagent iron ($^{57}Fe$) and zinc ($^{70}Zn$) was done in order to discriminate between reagent and sample derived iron and zinc in the different sample matrices since recovery of reagent iron and zinc was matrix dependent. In this regard, $^{57}Fe$ and $^{70}Zn$ was applied to complete digestion fluids i.e. simulated digestion fluids including specific enzymes and Milli-Q water to achieve required dilution as stipulated in the INFOGEST method, prior to the digestion. The complete digestion fluids were incubated in a shaking water bath at 20°C overnight, to allow for complete isotopic equilibration. The stable isotopes were added to each digestion solution at a level 10× their concentration in the respective solutions. (iii) dialysable iron and zinc were defined as bioaccessible iron and zinc meaning soluble, low molecular weight iron or zinc (< 12.4 kDa). Thirty minutes before the end of gastric digestion, dialysis tubes containing 17.5 mL of piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) buffer were inserted into the digestion vessels and the digestion was continued. Intestinal digestion followed with the addition of simulated intestinal digestion fluids with the dialysis tubes inside. After intestinal digestion, the dialysis tubes were carefully removed, and the dialysate (bioaccessible fraction) was transferred into clean storage tubes. The solution outside the dialysis tubes was centrifuged at 4,500 × *g* for 30 minutes and the supernatant, termed the soluble non dialysed fraction (SND), was further filtered through a 5μm syringe filter. The dialysate (or soluble dialysed (SD)) and soluble non-dialysed (SND) fractions were analysed for iron and zinc using ICP-MS as described in the next section. Bioaccessibility of iron ($Fe_{bio}$%) and zinc ($Zn_{bio}$%) was calculated using the equation below for iron as an example.

$$Fe_{bio} = \frac{Fe\_dialysate}{Fe_{tot}} * 100$$

Where $Fe_{dialysate}$ is the concentration of iron in the dialysate fraction (mg/kg) and $Fe_{tot}$ is the total iron concentration in the burger (mg/kg). Soluble non dialysed (SND) iron and zinc (%) was also calculated relative to the total iron or zinc in the burger. The iron and zinc concentration in the SND and dialysate were also summed up to give the total soluble iron and zinc, expressed in mg/kg.

### 3.7.3 Determination of iron and zinc in the dialysate and SND fractions

An aliquot of 3 ml of SND fraction was digested with 3 ml of $HNO_3$ (Primar Plus™), while 4 mL of the dialysate fraction was digested with 2 mL of 50% $HNO_3$. After microwave digestion, all samples were diluted to 20 mL with milliQ water. Prior to ICP-MS analysis, all samples were diluted accordingly to obtain an acid concentration of < 5%. An elemental analysis was done including the isotopes $^{57}Fe$ and $^{70}Zn$. Tomato leaves standard reference material (National Institute of Standards and Technology, Tomato leaves 1573a) was used to calculate iron and zinc recovery after ICP-MS.

## 3.8 Statistical analysis.

In order to identify significant differences between means, analysis of variance was performed using Genstat® (19$^{th}$ and 20$^{th}$ editions) statistical software. In the case of iron and zinc bioaccessibility, a 2-way ANOVA was used by setting burgers and digested fractions (SD and SND) as the independent variables. Similarly, for total minerals, total proteins, and total caloric content, a 2-way ANOVA was run with the product (burgers) and cooking method (raw or cooked) as independent variables. For the antinutritional factors, 1-way ANOVA was done to determine significant differences between products in the total phenolics content, phytic acid content, as well as in molar ratio of phytic acid and iron, and phytic acid and zinc. In all the statistical analyses, when significant differences were found, post-hoc Bonferroni tests were used to identify which groups differed. Statistical significance was accepted as $p<0.05$.

Additionally, correlation analyses were conducted to determine the relationship between antinutritional factors (i.e. phytic acid and phenolics), and zinc and iron SD and SND fractions. $R^2$ was calculated using Microsoft Excel, with the $R^2$ cut-off value at $p<0.05$ taken from Peacock et al (Peacock, 2011).

# 4 Results.

## 4.1 Energy content in cooked and raw burgers

There was a significant product*cooking interaction for energy content (p<0.001). Cooked and raw versions of every product differed significantly in their energy content. For all plant-based burgers, cooking increased the energy content, whereas cooking decreased the energy content of the beef burger. All raw burgers had significantly different energy contents, except for Vivera 1 and the Meatless Farm burgers. Within the raw burgers, the beef burger had the highest energy content, followed by Quorn, Vivera 1, The Meatless Farm and the two Sainsbury's burgers. All cooked burgers had significantly different energy contents, and the most energy dense burger was Quorn, followed by the Beef burger (figure **9**).

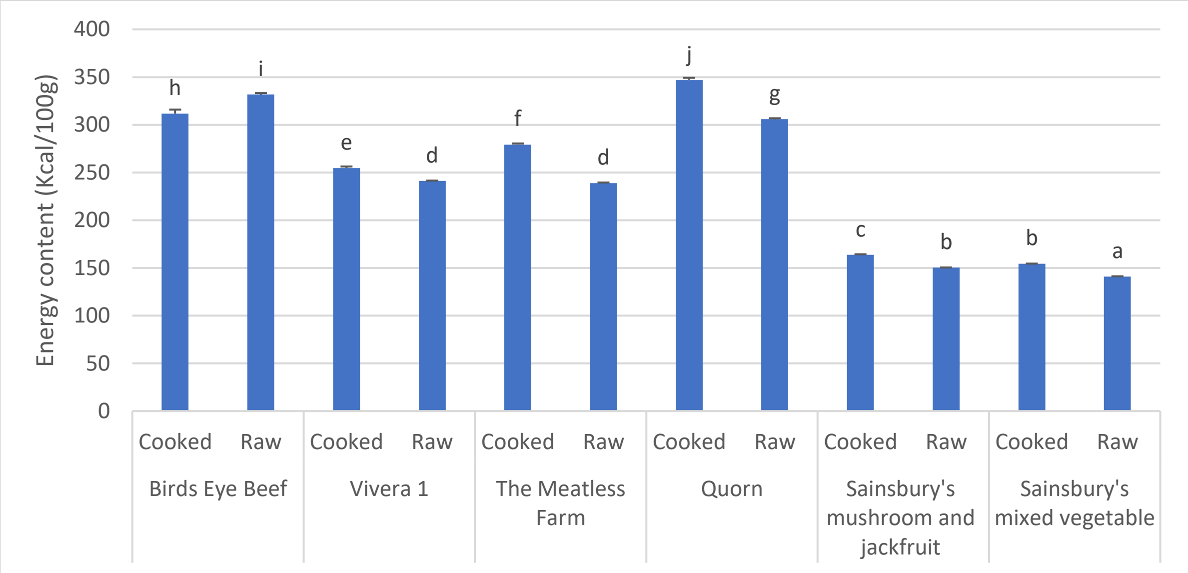



*Figure 9 Energy content in burgers. Letters above the blue bars represent differences in energy content across all cooked and raw burgers (p<0.001 for product*cooking interaction). The error bars represent standard deviation, and the number of replicates was 3.*

## 4.2 Protein content in cooked and raw burgers

There was a significant product*cooking interaction for protein content (p<0.001, figure **10**). None of Sainsbury's burgers, except the Sainsbury's Onion and Parsley, showed differences in their protein content and cooking did not have any effect. Besides, these burgers had a significantly lower protein content than the rest of burgers. In the rest of the burgers, cooking increased the protein concentration except for Vivera 2, for which protein levels did not change significantly with cooking. Within the raw versions, the Birds Eye Beef and the Sainsbury's Onion and Parsley burgers had the lowest protein contents. Vivera 1 and 2, The Meatless Farm and Quorn had a higher protein content and showed few differences between them. For the cooked burgers, The Meatless Farm and Quorn had the highest protein concentrations, with the rest of the burgers showing few differences between them.

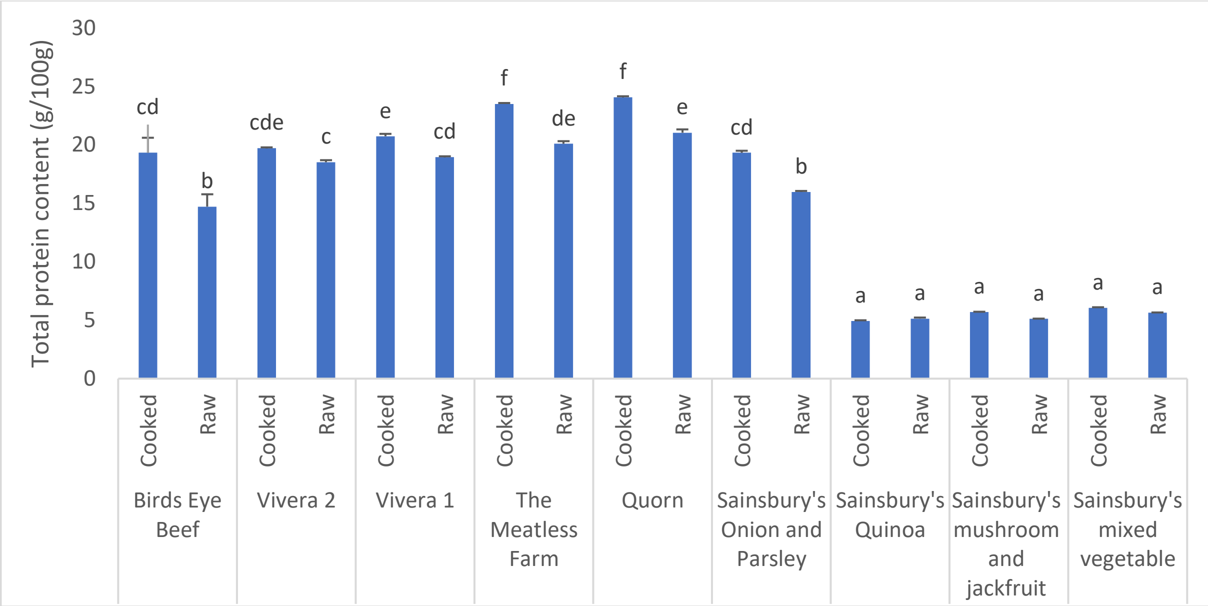



*Figure 10. Total protein content in burgers. Letters above the blue bars represent differences in protein content across all cooked and raw burgers (p<0.001 for product*cooking interaction). The error bars represent standard deviation, and the number of replicates was 3.*

## 4.3 Total mineral content in cooked and raw burgers

Iron. There was a significant product*cooking interaction for iron content (p<0.001, figure **11**). Cooking significantly increased the iron concentration for the Meatless Farm and Quorn burgers. In the rest of burgers there was no significant difference between the cooked and the raw versions. The Meatless Farm and Vivera 1 had the highest level of iron (in that order), both cooked and raw. There were few differences between the other burgers, with only the cooked Quorn burger having a higher iron content.

Zinc. There was a significant product*cooking interaction for zinc content (p<0.001, figure **11**). Cooking significantly increased the zinc content of Birds Eye Beef, The Meatless Farm and Quorn, which, in addition, are the burgers with the highest levels of zinc. Again, the Meatless Farm burger showed the greatest zinc content, followed by the beef and mycoprotein burgers.

Manganese. There was not a significant product*cooking interaction for manganese content (p=0.069, figure **11**), but there were significant differences for cooking (p=0.009), which increased manganese content, and for product (p<0.001). The meat burger was significantly the poorest in this mineral, followed by the Sainsbury’s burgers. Vivera 1, the Meatless Farm and Quorn burgers had the highest values for manganese content, which did not differ between them.

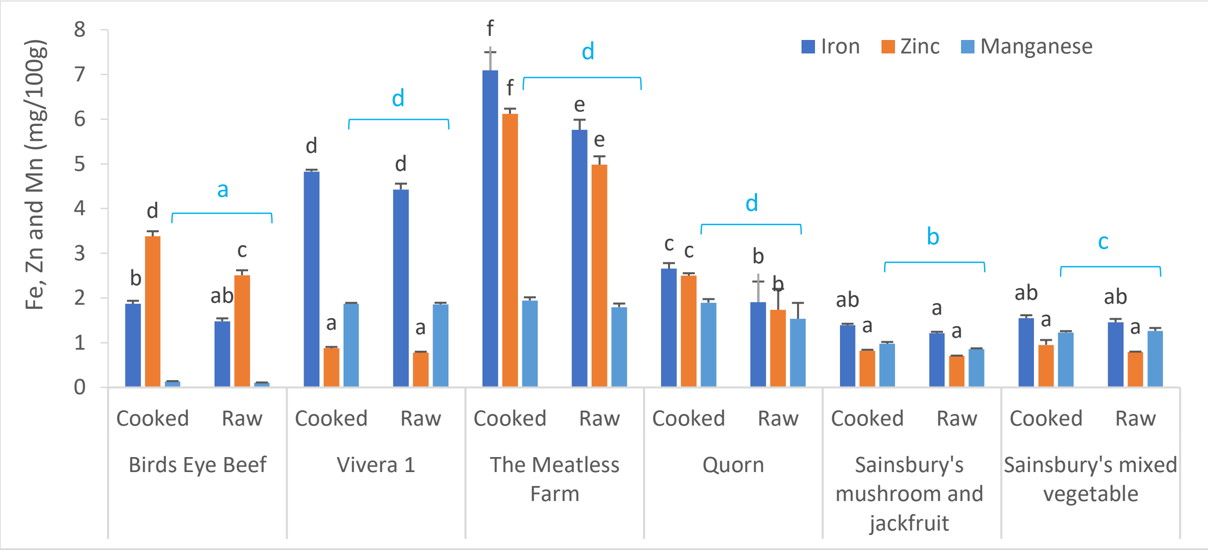



*Figure 11. Iron, zinc and manganese content in burgers. Letters above the blue horizontal lines represent differences for manganese between products (product p<0.001, product*cooking interaction p=0.069 and cooking p=0.009). Letters above the vertical bars represent differences for iron and zinc across all cooked and raw burgers: iron (p<0.001 for product*cooking interaction); Zinc (p<0.001 for product*cooking interaction). In all cases the error bars represent standard deviation, and the number of replicates was 3.*

Magnesium. There was a significant product*cooking interaction (p=0.027, figure **12**). The Birds Eye beef and Quorn burgers had the lowest magnesium contents, with the raw appearing to be slightly lower than the cooked burgers. The other burgers differed significantly in their magnesium levels, but there were no differences between the cooked and raw versions. Magnesium levels were highest in The Meatless Farm burger and decreased in the order: Vivera 1> Sainsbury's mixed vegetable > Sainsbury's mushroom and jackfruit.

Calcium. There was a significant product*cooking interaction (p=0.041, figure **12**). The beef burger was the poorest source of calcium, while The Meatless Farm burger was the richest. Within the plant-based burgers, the two Sainsbury's burgers had a lower calcium concentration, while Vivera, Quorn and The Meatless Farm burgers contained similar higher levels of calcium. Cooking only impacted (increased) the calcium concentration for the Quorn burger, with no differences between cooked and raw for the other products.

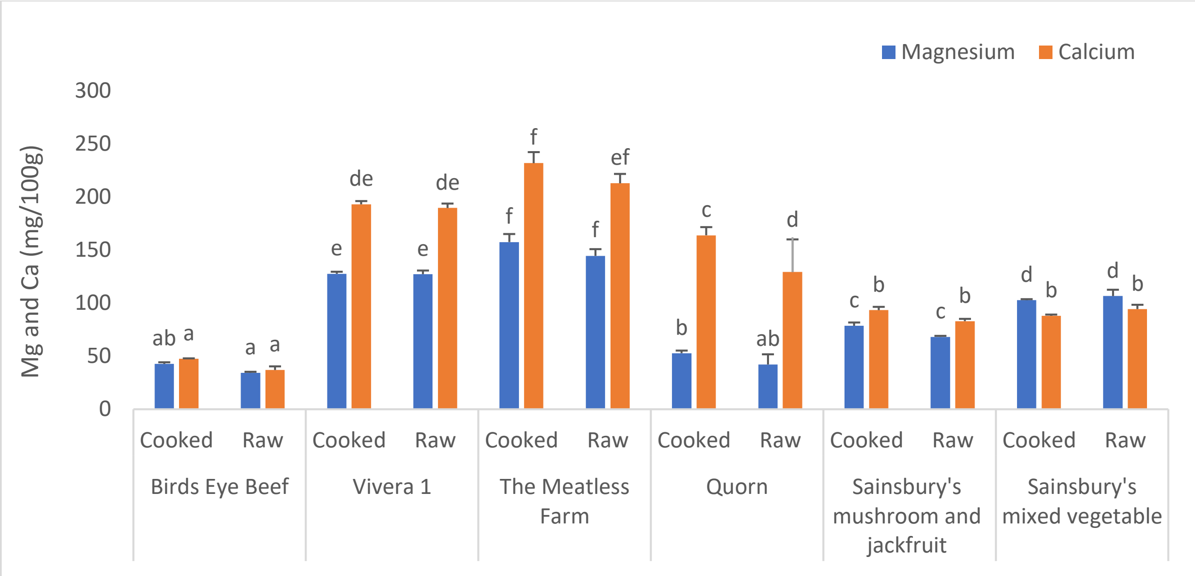



*Figure 12 Magnesium and calcium content in cooked and raw burgers. Letters represent differences for the same mineral across all cooked and raw burgers: magnesium (p=0.027 for Product*Cooking interaction); calcium (p=0.041 for Product*Cooking interaction). In all cases the error bars represent standard deviation, and the number of replicates was 3.*

Sodium. The product*cooking interaction was not quite significant (p=0.051, figure **13**) but there were differences between products for sodium (p<0.001). All plant-based burgers had significantly higher sodium contents than the beef burger. Vivera 1 and Sainsbury's mushroom jackfruit burgers were the richest in sodium with no differences between them, while Quorn, The Meatless Farm and Sainsbury's mixed vegetable burgers had smaller and similar amounts of sodium. Cooked burgers had greater concentrations of sodium than the raw versions (p<0.001).

Potassium. There was a significant product*cooking interaction for potassium (p=0.017, figure **13**). Vivera 1 had the highest potassium content, followed by The Meatless Farm, the two Sainsbury's burgers and the Birds Eye Beef burger, with the Quorn burger being the poorest source of potassium. Cooking only significantly increased the potassium concentration in the Birds Eye Beef and Sainsbury's mushroom and jackfruit burgers, with no differences seen for the other burgers.

Phosphorus. There was a significant product*cooking interaction for phosphorus (p=0.036, figure **13**). The concentration of phosphorus was highest for The Meatless Farm burger, but there were few differences between the meat and other plant-based burgers. Cooking slightly increased the phosphorus content of the Birds Eye Beef, Quorn and Sainsbury's mushroom and jackfruit burgers.

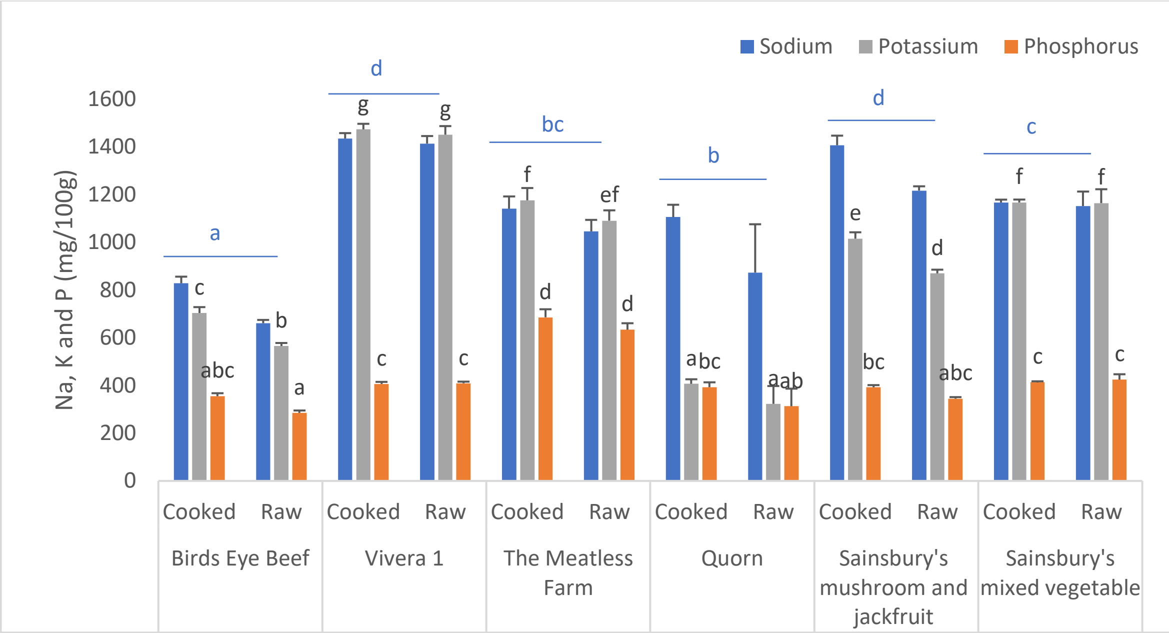



*Figure 13 Sodium, potassium and phosphorus content in cooked and raw burgers. Letters above the horizontal lines in blue represent differences for sodium between products (product p<0.001; product*cooking interaction p=0.051; cooking p<0.001). Letters above the vertical bars represent differences for potassium and phosphorus across all cooked and raw products: potassium (product*cooking interaction, p=0.017); phosphorus (product*cooking interaction p=0.036). In all cases the error bars represent standard deviation, and the number of replicates was 3.*

Selenium. There was a significant product*cooking interaction for selenium (p<0.001, figure **14**). The Meatless Farm was the highest, while Quorn and Sainsbury's mixed vegetable had the lowest selenium levels. Vivera 1, Birds Eye Beef and Sainsbury's mushroom and jackfruit burgers contained similar concentrations of selenium. Cooking significantly increased selenium levels for the meat burger.

Chromium. There was a significant product*cooking interaction for chromium (p=0.015, figure **14**). The raw and cooked Birds Eye Beef burger had the lowest content of chromium. The cooked Vivera 1 had the highest levels of chromium, followed by its raw version, and the cooked The Meatless Farm burger. There were not many differences between the other products. Cooking significantly increased chromium levels of The Meatless Farm and Vivera 1 burgers.

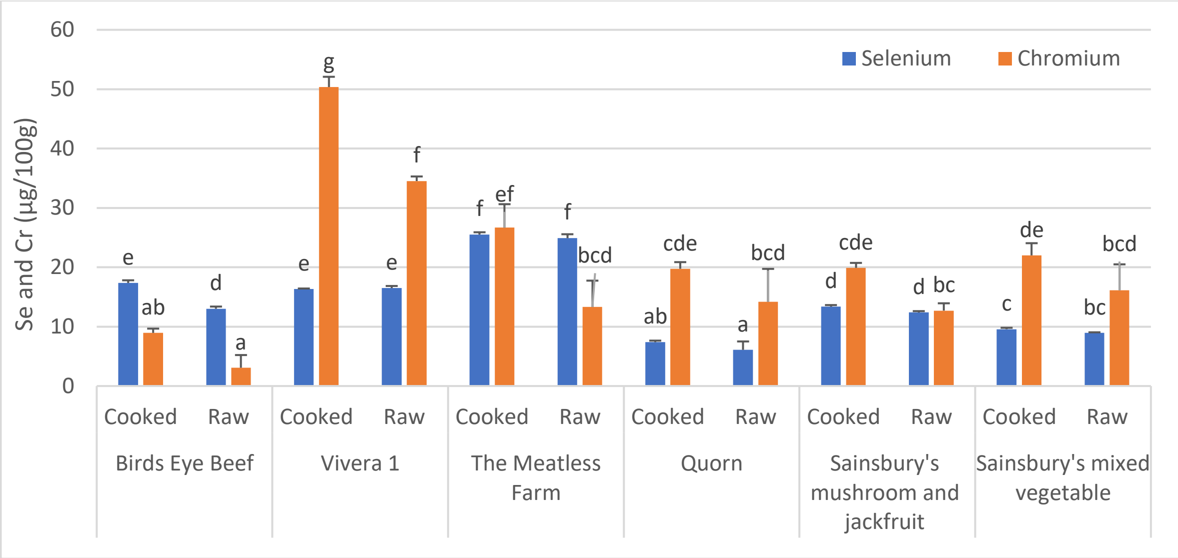



*Figure 14 Selenium and chromium content in cooked and raw burgers. Letters above the bars show differences for each mineral in raw and cooked burgers. Selenium (product*cooking interaction, p<0.001). Chromium (product*cooking interaction, p=0.015). In all cases the error bars represent standard deviation, and the number of replicates was 3.*

Copper and Molybdenum. In both cases there was no significant product*cooking interaction (p=0.08 for copper and p=0.327 for molybdenum, figure **15**). However, p values showed significance for product (p<0.001) and cooking (p<0.001) for the two minerals. All burgers had significantly different levels of copper, with the beef burger being the lowest and the Meatless Farm the highest. The other burgers had decreasing amounts of copper in this order: Sainsbury's mixed vegetable > Sainsbury's mushroom jackfruit >Vivera 1> Quorn. For molybdenum, the beef and the mycoprotein burgers had the lowest concentration for this mineral with no difference between them. Molybdenum was significantly higher in the two Sainsbury's and Vivera 1, and finally The Meatless Farm was significantly the richest burger in molybdenum. Cooking increased both copper and molybdenum concentrations in all burgers.

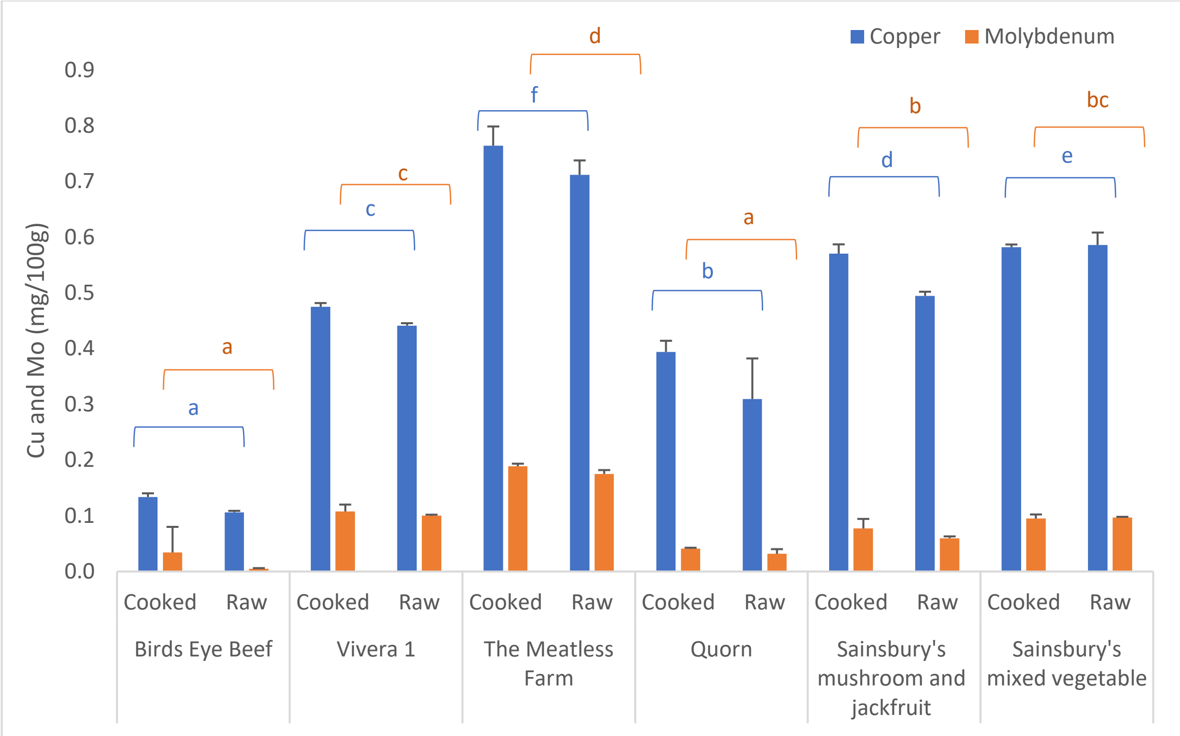



*Figure 15 Copper and molybdenum content in cooked and raw burgers. Letters in blue represent differences between products for copper and letters in orange represent differences between products for molybdenum. Copper: p=0.08 for product*cooking interaction, and p<0.001 for product and for cooking. Molybdenum: p=0.327 for product*cooking interaction, and p<0.001 for product and for cooking. In all cases the error bars represent standard deviation, and the number of replicates was 3.*

Non-essential minerals. Results for non-essential minerals in cooked and raw burgers are shown in Appendix 7. For some minerals there were negative values after ICP-MS analysis, meaning that there was no detectable mineral in the sample. Hence in the table shown in appendix 8 those data appear as "0.0000". Further calculations such as means, standard deviations and statistical analysis were done with data adjusted to zero.

## 4.4 Antinutritional factors

### 4.4.1 Total phenolics (tannins equivalent)

There were significant differences between products for total phenolics (p<0.001, figure **16**). The Meatless Farm contained the highest level of phenolics, but did not differ from that in Quorn, whereas Sainsbury's Onion and Parsley and Vivera 2 were lower. The lowest contents of phenolics were found in Sainsbury's Quinoa and Birds Eye Beef burgers.

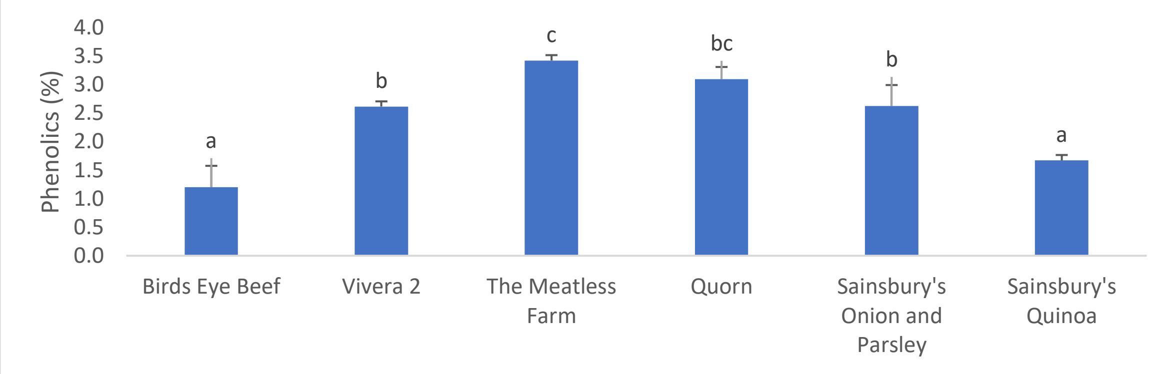



*Figure 16. Phenolics (tannins equivalent) content in cooked burgers. Letters show the differences between products (p<0.001 ANOVA). The error bars represent standard deviation, and the number of replicates was 3.*

### 4.4.2 Phytic acid and its mineral molar ratios.

Although significant differences between products for phytic acid were found (p=0.007, figure **17**), only The Meatless Farm burger was significantly higher than the Birds Eye beef and Sainsbury's Quinoa burgers. It might be worth indicating that there was a large variation between the three replicates of the Sainsbury's Onion and Parsley, as two of the values were considerable higher (average 548.13 mg/100g) than the third replicate (107.99 mg/100g).

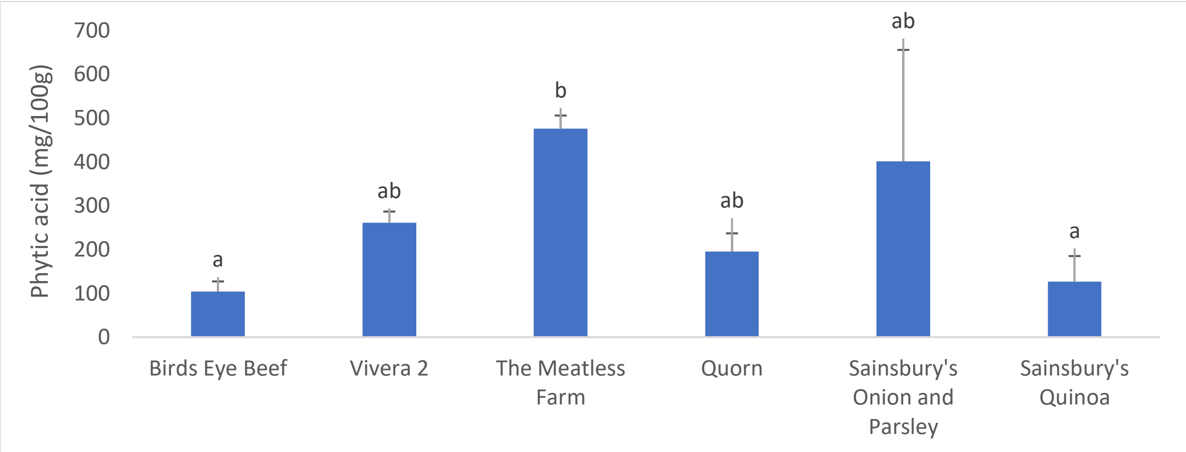



*Figure 17. Phytic acid content in cooked burgers. Letters show the differences between products (p=0.007 ANOVA). The error bars represent standard deviation, and the number of replicates was 3.*

There were no significant differences between products for the phytic acid-to-iron molar ratio (p=0.280, figure **18**), but there was a significant difference in phytic acid-to-zinc molar ratio (p=0.002, figure **18**), with the Birds Eye beef burger having a lower ratio than the two plant-based burgers .

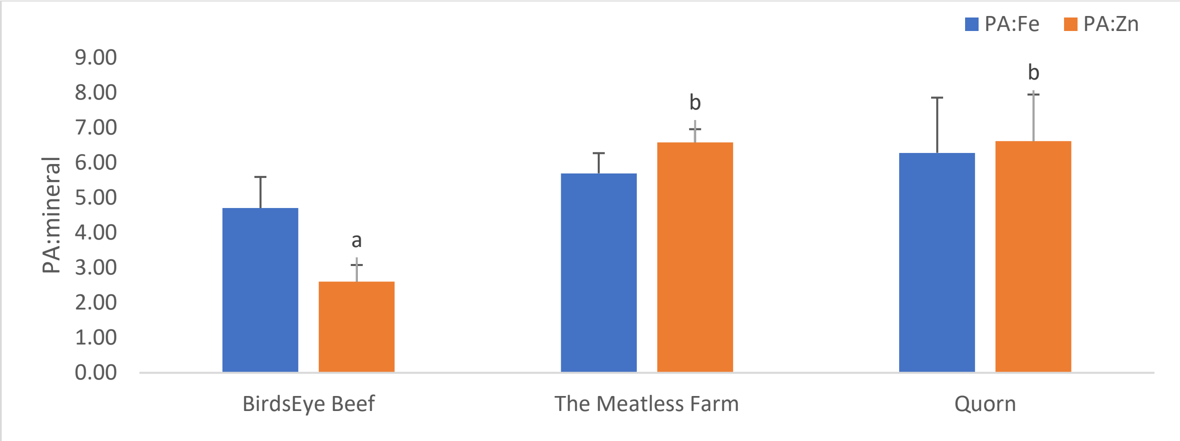



*Figure 18. Phytic acid-to-iron molar ratio (PA:Fe, p=0.280 ANOVA) and phytic acid-to-zinc molar ratio (PA:Zn, p=0.002 ANOVA). Letters show the differences for phytic acid-to-zinc molar ratio between products. In all cases the error bars represent standard deviation, and the number of replicates was 3.*

## 4.5 Analysis of protein digestibility (in vitro digestion).

There were significant differences between products for protein digestibility (p<0.001, figure **19**), with the Birds Eye beef burger having a significantly higher digestibility than any of the plant-based burgers. Differences between the plant-based burgers were not as apparent, with the two Sainsbury's burgers having significantly lower protein digestibility than Quorn, which had the highest values within the plant-based group, but did not differ greatly from The Meatless Farm and Vivera 2.

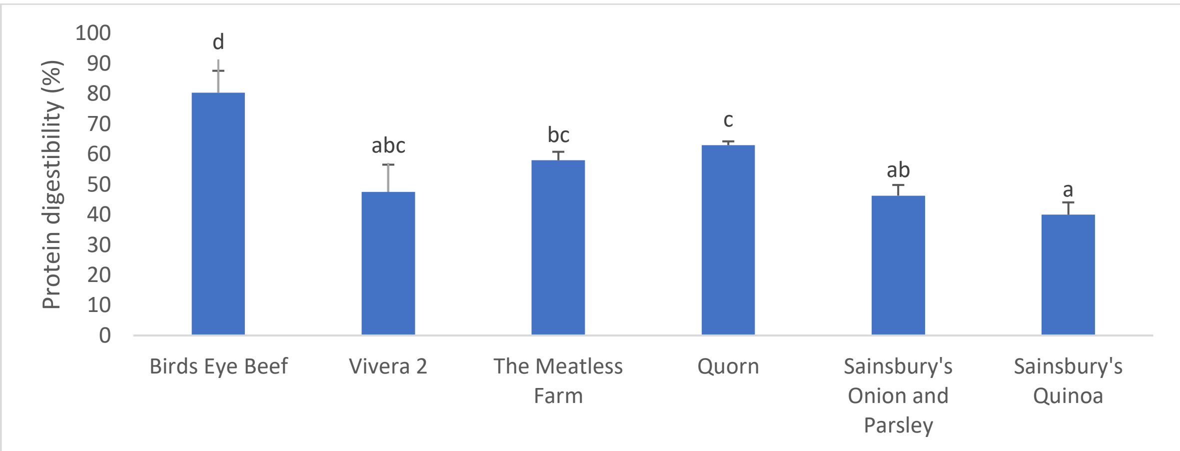



*Figure 19. Protein digestibility after in vitro digestion. The letters represent the differences between burgers (p<0.001 ANOVA). The error bars represent standard deviation, and the number of replicates was 3.*

## 4.6 Analysis of mineral bioaccesibility (in vitro digestion).

### 4.6.1 Iron bioaccesibility

There was a significant product*fraction interaction for iron bioaccessibility (p<0.001, figure **20**). There were few differences between products in the SD fraction, with The Meatless Farm burger having the highest content, but it was only significantly higher than the two Sainsbury's and the Birds

Eye Beef burgers.  There were more differences between products in the SND fraction though, with The Meatless Farm burger again had the highest content and Sainsbury's Quinoa the lowest. These were significantly higher or lower than the other burgers, but there were no differences in SND iron between Quorn, Vivera 2 and Sainsbury's onion and parsley. In all burgers, the SND iron fraction was significantly higher than the SD iron fraction.

There were significant differences in total soluble fraction between products (p<0.001, figure **20**). Again, The Meatless Farm was significantly the highest total soluble iron fraction, and Sainsbury's Quinoa the lowest. There were no significant differences between the total soluble iron fractions of Vivera 2, Quorn and Sainsbury's Onion and Parsley, but they were all significantly higher than the Birds Eye Beef burger. The non-soluble iron was calculated based on the total content of iron, that was measured on burgers from set 1. Because only 3 burger products were in both sets (i.e. Birds Eye beef, Quorn and The Meatless Farm), non-soluble iron was only calculated for those three burgers. The Meatless Farm had the highest non-soluble iron (p<0.001, figure **20**), while Quorn and the Birds Eye beef burger were similar.

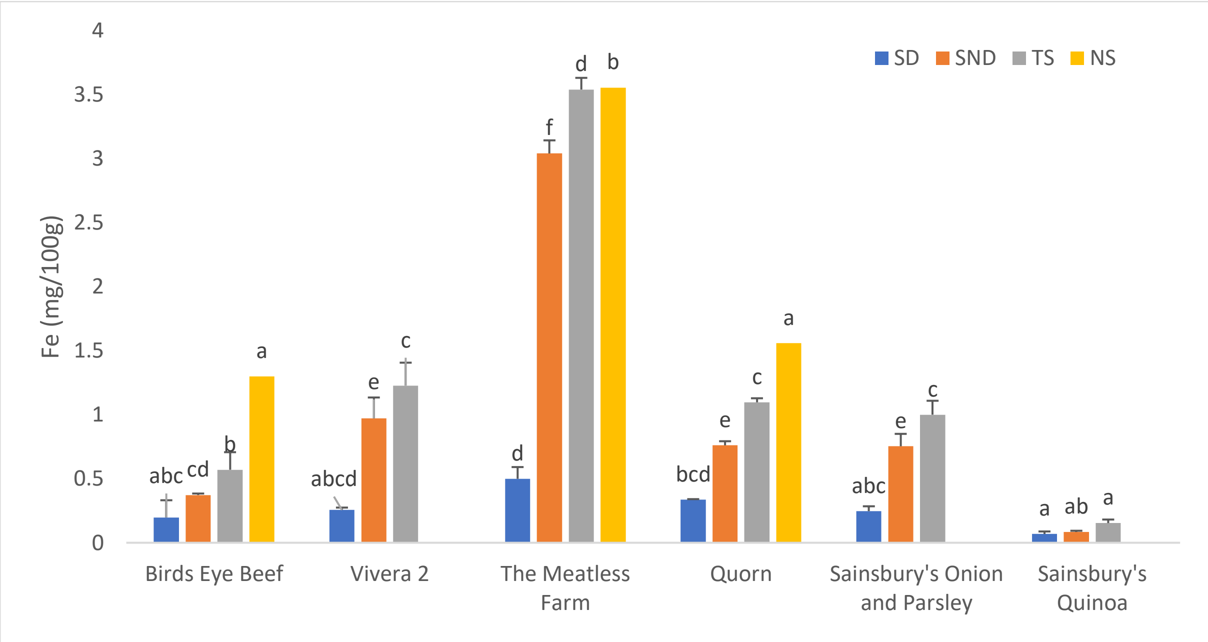



*Figure 20. Soluble Dialysable (SD), Soluble Non-Dialysable (SND), total soluble (TS) and non-soluble (NS) fractions of iron after in vitro digestion. Letters on the SD and SND bars represent differences between these two fractions across all burgers (p<0.001 for Product*Fraction interaction). Letters on the total soluble and non-soluble bars represent differences of each fraction between products (1-way ANOVA, p<0.001). In all cases the error bars represent standard deviation, and the number of replicates was 3.*

### 4.6.2 Zinc bioaccessibility.

There was no product*fraction interaction (p=0.782, figure **21**) for zinc bioaccessibility and also no difference between the SD and SND fractions (p=0.070), but there was a significant difference between the products (p<0.001), with the Birds Eye Beef and The Meatless Farm burgers being significantly higher than the other burgers. Similarly, there was a significant difference between products for the total soluble zinc ($p<0.001$, figure **21**), again with the Birds Eye Beef and The Meatless Farm having the highest total soluble zinc. The non-soluble zinc was again calculated based on the total content of zinc that was measured on burgers from set 1. Because only 3 burgers were in both sets (i.e. Birds Eye beef, Quorn and The Meatless Farm), non-soluble zinc was only calculated for those three burgers. The Meatless Farm had the highest non-soluble zinc ($p<0.001$, figure **21**), while Quorn and the Birds Eye beef burger did not differ.

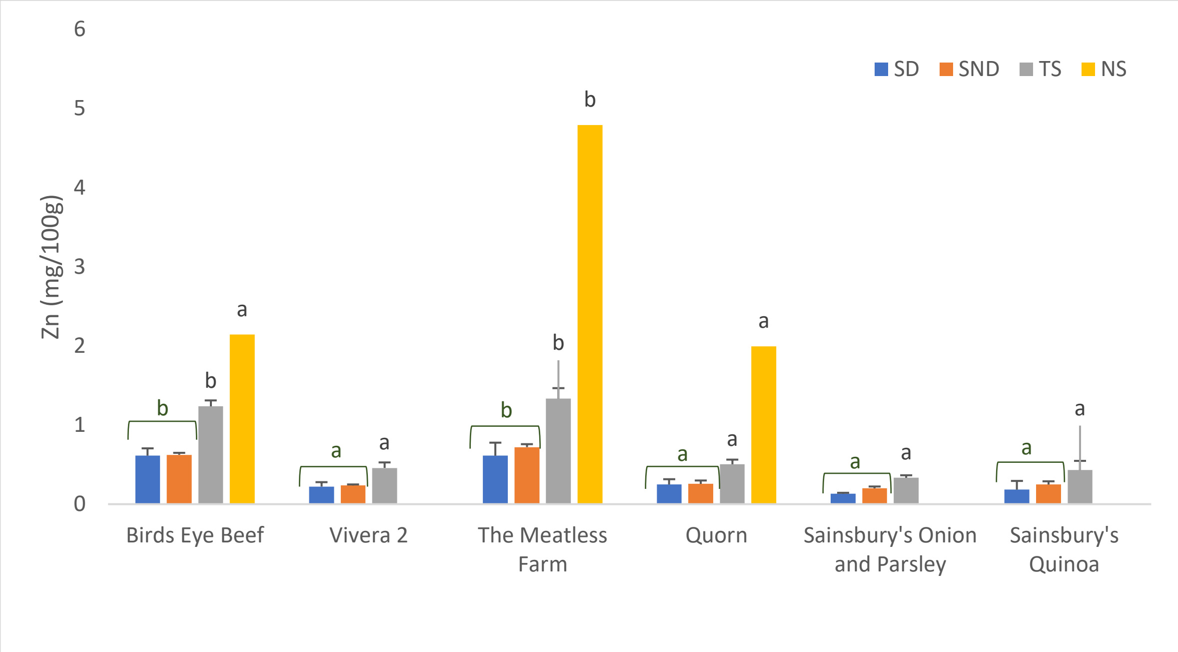
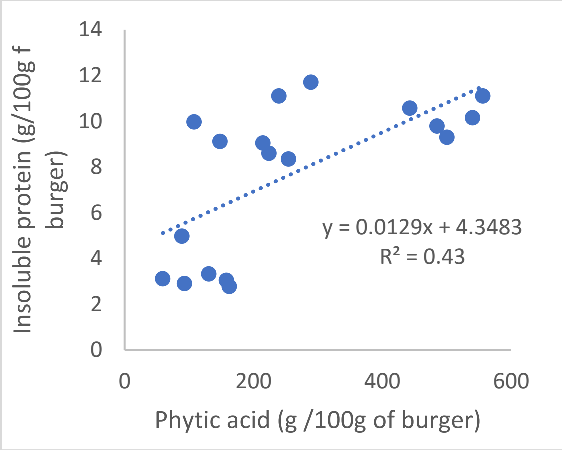



*Figure 21. Soluble Dialysable (SD), Soluble Non-Dialysable (SND), total soluble (TS) and non-soluble (NS) fractions of zinc after in vitro digestion. p values for 2-way ANOVA of dialysate and SND fractions are: 0.782 (product*fraction interaction), 0.070 (fraction) and <0.001 (product). Letters in dark green above the horizontal brackets correspond to differences between products for mean zinc. Letters on the total soluble and non-soluble bars represent differences of each fraction between products (1-way ANOVA, p<0.001). In all cases the error bars represent standard deviation, and the number of replicates was 3.*

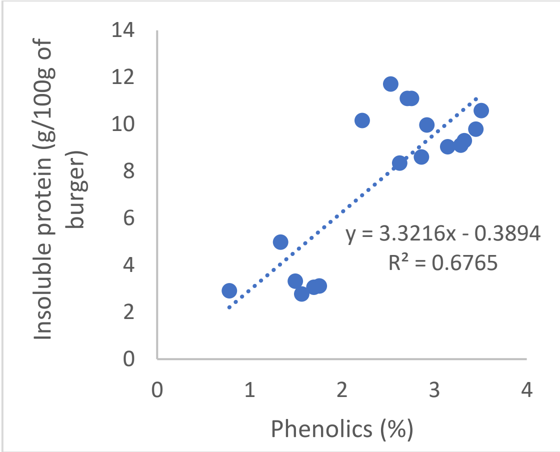
## 4.7 Correlation analyses.

### 4.7.1 Correlation of phenolics, phytic acid and insoluble proteins in burgers.

Interestingly, positive correlations were observed between the two anti-nutritional factors analysed and the insoluble protein fraction obtained after in vitro digestion (figure **22**), with the correlation for total phenolics being higher ($R^2$=0.68) than that for phytic acid ($R^2$=0.43).




(A)

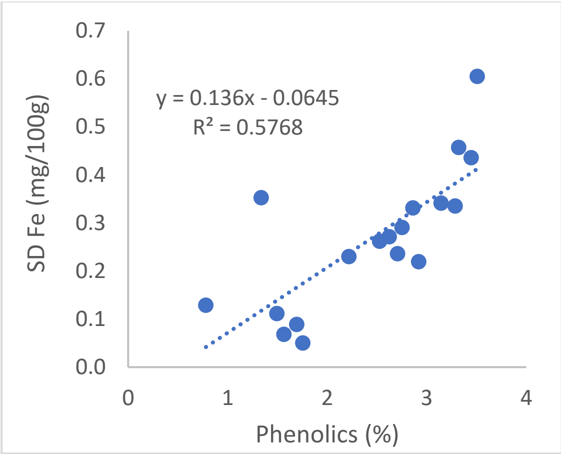



(B)

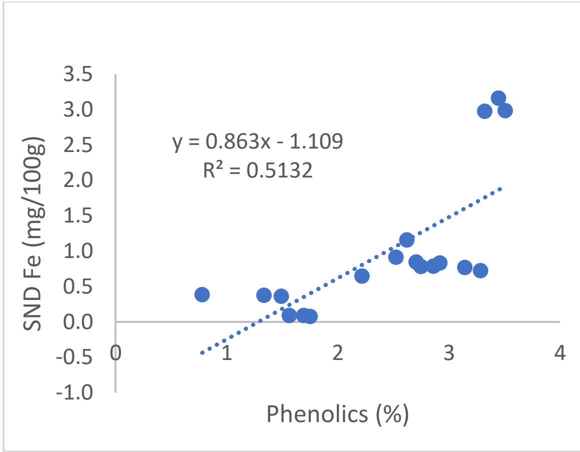
*Figure 22 Correlation of anti-nutritional factors and insoluble protein (A) Correlation of phytic acid and insoluble protein. (B) Correlation of phenolics (tannins) and insoluble protein. In all cases the number of replicates was 3.*

### 4.7.2 Correlation of phenolics (tannin equivalent) and iron.

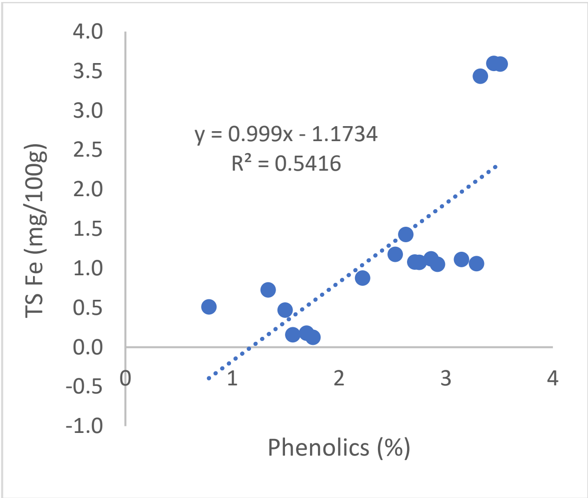
In order to investigate potential effects of ANFs on mineral bioaccessibility (e.g. due to binding), correlation analyses were performed between the phenolic and phytic acid contents (i.e. the ANFs) and the different measures of iron and zinc bioaccessibility. Total phenolic content was positively correlated to all 4 fractions of iron (i.e. SD (bioaccessible), SND, total soluble and non-soluble, $R^2$>0.4 in all cases, figure **23**). These correlations were slightly stronger for the bioaccesible (SD) and the total soluble iron.



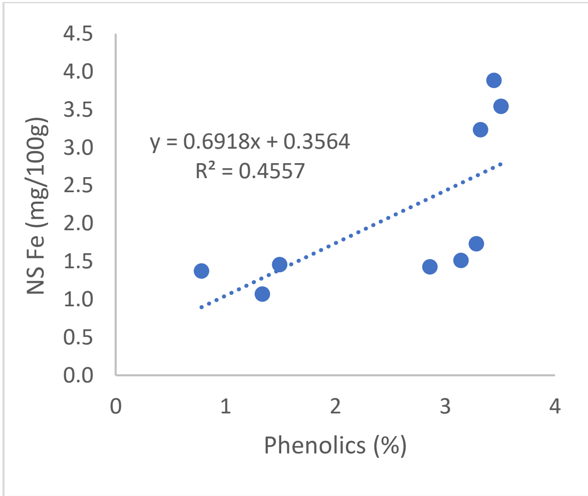

(A)




(B)




(C)




(D)

*Figure 23 Correlation of phenolics (%) and iron fractions. (A) Soluble Dialysable (SD) iron (B) Soluble Non-Dialysable (SND) iron. (C) Total soluble (TS) iron (D) Non-soluble (NS) iron. In all cases the number of replicates was 3.*

### 4.7.3 Correlation of phytic acid and iron

Similarly, Phytic acid was positively correlated with all 4 fractions of iron (figure **24**) and there was a particularly strong relationship with the non-soluble fraction ($R^2 = 0.9$, figure 23D). In all cases, $R^2$ was above 0.4 except for the bioaccessible (SD) fraction.

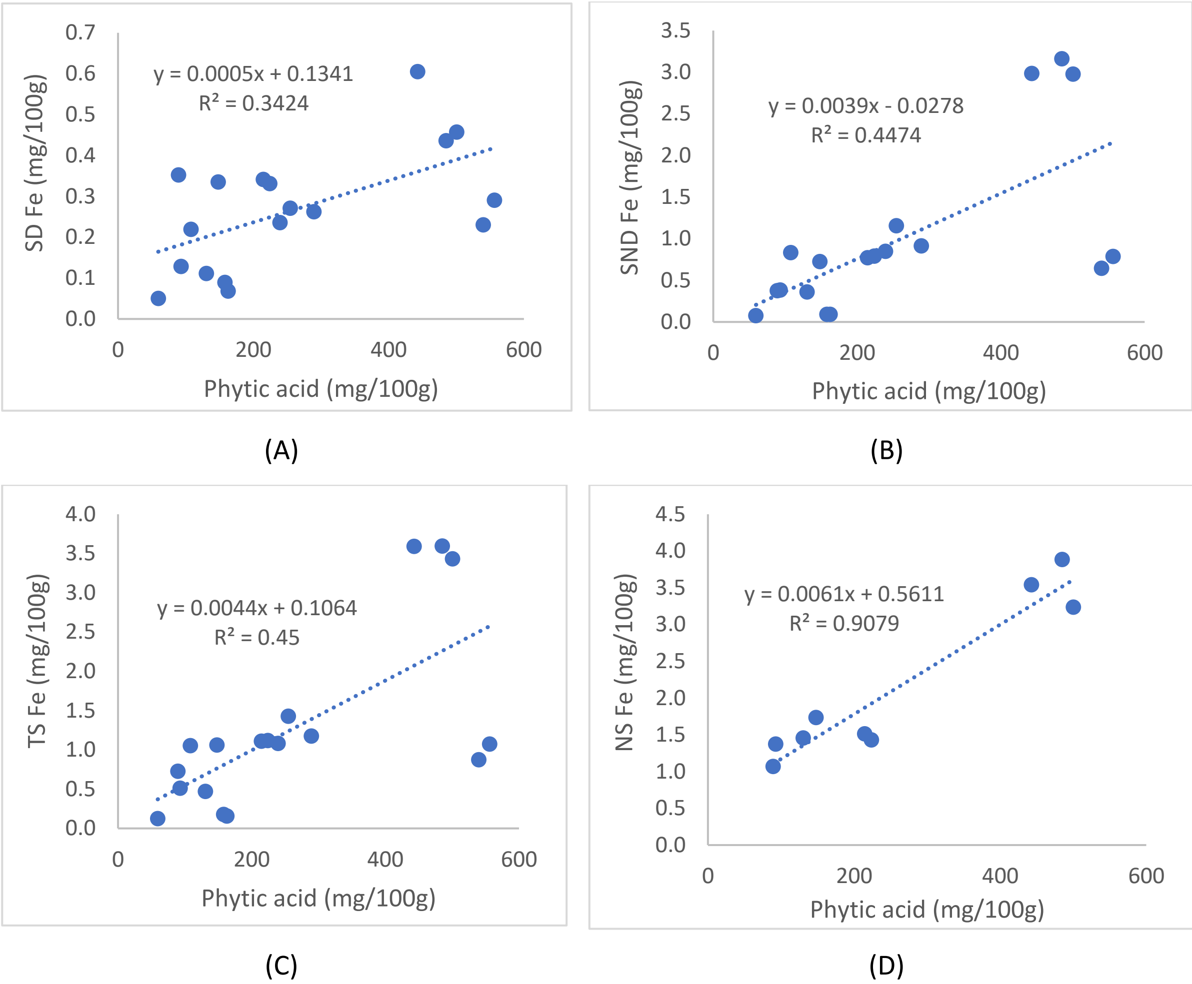



*Figure 24 Correlation of phytic acid and iron fractions. (A) Soluble Dialysable (SD) iron (B) Soluble Non-Dialysable (SND) iron. (C) Total soluble (TS) iron (D) Non-soluble (NS) iron. In all cases the number of replicates was 3.*

### 4.7.4 Correlation of phenolics (tannin equivalent) and zinc fractions.

The correlation analysis between the total phenolics and zinc fractions only showed weak or no correlations, with $R^2<0.4$ in all cases (figure **25**).

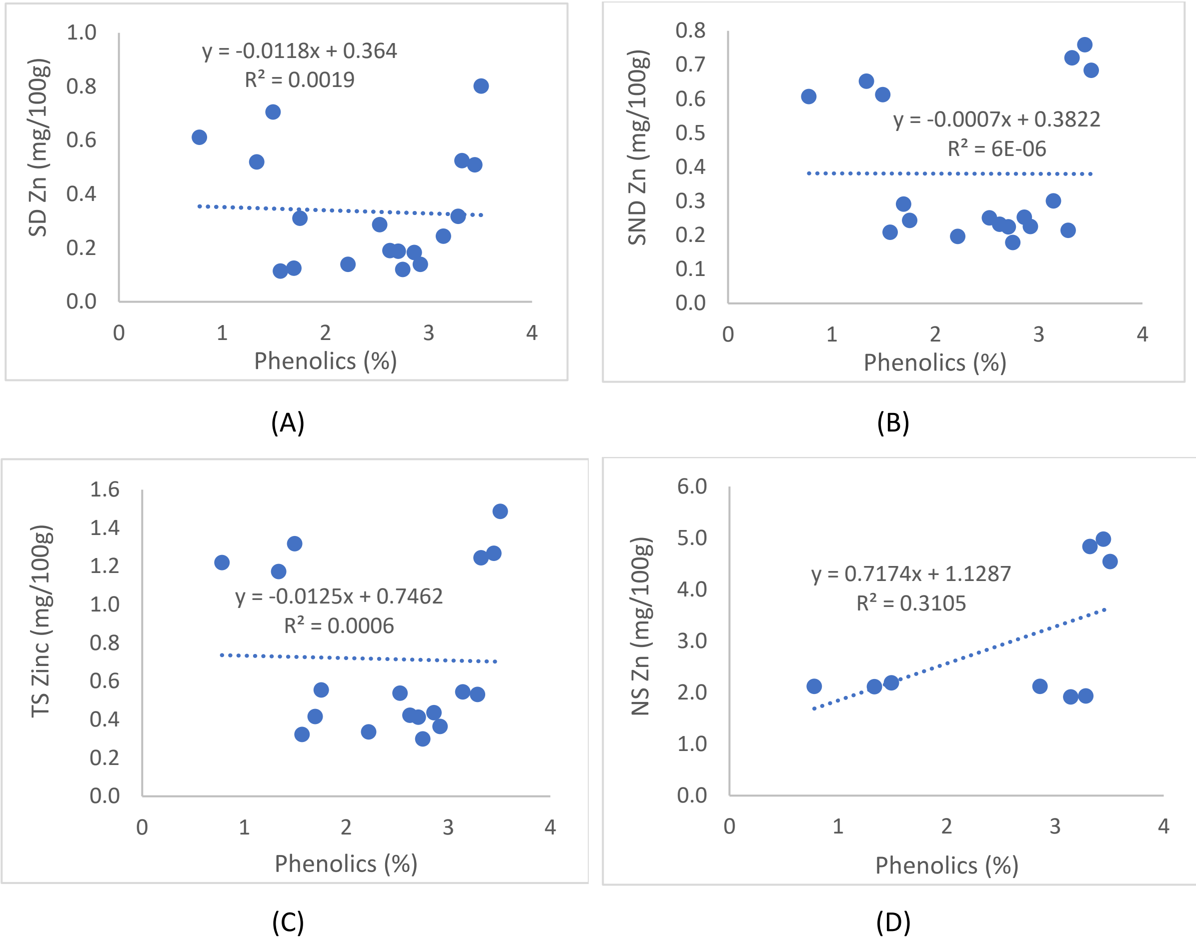



*Figure 25 Correlation of phenolics and zinc fractions (A) Soluble Dialysable (SD) zinc (B) Soluble Non Dialysable (SND) zinc (C) Total soluble (TS) zinc (D) Non-soluble (TS) zinc. In all cases the number of replicates was 3.*

### 4.7.5 Correlation of phytic acid and zinc fractions

In contrast, there was a strong positive correlation between phytic acid and non-soluble zinc ($R^2$=0.9065, figure **26**). Correlations for the rest of the zinc fractions were all much weaker ($R^2$<0.1).

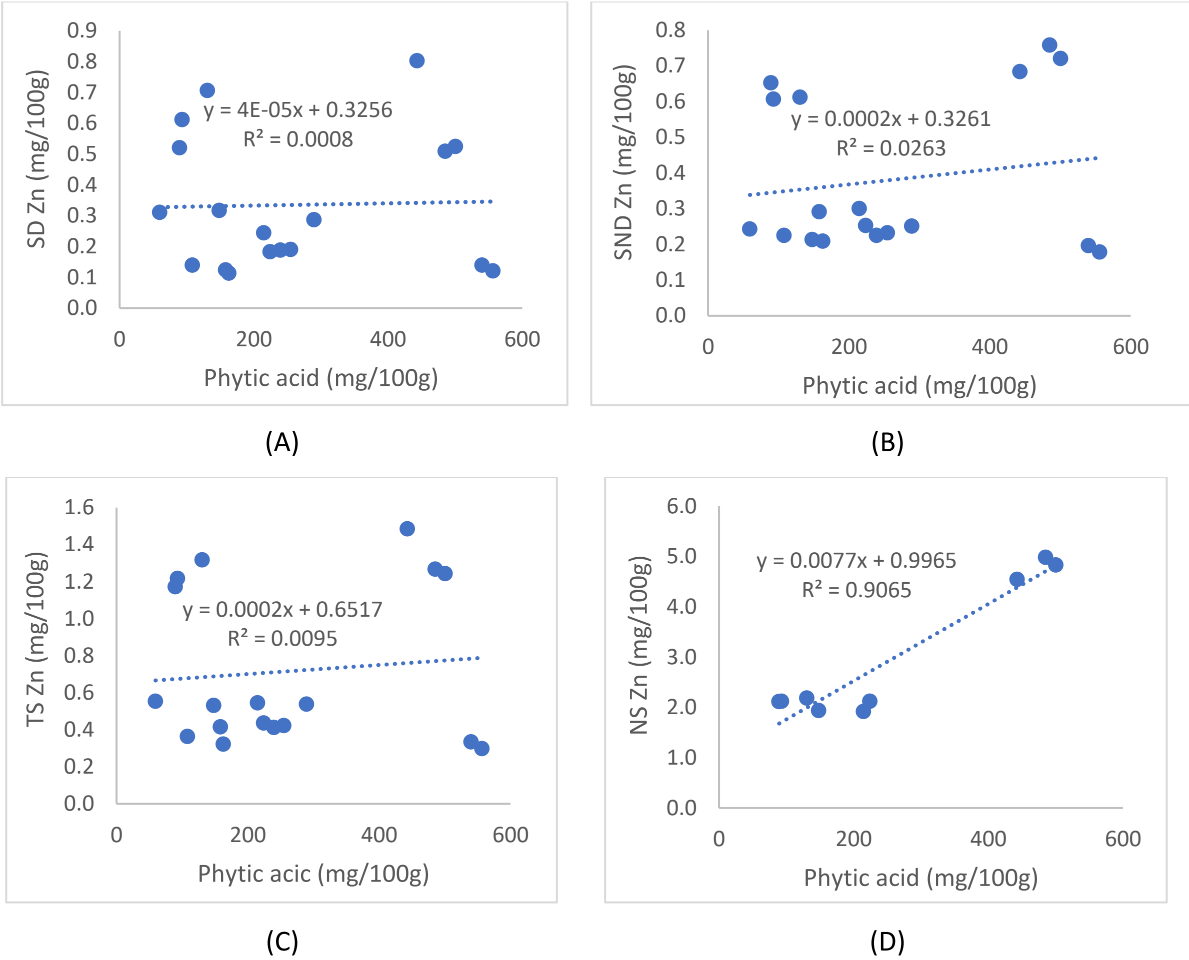



*Figure 26. Correlation of phytic acid (PA) and zinc fractions. (A) Soluble Dialysable (SD) zinc. (B) Soluble Non Dialysable (SND) zinc, (C) Total soluble (TS) zinc (D) Non-Soluble (NS) zinc. In all cases the number of replicates was 3.*

# 5 Discussion

The first objective of this project was to investigate the nutritional composition of plant-based burgers in comparison with a meat burger, and to assess the effect of cooking. Plant-based burgers composed of isolated, concentrated or extruded plant proteins (i.e. Vivera 1 and 2, The Meatless Farm, Quorn, and Sainsbury's Onion and Parsley) tended to have slightly lower levels of energy and similar or even higher amounts of protein compared to the beef burger. Meanwhile, plant-based burgers made of whole vegetables and/or less processed grains and pulses (i.e. Sainsbury's mushroom and jackfruit, Sainsbury's mixed vegetable, and Sainsbury's Quinoa) were much less protein- and energy-dense than the meat and the other plant-based products. The beef burger tended to contain less minerals than the high protein plant-based burgers, with burgers based on processed plant proteins (especially The Meatless Farm and Vivera 1 burgers) being generally richer in minerals than the whole vegetable-based burgers. There is nothing in the list of ingredients of The Meatless Farm and Vivera 1 burgers that can explain their increased mineral content. Because water can be a source of minerals (Azlan et al., 2012, Maraver et al., 2015, Szklarska and Rzymski, 2019) we suggest that the mineral content of the water used in the manufacture of these products might impact their final mineral concentration. The Meatless Farm, Vivera 1 and Quorn had higher iron levels than the beef burger. In the case of The Meatless Farm and Vivera 1 burgers, this may be explained by the fortification (claimed in their packaging, see appendix 1a). The Meatless Farm showed the greatest levels of Zinc (even above the RNI for zinc, see appendix 6), again probably due to its fortification as declared on its packaging (see appendix 1b). The beef burger had the second highest zinc content with one cooked burger covering most of the daily zinc needs (see appendix 6). This is due to beef being naturally rich in zinc (Van der Weele et al., 2019, England, 2019.). Zinc levels in Quorn burger were close to those in the beef burger. Indeed, Quorn pieces containing 95% mycoprotein (https://www.quorn.co.uk/products/chicken-style-pieces) have been found to contain notable amounts of zinc (7 mg/100) as sold (England, 2019.). Quorn burger only had 1.73mg/100 of zinc (raw) and this difference with Quorn pieces may be explained because burgers only had a 10% of mycoprotein. The other burgers tested provided approximately 30% of zinc RNI. One beef burger provided approximately half of the RNI for sodium, while eating any of the plant-based burgers would exceed 50% of sodium RNI (see appendix 6). In fact, the claimed salt content on the packaging is higher for all the plant-based burgers than for the beef burger (see appendix 1b). Given the importance of reducing sodium intake (Organisation, 2012a, Organisation, 2012b, Weaver, 2013, Jayedi et al., 2019, Iwahori et al., 2017, Oliveira et al., 2019), plant-based burgers would need to improve this aspect. For potassium, Quorn had the smallest amount of potassium, followed by the beef burger. No burger contributed more than 42% to potassium RNI (see appendix 6). The burgers were not a particularly rich source of calcium as their contribution to RNI ranged from 2.84% to

23.19% (see appendix 6). In addition, due the presence of phytic acid, the calcium might not be fully bioaccessible (WHO, 2004). In contrast, all burgers contained relevant amounts of phosphorus (especially the plant-based burgers) and contributed more than 50% of the RNI (see appendix 6). This might be caused by the use of phosphorus-rich additives, commonly found in processed foods (Trautvetter et al., 2018). Selenium is more commonly found in animal than in plant products (WHO, 2004). In line with this, the plant-based burgers had less selenium than the beef burger, except for The Meatless Farm. A single beef burger accounted for approximately half of the selenium RNI, while 2-3 plant-based burgers would be needed to cover selenium daily needs (see appendix 6). Quorn burger was the poorest source of selenium, this might be caused by the absence of soy in this burger, which is a known source of selenium (WHO, 2004). Finally, among the non-essential minerals (shown in appendix 8), we found a remarkably high content of lithium in Vivera 1 burger. This might be explained by its high content of rich sources of lithium such as wheat and potato, but also the water used for the manufacture of the burger can be an important source of lithium (Szklarska and Rzymski, 2019, Schrauzer, 2002). Due to the weight loss that occurred during cooking (appendix 3), cooked burgers had an increased energy, protein and mineral content. It is remarkable though, that this did not happen for the caloric content of the beef burger, whose energy values decreased with cooking. This coincides with the nutritional facts displayed in its packaging, where a decreased value for energy is also shown for the cooked product (see appendix 1b). The reduction in energy content is probably due to a loss in fat content during cooking as suggested on the label of this burger (see appendix 1b).

The second objective of this project was to determine the content of ANFs such as total phenolics (tannin equivalent) and phytic acid (PA). Although meat is free of anti-nutritional factors, we found both phenolics and PA in the beef burger. This is because in addition to meat, this burger contained other ingredients such as onions, wheat flour and rosemary, known to have polyphenols and PA (Rothwell JA, 2013, Dahdouh et al., 2019, FAO/IZiNCG, 2018). Unexpectedly, Sainsbury's Quinoa contained low levels of these ANFs despite being composed of less processed plant ingredients such as bulgur and lentils (see appendix 1a), which have been reported to contain phenolics (Rothwell JA, 2013, Xu and Chang, 2008, Smeriglio et al., 2017) and PA (Dahdouh et al., 2019). It was also remarkable that the Quorn burger had a low concentration of PA despite its content in by-products of wheat and pea (see appendix 2a), which are sources of PA (Dahdouh et al., 2019). Different processing techniques (dehulling, milling, soaking, germinating, fermenting and cooking) and even storage have been shown to decrease phytic acid in food through IP6 dephosphorylation to lower forms of IPs (Dahdouh et al., 2019, EFSA, 2015, Hurrell and Egli, 2010, Filho et al., 2017). Similarly, dehulling, soaking and boiling reduce phenolic content in foods (Sarwar Gilani et al., 2012, Xu and Chang, 2008). Hence, we speculate that the unexpected low levels of PA and phenolics in Sainsbury's Quinoa and Quorn burgers might be due to the different processing techniques to which they have been subjected during the manufacture. The calculation of phytate to iron and zinc molar ratios was used to estimate the effect of PA on mineral absorption. The phytate to iron molar ratio in plain cereal or legume-based meals that do not contain any enhancers should be 1 or, preferably, 0.4 to significantly improve iron absorption (Hurrell and Egli, 2010). However, in composite meals with certain vegetables that contain ascorbic acid and meat as enhancers, the ratio should be 6 (Hurrell and Egli, 2010). Hence this applies to the beef burger (as it contains meat), The Meatless Farm burger (as it contained added vitamin C), and to the Quorn burger (as it contained lemon juice concentrate) (see Appendix 2a). The beef burger phytate to iron ratio was 4.7, which is clearly below the 6 threshold. However, the ratios of The Meatless Farm (5.7) and Quorn (6.28) burgers were very close to 6, therefore PA would have an impact on iron bioavailability in these products. Ratios >15 are likely to compromise zinc bioavailability, while ratios from 5-15 are associated with a moderate zinc bioavailability, and ratios <5 are considered of high bioavailability (Dahdouh et al., 2019, WHO, 2004). Hence, the beef burger, which had a ratio of 2.6, would be expected to have a high zinc bioavailability, and The Meatless Farm and Quorn burgers (ratios were 6.58 and 6.61, respectively) would have a moderate zinc bioavailability. Nevertheless, the technique we used to measure phytate overestimates the total amount as it assumes that any phosphate containing compound in the sample is phytate, and thus this leads to a reduced estimation of mineral absorption (Dahdouh et al., 2019, FAO/IZiNCG, 2018). Furthermore, this method does not distinguish IP6 from lower forms of inositol phosphate (IP), which have a reduced mineral binding capacity (Dahdouh et al., 2019, FAO/IZiNCG, 2018). Hence, the use of more specific methods to measure the various IPs is recommended, especially for processed foods where IP6 is degraded to a greater extent to lower IPs, and this often involves high-performance liquid chromatography (Dahdouh et al., 2019).

The third and final objective of this project was to study the protein digestibility and the mineral bioaccessibility of plant-based burgers compared with a beef burger. The low levels of phenolics and PA found in the beef burger can explain its better protein digestibility compared to the plant-based burgers. Indeed, both ANFs have been shown to form complexes with proteins, thereby reducing their solubility and, hence, their digestibility (Sa et al., 2019, Rosa-Sibakov et al., 2018, Filho et al., 2017, Sarwar Gilani et al., 2012). In line with this, we found that total phenolics and PA both correlated with insoluble protein. In addition, the low levels of dietary fibre reported for the beef burger (see appendices 1b and 2b) might have contributed to its higher protein digestibility, as dietary fibre has been reported to hamper proteolysis (Sa et al., 2019). After the beef burger, Quorn burger had the best protein digestibility, which might be explained by its low phytate content. Indeed, previous reports have observed a positive correlation between phytic acid and plant protein solubility (Rosa-Sibakov et al., 2018). Trypsin inhibitors are another kind of anti-nutritional factor with known impact on protein digestibility (Avilés-Gaxiola et al., 2018, Filho et al., 2017, Sa et al., 2019) that hinder protein and amino acid digestibility up to 50% in rats and/or pigs (Sarwar Gilani et al., 2012). Soybeans are the richest source of trypsin inhibitors (Sarwar Gilani et al., 2012). All the plant-based burgers under study contained soy except for Quorn burger, and this might also explain its higher values for protein digestibility compared with the other plant-based burgers. Indeed, there is evidence suggesting that as soy protein content of a product increases, protein digestibility is reduced (Galán and Drago, 2014). In the case of Sainsbury's Quinoa burger, its low levels of phenolics and PA do not explain its poor protein digestibility. We consider that other factors such as the presence of edamame soy beans (and the potential presence of trypsin inhibitors), along with the absence of extruded, concentrated or isolated plant ingredients (see appendix 2a), which have been shown to have improved protein digestibility (Duque-Estrada et al., 2019, Sa et al., 2019), might be associated with the low protein digestibility of this burger. Also, other ANFs not analysed in this project such as Maillard reaction products, oxidized forms of sulphur amino acids, D-amino acids and lysinoalanine are all known to impact protein digestibility (Sarwar Gilani et al., 2012) and have not been analysed here. Finally, until 2019 Infogest had not been used to determine plant protein digestibility (Sa et al., 2019). This in vitro technique has some advantages such as reproducibility, simplicity, and low-cost assessment (Sa et al., 2019). However, the Digestible Indispensable Amino Acid Score (DIAAS) has been endorsed by FAO as the gold standard method to assess protein digestibility (Sa et al., 2019, FAO, 2013, FAO, 2014). Given the variety of factors that can impact protein digestibility, it would be of interest to develop a pool of data of plant protein digestibility using Infogest. This would be helpful for comparative analyses of protein digestibility, initial assessment and screening, as well as for hypothesis building, that can then be tested with more complex techniques such as the DIAAS gold standard.

In this study we defined the soluble dialysable fraction (SD) as the bioaccessible mineral, meaning soluble, low molecular weight iron or zinc (<16.4 kDa). The soluble non-dialysable fraction (SND) was soluble, high molecular weight iron or zinc (>16.4 kDa). Surprisingly, the beef burger had similar levels of SD iron to the concentrated plant protein-based burgers (i.e. Vivera 2, Quorn and Sainsbury's Onion and Parsley), except for The Meatless Farm whose concentration of bioaccessible iron was the highest, probably due to its fortification (see appendix 2a). To explain this discrepancy, it is necessary to clarify that haem iron, the main form of iron in beef (EFSA, 2015), is quantified with the dialysis technique we conducted as SND iron instead of SD iron, because of its molecular weight is >16 kDa (Maheswarappa et al., 2016, Whitaker, 1963, Zaia et al., 1992). However, this form of iron has been reported to be very bioavailable (EFSA, 2015, SACN, 2010), with values around 25% (EFSA, 2015). As mentioned earlier, The Meatless Farm had the highest levels of bioaccessible (SD) iron within the plant-based burgers due to its fortification. However, this burger also had the greatest content of SND, total soluble and non-soluble iron, which shows that fortification with iron does not necessarily lead to a greater bioaccessible iron. Non-absorbed iron can damage intestinal mucosa (Tiekou Lorinczova et al., 2020), modify the colonic microbiota equilibrium and favour growth of pathogenic strains over 'barrier' strains (Jaeggi et al., 2015, Ng, 2016, Paganini et al., 2017). The high levels of phenolics and PA in this burger might be binding the added iron and thereby hindering the solubility. Vivera 2, Quorn and Sainsbury's onion and parsley had similar amounts of SND and total soluble iron, which were higher than those fractions in the beef burger. However, the phytate-to-iron molar ratio estimated a lower impact of PA on iron bioavailability for the beef burger. Sainsbury's Quinoa, based on less processed plant foods (bulgur wheat, lentils, quinoa, etc, see appendix 2a), had the lowest SD, SND and total soluble iron. Nevertheless, this burger had the lowest concentrations of phenolics and PA, so it might contain other ANFs binding iron and reducing its solubility. Soy protein conglycinin fraction has been reported to reduce iron bioavailability (Galán and Drago, 2014, EFSA, 2015, Lynch et al., 1994). All plant-based burgers contained soy, except for the Quorn burger. However, Quorn burger did not show a higher iron bioaccessibility or solubility compared to the soy-based burgers. This might be explained by the presence of other ANFs and the variety of processes to which burgers have been subjected. Also, the role of soy protein on iron bioavailability in processed PBMAs should be further investigated. Interestingly, a pioneering private initiative has developed a soy haem iron (LegH) produced by genetically engineered yeast, which is then added to a soy and potato-based burger (Rachel Fraser, 2017). The bioavailability of this haem iron was previously shown to be similar to iron from bovine haemoglobin when supplemented to a food matrix (maize tortillas) using a Caco-2 cell culture model (Proulx and Reddy, 2006). However, it would be of interest to investigate iron bioavailability from LegH in food matrices such as soy based PBMAs.

There was no significant product*fraction interaction in zinc content and there were no differences between the bioaccessible (SD) and the SND fractions, but there were differences between products. The Meatless Farm and the beef burger had a greater amount of soluble zinc than the other burgers. For The Meatless Farm burger this is related to its fortification, as declared on the label (see appendix 2a). For the beef burger, this is due to beef's natural richness in zinc (Barnett et al., 2019, Rohrmann and Linseisen, 2016). However, the non-soluble zinc of The Meatless Farm was higher than the non-soluble zinc of the beef burger. This means that most of the added zinc to The Meatless Farm burger was insoluble and hence not available for absorption. This may be due to the higher levels of PA in The Meatless Farm burger, as indicated by the higher PA-to-zinc molar ratio compared to the beef burger. Similarly, there was a strong positive correlation between PA and non-soluble zinc. In contrast to PA, phenolics showed a weak or no correlation with all zinc fractions. Interestingly, a study indicated that the structure of phenolics might be more important than the total phenolics (Gabaza et al., 2018). Hence, for future research, the role of oligomeric and condensed tannins should be investigated separately. Besides, fibre might have decreased zinc bioaccessibility (Platel and Srinivasan, 2016, WHO, 2004). Certainly, dietary fibre values were lower in the beef burger than in the plant-based burgers (see Appendix 2b). Another major determinant of zinc absorption is the level and source of protein. Animal protein has been reported to improve zinc absorption from a phytate-containing diet (WHO, 2004), which explains the good bioaccesibility of zinc in the beef burger. For soy protein, studies are contradictory, with some reporting an increase (Platel and Srinivasan, 2016) and others a decrease in zinc bioaccessibility (Galán and Drago, 2014). As soy is the most common source of protein in PBMAs its role on zinc bioaccessibility should be further investigated.

Infogest in vitro digestion is a reproducible, simple and relatively low cost method (Brodkorb et al., 2019), but bioaccessibility data obtained with in vitro techniques needs to be confirmed (Turnlund, 2006). So, further research using other techniques such as stable isotope tracers in humans are needed for a full assessment of iron and zinc bioavailability (Turnlund, 2006, Collings et al., 2013). On top of this, it has been suggested that iron bioavailability depends on the systemic needs for iron, and it is not determined by the nature of the iron compound, nor the presence of enhancers and inhibitors (EFSA, 2015, SACN, 2010). In fact, it has been observed that when iron status (in terms of serum ferritin) is lower, iron absorption is more sensitive to diet, with enhancers exerting a greater effect and inhibitors having no significant impact (Collings et al., 2013). Also, the impact of antinutritional factors on iron bioavailability, in the context of a whole diet in the long term, seems to be weaker than has been reported in single meal studies (Delimont et al., 2017, EFSA, 2015, Collings et al., 2013). The homeostatic mechanisms to control iron uptake according to the body iron status take a few days to modify iron uptake and transfer (EFSA, 2015). Therefore, the effect of enhancers and inhibitors is greater in the context of a single meal study, as homeostatic mechanisms may not be able to adapt within a short period of time (EFSA, 2015). Also, in spite of the potential negative effect of tannins and PA on mineral bioaccesibility, these compounds are also associated with beneficial effects on human health (Sa et al., 2019). PA has been considered a natural antioxidant because it forms an iron chelate that suppresses iron-catalysed oxidative reactions (FAO/IZiNCG, 2018). Also, it has been associated with prebiotic activity due to the ability to bind enzymes such as amylases, so that a portion of the undigested starch reaches the intestine (Margier et al., 2018). Complexed unabsorbed tannins reach the colon where the gut microbiota metabolises them into compounds that counteract the effects of pro-oxidants (Smeriglio et al., 2017). Furthermore, microbiota can increase the availability of dietary iron by converting ellagic acid (an hydrolysable tannin) to urolithin A (Skrypnik and Suliburska, 2018). Therefore, our analyses of ANFs and mineral bioaccessibility could be considered preliminary data for initial assessment but other aspects need to be analysed to gain a more comprehensive knowledge of the role of ANFs in health and the adequacy of PBMAs as sources of proteins and minerals.

Finally, once the objectives of this project have been addressed, we consider important to mention a more practical limitation of this research, and it is not having been able to use the same burgers for all analyses. This would have allowed us to have a more complete set of data and assessment of the burgers.

# 6 Conclusions

Plant-based burgers composed of highly concentrated plant proteins had slightly less energy and similar or even higher protein contents than a beef burger. In addition, plant-based burgers were a richer source of minerals than the beef burger, but they tended to have higher levels of sodium. Cooking tended to increase the concentration of minerals, protein and energy. Plant-based burgers also had higher levels of total phenolics (tannin equivalent) and phytic acid than the beef burger, for which protein digestibility was greater. Iron solubility was similar between the beef burger and the plant-based burgers containing plant protein concentrates. However, zinc solubility was greater for the beef burger than for the plant-based burgers. The Meatless Farm burger (fortified with iron and zinc) had similar soluble iron and zinc than the beef burger. However, this burger also had higher amounts of non-soluble iron and zinc, which shows that most of these added minerals are not available for absorption.

As mentioned in the introduction, there is a global overconsumption of protein, and animal-sourced food intake in developed countries can be reduced by at least one third without any replacement and this would not result in protein deficiency (Van der Weele et al., 2019). In this context, protein needs could still be met by replacing meat with PBMAs, despite their lower protein digestibility. In addition, including PBMAs in the diet would improve the balance between animal and plant protein intake, which would be beneficial for both health and environment. Interestingly, the plant-based burger with the best protein digestibility was Quorn, the only soy-free, mycoprotein containing burger. The digestibility of mycoprotein has been shown to be comparable to milk protein (Dunlop et al., 2017, Monteyne et al., 2020), but Quorn burger contained only a 10% of mycoprotein. Hence, it would be of interest to further investigate whether soy-free PBMAs and mycoprotein-based meat alternatives can deliver more digestible protein. Also, attention should be paid to essential amino acid intake, that can be achieved by including a variety of pulses in the diet (Messina, 2014, Melina et al., 2016). Hence, to study PBMAs amino acid profile and digestibility would be of interest to create products with maximised amino acid quality. Our results indicate that the plant-based burgers can provide more soluble iron than the beef burger. In line with this, it has been found that serum ferritin in vegetarians is usually within the reference ranges (SACN, 2010). Also, it has been reported that non-haem iron absorption is 10 times higher and haem iron is 4 times higher in iron-deficient compared with iron-replete individuals (Reed Mangels, 2011). Therefore, we predict that replacing meat with PBMAs would not have a detrimental impact on anaemia prevalence, but this needs to be confirmed. Besides, due to the positive effects of ANFs reported in previous studies and their lower impact on iron absorption in long term studies, their role on nutrient uptake from plant-based burgers and in the diet in general is unclear. The concentration of PA and phenolics in the plant-based burgers did not always show the expected higher or lower values for mineral bioaccessibility and protein digestibility. Hence other factors such as the presence of trypsin inhibitors (soy) and the processing to which burgers had been subjected might be involved. The burgers based on plant protein concentrates/isolates and extrudates had better values for protein digestibility and iron solubility than those based on less processed plant foods. Thus, PBMAs subjected to these processing techniques could contribute to a greater extent to achieve protein and mineral recommended intake than PBMAs based on whole plant foods. Finally, modelling different degrees of meat replacement with various proportions of PBMAs and non-processed plant-foods would be of interest to assess the ideal role of meat alternatives in a transition to a healthy, sustainable plant-based diet.

# 7 Note on the impact of Covid-19 on the present project.

Some of the analyses initially planned for this Mres project were not possible due to closure of the University laboratories in response to the Covid-19 pandemic. These included:

- Determination of amino acids in cooked, raw and digested burgers.
- Protein digestibility:
    - Determination of amino acids in soluble phase of burgers after digestion
    - SDS page for qualitative protein analysis on digested burgers.
    - Determination of the degree of hydrolysis on digested burgers.
- Determination of trypsin inhibitor contents

# 8 Appendices

## 8.1 Appendix 1a. Ingredients of burgers in set 1

*Table 4 List of ingredients of burgers in set 1. VIV1: Vivera burger; SMJ: Sainsbury Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury Mixed Vegetable burger.*

| BEB | VIV1 | TMF | QUO | SMJ | SMX |
|---|---|---|---|---|---|
| Beef (77%),<br>Onion (14%),<br>Beef fat,<br>Fortified wheat flour (wheat, calcium carbonate, iron, niacin, thiamine),<br>Salt,<br>Water,<br>Onion powder,<br>Yeast extract,<br>Spices,<br>Natural rosemary flavouring. | Rehydrated soy and wheat protein (77%),<br>Red onion (5%),<br>Sunflower oil,<br>Potato starch,<br>Thickner: methyl cellulose,<br>Natural flavourings,<br>Flavourings,<br>Sea salt,<br>Spices (cardamom, cumin, turmeric, white pepper, nutmeg, onion powder, paprika powder, allspice, chilli powder, mace),<br>Dried glucose syrup,<br>Onion,<br>Dextrose,<br>Potato fibre,<br>Garlic,<br>Maltodextrin,<br>Onion extract,<br>Vitamins and minerals (Iron and vitamin B12). | Water,<br>Soya protein concentrate,<br>Pea protein,<br>Soya protein isolate,<br>Rapeseed oil,<br>Shea oil,<br>Coconut oil,<br>Chicory root fibre,<br>Thickener: methyl cellulose,<br>Caramelised carrot concentrate,<br>Carrot fibre,<br>Rice protein,<br>Salt,<br>Flavouring,<br>Vegetable and fruit extract (beetroot, radish, tomato),<br>Yeast extract,<br>Carrot concentrate,<br>Emulsifier: soya lecithin,<br>Antioxidant: ascorbic acid, | Water,<br>Textured proteins (wheat gluten, pea protein, wheat starch, wheat flour, pea protein isolate),<br>Vegetable oils (sunflower, palm, coconut),<br>Mycoprotein (10%),<br>Natural flavouring,<br>Red Beet juice (4%) (red beet, lemon juice concentrate),<br>Stabiliser: methyl Cellulose,<br>Potato protein,<br>Barley Malt Extract. | Water,<br>Mushroom (22%),<br>Jackfruit (15%),<br>Pea flour,<br>Fried onions (onion, rapeseed oil),<br>Vegetable suet (sustainable palm oil, rice flour),<br>Breadcrumb (rice flour, maize flour, maize starch, salt, dextrose),<br>Pea fibre,<br>Pea starch,<br>Stabiliser: methyl cellulose,<br>Pea protein,<br>Dried onion,<br>Light Brown Sugar (sugar beet, cane molasses),<br>Salt,<br>Yeast extract,<br>Natural flavouring,<br>Caramel powder,<br>Cracked black pepper, | Mushroom (24%)<br>Pea flour (11%)<br>Water<br>Fried onion (9%) (Onion, rapeseed oil)<br>Peas (8%)<br>Sweet corn (7%)<br>Cooked brown rice (water, brown rice, salt)<br>Cooked red kidney beans (water, red kidney beans)<br>Carrot (5%)<br>Coriander<br>Pea fibre (2.5%)<br>Roast garlic puree<br>Breadcrumb (rice flour, maize flour, maize starch, salt, dextrose)<br>Red chilli puree<br>Salt<br>Stabiliser: methyl cellulose<br>Cumin powder<br>Cracked black pepper |

| | | | | | |
|---|---|---|---|---|---|
| | | Vitamins and minerals (Niacin, Zinc, Iron, Vitamin B6, Vitamin B2, Vitamin B1, Vitamin B12). | | Preservative: sodium metabisulphite. | Colour: paprika extract Preservative: sodium metabisulphite |

## 8.2 Appendix 1b. Nutritional facts (as on the label) of burgers in set 1.

*Table 5 Nutritional facts (as on the label) of burgers in set 2. All values are per 100g. VIV1: Vivera burger; SMJ: Sainsbury's Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury's Mixed Vegetable burger.*

| Nutrient | BEB (as sold) | BEB (grilled) | VIV 1 (as sold) | TMF (as sold) | QUO (pan fried) | SMJ (pan fried) | SMX (pan fried) |
|---|---|---|---|---|---|---|---|
| Energy (KJ) | 1226 | 916.67 | 706 | 846 | 1070 | 591 | 556 |
| Energy (Kcal) | 296 | 220.18 | 169 | 203 | 255 | 141 | 132 |
| Fats g | 25 | 16.67 | 7.3 | 10.5 | 14 | 4.2 | 1.8 |
| of which | | | | | | | |
| saturated g | 8.4 | 5.70 | 0.86 | 3.3 | 3 | 1.9 | 0.2 |
| Carbohydrates g | 2.7 | 2.72 | 4.8 | 3.3 | 10 | 16.9 | 20.3 |
| of which | | | | | | | |
| sugars g | 1.1 | 1.05 | <0.4 | 0.7 | 3 | 1.9 | 2.1 |
| starch g | | | | | | 15 | 18.2 |
| Dietary fibre g | <0.5 | <0.44 | 5 | 4.5 | 3.1 | 5.4 | 5.3 |
| Protein g | 15 | 14.91 | 18.5 | 21.8 | 21 | 6.2 | 6 |
| Salt g | 0.73 | 0.73 | 1.55 | 1.41 | 1.2 | 1.19 | 1.13 |
| Iron mg | | | 2.1 | 2.25 | | | |
| Zinc mg | | | | 4 | | | |
| Vitamin B12 µg | | | 0.38 | 1.55 | | | |
| Vitamin B3 mg | | | | 4 | | | |
| Vitamin B6 mg | | | | 1.8 | | | |
| Vitamin B2 mg | | | | 0.4 | | | |
| Vitamin B1 mg | | | | 0.3 | | | |

## 8.3 Appendix 2a. Ingredients of samples from set 2.

*Table 6 List of ingredients of burgers in set 2. VIV 2: Vivera burger 2; SOP: Sainsbury's Onion and Parsley burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SQU: Sainsbury's Quinoa burger.*

| BEB | VIV 2 | TMF | QUO | SOP | SQU |
|---|---|---|---|---|---|
| Beef (77%),<br>Onion (14%),<br>Beef fat,<br>Fortified wheat flour (wheat, calcium carbonate, iron, niacin, thiamine),<br>Salt,<br>Water,<br>Onion powder,<br>Yeast extract,<br>Spices,<br>Natural rosemary flavouring. | Rehydrated soy and wheat protein (72%),<br>Red onion,<br>Sunflower oil,<br>Natural flavourings,<br>Thickener: methyl cellulose,<br>Hydrolysed wheat protein,<br>Potato starch,<br>Flavouring,<br>Dried glucose syrup,<br>Spices,<br>Sea salt,<br>Onion,<br>Potato fibre,<br>Dextrose,<br>Maltodextrin,<br>Garlic,<br>Onion extract,<br>Vitamins and minerals (iron and vitamin b12). | Water,<br>Soya protein concentrate,<br>Pea protein,<br>Soya protein isolate,<br>Rapeseed oil,<br>Shea oil,<br>Coconut oil,<br>Chicory root fibre,<br>Thickener: methyl cellulose,<br>Caramelised carrot concentrate,<br>Carrot fibre,<br>Rice protein,<br>Salt,<br>Flavouring,<br>Vegetable and fruit extract (beetroot, radish, tomato),<br>Yeast extract,<br>Carrot concentrate,<br>Emulsifier: soya lecithin,<br>Antioxidant: ascorbic acid, | Water,<br>Textured proteins (wheat gluten, pea protein, wheat starch, wheat flour, pea protein isolate),<br>Vegetable oils (sunflower, palm, coconut),<br>Mycoprotein (10%),<br>Natural flavouring,<br>Red Beet juice (4%) (red beet, lemon juice concentrate),<br>Stabiliser: methyl Cellulose,<br>Potato protein,<br>Barley Malt Extract. | Rehydrated textured soya protein (56%),<br>Water,<br>Onion (7%),<br>Onion puree (6%),<br>Rapeseed oil,<br>Soya protein, concentrate<br>Yeast extract,<br>Chickpea flour,<br>Stabiliser: methyl cellulose,<br>Tomato puree,<br>Garlic puree,<br>Parsley,<br>Onion powder,<br>Malted barley extract,<br>Maltodextrin,<br>Garlic powder,<br>Salt,<br>Dextrose,<br>Black pepper,<br>White pepper,<br>Tomato powder, | Bulgur wheat (15%),<br>Onion,<br>Green lentils,<br>Quinoa (10%),<br>Sweet potato (9%),<br>Red peppers,<br>Wheat flour (calcium carbonate, iron, niacin, thiamine),<br>Edamame bean (soya),<br>Courgette,<br>Sunflower oil,<br>Rice starch,<br>Spiced seasoning (salt, onion powder, cumin, yeast extract, chilli powder, black pepper, coriander, oregano, sunflower oil),<br>Garlic,<br>Seasoning (breadcrumb rusk (wheat flour, calcium carbonate, iron, niacin, thiamine, salt), salt, sugar, |

| | | | | | |
|---|---|---|---|---|---|
| | | Vitamins and minerals (Niacin, Zinc, Iron, Vitamin B6, Vitamin B2, Vitamin B1, Vitamin B12). | | Flavouring. | pepper, ginger, nutmeg, cloves, cinnamon),<br>Sugar,<br>Turmeric. |

## 8.4 Appendix 2b. Nutritional facts (as on the label) of samples in set 2.

*Table 7 Nutritional facts (as on the label) of burgers in set 2. All values are per 100g. VIV 2: Vivera burger 2; SOP: Sainsbury's Onion and Parsley burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SQU: Sainsbury's Quinoa burger.*

| Nutritional information | BEB (as sold) | BEB (grilled) | VIV 2 | TMF (as sold) | QUO (pan fried) | SOP (grilled) | SQU (oven cooked) |
|---|---|---|---|---|---|---|---|
| Energy (KJ) | 1226 | 916.67 | 686 | 846 | 1070 | 627 | 802 |
| Energy (Kcal) | 296 | 220.18 | 164 | 203 | 255 | 149 | 192 |
| Fats g | 25 | 16.67 | 5.4 | 10.5 | 14 | 4 | 9.4 |
| of which | | | | | | | |
| saturated g | 8.4 | 5.70 | 0.6 | 3.3 | 3 | 0.3 | 1 |
| monounsaturated g | | | | | | 2.4 | 2.6 |
| polyunsaturated g | | | | | | 1.1 | 5.3 |
| Carbohydrates g | 2.7 | 2.72 | 8.9 | 3.3 | 10 | 6.4 | 19.3 |
| of which | | | | | | | |
| sugars g | 1.1 | 1.05 | 1.8 | 0.7 | 3 | 1.4 | 1.6 |
| starch g | | | | | | 5 | 17.7 |
| Dietary fibre g | <0.5 | 0.44 | 6 | 4.5 | 3.1 | 5.6 | 5.8 |
| Protein g | 15 | 14.91 | 17 | 21.8 | 21 | 19.1 | 4.7 |
| Salt g | 0.73 | 0.73 | 1.2 | 1.41 | 1.2 | 1.16 | 0.75 |
| Iron mg | | | 2.1 | | | | |
| Zinc mg | | | 0.38 | | | | |

## 8.5 Appendix 3. Cooking weight losses of burgers.

*Table 8 Burgers weight loss during cooking.*

| Burger | Average weight loss (g) |
|---|---|
| Birds Eye Beef | 25.59 |
| Vivera 2 | 13.24 |
| Sainsbury’s Mushroom and Jackfruit | 12.69 |
| Sainsbury’s Onion and Parsley | 11.17 |
| Sainsbury’s Mixed Vegetable | 10.68 |
| Vivera 1 | 10.21 |
| Quorn | 10.13 |
| The Meatless Farm | 9.33 |
| Sainsbury’s Quinoa | 7.19 |

## 8.6 Appendix 4. Moisture and dry content of burgers.

*Table 9 Freeze-drying 1. Burgers were subjected to determination of energy, protein and mineral content. VIV1: Vivera burger; SMJ: Sainsbury's Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury's Mixed Vegetable burger.*

| *Cooking* | *Sample ID* | *Weight before freeze-drying (g)* | *Weight after freeze-drying (g)* | *Freeze-drying water loss (g)* | *Moisture* | *Dry content* |
|---|---|---|---|---|---|---|
| *Cooked* | VIV 1 | 95.26 | 46.56 | 48.70 | 0.51 | 0.49 |
| | SMJ | 103.30 | 36.32 | 66.98 | 0.65 | 0.35 |
| | TMF | 99.72 | 48.94 | 50.78 | 0.51 | 0.49 |
| | QUO | 97.05 | 54.99 | 42.06 | 0.43 | 0.57 |
| | BEB | 85.61 | 38.37 | 47.24 | 0.55 | 0.45 |
| | SMX | 107.56 | 40.04 | 67.52 | 0.63 | 0.37 |
| *Raw* | VIV 1 | - | 48.14 | 57.53 | 0.54 | 0.46 |
| | SMJ | - | 39.99 | 85.04 | 0.68 | 0.32 |
| | TMF | - | 49.34 | 68.36 | 0.58 | 0.42 |
| | QUO | - | 55.86 | 56.22 | 0.50 | 0.50 |
| | BEB | - | 51.30 | 63.55 | 0.55 | 0.45 |
| | SMX | - | 38.66 | 75.30 | 0.66 | 0.34 |

*Table 10 Freeze-drying 2. Burgers were subjected to protein determination. VIV2: Vivera 2; SOP: Sainsbury's Onion and Parsley; SQU: Sainsbury's Quinoa.*

| | *Sample ID* | *Weight before freeze-drying* | *Weight after freeze-drying (g)* | *Freeze-drying water loss (g)* | *Moisture* | *Dry content* |
|---|---|---|---|---|---|---|
| *Cooked* | VIV 2 | 94.08 | 42.21 | 51.87 | 0.55 | 0.45 |
| | SOP | 47.9 | 18.74 | 29.16 | 0.61 | 0.39 |
| | SQU | 85.5 | 35.29 | 50.21 | 0.59 | 0.41 |
| *Raw* | VIV 2 | 101.94 | 45.10 | 56.84 | 0.56 | 0.44 |
| | SOP | 57.31 | 18.52 | 38.79 | 0.68 | 0.32 |
| | SQU | 87.49 | 34.43 | 53.06 | 0.61 | 0.39 |

*Table 11 Freeze-drying 3. Burgers were cooked and subjected to anti-nutritional factors determination (i.e. Total phenolics (tannins equivalent) and phytic acid). VIV2: Vivera 2; SOP: Sainsbury's Onion and Parsley; TMF: The Meatless Farm; QUO: Quorn; BEB: Birds Eye Beef; SMX: Sainsbury's Mixed Vegetables*

| *Sample ID* | *Weight before freeze-drying (g)* | *Weight after freeze-drying (g)* | *Freeze-drying water loss (g)* | *Moisture* | *Dry content* |
|---|---|---|---|---|---|
| *VIV 2* | 26.54 | 13.23 | 13.31 | 0.50 | 0.50 |
| *SOP* | 10.55 | 4.16 | 6.39 | 0.61 | 0.39 |
| *TMF* | 31.50 | 14.86 | 16.63 | 0.53 | 0.47 |
| *QUO* | 32.06 | 17.21 | 14.85 | 0.46 | 0.54 |
| *BEB* | 23.49 | 10.16 | 13.33 | 0.57 | 0.43 |
| *SQU* | 24.58 | 10.83 | 13.75 | 0.56 | 0.44 |

## 8.7 Appendix 5. Recommended Nutrient Intake (RNI) for minerals (mg/day)

*Table 12 Recommended Nutrient Intake (RNI) for minerals (mg/day)*

| | *Source* | *Males* | *Males 19-65* | *Males >65* | *Females* | *Females 19-50* | *Females 50-65* | *Females >65* |
|---|---|---|---|---|---|---|---|---|
| *Zinc* | WHO,2004 | 4.2 | | | 3 | | | |
| *Iron* | WHO,2004 | 9.1 | | | | 19.6 | 7.5 | 7.5 |
| *Selenium* | WHO,2004 | | 0.034 | 0.033 | | 0.026 | 0.026 | 0.025 |
| *Calcium* | WHO, 2004 | | 1000 | 1300 | | 1000 | 1300 | 1300 |
| *Sodium* | WHO, 2012 | 2000 | | | 2000 | | | |
| *Potassium* | WHO, 2012 | 3510 | | | 3510 | | | |
| *Magnesium* | WHO,2004 | | 260 | 224 | | 220 | 220 | 190 |
| *Phosphorus* | EFSA, 2015 | 550 | | | 550 | | | |
| *Chromium* | WHO, 1996 | 0.033 | | | 0.033 | | | |
| *Copper* | WHO, 1996 | 0.8 | | | 0.7 | | | |
| *Molybdenum* | WHO, 1996 | 0.025 | | | 0.025 | | | |
| *Manganese* | WHO, 1996 | 3.5 | | | 3.5 | | | |
| *Sulphur* | COMA, 1991 | 0.7 | | | 0.7 | | | |

## 8.8 Appendix 6. Contribution of burgers to mineral RNI (%)

*Table 13 Contribution of 100g of burgers to iron, zinc and manganese RNIs (%): >50%, grey; >100%, green; >150, blue. * WHO, 2004 (WHO, 2004). ** WHO, 1996 (Organisation, 1996). ***WHO, 2012 (Organisation, 2012a, Organisation, 2012b) **** EFSA, 2015 (EFSA, 2015).*

| Product | Cooking | Iron* | | | Zinc* | | Manganese** |
|---|---|---|---|---|---|---|---|
| | | Males | Females 19-50y | Females >50y | Males | Females | Adults |
| Birds Eye Beef | Cooked | 20.55 | 9.54 | 24.94 | 80.47 | 112.66 | 3.86 |
| | Raw | 16.26 | 7.55 | 19.73 | 59.79 | 83.71 | 3.05 |
| Vivera 1 | Cooked | 53.07 | 24.64 | 64.39 | 20.82 | 29.15 | 53.57 |
| | Raw | 48.63 | 22.58 | 59.01 | 18.43 | 25.80 | 53.08 |
| The Meatless Farm | Cooked | 77.95 | 36.19 | 94.58 | 145.74 | 204.04 | 55.58 |
| | Raw | 63.32 | 29.40 | 76.83 | 118.58 | 166.02 | 51.31 |
| Quorn | Cooked | 29.18 | 13.55 | 35.41 | 59.50 | 83.31 | 54.19 |
| | Raw | 20.97 | 9.74 | 25.45 | 41.36 | 57.91 | 43.91 |
| Sainsbury's mushroom and jackfruit | Cooked | 15.30 | 7.10 | 18.56 | 19.56 | 27.39 | 27.93 |
| | Raw | 13.36 | 6.20 | 16.21 | 16.72 | 23.41 | 24.51 |
| Sainsbury's mixed vegetable | Cooked | 17.04 | 7.91 | 20.67 | 22.66 | 31.73 | 35.11 |
| | Raw | 16.01 | 7.43 | 19.43 | 18.89 | 26.45 | 36.08 |

Contribution of burgers to mineral RNI (%) - continuation

*Table 14 Contribution of 100g of burgers to calcium, magnesium, sodium potassium and phosphorus RNIs (%): >50%, grey; >100%, green; >150, blue. * WHO, 2004 (WHO, 2004). ** WHO, 1996 (Organisation, 1996). ***WHO, 2012 (Organisation, 2012a, Organisation, 2012b). **** EFSA, 2015 (EFSA, 2015).*

| Product | Cooking | Calcium* | | Magnesium* | | | | Sodium*** | Potassium*** | Phosphorus**** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Adults 19-65 y | Adults >65y | Males 19-65y | Males >65 | Females 19-65y | Females >65y | Adults | Adults | Adults |
| Birds Eye Beef | Cooked | 4.76 | 3.67 | 16.44 | 19.08 | 19.43 | 22.50 | 41.42 | 20.0532 | 64.54 |
| | Raw | 3.70 | 2.84 | 13.19 | 15.31 | 15.58 | 18.05 | 33.04 | 16.1099 | 51.84 |
| Vivera 1 | Cooked | 19.31 | 14.85 | 49.11 | 57.01 | 58.04 | 67.21 | 71.76 | 41.9764 | 73.91 |
| | Raw | 18.96 | 14.59 | 48.97 | 56.84 | 57.88 | 67.02 | 70.69 | 41.3442 | 74.39 |
| The Meatless Farm | Cooked | 23.19 | 17.84 | 60.61 | 70.35 | 71.63 | 82.93 | 57.03 | 33.5071 | 124.67 |
| | Raw | 21.29 | 16.38 | 55.57 | 64.50 | 65.68 | 76.05 | 52.29 | 31.0682 | 115.21 |
| Quorn | Cooked | 16.39 | 12.61 | 20.33 | 23.60 | 24.03 | 27.83 | 55.29 | 11.5936 | 71.38 |
| | Raw | 12.94 | 9.95 | 16.21 | 18.81 | 19.15 | 22.18 | 43.67 | 9.2030 | 56.89 |
| Sainsbury's mushroom and jackfruit | Cooked | 9.34 | 7.18 | 30.26 | 35.13 | 35.77 | 41.41 | 70.34 | 28.9219 | 71.33 |
| | Raw | 8.30 | 6.39 | 26.23 | 30.45 | 31.00 | 35.90 | 60.85 | 24.7835 | 62.66 |
| Sainsbury's mixed vegetable | Cooked | 8.81 | 6.78 | 39.54 | 45.89 | 46.72 | 54.10 | 58.33 | 33.2478 | 75.22 |
| | Raw | 9.44 | 7.26 | 41.01 | 47.61 | 48.47 | 56.12 | 57.61 | 33.1679 | 77.14 |

Contribution of burgers to mineral RNI (%) - continuation

*Table 15 Contribution of 100g burgers to selenium, chromium, copper and molybdenum RNIs (%): >50%, grey; >100%, green; >150, blue. * WHO, 2004 (Organization, 2004). ** WHO, 1996 (Organisation, 1996). ***WHO, 2012 (Organisation, 2012a, Organisation, 2012b). **** EFSA, 2015 (EFSA, 2015).*

| Product | Cooking | Selenium* | | | | Chromium** | Copper** | | Molybdenum** |
|---|---|---|---|---|---|---|---|---|---|
| | | Males 19-65y | Males >65y | Females 19-65 y | Females >65y | Adults | Males | Females | Adults |
| Birds Eye Beef | Cooked | 51.06 | 52.61 | 66.77 | 69.44 | 27.13 | 16.71 | 19.09 | 136.31 |
| | Raw | 38.29 | 39.45 | 50.08 | 52.08 | 9.33 | 13.29 | 15.19 | 20.24 |
| Vivera 1 | Cooked | 48.09 | 49.54 | 62.88 | 65.40 | 152.60 | 59.39 | 67.87 | 430.43 |
| | Raw | 48.51 | 49.98 | 63.44 | 65.98 | 104.63 | 55.17 | 63.05 | 401.17 |
| The Meatless Farm | Cooked | 75.03 | 77.30 | 98.11 | 102.04 | 80.86 | 95.54 | 109.19 | 755.80 |
| | Raw | 73.22 | 75.44 | 95.75 | 99.58 | 40.32 | 88.97 | 101.68 | 700.25 |
| Quorn | Cooked | 21.74 | 22.40 | 28.43 | 29.57 | 59.85 | 49.26 | 56.29 | 163.49 |
| | Raw | 17.93 | 18.47 | 23.45 | 24.38 | 43.01 | 38.66 | 44.19 | 126.66 |
| Sainsbury's mushroom and jackfruit | Cooked | 39.31 | 40.50 | 51.40 | 53.45 | 60.32 | 71.31 | 81.49 | 308.61 |
| | Raw | 36.47 | 37.58 | 47.70 | 49.60 | 38.50 | 61.82 | 70.65 | 239.02 |
| Sainsbury's mixed vegetable | Cooked | 28.15 | 29.00 | 36.81 | 38.28 | 66.68 | 72.76 | 83.16 | 379.93 |
| | Raw | 26.30 | 27.10 | 34.39 | 35.77 | 48.93 | 73.24 | 83.71 | 387.54 |

## 8.9 Appendix 7. Content of non-essential minerals in burgers.

*Table 16 Content of cobalt, lithium, beryllium and Barium in cooked and raw burgers. Cobalt (product*cooking p=0.005), Lithium (product*cooking p=0.554, Product p<0.001. Cooking p=0.165), Beryllium (Product*Cooking p=0.7. Product p=0.6. Cooking p=0.0291) and Barium (Product*Cooking p=0.023). VIV1: Vivera burger; SMJ: Sainsbury's Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury's Mixed Vegetable burger.*

| Burger | Cooking | Co (mg/100g) | | | Li (mg/100g) | | | Be (mg/100g) | | | B (mg/100g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Letters | Mean | SD | Letters | Mean | SD | Letters | Mean | SD | Letters |
| BEB | Cooked | 0.0094 | 0.0001 | a | 0.0000 | 0.0000 | a | 0.1709 | 0.2961 | - | 0.0000 | 0.0000 | a |
| | Raw | 0.0147 | 0.0034 | ab | 0.0000 | 0.0000 | | 0.4470 | 0.7743 | - | 0.0000 | 0.0000 | a |
| VIV1 | Cooked | 0.0061 | 0.0001 | c | 72.8717 | 4.5740 | b | 0.5054 | 0.8754 | - | 0.6876 | 0.1007 | c |
| | Raw | 0.0060 | 0.0003 | c | 75.1933 | 3.9325 | | 0.6736 | 1.1667 | - | 0.6598 | 0.1242 | c |
| TMF | Cooked | 0.0025 | 0.0001 | d | 0.0000 | 0.0000 | a | 0.2966 | 0.5137 | - | 1.2188 | 0.1079 | d |
| | Raw | 0.0042 | 0.0003 | d | 1.7719 | 3.0691 | | 2.2717 | 3.9347 | - | 1.1416 | 0.0898 | d |
| QUO | Cooked | 0.0059 | 0.0001 | f | 1.7007 | 2.9457 | a | 0.3843 | 0.3361 | - | 0.0000 | 0.0000 | a |
| | Raw | 0.0055 | 0.0001 | e | 2.2603 | 0.2606 | | 0.0000 | 0.0000 | - | 0.0000 | 0.0000 | a |
| SMJ | Cooked | 0.0100 | 0.0005 | c | 1.3328 | 1.6451 | a | 0.0000 | 0.0000 | - | 0.6754 | 0.0395 | c |
| | Raw | 0.0191 | 0.0005 | bc | 0.0000 | 0.0000 | | 0.5489 | 0.5995 | - | 0.3881 | 0.0618 | b |
| SMX | Cooked | 0.0021 | 0.0001 | abc | 0.0000 | 0.0000 | a | 0.2522 | 0.4368 | - | 0.5964 | 0.0789 | bc |
| | Raw | 0.0044 | 0.0002 | abc | 3.8994 | 4.3450 | | 0.4097 | 0.3729 | - | 0.5987 | 0.1192 | bc |

Content of non-essential minerals in burgers (continuation)

*Table 17 Content of aluminium, sulphur, titanium and vanadium for cooked and raw burgers. Aluminium (Product*Cooking p=0.598. Product p<0.001. Cooking p=0.509). Sulphur (Product*cooking p=0.006). Titanium (Product*Cooking p<0.001). Vanadium (Product*Cooking p= 0.058. Product p<0.001. Cooking p=0.008). VIV1: Vivera burger; SMJ: Sainsbury's Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury's Mixed Vegetable burger.*

| Burger | Cooking | Al (mg/100g) | | | S (mg/100g) | | | Ti (mg/100g) | | | V (mg/100g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Letters | Mean | SD | Letters | Mean | SD | p | Mean | SD | Letters |
| BEB | Cooked | 587.3410 | 42.4650 | | 364.0316 | 22.0352 | cde | 0.0000 | 0.0000 | a | 0.0032 | 0.0006 | |
| | Raw | 505.7805 | 109.4995 | a | 273.4181 | 6.8647 | ab | 0.0000 | 0.0000 | a | 0.0023 | 0.0005 | ab |
| VIV1 | Cooked | 630.7554 | 52.7589 | | 455.5511 | 24.9765 | f | 0.0000 | 0.0000 | a | 0.0022 | 0.0002 | |
| | Raw | 669.6217 | 72.2842 | ab | 440.6023 | 24.6828 | ef | 0.0000 | 0.0000 | a | 0.0021 | 0.0002 | a |
| TMF | Cooked | 665.2081 | 59.0196 | | 458.6552 | 21.2278 | f | 0.0000 | 0.0000 | a | 0.0020 | 0.0002 | |
| | Raw | 606.9009 | 29.9358 | ab | 424.2842 | 16.6145 | def | 0.0000 | 0.0000 | a | 0.0018 | 0.0001 | a |
| QUO | Cooked | 770.4641 | 55.5077 | | 445.9021 | 14.4952 | ef | 0.0003 | 0.0001 | b | 0.0056 | 0.0005 | |
| | Raw | 696.8952 | 161.8905 | b | 344.5289 | 77.5341 | bcd | 0.0006 | 0.0002 | c | 0.0042 | 0.0010 | c |
| SMJ | Cooked | 590.1965 | 27.0800 | | 292.0492 | 17.9188 | abc | 0.0000 | 0.0000 | a | 0.0041 | 0.0001 | |
| | Raw | 620.3714 | 144.5844 | ab | 240.4017 | 6.3114 | a | 0.0000 | 0.0000 | a | 0.0040 | 0.0004 | c |
| SMX | Cooked | 1207.7011 | 44.9596 | | 275.5361 | 19.2706 | abc | 0.0000 | 0.0000 | a | 0.0030 | 0.0001 | |
| | Raw | 1241.1535 | 54.1362 | c | 305.8300 | 20.1175 | abc | 0.0000 | 0.0000 | a | 0.0031 | 0.0005 | b |

Content of non-essential minerals in burgers (continuation)

*Table 18 Content of nickel, germanium, arsenic, rubidium in cooked and raw burgers. Nickel (Product*Cooking p= 0.550. Product p<0.001. Cooking p=0.031). Germanium (Product*Cooking p= 0.161. Product p<0.001. Cooking p=0.017). Arsenic (Product*Cooking p= 0.215. Product p<0.001. Cooking p=0.079). Rubidium (Product*Cooking p<0.001). VIV1: Vivera burger; SMJ: Sainsbury's Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury's Mixed Vegetable burger.*

| Burger | Cooking | Ni (mg/100g) | | | Ge (mg/100g) | | | As (mg/100g) | | | Rb (mg/100g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Letters | Mean | SD | Letters | Mean | SD | Letters | Mean | SD | Letters |
| BEB | Cooked | 0.0119 | 0.0033 | a | 0.0000 | 0.0001 | a | 0.0007 | 0.0002 | a | 0.6343 | 0.0184 | f |
| | Raw | 0.0067 | 0.0018 | | 0.0000 | 0.0000 | | 0.0004 | 0.0001 | | 0.5115 | 0.0079 | d |
| VIV1 | Cooked | 0.0799 | 0.0299 | c | 0.0004 | 0.0001 | cd | 0.0009 | 0.0001 | a | 0.5851 | 0.0075 | e |
| | Raw | 0.0490 | 0.0004 | | 0.0002 | 0.0000 | | 0.0009 | 0.0003 | | 0.5724 | 0.0052 | e |
| TMF | Cooked | 0.0732 | 0.0034 | c | 0.0004 | 0.0001 | d | 0.0008 | 0.0001 | a | 0.5067 | 0.0108 | d |
| | Raw | 0.0692 | 0.0138 | | 0.0004 | 0.0002 | | 0.0006 | 0.0001 | | 0.4741 | 0.0185 | d |
| QUO | Cooked | 0.0249 | 0.0016 | ab | 0.0002 | 0.0001 | bc | 0.0020 | 0.0002 | b | 0.1622 | 0.0059 | a |
| | Raw | 0.0165 | 0.0039 | | 0.0002 | 0.0000 | | 0.0016 | 0.0005 | | 0.1295 | 0.0302 | a |
| SMJ | Cooked | 0.0626 | 0.0013 | bc | 0.0001 | 0.0001 | ab | 0.0031 | 0.0003 | c | 0.3894 | 0.0071 | b |
| | Raw | 0.0509 | 0.0015 | | 0.0001 | 0.0001 | | 0.0028 | 0.0003 | | 0.3496 | 0.0047 | b |
| SMX | Cooked | 0.1108 | 0.0731 | c | 0.0002 | 0.0001 | b | 0.0025 | 0.0003 | c | 0.3052 | 0.0024 | c |
| | Raw | 0.0644 | 0.0013 | | 0.0002 | 0.0000 | | 0.0028 | 0.0001 | | 0.3036 | 0.0049 | c |

Content of non-essential minerals in burgers (continuation)

*Table 19 Content of strontium, silver and cadmium in cooked and raw burger. Strontium (Product*Cooking p=0.049). Silver (Product*Cooking p=0.006). Cadmium (Product*Cooking p=0.419). VIV1: Vivera burger; SMJ: Sainsbury's Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury's Mixed Vegetable burger.*

| Burger | Cooking | Sr (mg/100g) | | | Ag (mg/100g) | | | Cd (mg/100g) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Letters | Mean | SD | Letters | Mean | SD | Letters |
| BEB | Cooked | 0.1774 | 0.0021 | a | 0.0000 | 0.0000 | a | 0.0016 | 0.0006 | ab |
| | Raw | 0.1388 | 0.0066 | a | 0.0000 | 0.0000 | a | 0.0001 | 0.0001 | a |
| VIV1 | Cooked | 0.5068 | 0.0024 | def | 0.0000 | 0.0001 | a | 0.0045 | 0.0007 | de |
| | Raw | 0.4911 | 0.0033 | de | 0.0000 | 0.0001 | a | 0.0028 | 0.0004 | bcd |
| TMF | Cooked | 0.4836 | 0.0085 | de | 0.0000 | 0.0000 | a | 0.0040 | 0.0001 | cde |
| | Raw | 0.4501 | 0.0210 | de | 0.0001 | 0.0000 | a | 0.0028 | 0.0002 | bcd |
| QUO | Cooked | 0.5169 | 0.0187 | ef | 0.0000 | 0.0000 | a | 0.0054 | 0.0018 | e |
| | Raw | 0.4153 | 0.0969 | cd | 0.0001 | 0.0001 | a | 0.0026 | 0.0007 | bcd |
| SMJ | Cooked | 0.5894 | 0.0112 | f | 0.0018 | 0.0001 | c | 0.0037 | 0.0006 | bcde |
| | Raw | 0.5333 | 0.0139 | ef | 0.0016 | 0.0001 | b | 0.0019 | 0.0003 | abc |
| SMX | Cooked | 0.3191 | 0.0048 | b | 0.0017 | 0.0001 | bc | 0.0042 | 0.0007 | de |
| | Raw | 0.3352 | 0.0074 | bc | 0.0017 | 0.0001 | bc | 0.0024 | 0.0002 | bcd |

Content of non-essential minerals in burgers (continuation)

*Table 20 Content of cesium, barium and tallion in cooked and raw burgers. Cesium (Product*Cooking p=0.021). Barium (Product*Cooking p=0.483). Thallium (Product*Cooking p=0.971. Product p<0.001. Cooking p=0.749). VIV1: Vivera burger; SMJ: Sainsbury's Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury's Mixed Vegetable burger.*

| Burger | Cooking | Cs (mg/100g) | | | Ba (mg/100g) | | | Tl (mg/100g) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Mean** | **SD** | **Letters** | **Mean** | **SD** | **Letters** | **Mean** | **SD** | **Letters** |
| BEB | Cooked | 0.0019 | 0.0001 | c | 0.0687 | 0.0442 | ab | 0.0000 | 0.0000 | a |
| | Raw | 0.0015 | 0.0000 | b | 0.0291 | 0.0018 | a | 0.0000 | 0.0000 | |
| VIV1 | Cooked | 0.0028 | 0.0002 | e | 0.2989 | 0.0039 | fg | 0.0006 | 0.0000 | b |
| | Raw | 0.0028 | 0.0001 | e | 0.2876 | 0.0044 | fg | 0.0006 | 0.0000 | |
| TMF | Cooked | 0.0010 | 0.0001 | a | 0.3156 | 0.0021 | g | 0.0000 | 0.0000 | a |
| | Raw | 0.0009 | 0.0001 | a | 0.2919 | 0.0125 | fg | 0.0000 | 0.0000 | |
| QUO | Cooked | 0.0010 | 0.0001 | a | 0.1710 | 0.0030 | d | 0.0000 | 0.0000 | a |
| | Raw | 0.0009 | 0.0002 | a | 0.1327 | 0.0324 | cd | 0.0000 | 0.0000 | |
| SMJ | Cooked | 0.0027 | 0.0000 | de | 0.2613 | 0.0031 | ef | 0.0000 | 0.0000 | a |
| | Raw | 0.0024 | 0.0002 | d | 0.2286 | 0.0045 | e | 0.0000 | 0.0000 | |
| SMX | Cooked | 0.0009 | 0.0000 | a | 0.1218 | 0.0101 | dc | 0.0000 | 0.0000 | a |
| | Raw | 0.0009 | 0.0000 | a | 0.1119 | 0.0006 | bc | 0.0000 | 0.0000 | |

Content of non-essential minerals in burgers (continuation)

*Table 21 Content of lead and uranium in cooked and raw burgers. Lead (Product*Cooking p=0.424. Product p=0.376. Cooking p=0.992). Uranium (Product*Cooking p=0.037). VIV1: Vivera burger; SMJ: Sainsbury's Mushroom and Jackfruit burger; TMF: The Meatless Farm burger; QUO: Quorn burger; BEB: Birds Eye Beef burger; SMX: Sainsbury's Mixed Vegetable burger.*

| Burger | Cooking | Pb (mg/100g) | | U (mg/100g) | | |
|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Letters |
| BEB | Cooked | 0.0054 | 0.0011 | 0.0000 | 0.0000 | a |
| | Raw | 0.0045 | 0.0005 | 0.0000 | 0.0000 | a |
| VIV1 | Cooked | 0.0035 | 0.0002 | 0.0000 | 0.0000 | a |
| | Raw | 0.0018 | 0.0001 | 0.0000 | 0.0000 | a |
| TMF | Cooked | 0.0028 | 0.0002 | 0.0002 | 0.0000 | a |
| | Raw | 0.0016 | 0.0001 | 0.0002 | 0.0000 | a |
| QUO | Cooked | 0.0031 | 0.0003 | 0.0021 | 0.0001 | d |
| | Raw | 0.0093 | 0.0122 | 0.0016 | 0.0004 | c |
| SMJ | Cooked | 0.0026 | 0.0004 | 0.0011 | 0.0000 | b |
| | Raw | 0.0024 | 0.0005 | 0.0010 | 0.0000 | b |
| SMX | Cooked | 0.0042 | 0.0041 | 0.0000 | 0.0000 | a |
| | Raw | 0.0021 | 0.0001 | 0.0000 | 0.0000 | a |

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