How traditional preparation methods affect the nutritional composition of Lupin and Soy.

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Dissertation Declaration

The work presented in this dissertation is my own work except where stated in the text. Technical assistance, where relevant, has been acknowledged. I understand the nature of plagiarism and that it is a serious academic offence. I confirm that no material in this project has been plagiarised.

Abstract

Awareness of the health benefits associated with reducing the consumption of animal products has led to an increase in the development of meat alternatives. Many of these alternatives rely on soybean because of its adaptability in producing a palatable meat alternative, such as Tempeh and Tofu. However, many are falling out of favour of soy as its consumption is linked to deforestation and loss of biodiversity. Furthermore, most of soy is imported into the UK therefore carbon footprint has to be taken into consideration. Lupin bean is deemed a possible alternative for soy as they can be cultivated in the UK and have many environmental benefits including nitrogen fixing, but its nutritional composition in comparison to soy is unknown particularly following processing by fermentation to produce tempeh and the coagulating to produce tofu. Thus the aim of this project was to compare the nutritional profile, focussing on protein and amino acid content, of soy and lupin beans. We observed that boiling the raw soy and lupin beans significantly increased the water content but decreased in nutritional content (energy, fats and protein). However while fermentation (process for the production of tempeh) increased the protein content in both beans the amino acid content was different. Fermentation increased the levels of branch chain amino acids (BCAA) in lupin while decreasing them in soy, although further frying of the soy increased it to greater levels of that of lupin where no further increase was observed. It was found that a processing method can influence the nutritional content of these products, however it was found that the Soy tempeh BCAA actually adapted best to the frying.

Acknowledgments

I would like to thank my supervisors Preeti Jethwa and Andy Salter for their support at each stage of this project, particularly in formulating research questions and methodology. I would also like to thank John Stubberfield for the amazing work helping me to perform my proximal analysis.

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Abbreviations

AAA – Aromatic amino acids

BCAA – Branch chain amino acids

EAA – Essential amino acids

HPLC - high performance liquid chromatography

LCMSMS - Liquid chromatography triple quad mass spectrometer

LDL - low density lipoprotein

LEDC – Less economically developed countries

LT – Lupin Tempeh

LTF – Lupin tempeh fried

MEDC – More economically developed countries

mTORC1 - mammalian target of rapamycin complex 1

NEAA – non essential amino acids nHDL - non-high-density lipoprotein cholesterol

NSP - non-starch polysaccharides

PDCAAS - Protein Digestibility Corrected Amino Acid Score

PER - protein efficiency ratio

PUFA - polyunsaturated fats

QC - Quality control

RDA – Recommended daily allowances

RL – Raw Lupin

RLB – Raw Lupin Boiled

RSB – Raw Soy boiled

RT – Raw Tofu

RTF – Raw Tofu Fried

ST – Soy tempeh

STF – Soy tempeh fried

1. Protein and the environment

The ever increasing in population coinciding with protein consumption, has resulted in the resources such as land and water becoming scarcer (Rulli et al., 2013, Godfray et al., 2010, Yu et al., 2013). Protein can come from two sources, animal, and plant, however there are environmental risks associated with both.

With the increased demand of global protein, especially that of high-quality animal protein, comes sustainability and environmental issues. For example rearing livestock produces large amounts of methane and other greenhouse gases, there is also sustainability issues due to the fact that large amounts of water and land will need to be used in order to rear these animals. For example, it is found that 6kg of plant protein is used to rear 1kg of high-quality animal protein (Henchion et al., 2017, Pimentel and Pimentel, 2003).

One of the plant sources commonly used is used to feed livestock soybean (Boerema et al., 2016). Which from 1970 has seen its consumption to increase around 200 million tons (Garrett et al., 2013). Besides livestock feed, one of the main reasons for soy staggering popularity increase, is because of the versatility. For example it can converted into a vegetable oil, used as biofuel, used as a precursor for many soy-based products e.g. (tempeh and tofu). The resulted "soybean boom" has been mainly associated with South American countries with suitable climates. However recently it has been noted that the increase in soybean production has led to many environmental impacts, such as loss of ecosystem and deforestation (Garrett et al., 2013, Nepstad et al., 2006, Hecht, 2005, Steward, 2007). The deforestation comes has come in recent years as a result of soybean production occurring on uncultivated ecosystems.

Therefore with soybean and meat demand every increasing it is found that more economically developed countries (MEDCs) have been using the land of less economically developed countries (LEDC) in order to expand their resources. This phenomenon is referred to as “displaced land used” and has many consequences. For example, the associated land and water of the LEDC will be harvested, as well as leaving behind the MEDCs carbon footprint and other environmental impacts (D’Odorico et al., 2013). These environmental impacts include, soil and land degradation, reduction in plant and animal diversity, deforestation, leaching and pollution into open water sources, and loss of own natural resources (Fearnside, 1999). It has been consistently shown that there is a lack of environmental understanding associated with “displaced land used”. Therefore there is a need for an increased awareness and decision making in order to produce a more sustainable outcome when these practices go ahead in the future (Grote et al., 2005). On top of these there needs to be a complete re-evaluation of the current policies that are put in place as well as stricter fines imposed of the countries that fail to meet the requirements, for example monetising the impact of deforestation (Schmitz et al., 2012). So whether its sustainability issues, environmental impacts, or land displacement, they all have been linked to the growing importance and demand of protein. So what is protein?

1.1 Protein

Proteins are made of long chains of amino acids, called polymers, which are connected by α-peptide bonds. While they can be characterised by their structures (primary, secondary, tertiary and quaternary). For the purpose of nutrition, we will be focusing on the primary conformation considering this relates to the amino acids (Watford and Wu, 2018).

Protein consumption can come from a range of plant and animal sources such as fish, eggs, legumes, milk, nuts, and poultry (Delimaris, 2013). The Recommended Daily Allowance

(RDA) of protein is said to be 0.8g per kilo gram of body weight per day (Trumbo et al., 2002). However for people competing in recreational to intense physical activity it is found that the RDA should increase to 1.1-1.6g/kg, in order to meet the adaptions of the body (Carbone and Pasiakos, 2019). For example it is found that eating slightly above the RDA protein content for elderly people can lead to increases in lean body mass, increased bone density, as well as improved muscular strength and durability (Houston et al., 2008, Mitchell et al., 2017, Kerstetter et al., 2000, Park et al., 2018). It is important to keep within these ranges as malnutrition can lead to some devastating effects. For example, undernutrition can lead to weakened immunity, growth stunting, muscular fatigue, and anaemia. Whereas long-lasting overconsumption (>2g/kg) of protein can lead to vascular, renal and digestive problems (Wu, 2016). But how is protein digested in the body?

Digestion occurs in the stomach, these acidic conditions (pH 1-2) activate the zymogen form pepsinogen to the active pepsin. Pepsin is stomach enzyme that digests proteins by cleaving the peptide alpha bond of the aromatic amino acids (AAA). This results in combination of intermediates which are transported to the duodenum, where pancreatic proenzymes (trypsin, chymotrypsin, carboxypeptidase) act upon them and produce tripeptides, dipeptides, and amino acids (Kiela and Ghishan, 2016). These peptides and amino acids are transferred to the intestinal cells apical end where they are further broken down via Dipeptidase and amino peptidase. These tripeptides and dipeptides are then absorbed into the intestinal cells via a hydrogen co-transporter channel, and then is broken down by peptidase to form amino acids. As for amino acids, they are absorbed into the intestinal cells via a sodium co-transporter channel. Following this, the amino acids is able to diffuse across the into the blood stream where it is taken up by the liver to synthesise new proteins or for storage.

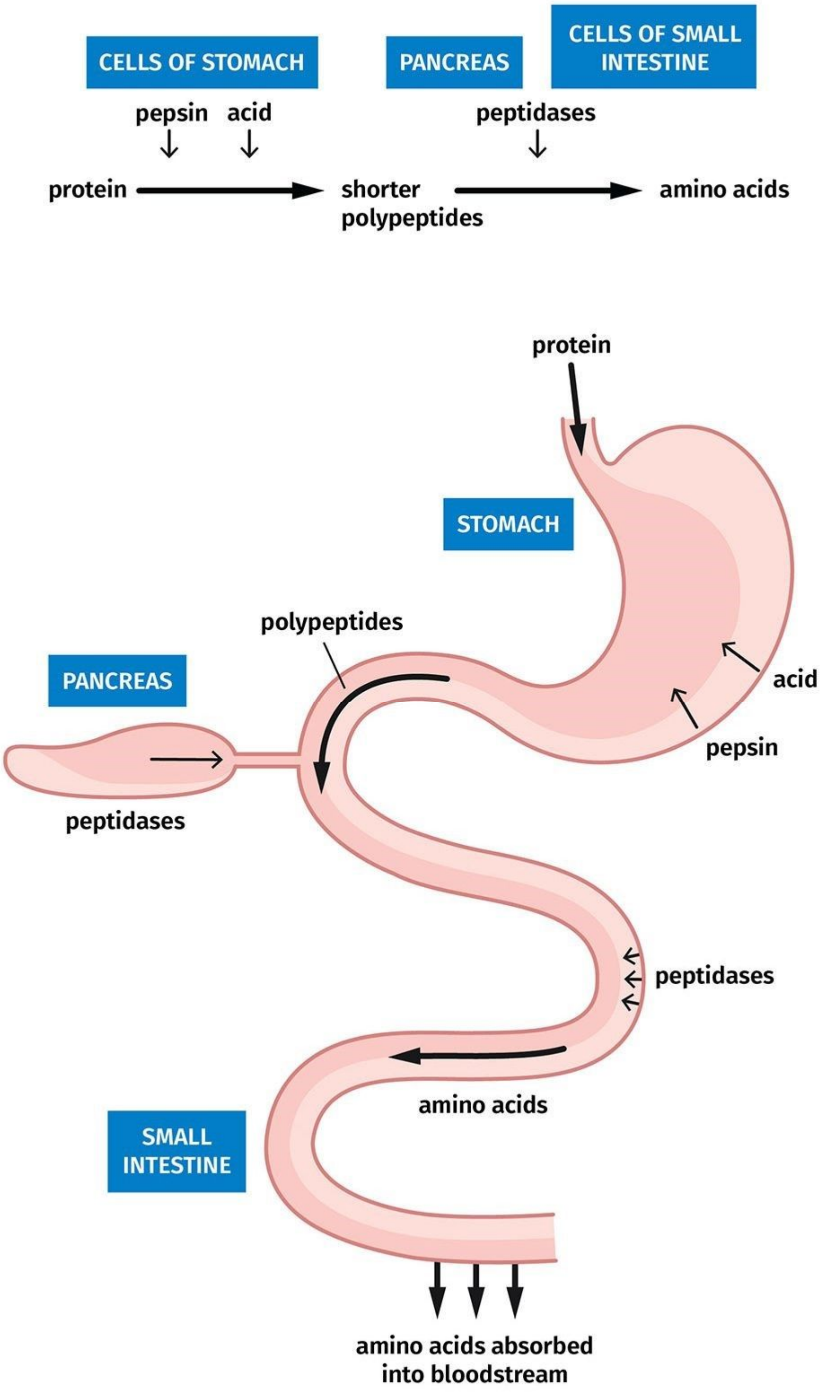

Figure 1- How proteins are absorbed in the digestive system. Protein enters the stomach, where it is broken down into small polypeptides by pepsin and acid. After that the polypeptides travel to the small intestine to be broken down by peptidases, which are released by the pancreas, once in the intestine the peptides will be broken down into amino acids which can be absorbed into the blood stream using co-transporters (2020)

1.1.1 What are amino acids

Amino acids are an organic compound that consist of an amino ($-NH_2$) and carboxylic acid ($-COOH$). An amino acids R-group or side change property can determine the unique properties of said amino acid. Thus, depending on which amino acid is coded, the order of these amino acids and other mitigating factors results in the protein synthesised (Watford and Wu, 2018). It is found muscle protein consists of 20 amino acids, nine are considered essential amino acids (EAA) (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and 11 which are non-essential amino acids (NEAA) (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, taurine, and tyrosine) for humans and most of other animals (Hou et al., 2015). The essential amino acids are exogenous, meaning that they must be supplied by the diet in sufficient amounts as they cannot be synthesised naturally within the body (Blomstrand et al., 2006). Whereas non-essential can be synthesised via transamination (Hou, 2018, Wu, 2013). Transamination is when the amino group of amino acid is cleaved and moved onto the acceptor keto-acid via transaminase, in order to produce a new amino acid and new keto-acid (Litwack, 2018).

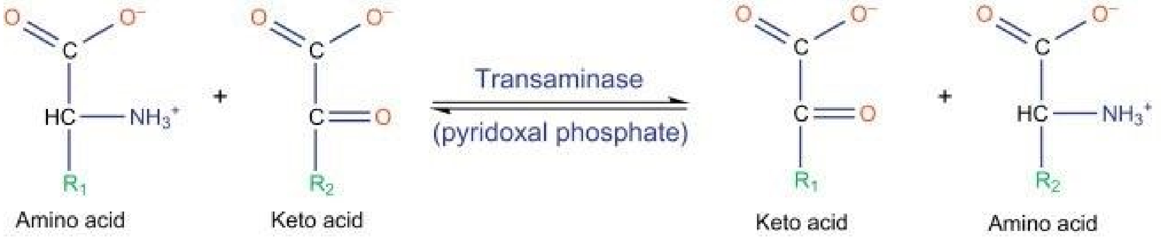

Figure 1.2 The transamination reaction. The amino group of the amino acid goes onto the keto-acid via transaminase (pyridoxal phosphate), producing a new amino acid and a new keto-acid (Litwack, 2018).

The regulator for transamination is protein turnover, therefore all 20 amino acids must be supplied for sufficient protein synthesis to take place. Of these 20 amino acids, there are a group called branched chain amino acids (BCAAs), comprised of Leucine, Isoleucine and Valine are essential for protein synthesis (Wolfe, 2017). Of these, leucine is seen as the most influential of the BCAAs as not only is it a precursor for muscle protein synthesis but also a regulator of a number of intracellular signals including mammalian target of rapamycin complex 1 (mTORC1). mTORC1 is directly involved in muscle protein synthesis and the regulation of glucose (Thomas et al., 2016, Jäger et al., 2017, Phillips, 2016, Lane et al., 2017). Whereas isoleucine causes muscular hypertrophy by increasing intramyocellular fat deposition as well as myogenesis, and valine enables tissue repair and correct nitrogen balance in body (Liu et al., 2021). The BCAAs most be consumed from the diet because they are essential, they can be sourced from a range of products and recently have been refined to be incorporated into nutritional supplements because of their anabolic affects (Wolfe, 2017). Therefore insufficient consumption of these can cause lack of protein synthesis, where as a lack in non-essential is easily compensated by having an increased de-novo production (Volpi et al., 2003).

1.2 Animal protein

It is found that animal proteins such as "whey" are digested quicker than that of plant proteins e.g. soy (Bos et al., 2003, Tang et al., 2009). This is because animal proteins are highly soluble in acidic conditions, therefore are able to pass from the stomach to the duodenum are a quicker rate (Boirie et al., 1997). It has also been found that the postprandial muscle protein synthesis rate is greater in animal protein than it is plant protein (Yang et al., 2012). One of the main reasons for this is because, animal protein contains a large proportion of leucine, which acts to inhibit protein breakdown as well as activates protein synthesis (Anthony et al., 2001, Suryawan et al., 2011). Due to the fact animal protein usually contains all of the essential amino acids, there is very little limiting amino acids, meaning that deamination and oxidation rates are lower (ProQuest, 2007).

Therefore there will be greater protein utilisation as the amino acids will not be eliminated (Tujioka et al., 2011). Also it is found that in most animal sources such as casein, milk beef, eggs, chicken, that the Protein Digestibility Corrected Amino Acid Score (PDCAAS) is 1.00 or close to this score (FAO, 1991, Hoffman and Falvo, 2004). The (PDCAAS) was introduced in 1989 as a way of working out the digestibility of a protein source (FAO, 1991). The calculation for PDCAAS is:

$$\text{PDCAAS} \quad = \quad \frac{\text{mg limiting amino acid in 1g test protein}}{\text{mg of same amino acid in 1g reference protein}} \times \text{Faecal True Digestibility}$$

Figure 1.3 The PDCAAS equation. PDCAAS = (mg limiting amino acid in 1g test protein / mg of same amino acid in 1g reference protein) x faecal true digestibility.

The results are either expressed as a score out of 100, or as a decimal, for the purpose of this paper we will be using decimals. Therefore a closer score to 1.00 means the greater protein quality.

1.3 Plant proteins

Plant proteins have demonstrated positive affects to the cardiovascular over that of animal protein consumption. For example a meat analysis study conducted by (Li et al., 2017) found that when participants consumed plant protein sources compared to animal protein their blood lipid content reduced, as well apolipoprotein B, non-high-density lipoprotein (nHDL) cholesterol, and low density lipoprotein (LDL) cholesterol. Thus improving cardiac health and lower potential cardiovascular disease risk. On top of this study, another study found adolescents consuming a higher proportion of animal protein compared to plant protein displayed a greater BMI and body fat percentage. Therefore suggesting that the replacement of animal protein to plant protein could be an aiding factor in reducing adolescent obesity (Lin et al., 2015). Overall it is found that plant based products are less digestible compared to that of animal base (Leser, 2013). The structural conformation of plants has huge impacts on absorption, for example plants contain a low α-helix compared to animal, as well as a high β-sheet content (Carbonaro et al., 2012). This high β-sheet actually reduces protein digestibility, as it inhibits proteolysis in the gastrointestinal tract (Carbonaro et al., 2012, Nguyen et al., 2015). On top of this, plants contain non-starch polysaccharides (NSP), which can bind to the binding sites of protein-cleaving enzymes, thus also reducing digestibility (Duodu et al., 2003). Plants are known for containing large amounts of antinutritional factors that reduce protein uptake, for example, tannins, hemagglutinins, gossypol, glucosinolates, protease inhibitors and phytic acid all have an affect (Sarwar Gilani et al., 2012). Phytic is acid is found to chelate minerals as well as interacting with proteins, thus reducing their bioavailability (Multari et al., 2015). It has been found that processes such as soaking, can activate phytase which causes the breakdown of phytic acid (Chouchene et al., 2018, Wang and Guo, 2021). Other preparation methods such as heat treatment will improve the bioavailability of food products by disrupting activity of protease inhibitors such as trypsin and chymotrypsin (Sarwar Gilani et al., 2012, Sarwar, 1997). It has been found that treated plant protein sources have about 18% greater bioavailability compared to its untreated counterpart (Sarwar, 1997, Rutherfurd et al., 2015). Overall it is found the PDCAAS in plants is found to be more variable with plants such as quinoa, pea, potato and canola having around 0.75, whereas Soy is found to be closer to 1.00. The variability and generally lowering scoring plant protein comes from insufficient indispensable amino acid content. For example it is found that in grains, lysine is generally a limiting amino acid, and in lowsulphur containing plants proteins such as Legumes there is a reduced methionine and cysteine content (Young VR, 1994).

1.3.1 Soy

Soy is a high protein bean, native to South East Asia (Synder HE, 1987). Soybean cultivation within Asian populations has been around for hundreds of years, whereas within western population is has been quite a new phenomenon. This was in the form of tofu, as a new health-conscious generation arose from the 1970s, enabling people to look for high protein, low fat alternatives. Ever since then, there has been a rapid increase in soy consumption and research, with many scientists finding independent nutritional benefits

(Messina, 2010). Soybean is one of the main plant protein sources used globally, it has been heavily researched and is found to have a much greater nutritional composition compared to that of other plant proteins (Ju, 1985). Soybean is converted into two commercially available products, tofu and tempeh. Tofu is made from curdling soymilk with coagulants until a solid block is formed (Eze et al., 2018). It's a valuable plant source containing minerals such as phosphorus, zinc, selenium and magnesium, as well as an excellent protein source as it contains all nine essential amino acids (Lewin, 2017).

It is found that tofu can contribute a significant amount of protein, with around 6g per serving (Adams, 2017b). Along with these nutritional benefits, it is considered extremely palatable and relatively cheap. Thus, tofu consumption is spreading to western populations and is becoming more popular in the United States and UK (H.L, 1984, DEY, 2017). It is commonly known as one of the highest yielding plant proteins, as well as having similar PDCAAS compared to that of animal products, with around 0.9 to 1.0 (Hughes et al., 2011, Rutherfurd et al., 2015). Due to the high PDCAAS score, the FAO has made claims for soy to be considered as a high-protein source (FAO, 2013). With regards to fat content, it is found that soy-based products are some of the only foods to contain both essential fatty acids. As the polyunsaturated fats (PUFA) is comprised of omega-6 and omega-3 essential fatty acids (Messina, 2016).

1.3.2 Lupin

Lupin is a high protein legume, that has been cultivated for more than 6000 years (Schindler et al., 2011). It is primarily grown in Western Australia (80%), with only 4% of all Lupin grown globally used for human consumption (Belski et al., 2011). Lupin seed's protein content is found to be around 32.2-42.0 g/ 100 g-1 (dry basis), depending on the species of Lupin used (Roy et al., 2010). The overall amino acid profile is of Lupin is not complete with their being a shortage of sulphur containing amino acids such as cysteine and methionine (da Silva et al., 2011). Depending on the species, the Lipid content is said to be around 5.5-13.0 g/ 100-1 (Fleetwood, 1982). Due to recent publications finding that it has anti-hypersensitive anti-dyslipidaemic, anti-diabetic and cardiovascular benefits, it is now considered as a functional food source (Sirtori et al., 2004, Sirtori et al., 2005, Marchesi et al., 2008). It is found that the low cost of Lupin compared to the Soy, as well as the comparable protein contents between the two, have allowed for increase interest within Lupin (Johnson, 2017)

1.4 Manipulation of plant based proteins into meat alternatives

1.4.1 Tempeh

Tempeh is a fermented soybean originating from Indonesia, it is commonly fermented using the bacteria Rhizopus Spp (Nakajima et al., 2005). Despite being a high protein yielding food, tempeh consumption is often associated with the lower class in Indonesia. The common misconception may be as a result of the price. Tempeh is a considerably versatile food stuff, as it can be eaten as a snack or a complementary protein source to a meal, a sauce, or be used tempeh barbeque (sate tempeh). It is found that frying Is the general cooking method of Tempeh (Karyadi and Lukito, 1996). Tempeh is a traditional product originated from Indonesia, tempeh is produced from a fermentation process in which the soybean is inoculated in Rhizopus Oligoporous bacteria in order to form a cakelike product (Handoyo and Morita, 2006). This fermentation process increases the bioavailability of a number of nutrients when compared to the unfermented soybean

(Jauhari et al., 2013) including conversion of glycosides to aglycones (Kuligowski et al., 2017), lipids into fatty acids (Ruiz-Terán and David Owens, 1996) and oxidation occurring between iron (II) to iron (III) (Tawali and Schwedt, 1998). Previously it was stated that there were three main steps in tempeh, which were soaking, boiling and fermenting (M., 1992). However it was lately corrected that there are actually nine steps (soaking, dehulling, washing, boiling, draining, cooling, inoculating (with Rhizopus spp), packaging, and then incubating). First of all the dehydrated soybeans are left to soak in clear water for around 24 hours. After that are they are dehulled, this can be done manually or with the aid of machinery. The dehulled beans are then boiled for around 30 minutes and then left to dry, in which they are left to subside to around 25 degrees. Once cooled to a suitable temperature. the bacterium, Rhizopus Spp is added to the soybean, mixed, and then transferred over to perforated bag for fermentation allowing to take place. The fermentation phase allows conformational changes within the soybean that will improve taste, nutritional bioavailability, and texture. Enzymes within the bacteria Rhizopus Spp will digest the macro nutrients to more digestible products. For example soy contains stachyose and raffinose, these complex sugars that cause flatulence will be broken down into more bioavailable products. Also in the fermentation phase, the phytates that are usually associated with inhibiting mineral absorption are destroyed, thus improving bioavailability. There is an improved vitamin B content, post fermentation which improves chemical and functional properties of the product (Furlan Bavia et al., 2012). It is also found that there is an increased aglycones content, due to the fact in fermentation there is the presence of βglycosidase which breakdown β-glycosides to the active form (aglycones). After the fermentation phase, the bag is then left at around 27-30 degrees, with 75-75% relative humidity for around two days of incubation. Once incubated, the tempeh should have a white cake-like mould to it (Ahnan-Winarno et al., 2021). Tempeh's popularity is ever increasing, due to the fact that it is simple to produce, inexpensive, and has a range of beneficial nutritional properties (Jauhari et al., 2013). For example, it is found that the bioavailability in the amino acid content of tempeh is said to be around 310 times greater than that of its precursor, soybean (Hwa L. Wang, 1968). On top of this it has a high proportion of BCAA (isoleucine, leucine and valine), which are commonly used to aid protein synthesis in respiring muscles (HERMANA, 2001). Due to the high amino acid and protein profile of tempeh, it has been found to have cholesterol-lowering properties, and therefore reduce the risk of a myocardial infarction (Kao and Chen, 2006, Cederroth and Nef, 2009, Wang et al., 2013). Besides the amino acid content, tempeh is primarily known for having a large isoflavone capacity, therefore can be associated as having anti-cancer and antioxidant properties which are beneficial to human health (Veldman et al., 2001, Kao et al., 2007, Messina, 2010).

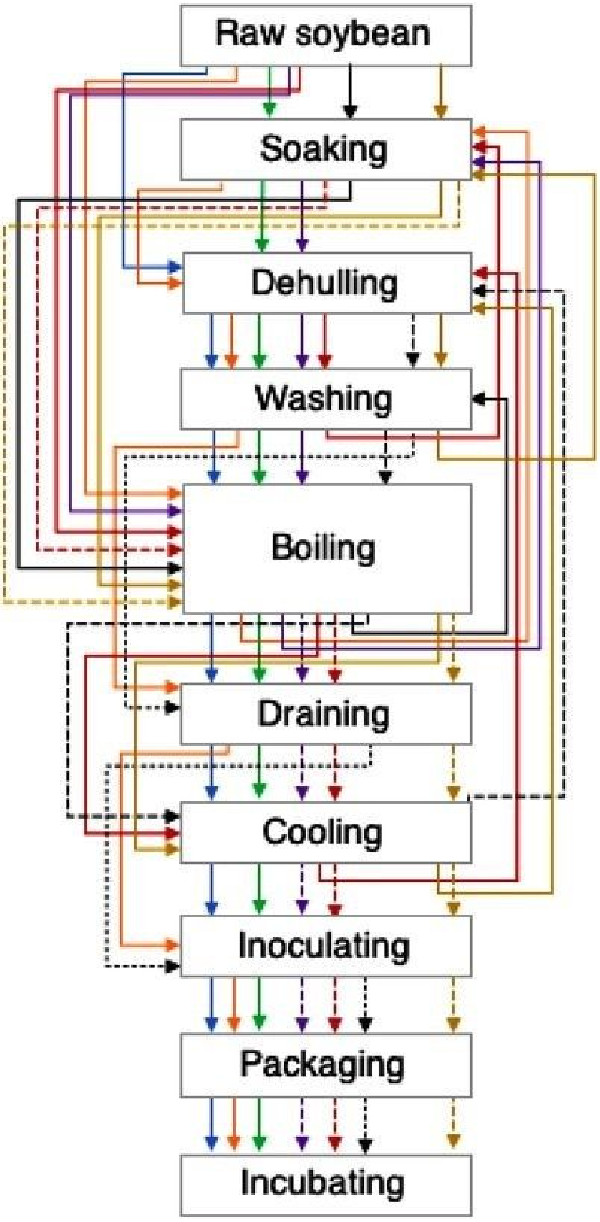

Figure 1.4 The variation in tempeh processing methods (Ahnan-Winarno et al., 2021).

1.4.2 Tofu

Tofu is a food stuff that comes from soy. It is produced by using the condensed version of Soymilk to create compact blocks. Tofu like tempeh, can be used in a range of recipes, the preparation cooking method of Tofu is often frying, boiling or steaming (Eze et al., 2018). Prior to soaking Soybean hulls are removed, removing the hulls actually decreases the beany flavour as well as improving colour (Kang, 2014). Once the beans have been soaked for around 10 hours at 22 degrees, they are then ground into a slurry and cooked for 5 minutes at 100 degrees (T McHugh, 2016). The soy milk is then filtered from the solid soy product through centrifugation and left to coagulate. Finally the tofu is pressed and pasteurised in order to extend shelf life. Tofu is found to have all the nine essential amino acids as well as an abundance of micronutrients including iron, copper, zinc, magnesium, vitamin Bs and phosphorus (Petre, 2017, J., 2017). Tofu contains vast amounts of isoflavones which can be useful when protected against inflamed blood vessels (Beavers et al., 2010). In terms of other non-nutritional aspects, Tofu contains a lot of antioxidant properties which will to defend against the risk of heart disease and some cancers (J., 2017).

1.5 Sustainable diet

A sustainable diet is defined as *"a diet comprised of foods brought to the market with production processes that have little environmental impact, is protective and respectful of biodiversity and of ecosystems, and is nutritionally adequate, safe, healthy, culturally acceptable, and economically affordable"* (Pimentel and Pimentel, 2003, Aleksandrowicz et al., 2016, Chai et al., 2019). This would usually be heavily related to a vegetarian/vegan diet. However it is found that only about 22% of the world are vegetarian. It is found that conversion to a vegetarian diet maybe associated with many cultural, social, and psychological issues (Schmidt and Mouritsen, 2020). On top of this human evolution has been conditioned to an *Unami* flavour which is generally related to animal-meat products (Wrangham, 2010). In order to slowly reduce animal-meat consumption, initiatives such as meat-free Mondays, and Veganuary have been proposed. These initiatives will hopefully cause people to consume a sustainable diet, in an accordance with the estimations and research from EAT-Lancet report. The report states a sustainable diet will include ones high in fruit, legumes, whole grains, unsaturated fats, and most importantly vegetables, coinciding with diets low in artificial sugars, starchy vegetables, red meat, and processed products (Willett et al., 2019). With these recommendations, there is a possibility that the United Nations Sustainable Development goals will be met by 2050, as long as drastic changes are made ((UN), 2019). A possible solution to meet the development goals and combat the exponential growth in the production of animal protein (H. Steinfeld, 2006), is to use alternative protein sources. The hope is these alternative plant protein sources could replace animal-based proteins, in a way that is environmentally friendly, mitigate global warming and treat malnutrition (F. Wild, 2014).

1.6 Aims and objectives

Soybean is one of the main plant protein sources used globally, it has been heavily researched and is found to have a much greater nutritional composition compared to that of other plant proteins (Ju, 1985). Soy's adaptability to act as the precursor to tofu and tempeh have made it a popular product in western populations and is becoming more popular in the United States (H.L, 1984, DEY, 2017). However the demand on importing soybean has lead to huge environmental costs, thus there has been an increase in trying to find alternative legumes which are more cost effective, but still able to create commercially favourable products similar to Tofu and soy Tempeh. One of the plant protein sources with the most potential is that of Lupin.

Lupin is a native European legume and has been used in food and livestock for more than 3000 years (Wäsche et al., 2001). Lupin has recently been noted as a functional food due to its high protein content and amino acid capacity (Wickramasinghe, 2017, Abeshu, 2017). The protein content and functional nutritional properties of Lupin are similar to that of

Soybean (Johnson et al., 2007) (JAYASENA, 2004) but has a lower fat content (KYLE, 1994). Furthermore is half the price of Soy (Jayasena et al., 2010)(Jayasena et al., 2010)and can be manipulated to produce tofu and tempeh (Wickramasinghe, 2017) . Therefore has the potential to be a more environmental and economical alternative to soy.

Here we evaluated whether Lupin could be a potential alternative plant based protein to Soy by assessing the nutritional composition (energy, fat, protein) and branch chain amino acid (isoleucine, leucine and valine) content. We are focusing on the BCAA content because of the impact these amino acids have on stimulating anabolism in the muscles (Kobayashi et al., 2006). Furthermore, as Tempeh and Tofu are generally consumed following cooking, we assessed whether steaming and frying can influence the composition.

1.6.1 Objectives

1. Perform a proximal analysis on lupin and soy in different preparation methods.
2. Proximal analysis including fat content, energy content, protein content and BCAA content.
3. Describe the differences how and why different preparation methods influence the nutritional profile of the product.
4. Determine whether lupin is a suitable replacement for soy in terms of different processing methods.

   Determine whether the BCAA content of both the products is suitable for a recreational athlete.

2. **Methods**

2.1 Materials

Raw soybean, Lupin and tempeh were gifted from BetterNatureUK. Tofu was sourced from The Tofoo Co Naked Tofu. All chemicals were petroleum ether (Fisher, UK), Benzoic acid (Parr USA), Aspartic acid (Fisher, UK). Deionised water (prepared in-house), Hydrogen peroxide, 30 % w/v, AR, (Fisher, UK) Formic acid, 98-100%, AR (Fisher, UK) Phenol, SLR (Fisher, UK) Sodium metabisulphite, AR, (Fisher, UK), Hydrochloric acid, S.G.1.18, AR, (Fisher, UK), Ammonium Formate (Fisher, UK), Cell Free Amino Acid Mixture - 13C,15N (Sigma-Aldrich, USA).

2.2 Preparation of material

2.2.1 Soaking

Raw soy and raw lupin (100g) was placed into a container and then filled with 300ml of clean, cold water and left to soak for 24 hours. Soaked beans were drained using a sieve to remove the hulls and weighed in order to calculate water uptake.

2.2.2 Boiling

It has been found among legumes that boiling can have significant effects on the nutritional composition (Ertas and Bilgicli, 2012). The soaked beans were placed into a pan filled with 1500ml of water at 150°C for 30 minutes. Once boiled the beans were drained, cooled, and weighed again.

2.2.3 Frying

The tofu and tempeh were cut into 100g blocks before being fried in 10ml of preheated sunflower oil for 5 minutes on heat level 7. The product was then transferred to a plate to cool down prior to being weighed.

2.3 Nutritional composition

Proximate analysis was performed on the lupin and soy products, the samples comprised of Raw lupin (RL), Raw Soy (RS), Raw Lupin Boiled (RLB), Raw Soy Boiled (RSB), Lupin Tempeh (LT), Soy Tempeh (ST), Lupin Tempeh Fried (LTF), Soy Tempeh Fried (STF), RT (Raw Tofu), Raw Tofu Fried (RTF).

2.3.1 Water content

Cooled samples were weighed, transferred to an aluminium foil catering tray and fitted with a lid. Samples were placed in -20°C for 24 hours and then in -80°C for 12 hours, prior to being put in a freeze dryer (Christ gamma LSC1-16+ (Christ, Germany)) to remove all moisture for 48-72 hours, until sample weight was stable. The samples were weighed, labelled, and then milled for further analysis.

2.3.2 Energy content

The Parr™ Automatic isoperbol calorimeter (RS Components, UK) was used to determine gross energy (MJ/Kg) for the samples. To do this 1-2g of sample was weighed in a crucible, compressed and placed in the bomb head with the ignition wire attached, the oxygen and nitrogen gas cylinders pressures set to approximately 450psi and 80psi respectively, and run using the set programme. Benzoic acid tablets (26.454MJ/Kg) were used as quality control.

2.3.3 Lipid content

Lipid content was measured using soxhlet method using Gerhardt Soextherm (Gerhardt, Germany). For this 1g of sample was enclosed in filter paper prior to being placed in a cellulose thimble; cotton wool was placed on top to keep contents contained. The soxhlet cellulose thimbles were placed into oven dried glass jars (type), with 3 boiling stones, and weighed ($Flask_A$). The solvent petroleum ether (140ml) was then poured into the jars and boiled at 150°C for 30 minutes for 2-3 hours until that. solvent level is reduced below thimble. The lipid content is extracted by the refluxing the solvent back into the beaker and the thimbles are removed. The solvent is then condensed by placing the jars into the oven at 103°C for 1 hour, cooled and weighed ($Flask_B$). The lipid percentage was calculated by using the equation: (weight of $Flask_A$ – weight of $Flask_B$) / sample weight) * 100.

2.3.4 Total protein content

Protein content was collected using the Flash EA1112 (Thermo Scientific - USA). 50mg of sample, standards (aspartic acid), QCs (quality control) (aspartic acid) and blanks (0mg) were weighed into foil capsules before being placed in the auto sampler. The Eager Xperience software was used to run the samples under the following conditions; furnace temperatures were set to 900°C and 680°C, respectively, gas flows to 140ml/min (carrier - helium) and 100ml/min (reference - oxygen). The filament on the thermal conductivity detector was set at 1,000µV. Samples were loaded, including bypass, blanks, standards and quality control. Protein concentration was calculated by multiplying the nitrogen concentration by 6.25 (as the nitrogen content of protein is found to be 16%). The bypass was used to determine the location of the nitrogen peak and the blanks and standards were used to create a standard curve. QCs were compared to expected values of 10.52% nitrogen.

2.3.5 Amino acid

The composition of amino acids in the samples was determined by Liquid chromatography triple quad mass spectrometer (LCMSMS), (Thermo Fisher Vanquish and Altis – USA).

50mg (±10%) of sample was weighed into a glass vial. Samples were then chilled at -20C for 1 hour before adding 2.5ml of chilled oxidation solution and then returned to the fridge for 16-18h. Post oxidation, each sample is removed from fridge and 0.42g of sodium metabisulphite added (to decompose and additional oxidation reagent). 2.5ml 12M HCl (hydrochloric acid) and 0.5 ml of hydrolysis reagent (6M HCl with 1% Phenol) was added to each sample. Samples are crimped and put in the oven at 110°C for 24 hours. Post hydrolysis, samples were cooled, transferred to 50ml tubes and rinsed with ammonium formate buffer (pH2.8, 20mM). Samples were gently swirled and 4M ammonium formate and formic acid was added to adjust to pH2.8. The tubes were topped up to 50ml with ammonium formate buffer (pH2.8, 20mM). Samples were then centrifuged 3000 rpm for 10 minutes, and then the supernatant passed through a 0.22 μm filter into a HPLC (high performance liquid chromatography) vial. 200ul of internal standard, 150ul of buffer, 50ul from sample vial. Go on auto sampler in LCMSMS.

3. **Results**

3.1 Do preparation and cooking effect the nutritional content of Lupin and Soy

3.1.1 Assessment of energy content

We observed that boiling raw lupin or soy resulted in a reduction in the amount of energy by 71.06% and 60.34% respectively (Energy (kcal/100g): RL 456.0 ± 1.0 to RLB: 131 ± 1.8; RS, 534.6 ± 1.2, RSB: 212.0 ± 1.3, $P<0.0001$ raw vs. boiled, Figure 3.1). Interestingly fermentation of RLB led to a significant increase in energy content (165.7 ± 3.6, $P < 0.001$ vs. raw boiled, Figure 3.1A), while fermentation of the RSB further reduced the energy content (173.9 ± 1.6, $P < 0.001$ vs raw boiled, Figure 3.1B). While the fermentation of the proteins significantly increased fat content in both protein sources (LTF: 268.48±0.97kcal/100g, STF: 324.44 ± 2.80 kcal/100g (Figure 3.1B). However when compared to the raw product both of them were significantly lower ($p <0.0001$; figure 3.1).

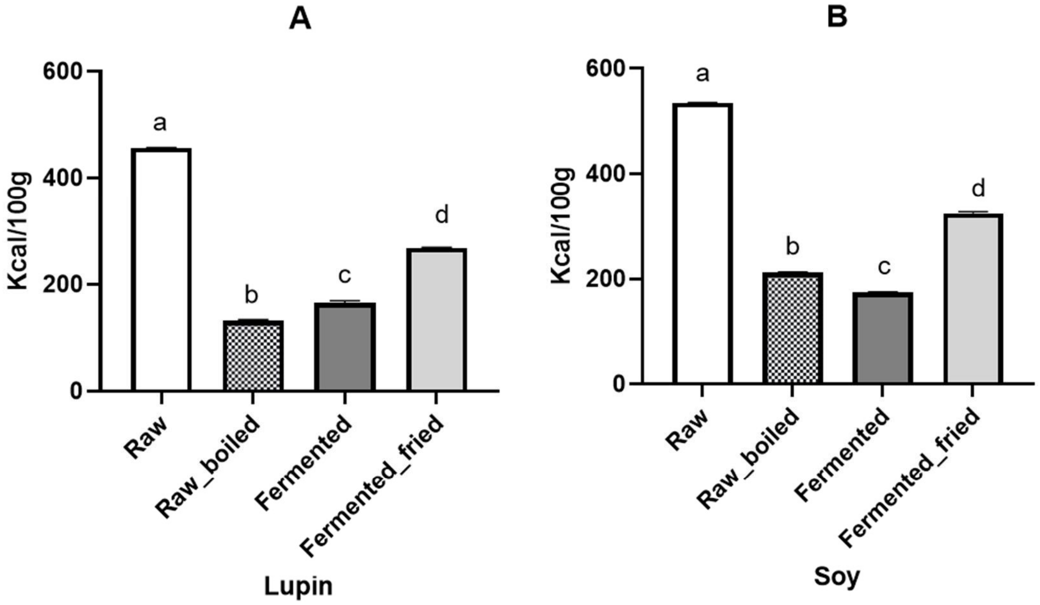

Figure 3.1. Cooking and preparation effects Energy content of Lupin and Soy. The energy (kcal/100g) of Lupin bean (A) and Soybean (B) under different preparation (boiling, fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment $p<0.0001$. Data letter "a" "b" "c" and "d" indicate significance.

3.1.2 Assessment of fat content

We observed that boiling RL or RS resulted in a reduction in the amount of fat by 65% and 54% respectively, (fat (g/100g): RS: 15.8 ± 0.56, RSB: 7.3 ±0.1, RL 10.2 ± 0.5, RLB 3.5 ± 0.1, $p < 0.0001$ raw vs. boiled; Figure 3.3). Fermentation did not further reduce fat content in RLB (fermented fat (g/100g): 4.8 ± 0.4; p= ns. vs raw boiled, Figure 3.2A, however fermentation of RSB further reduced the fat content (fermented fat (g/100: soy 5.70 ± 0.13, $P < 0.0001$ vs raw boiled, Figure 3.2B). Finally, we observed a significant increase in the fat content following the frying of the LT, not only higher than the fermented but also higher than the RL (11.68 ± 0.95g/100g and respectively $p < 0.0001$ vs. fermented, $p < 0.0001$ raw boiled and $p < 0.0001$ vs. raw figure 3.2A). Whereas in STF was significantly higher vs ST and vs RSB, however was significantly lower vs RS (Figure 3.2B).

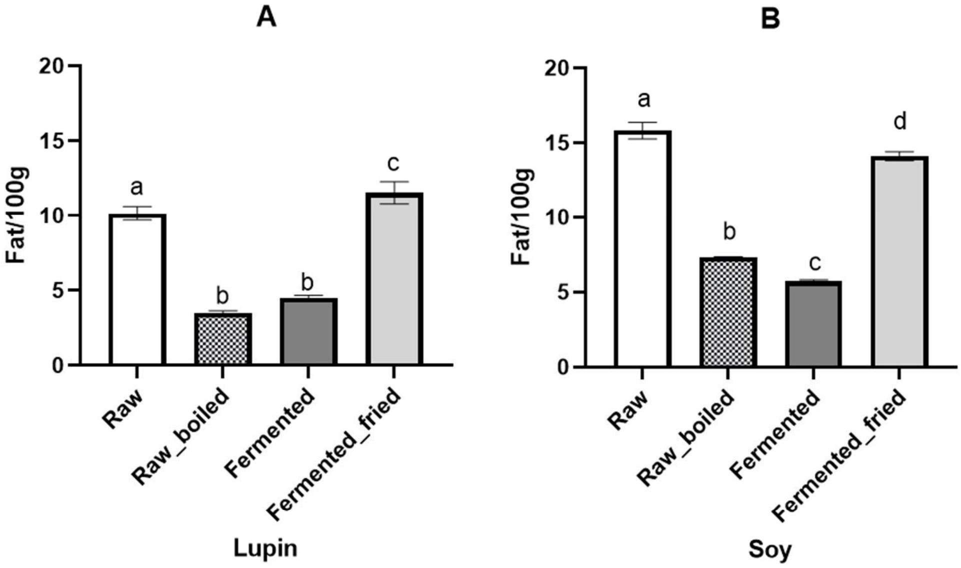

Figure 3.2. Cooking and preparation effects Fat content of Lupin and Soy. The fat (g/100g) of Lupin bean (A) and Soybean (B) under different preparation (boiling, fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment $p<0.0001$. Data letter "a" "b" "c" and "d" indicate significance.

3.1.3 Assessment of protein content

We observed that protein content was reduced following all types of processing (boiling and fermenting) in both proteins when compared to the raw content (Protein content (g/100g): RL: 37.3 ± 0.4, RLB 12.2 ±0.1, LT 16.3 ± 0.7, LTF 20.2 ± 0.2; $p < 0.001$ vs. raw; RS: 39.6 ± 0.8 RSB 16.2 ± 0.3, ST 18.3 ±0.3, STF 26.1 ± 0.3, $p < 0.001$; Figure 3.3). Interestingly fermenting the RLB and RSB led to an increase in protein content by 34.15% and 12.74% ($p < 0.001$), which was further enhanced by 23.32% and 42.05% frying in both LT and ST respectively ($P < 0.001$; Figure 3.3)

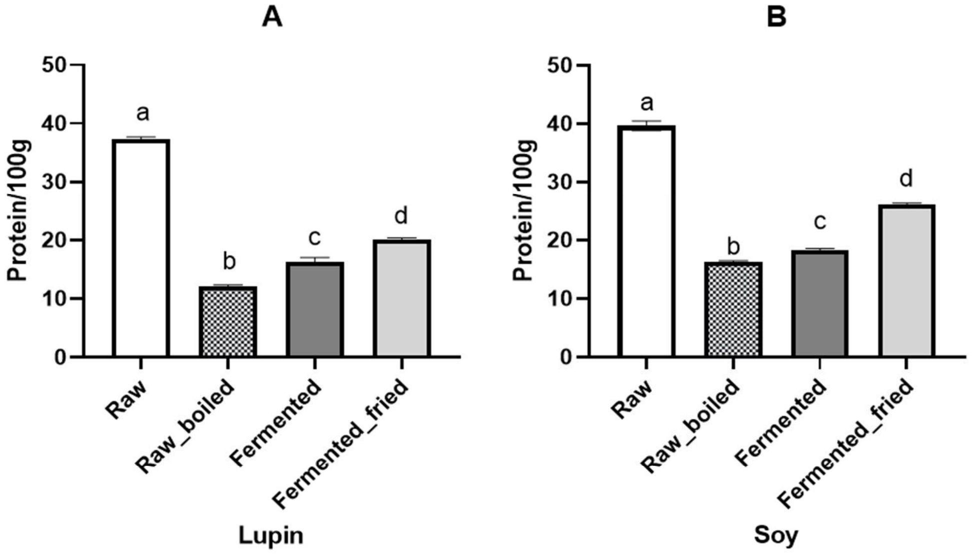

Figure 3.3. Cooking and preparation effects Protein content of Lupin and Soy. The protein (g/100g) of Lupin bean (A) and Soybean (B) under different preparation (boiling, fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment p<0.0001. Data letter "a" "b" "c" and "d" indicate significance.

3.1.4 Assessment of BCAA

3.1.4.1 Isoleucine content

We found that isoleucine content of RL and RS was significantly decreased following boiling (RL: 1.73±0.14g/100g to RLB: 0.47±0.06g/100g (Figure 3.4A) and RS: 1.80±0.20g/100g to RSB: 0.85±.011g/100g, P <0.0001), but fermentation of RLB significantly increased the levels of isoleucine while fermentation of RSB increased isoleucine levels (isoleucine levels: LT 0.79±0.01g/100g; P < 0.001 vs. RL; ST 0.77±0.04g/100g, P < 0.001 vs. RL; Figure 3.4 (P <0.0001) to Interestingly frying the fermented product had no effect on the isoleucine content of LT but significantly increased the protein content in ST (LTF: 0.79±0.03g/100g, p = ns. vs. LT, STF 1.08±.0.10g/100g, p < 0.0001 vs ST; Figure 3.4)

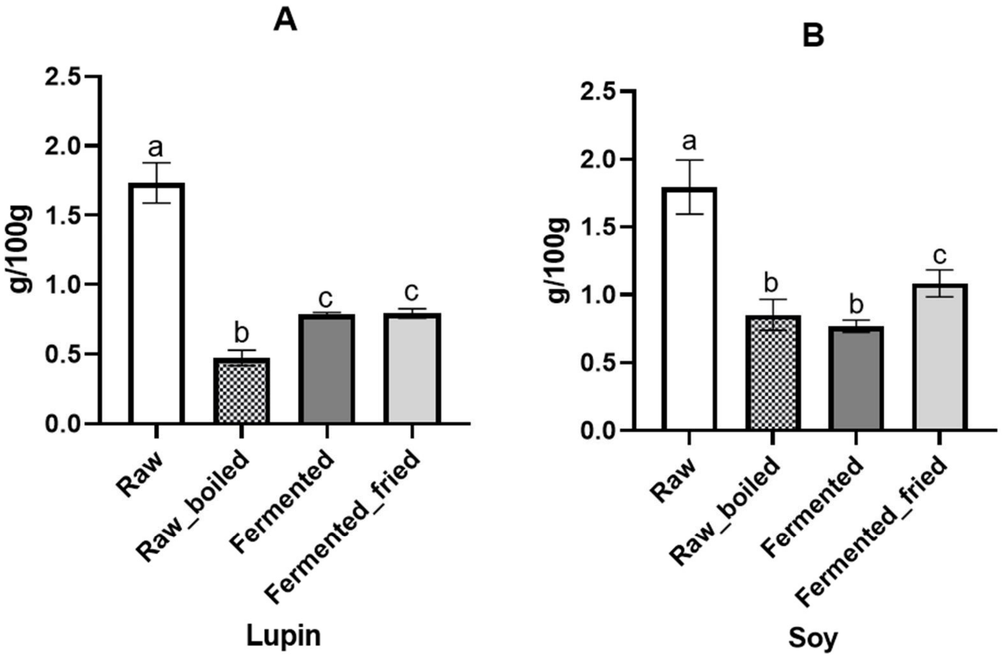

Figure 3.4. Cooking and preparation effects Isoleucine content of Lupin and Soy. The isoleucine (g/100g) of Lupin bean (A) and Soybean (B) under different preparation (boiling, fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment $p<0.0001$. Data letter "a" "b" and "c" indicate significance.

3.1.4.2 Leucine content

It was found when the protein source is from raw to boiled there is a decrease in leucine content. In RL: 2.61 ± 0.35g/100g, RLB: 0.75 ± 0.08g/100g $p < 0.0001$ raw vs boiled. In RS: 2.8 ± 0.26g/100g, RSB: 1.38 ± 0.13g/100g. When the products were fermented it was found that the p= ns. boiled product vs fermented, the Lupin value increased to 1.21 ± 0.02g/100g, whereas the soy value actually decreased to 1.13 ± 0.09g/100g. There were conflicting results when the fermented protein sources were fried as in LTF (1.19 ± 0.09g/100g there was p= ns. Fermented vs fermented fried (figure 3.5A), but for Soy (1.66 ± 0.16g/100g) it was $p <0.0001$ fermented vs fermented fried (figure 3.5B). It was found that for both products that the raw was significantly higher than the fermented fried ($P<0.0001$).

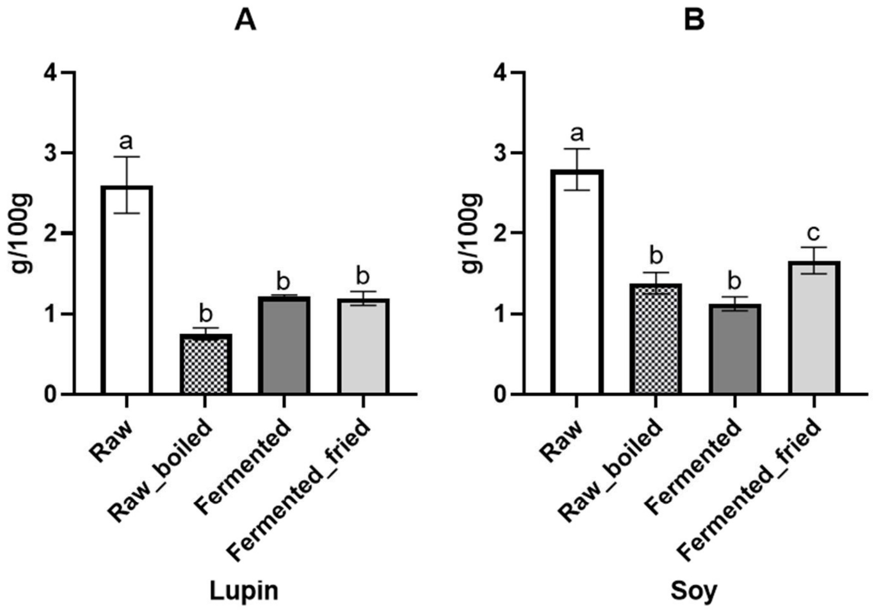

Figure 3.5. Cooking and preparation effects Leucine content of Lupin and Soy. The leucine (g/100g) of Lupin bean (A) and Soybean (B) under different preparation (boiling, fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment p<0.0001. Data letter "a" "b" and "c" indicate significance.

3.1.4.3 Valine content

The highest valine came from the RL and RS with 1.65 ± 0.36g/100g and 2 ± 0.27, respectively (Figure 3.6). The valine content dropped significantly in Lupin and Soy to 0.48 ± 0.06g/100g and 0.94 ± 0.16g/100g, both p < 0.0001 raw vs boiled. It was also found that there was p = ns. Raw boiled vs fermented vs fermented fried, for both protein sources (Figure 3.6). Although there was p= ns found between the rest of the results it was interesting to see the conflicting valine contents. LT valine content increased to 0.78 ± 0.01 g/100g, whereas the soy decreased to 0.8 ± 0.04g/100g. As for frying the fermented product, both valine contents increased, however there a much greater increase found in STF 1.15 ± 0.1g/100g (Figure 3.7B), compared to LTF 0.8 ± 0.05g/100g (Figure 3.6A).

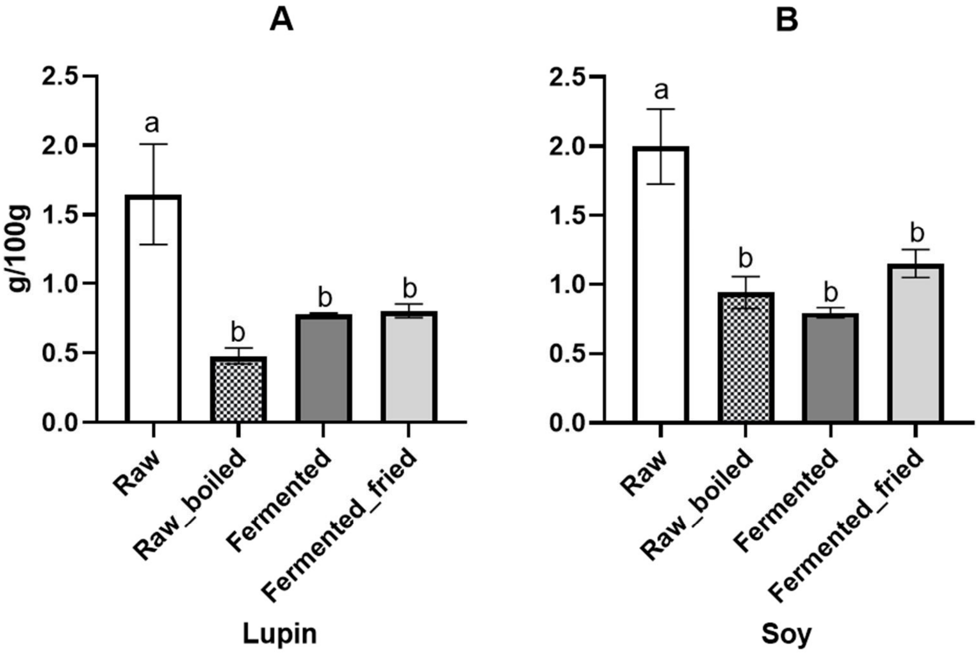

Figure 3.6. Cooking and preparation effects Valine content of Lupin and Soy. The valine (g/100g) of Lupin bean (A) and Soybean (B) under different preparation (boiling, fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment p<0.0001. Data letter "a" "b" indicate significance.

3.2 How does cooking effect the nutritional content of fermented soy and lupin when compared to soy tofu.

3.2.1 Assessment of energy content

It was found that soy had the highest energy content to the other proteins sources when fermented 173.86±1.55kcal/100g or fermented fried 324.44±2.8, these were found to be significant to each other p <0.0001 fermented vs fermented fried. As well as being significant to all of the other protein sources whether fermented or fried (Figure 3.7). The tofu was found to have the lowest energy content compared to the other protein sources when either fermented: 132.24 ± 2.79kcal/100g or fermented fried: 227.44 ± 1.86. With regards to tofu it was also found p <0.0001 fermented vs fermented fried, there was also significant difference found with both protein sources (Figure 3.7). Meaning that lupin was found to be significant to all other the protein sources P <0.0001, as well as being significantly different when fried, P <0.0001 fermented vs fermented fried. LT fermented energy content was close to that of ST with 165.68 ± 3.56 kcal/100g, however post frying it showed a greater difference with 268.48 ± 0.97 kcal/100g (Figure 3.7).

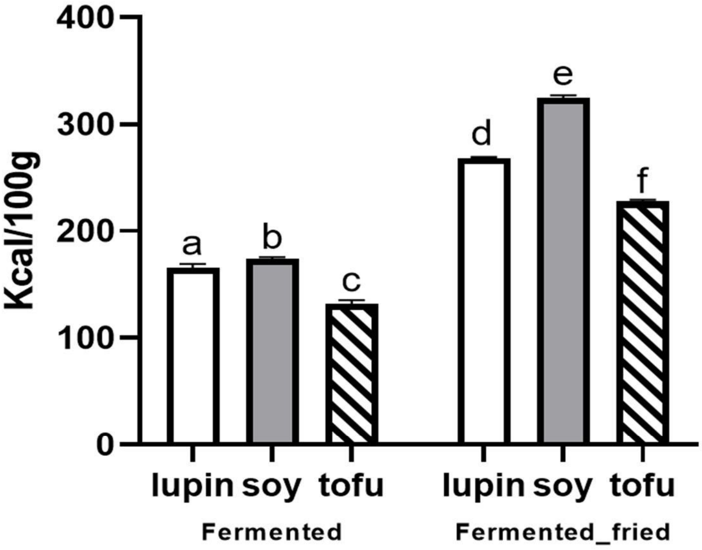

Figure 3.7. Cooking and preparation effects Energy content of Lupin, Soy and Tofu. The energy (kcal/100g) of Lupin bean, Soy and Tofu under different preparation (fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment $p<0.0001$. Data letter "a" "b" "c" "d" "e" and "f" indicate significance.

3.2.2 Assessment of fat content

It was found that all of the fermented protein sources were significant to each other $P< 0.0001$, with RT having the highest fat content 7.24 ± 0.28g/100g, followed by ST 5.70 ± 0.13g/100g and then LT having the lowest content with 4.8 ± 0.4g/100g (Figure 3.8). All of the fermented protein sources were $P <0.0001$ fermented vs fermented fried, to their counterpart. In the fermented fried results, although STF had the highest fat content with 16.98 ± 0.7g/100g, it was p = ns. STF vs RTF, with tofu fat content being slightly less with 13.69 ± 0.05g/100g. As for the LTF, this was significant to both STF and RTF, as well as having the lowest fat content with 11.68 ± 0.95g/100g (figure 3.8)

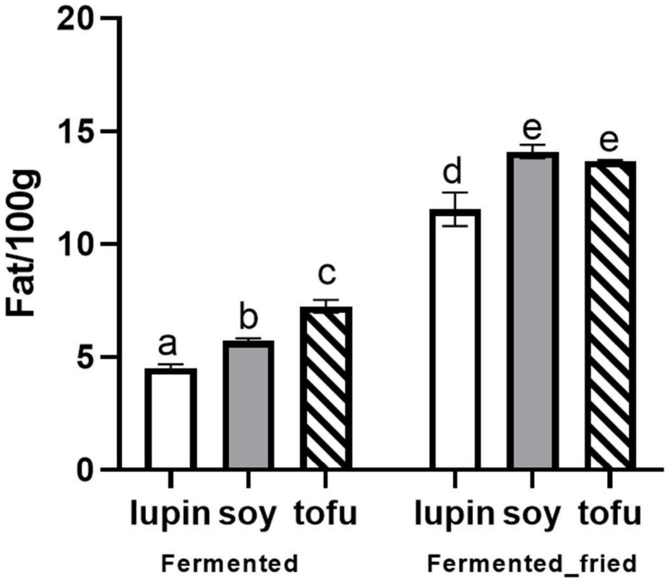

Figure 3.8. Cooking and preparation effects Fat content of Lupin, Soy and Tofu. The fat (g/100g) of Lupin bean, Soy and tofu under different preparation (fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment p<0.0001. Data letter "a" "b" "c" "d" and "e" indicate significance.

3.2.3 Assessment of protein content

The results observed in Figure 3.10 show that soy had the highest protein when fermented: 18.31 ± 0.26g/100g, as well as when fermented fried: 26.01 ± 0.32g/100g. These results were also found to be significantly different from one another P <0.0001 fermented vs fermented fried. Lupin followed this trend, as there was an increase from LT content: 16.34 ± 0.66g/100g to LTF: 20.16 ± 0.22g/100g, with P<0.0001 fermented vs fermented fried. The LT value was seen to be similar to that of RTF: 15.59 ± 0.19g/100g, however there was no significance found, p = ns, RTF vs LT. Whereas the RTF was found to be significant P<0.0001 vs RT protein content: 13.06g/100g ± 0.17g/100g (Figure 9).

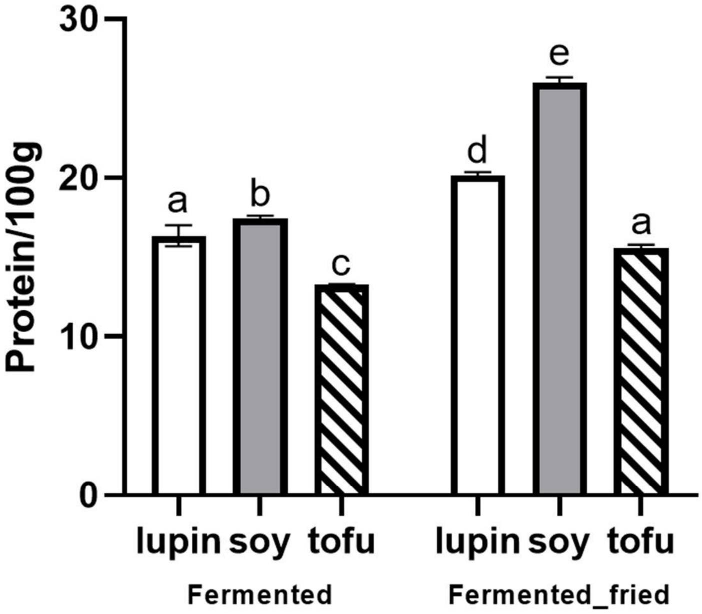

Figure 3.9. Cooking and preparation effects Protein content of Lupin, Soy and Tofu. The protein (g/100g) of Lupin bean, Soy and tofu under different preparation (fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment $p<0.0001$. Data letter "a" "b" "c" "d" and "e" indicate significance.

3.2.4 Assessment of BCAA

3.2.4.1 Isoleucine content

When looking into the isoleucine content of LT, ST, RT, it was found that there was no significance between them p =ns. Fermented lupin vs fermented soy vs tofu (Figure 3.10). The RT had the highest isoleucine content 0.85 ± 0.07g/100g, followed by LT 0.79 ± 0.01g/100g and then ST with 0.77 ± 0.04g/100g. Although ST had the lowest isoleucine content, when fried it increased significantly to 1.08 ± 0.10g/100g. This was not only $P<0.0001$ vs ST, but also significant compared to the other protein sources. As for LT and RT, there were marginal differences when fried, showing LTF with 0.79 ± 0.03g/100g and RTF with 0.79 ± 0.01g/100g, respectively, due to the low changes it was found there was p =ns. Between its own counterpart (Figure 3.10).

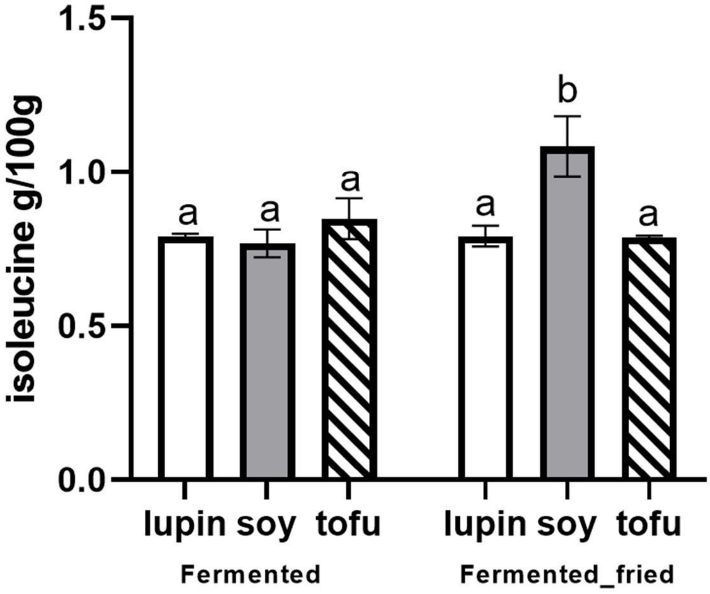

Figure 3.10. Cooking and preparation effects Isoleucine content of Lupin, Soy and Tofu. The Isoleucine (g/100g) of Lupin bean, Soy and tofu under different preparation (fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment $p<0.0001$. Data letter "a" and "b" indicate significance.

3.2.4.2 Assessment of leucine content

Similar to the isoleucine graph, it was found that the leucine values of the fermented protein sources showed p =ns (Figure 3.11). As well as RT leucine value being the highest for fermented 1.35 ± 0.06g/100g, and then losing leucine content after frying to 1.26 ± 0.04g/100g, p =ns. RT vs RTF. The RTF along with the LTF (1.19 ± 0.09g/100g), was found to be significantly different $P <0.0001$ vs the STF (1.66 ± 0.16g/100g). Although the STF value was the highest, it was observed that the ST had the lowest value out of all the protein sources with 1.13 ± 0.09g/100g, this was also significant $p<0.0001$ STF vs ST. Despite being the lowest it was not significant to the other values, including lupin with 1.21 ± 0.02g/100g (Figure 3.12).

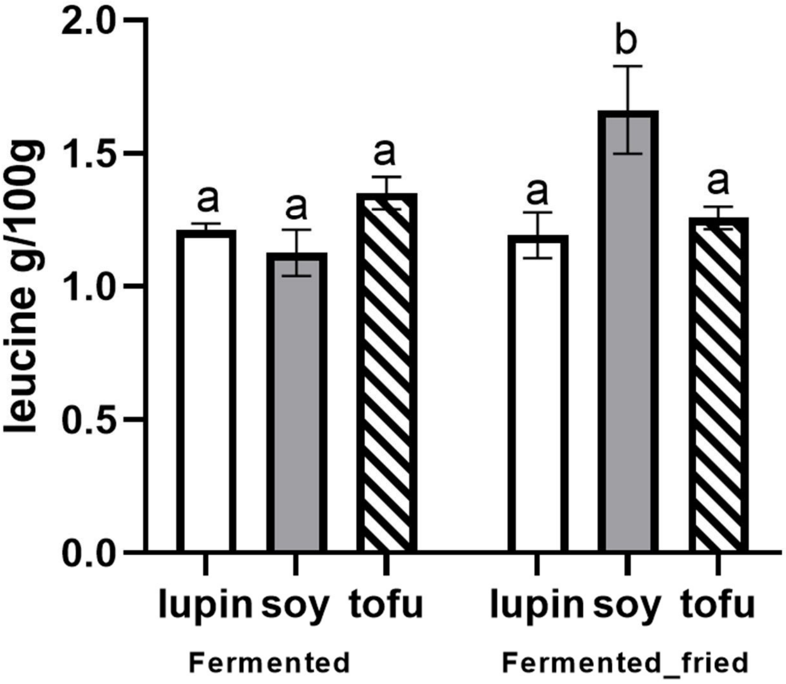

Figure 3.11. Cooking and preparation effects Leucine content of Lupin, Soy and Tofu. The Leucine (g/100g) of Lupin bean, Soy and tofu under different preparation (fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment p<0.0001. Data letter "a" and "b" indicate significance.

3.2.5 Valine content

Figure 3.12 shows that in the fermented results, the highest valine contents come from the RT with 0.87 ± 0.02g/100g, followed by ST with 0.8 ± 0.04g/100g and then LT with 0.78 ± 0.01 g/100g. These results all showed p =ns. Post frying the greatest increase came from the STF, as the valine value went to 1.15 ± 0.1g/100g, this also showed significance between p <0.0001 vs ST, as well as the other protein sources. Despite there being p =ns. Between the counterparts of fermented vs fermented fried in lupin and tofu, conflicting results were shown. As after frying, the valine content in lupin increased slightly to 0.8 ± 0.05/100g, whereas in tofu there was a decrease to 0.83 ± 0.01 when fried (Figure 3.12).

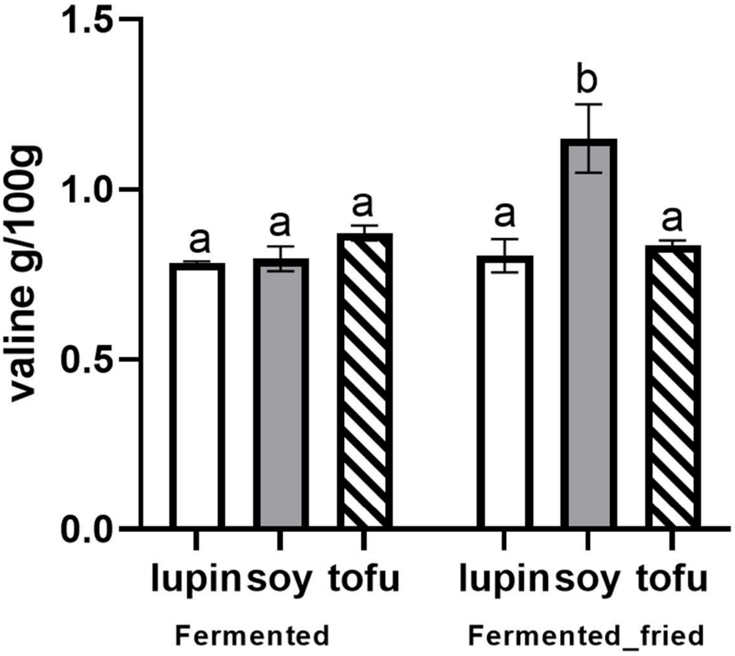

Figure 3.12. Cooking and preparation effects Valine content of Lupin, Soy and Tofu. The Valine (g/100g) of Lupin bean, Soy and tofu under different preparation (fermentation and frying). Values are group mean ±SEM, n = 3. Effect of treatment $p<0.0001$. Data letter "a" and "b" indicate significance.

4. **Discussion**

In this study we observed how different preparation methods affect the nutritional quality of the products tested, by observing a range of preparation techniques such as boiling, fermentation and frying. It was found overall that the boiling method had a detrimental effect on the nutritional profile, a potential reason for this could be the water content of the product increasing thus decreasing the contents of other nutritional products. Whereas the fermented had conflicting results on the lupin and soy. Besides the protein content, the Soy actually decreased in every proximal analysis test, where as the Lupin actually increased significantly in all besides leucine and valine. When frying, the ST, LT and RT all significantly increase in the energy, fat and protein department, however it was found that only the ST increased significantly when fried with regards to the BCAA content. Precooked the tofu was found to have a lower energy and protein content to that of the tempeh, whereas it had a significantly higher fat content. On top of this, it was found that the precooked BCAA content was similar to that of the other two tempeh products, however when fried it was significantly less than the STC.

Do preparation and cooking alter the nutrient content of soy or lupin?

4.1.1. Boiling

We found that raw soybean had a higher energy content, lower fat content and comparable protein content when compared to raw lupin, but boiling lead to a reduction in these by 60-70%, with a greater reduction observed in lupin. Unfortunately boiling is necessary for most legumes to improve the palatability as well as protein digestibility by the elimination of many anti-nutritional factors that are heat-labile (Chau et al., 1997). There are a number of reasons for the difference between the two proteins; it is well known that Lupin contains a greater amount of carbohydrates(40.2/100g > 30.2/100g (Data, 2021)) which is greatly affected in boiling as there is a rupturing of the carbohydrate molecule and then amylolysis occurs (Jood et al., 1988) and this increase in water absorption by lupin replaces the fat and protein content (Fabbri and Crosby, 2016). Interestingly while the results we obtained were in line with others for soybean protein (Abeshu, 2017, Ertas and Bilgicli, 2012) but differed for lupin, in which no significant differences were observed (Niyibituronsa et al., 2019). Many results have shown that the boiling process can decrease protein content, possibly due to the high temperatures leading to denaturation of the protein structure and amino acid dissolving into the surrounding water, thus leading to amino acid extraction within the protein source (Venugopal, 2006) (Ertas and Bilgicli, 2012) (Barampama, 1995). Our results were no different, the BCAA levels were reduced in both proteins however the reduction was greater in lupin than soy by almost 20%. A reason for this could be that there was a greater amount of water absorbed in the lupin compared to soy, therefore hydrolysis could have a occurred at a greater rate.

4.1.2. Fermenting

The fermentation process showed conflicting results between the two protein sources. For example it was found that the Energy and Fat content increase by around 25-29% in the Lupin. A reason for the increased Energy and Fat content increasing in the Lupin could be because the content of free sugars, polysaccharides and oligosaccharides all decreasing in fermentation, thus fat content prevailing (N. Fudiyansyah, 1995). The fat content increasing was found to not be consistent with results from (Wickramasinghe, 2017) as they found fat content actually decreased. A reason for this could be that the fermentation experiment was not actually carried out in this experiment.

However decreased by around 17-22% in the Soybean for Energy and Fat content. This could be because the rhizoprous oligoporous uses, linoleic acid, palmitic acid, stearic acid in fermentation as energy for the mould (Astuti et al., 2000). The results from this paper are consistent with papers online as it found that post-fermentation in tempeh, that the lipid content is to have been reduced by around 25% (Murata et al., 1967).

When fermented protein levels significantly increased in both protein sources, which is consistent from (Wickramasinghe, 2017), which also stated that significant increases were shown in their lupin tempeh. It is found that the proximate composition is unchanged in fermentation however protein percentage increase occurs because of carbohydrate levels reducing. This is as a result of the carbohydrate being broken down for energy in order for the fermentation reaction to occur (Sarkar, 1993).

Despite both protein sources significantly increasing, when looking into the BCAA there were again conflicting results. Overall it was found the Lupin performed better than the Soy. For example the Branch chain amino acid content of the Lupin was found to increase around 60-70%, which is similar to results found from (Handoyo and Morita, 2006). Whereas for Soy it was found to actually decrease around 9-19%, with greatest decrease found in leucine. The decrease in contents for Soy was of interest considering you would expect all the amino acids to increase post fermentation because Rhizoporous oligoporous would hydrolyse the large polypeptides into small more bioavailable amino acids (Sparringa and Owens, 1999). On top of this the fermentation process increases the protein digestibility corrected amino acid score (PDCAAS), and protein efficiency ratio (PER), and a number of amino acids, including Isoleucine, leucine and valine (ReyesMoreno et al., 2004, Handoyo et al., 2006).

4.1.3. Frying

In order to increase palatability, as well as to kill off bacteria, frying is a common preparation method used among tempeh and tofu. It was found that there were all significant increases within the Energy, Fat and Protein content of the three protein sources used. However it was seen that the Soy had the greatest increase of nutritional content followed by Lupin and then Tofu. The fat content significantly increase within all three protein sources was not surprising because foods of plant origin are more water and less fat. Therefore the oil absorbed into intracellular space filled with air, due to evaporated water, leading to a greater fat absorption (Fillion and Henry, 1998, Ghidurus et al., 2010). A bi-product of increased fat absorption is increased energy content (Fillion and Henry, 1998). Despite having the lowest percentage increase in fat absorption, it was found that out of the protein sources, tofu had the highest lipid content pre-fried. This was expected as it is found to have a high source of omega-3, omega-3 fatty acids are beneficial to the diet as they provide energy as well as aiding recovery (Simopoulos, 2007) (Adams, 2017a).

Post-frying the protein content within the protein sources increased from around 1743%. Which is conflicting with previous papers, as this high temperature cooking method is expected to decrease the nutritional content and antioxidant capacity (Setiawan, 2016). As high temperature frying will allow the oil in frying will occupy any free gaps left by the evaporating water (Damanik et al., 2018). These thermally conducted oil molecules, will have staggering effects on the protein. Although protein breakdown usually increases protein digestibility, as it is being broken down into amino acids which are easier to assimilate into the body (Afifah et al., 2019), temperatures over 100 degrees will cause the protein and amino acid to denature and no longer be a functional soluble protein (Zakaria, 2015, Sbroggio et al., 2016). This is as a result of the frying method causing a maillard reaction within the tempeh matrix, causing crosslinking within the protein substrate binding site, resulting in conformation and therefore leading to inhibition of complementary enzyme (S. Sulthoniyah, 2013).

Yet it was found in one paper that the Tofu protein content increased by 87.87% (Dina and Ghadir, 2019). Reasons for this could be because of different frying methods, different variations of tofu and ingredients used. On top of this you would expect the protein percentage of tofu to increase because of the high water content within the tofu decreasing therefore allowing the protein to prevail (Dina and Ghadir, 2019). This is what could have been occurring in the paper.

It was found that the amino acids in Soy all significantly increased from around 41-47%. This is conflicting with results from (Afifah, 2019), which found there to be decreases around 69-71% in soy tempeh fried. As mentioned before, decreases are expected due to the high temperature method causing irreversible denaturing of the amino acid meaning it can no longer be considered as functional for absorption (Damanik et al., 2018). On top of this, these results were completely conflicting with lupin tempeh and tofu which found marginal changes within all three amino acids, which showed no significance.

Decreases in Tofu’s isoleucine, leucine and valine in this paper were found to be 0.06g/100g, 0.09g/100g and 0.04/100g, respectively. When comparing results to that of (Dina and Ghadir, 2019), it was found that the isoleucine was consistent with, 0.06g/100g, however leucine and valine showed a much greater decrease of 0.53g/100g and 0.14g/100g.

4.1 Limitations

The first limitation was that the lupin tofu was not performed as this would have allowed for a greater comparison when comparing the full nutritional profile of all three products. Another area was that the processing methods of the tofu and tempeh was not actually performed in the laboratory. As previously mentioned in the methods, the end products were sourced from Better Nature and Tofoo, which lacked basic sample information (of the precursors) regarding batch numbers. Since it is not known whether the end products were produced from the same batch of precursors, inherent variation between batches could not be accounted for in these results which included tempeh and tofu product. Meaning that it is difficult to draw conclusions when going through the proximal analysis techniques. Another area of limitation could be that the anti-nutrition profile was not tested, as previously mentioned there are anti-nutritional content found in plants reduce the bioavailability of protein. Therefore the results in this paper provide a hypothetical protein content and not an accurate representation of the official bioavailability.

4.2 Conclusion

While the use and consumption of plant-based protein is increasing, it is was importance to assess how new products nutritional quality is affected among a range of preparation methods. Frying and boiling are able to increase the palatability and acceptability. However the high temperature methods are found to have irreversible effects on the quantity of key nutrients. It was found that preparation method was also key as it was found when Soy was transformed into tempeh and not tofu, the maintenance of nutritional quality was better held.

For boiling it was found to be more suited towards an athlete when boiled compared to the lupin because of it having a higher nutritional content in all areas assessed. For fermenting process it was found that the lupin performed much better than the soy as many of the nutritional markers increased. However as lupin is not consumed raw, it is hard to conclude these results. It would however be of interest to find a way of cooking the lupin in order to maintain its fermented nutritional advantage of soy as a higher nutritional profile (particularly BCAA) means it could ask as a potential replacement to soy. As for frying, the soy tempeh performed best as all of the nutritional contents tested was found to significantly increase, whereas within the lupin tempeh and tofu the amino acid section was found to show no improvements. Therefore due to the high BCAA contents found post frying in the soy tempeh, it could be seen that this would be the most appropriate for the recreational athlete.

4.1.1 Future research

In future research it would be of interest to see how these different products act upon muscle cell lines and measure the differentiation or to look at muscular hypertrophy through a controlled diet. As this paper primarily focused on the quantity of the nutritional profile of the product, as there could be mitigating factors that may affect the digestibility of the product and therefore it would be interesting to see the true digestibility of these products.

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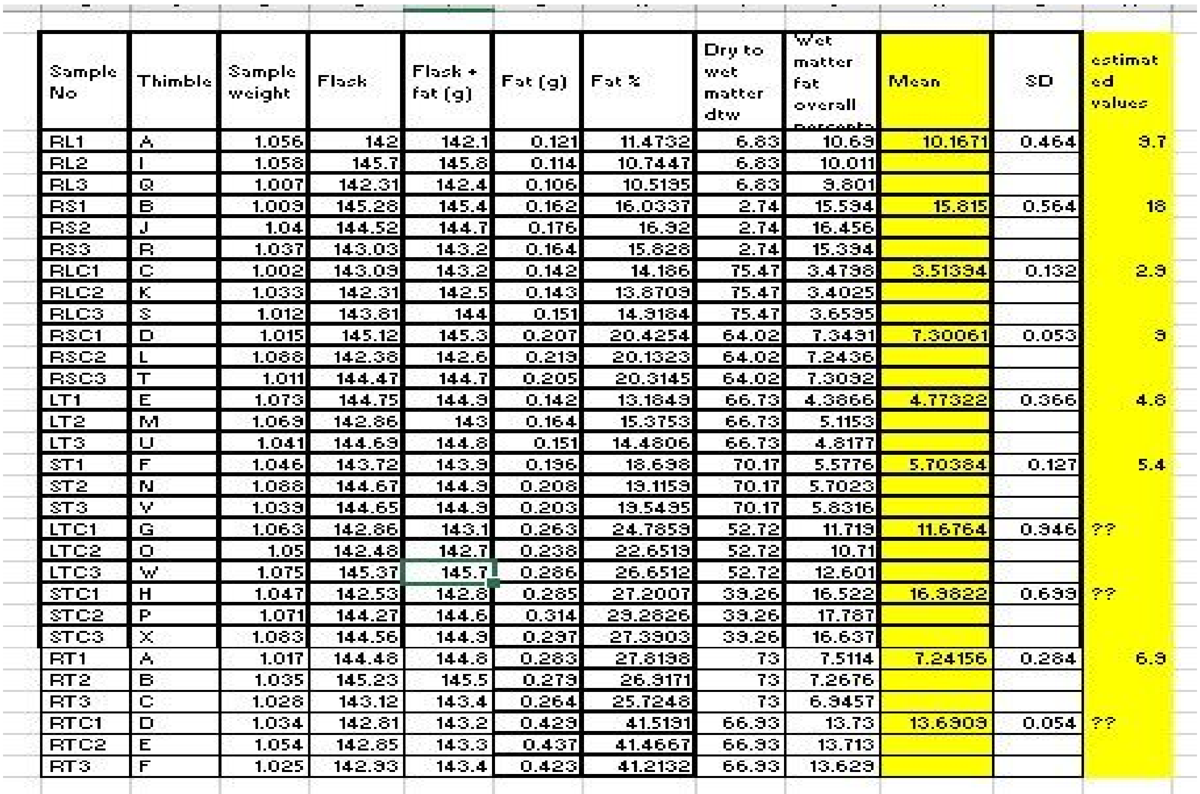
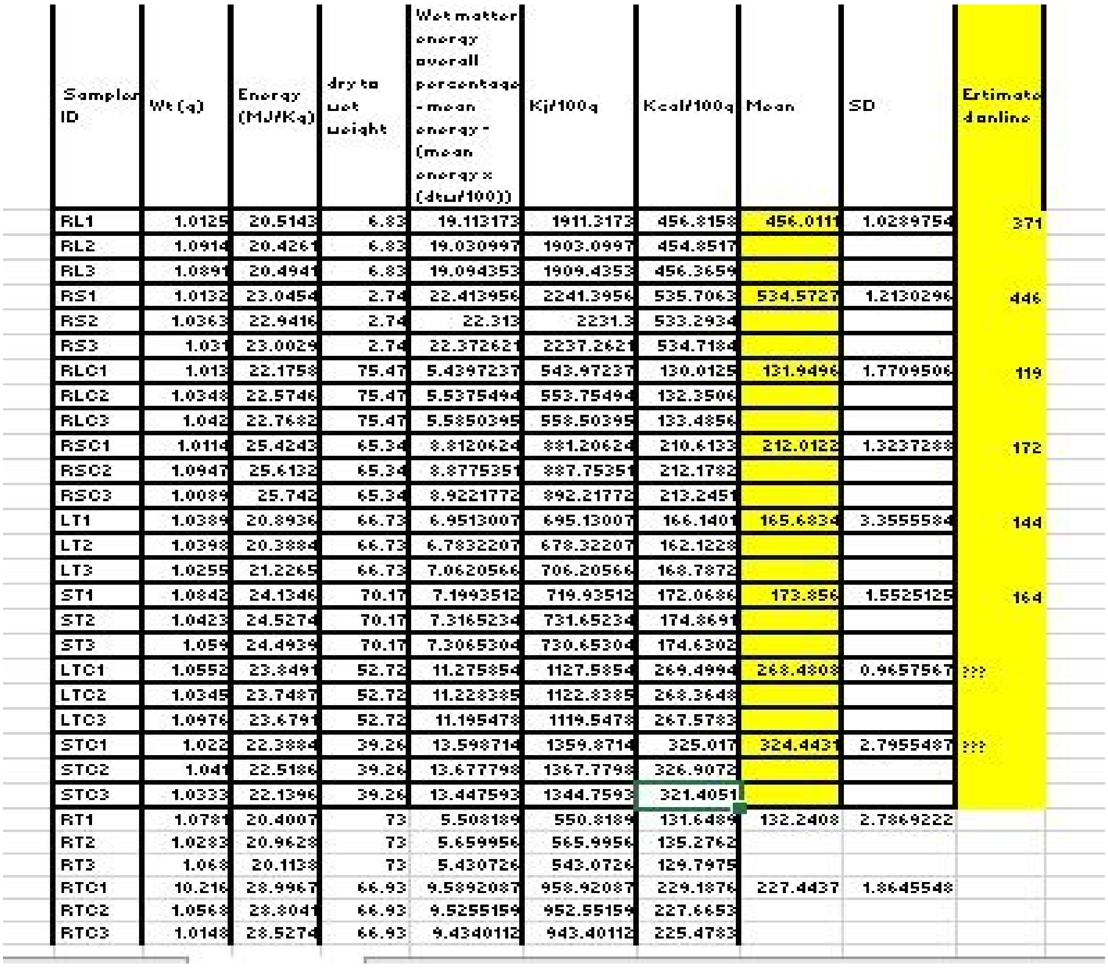
Appendices



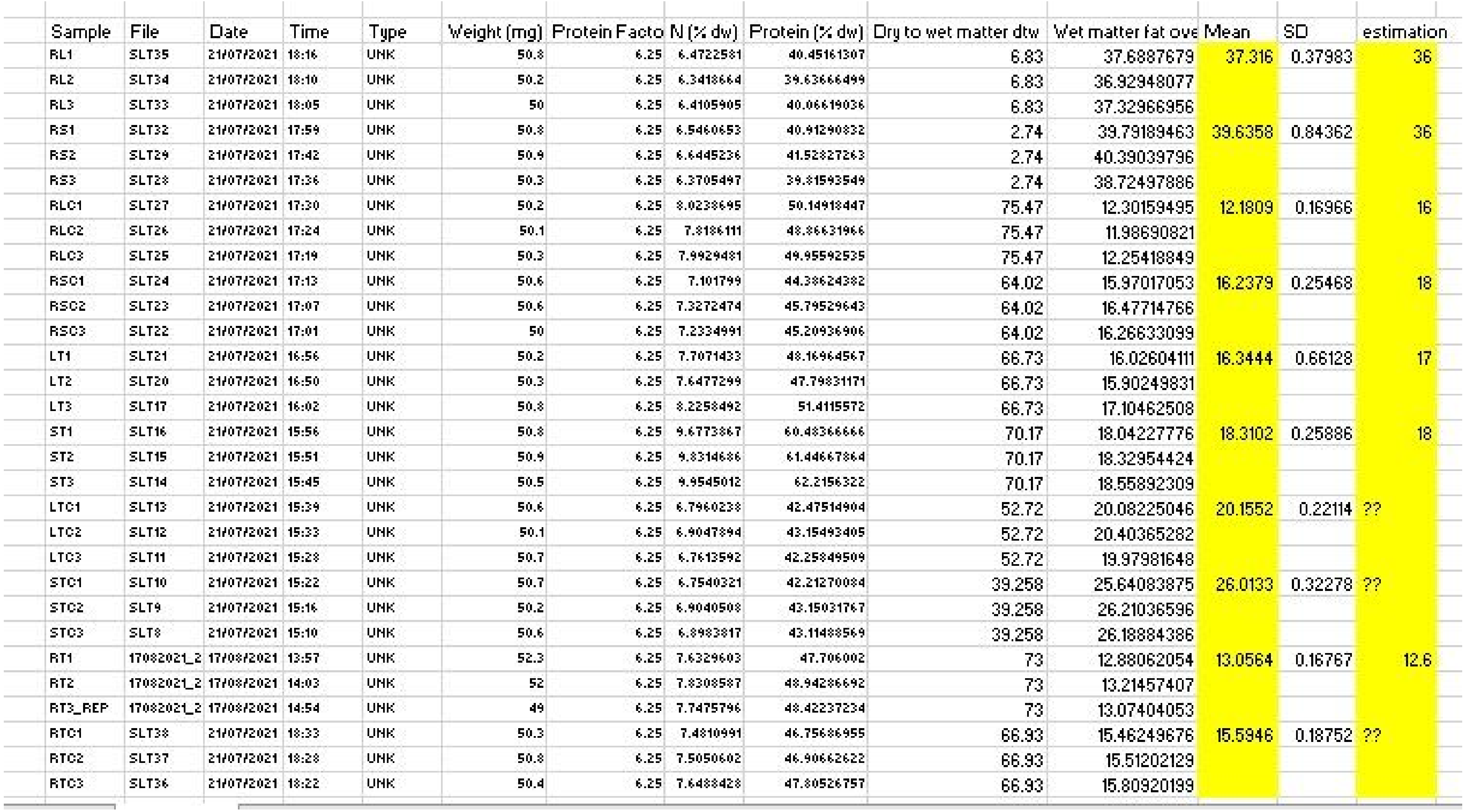
Sample No	Thimble	Sample weight	Flask	Flask + fat (g)	Fat (g)	Fat %	Dry to wet matter dtw	Wet matter fat overall percenta	Mean	SD	estimated values
RL1	A	1.056	142	142.1	0.121	11.4732	6.83	10.69	10.1671	0.464	9.7
RL2	I	1.058	145.7	145.8	0.114	10.7447	6.83	10.011			
RL3	Q	1.007	142.31	142.4	0.106	10.5195	6.83	9.801			
RS1	B	1.009	145.28	145.4	0.162	16.0337	2.74	15.594	15.815	0.564	18
RS2	J	1.04	144.52	144.7	0.176	16.92	2.74	16.456			
RS3	R	1.037	143.03	143.2	0.164	15.828	2.74	15.394			
RLC1	C	1.002	143.09	143.2	0.142	14.186	75.47	3.4798	3.51394	0.132	2.9
RLC2	K	1.033	142.31	142.5	0.143	13.8709	75.47	3.4025			
RLC3	S	1.012	143.81	144	0.151	14.9184	75.47	3.6595			
RSC1	D	1.015	145.12	145.3	0.207	20.4254	64.02	7.3491	7.30061	0.053	9
RSC2	L	1.088	142.38	142.6	0.219	20.1323	64.02	7.2436			
RSC3	T	1.011	144.47	144.7	0.205	20.3145	64.02	7.3092			
LT1	E	1.073	144.75	144.9	0.142	13.1849	66.73	4.3866	4.77322	0.366	4.8
LT2	M	1.069	142.86	143	0.164	15.3753	66.73	5.1153			
LT3	U	1.041	144.69	144.8	0.151	14.4806	66.73	4.8177			
ST1	F	1.046	143.72	143.9	0.196	18.698	70.17	5.5776	5.70384	0.127	5.4
ST2	N	1.088	144.67	144.9	0.208	19.1159	70.17	5.7023			
ST3	V	1.039	144.65	144.9	0.203	19.5495	70.17	5.8316			
LTC1	G	1.063	142.86	143.1	0.263	24.7859	52.72	11.719	11.6764	0.946	??
LTC2	O	1.05	142.48	142.7	0.238	22.6519	52.72	10.71			
LTC3	W	1.075	145.37	145.7	0.286	26.6512	52.72	12.601			
STC1	H	1.047	142.53	142.8	0.285	27.2007	39.26	16.522	16.9822	0.699	??
STC2	P	1.071	144.27	144.6	0.314	29.2826	39.26	17.787			
STC3	X	1.083	144.56	144.9	0.297	27.3903	39.26	16.637			
RT1	A	1.017	144.48	144.8	0.283	27.8196	73	7.5114	7.24156	0.284	6.9
RT2	B	1.035	145.23	145.5	0.279	26.9171	73	7.2676			
RT3	C	1.028	143.12	143.4	0.264	25.7248	73	6.9457			
RTC1	D	1.034	142.81	143.2	0.429	41.5191	66.93	13.73	13.6909	0.054	??
RTC2	E	1.054	142.85	143.3	0.437	41.4667	66.93	13.713			
RT3	F	1.025	142.93	143.4	0.423	41.2132	66.93	13.629			

<u>Appendices A: Full lipid content of all the products tested.</u> The lipid content of lupin and soy under different preparation techniques and quantified using the Soxhlet method.



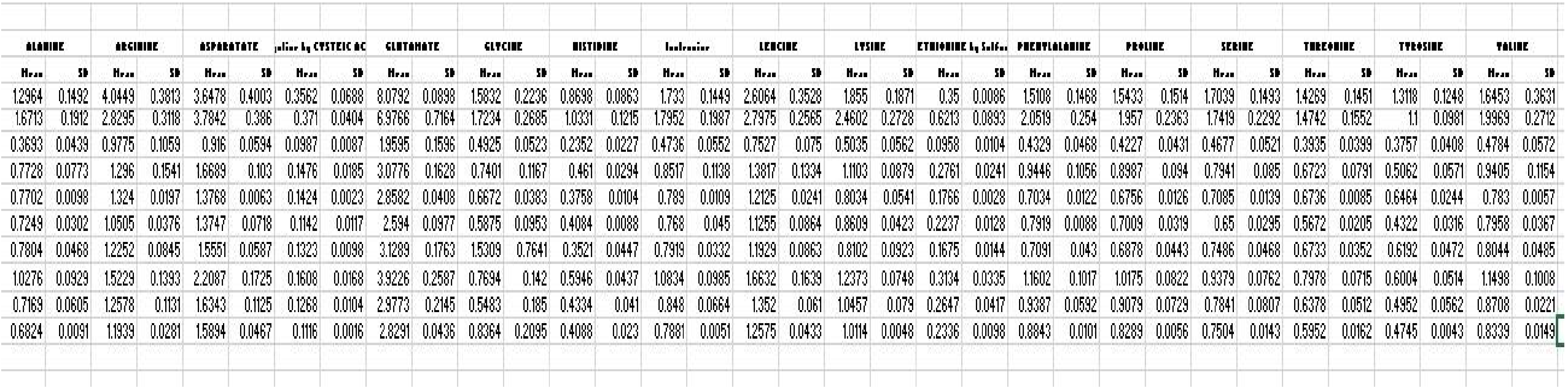
Samples ID	Wt (g)	Energy (MJ/Kg)	dry to wet weight	Wet matter energy overall percentage - mean energy - (mean energy x (dtw/100))	Kj/100g	Kcal/100g	Mean	SD	Estimated online
RL1	1.0125	20.5143	6.83	19.113173	1911.3173	456.8158	456.0111	1.0289754	371
RL2	1.0914	20.4261	6.83	19.030997	1903.0997	454.8517			
RL3	1.0891	20.4941	6.83	19.094353	1909.4353	456.3659			
RS1	1.0132	23.0454	2.74	22.413956	2241.3956	535.7063	534.5727	1.2130296	446
RS2	1.0363	22.9416	2.74	22.313	2231.3	533.2934			
RS3	1.031	23.0029	2.74	22.372621	2237.2621	534.7184			
RLC1	1.013	22.1758	75.47	5.4397237	543.97237	130.0125	131.9496	1.7709506	119
RLC2	1.0348	22.5746	75.47	5.5375494	553.75494	132.3506			
RLC3	1.042	22.7682	75.47	5.5850395	558.50395	133.4856			
RSC1	1.0114	25.4243	65.34	8.8120624	881.20624	210.6133	212.0122	1.3237288	172
RSC2	1.0947	25.6132	65.34	8.8775351	887.75351	212.1782			
RSC3	1.0089	25.742	65.34	8.9221772	892.21772	213.2451			
LT1	1.0389	20.8936	66.73	6.9513007	695.13007	166.1401	165.6834	3.3555584	144
LT2	1.0398	20.3884	66.73	6.7832207	678.32207	162.1228			
LT3	1.0255	21.2265	66.73	7.0620566	706.20566	168.7872			
ST1	1.0842	24.1346	70.17	7.1993512	719.93512	172.0686	173.856	1.5525125	164
ST2	1.0423	24.5274	70.17	7.3165234	731.65234	174.8691			
ST3	1.059	24.4939	70.17	7.3065304	730.65304	174.6302			
LTC1	1.0552	23.8491	52.72	11.275854	1127.5854	269.4994	268.4808	0.9657567	???
LTC2	1.0345	23.7487	52.72	11.228385	1122.8385	268.3648			
LTC3	1.0976	23.6791	52.72	11.195478	1119.5478	267.5783			
STC1	1.022	22.3884	39.26	13.598714	1359.8714	325.017	324.4431	2.7955487	???
STC2	1.041	22.5186	39.26	13.677798	1367.7798	326.9072			
STC3	1.0333	22.1396	39.26	13.447593	1344.7593	321.4051			
RT1	1.0781	20.4007	73	5.508189	550.8189	131.6489	132.2408	2.7869222	
RT2	1.0283	20.9628	73	5.659956	565.9956	135.2762			
RT3	1.068	20.1138	73	5.430726	543.0726	129.7975			
RTC1	10.216	28.9967	66.93	9.5892087	958.92087	229.1876	227.4437	1.8645548	
RTC2	1.0568	28.8041	66.93	9.5255159	952.55159	227.6653			
RTC3	1.0148	28.5274	66.93	9.4340112	943.40112	225.4783			

Appendices B: Full energy content of all the products tested. The energy content of lupin and soy under different preparation techniques and quantified using the Bomb calorimeter method.



Sample	File	Date	Time	Type	Weight (mg)	Protein Facto	N (% dw)	Protein (% dw)	Dry to wet matter dtw	Wet matter fat ove	Mean	SD	estimation
RL1	SLT35	21/07/2021	18:16	UNK	50.8	6.25	6.4722581	40.45161307	6.83	37.6887679	37.316	0.37983	36
RL2	SLT34	21/07/2021	18:10	UNK	50.2	6.25	6.3418664	39.63666499	6.83	36.92948077			
RL3	SLT33	21/07/2021	18:05	UNK	50	6.25	6.4105905	40.06619036	6.83	37.32966956			
RS1	SLT32	21/07/2021	17:59	UNK	50.8	6.25	6.5460653	40.91290832	2.74	39.79189463	39.6358	0.84362	36
RS2	SLT29	21/07/2021	17:42	UNK	50.9	6.25	6.6445236	41.52827263	2.74	40.39039796			
RS3	SLT28	21/07/2021	17:36	UNK	50.3	6.25	6.3705497	39.81593549	2.74	38.72497886			
RLC1	SLT27	21/07/2021	17:30	UNK	50.2	6.25	8.0238695	50.14918447	75.47	12.30159495	12.1809	0.16966	16
RLC2	SLT26	21/07/2021	17:24	UNK	50.1	6.25	7.8186111	48.86631966	75.47	11.98690821			
RLC3	SLT25	21/07/2021	17:19	UNK	50.3	6.25	7.9929481	49.95592535	75.47	12.25418849			
RSC1	SLT24	21/07/2021	17:13	UNK	50.6	6.25	7.101799	44.38624382	64.02	15.97017053	16.2379	0.25468	18
RSC2	SLT23	21/07/2021	17:07	UNK	50.6	6.25	7.3272474	45.79529643	64.02	16.47714766			
RSC3	SLT22	21/07/2021	17:01	UNK	50	6.25	7.2334991	45.20936906	64.02	16.26633099			
LT1	SLT21	21/07/2021	16:56	UNK	50.2	6.25	7.7071433	48.16964567	66.73	16.02604111	16.3444	0.66128	17
LT2	SLT20	21/07/2021	16:50	UNK	50.3	6.25	7.6477299	47.79831171	66.73	15.90249831			
LT3	SLT17	21/07/2021	16:02	UNK	50.8	6.25	8.2258492	51.4115572	66.73	17.10462508			
ST1	SLT16	21/07/2021	15:56	UNK	50.8	6.25	9.6773867	60.48366666	70.17	18.04227776	18.3102	0.25886	18
ST2	SLT15	21/07/2021	15:51	UNK	50.9	6.25	9.8314686	61.44667864	70.17	18.32954424			
ST3	SLT14	21/07/2021	15:45	UNK	50.5	6.25	9.9545012	62.2156322	70.17	18.55892309			
LTC1	SLT13	21/07/2021	15:39	UNK	50.6	6.25	6.7960238	42.47514904	52.72	20.08225046	20.1552	0.22114	??
LTC2	SLT12	21/07/2021	15:33	UNK	50.1	6.25	6.9047894	43.15493405	52.72	20.40365282			
LTC3	SLT11	21/07/2021	15:28	UNK	50.7	6.25	6.7613592	42.25849509	52.72	19.97981648			
STC1	SLT10	21/07/2021	15:22	UNK	50.7	6.25	6.7540321	42.21270084	39.258	25.64083875	26.0133	0.32278	??
STC2	SLT9	21/07/2021	15:16	UNK	50.2	6.25	6.9040508	43.15031767	39.258	26.21036596			
STC3	SLT8	21/07/2021	15:10	UNK	50.6	6.25	6.8983817	43.11488569	39.258	26.18884386			
RT1	17082021_2	17/08/2021	13:57	UNK	52.3	6.25	7.6329603	47.706002	73	12.88062054	13.0564	0.16767	12.6
RT2	17082021_2	17/08/2021	14:03	UNK	52	6.25	7.8308587	48.94286692	73	13.21457407			
RT3_REP	17082021_2	17/08/2021	14:54	UNK	49	6.25	7.7475796	48.42237234	73	13.07404053			
RTC1	SLT38	21/07/2021	18:33	UNK	50.3	6.25	7.4810991	46.75686955	66.93	15.46249676	15.5946	0.18752	??
RTC2	SLT37	21/07/2021	18:28	UNK	50.8	6.25	7.5050602	46.90662622	66.93	15.51202129			
RTC3	SLT36	21/07/2021	18:22	UNK	50.4	6.25	7.6488428	47.80526757	66.93	15.80920199			

Appendices C: Full protein content of all the products tested. The protein content of lupin and soy under different preparation techniques and protein contents were collected using the EA1112 (Thermo Scientific - USA).



ALANINE		ARGININE		ASPARATATE		line by CYSTEIC AC		GLUTAMATE		GLYCINE		HISTIDINE		Isoleucine		LEUCINE		LYSINE		ETHIONINE by Sulfo		PHENYLALANINE		PROLINE		SERINE		THREONINE		TYROSINE		VALINE	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.2964	0.1492	4.0449	0.3813	3.6478	0.4003	0.3562	0.0688	8.0792	0.0898	1.5832	0.2236	0.8698	0.0863	1.733	0.1449	2.6064	0.3528	1.855	0.1871	0.35	0.0086	1.5108	0.1468	1.5433	0.1514	1.7039	0.1493	1.4269	0.1451	1.3118	0.1248	1.6453	0.3631
1.6713	0.1912	2.8295	0.3118	3.7842	0.386	0.371	0.0404	6.9766	0.7164	1.7234	0.2685	1.0331	0.1215	1.7952	0.1987	2.7975	0.2565	2.4602	0.2728	0.6213	0.0893	2.0519	0.254	1.957	0.2363	1.7419	0.2292	1.4742	0.1552	1.1	0.0981	1.9969	0.2712
0.3693	0.0439	0.9775	0.1059	0.916	0.0594	0.0987	0.0087	1.9595	0.1596	0.4925	0.0523	0.2352	0.0227	0.4736	0.0552	0.7527	0.075	0.5035	0.0562	0.0958	0.0104	0.4329	0.0468	0.4227	0.0431	0.4677	0.0521	0.3935	0.0399	0.3757	0.0408	0.4784	0.0572
0.7728	0.0773	1.296	0.1541	1.6689	0.103	0.1476	0.0185	3.0776	0.1628	0.7401	0.1167	0.461	0.0294	0.8517	0.1138	1.3817	0.1334	1.1103	0.0879	0.2761	0.0241	0.9446	0.1056	0.8987	0.094	0.7941	0.085	0.6723	0.0791	0.5062	0.0571	0.9405	0.1154
0.7702	0.0098	1.324	0.0197	1.3768	0.0063	0.1424	0.0023	2.8582	0.0408	0.6672	0.0383	0.3758	0.0104	0.789	0.0109	1.2125	0.0241	0.8034	0.0541	0.1766	0.0028	0.7034	0.0122	0.6756	0.0126	0.7085	0.0139	0.6736	0.0085	0.6464	0.0244	0.783	0.0057
0.7249	0.0302	1.0505	0.0376	1.3747	0.0718	0.1142	0.0117	2.594	0.0977	0.5875	0.0953	0.4084	0.0088	0.768	0.045	1.1255	0.0864	0.8609	0.0423	0.2237	0.0128	0.7919	0.0088	0.7009	0.0319	0.65	0.0295	0.5672	0.0205	0.4322	0.0316	0.7958	0.0367
0.7804	0.0468	1.2252	0.0845	1.5551	0.0587	0.1323	0.0098	3.1289	0.1763	1.5309	0.7641	0.3521	0.0447	0.7919	0.0332	1.1929	0.0863	0.8102	0.0923	0.1675	0.0144	0.7091	0.043	0.6878	0.0443	0.7486	0.0468	0.6733	0.0352	0.6192	0.0472	0.8044	0.0485
1.0276	0.0929	1.5229	0.1393	2.2087	0.1725	0.1608	0.0168	3.9226	0.2587	0.7694	0.142	0.5946	0.0437	1.0834	0.0985	1.6632	0.1639	1.2373	0.0748	0.3134	0.0335	1.1602	0.1017	1.0175	0.0822	0.9379	0.0762	0.7978	0.0715	0.6004	0.0514	1.1498	0.1008
0.7169	0.0605	1.2578	0.1131	1.6343	0.1125	0.1268	0.0104	2.9773	0.2145	0.5483	0.185	0.4334	0.041	0.848	0.0664	1.352	0.061	1.0457	0.079	0.2647	0.0417	0.9387	0.0592	0.9079	0.0729	0.7841	0.0807	0.6378	0.0512	0.4952	0.0562	0.8708	0.0221
0.6824	0.0091	1.1939	0.0281	1.5894	0.0467	0.1116	0.0016	2.8291	0.0436	0.8364	0.2095	0.4088	0.023	0.7881	0.0051	1.2575	0.0433	1.0114	0.0048	0.2336	0.0098	0.8843	0.0101	0.8289	0.0056	0.7504	0.0143	0.5952	0.0162	0.4745	0.0043	0.8339	0.0149

Appendices D: Full protein content of all the products tested. The full amino acid content of lupin and soy under different preparation techniques and amino acid content was determined by Liquid chromatography triple quad mass spectrometer (LCMSMS).

Appendices E: Paper to be submitted for publishing

Do alternative proteins sources have sufficient branched chain amino acids compared to common protein sources: A scoping review

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Keywords

Branch-chained amino acids (BCAA), neglected proteins, underutilised crops, isoleucine, leucine, valine

Abstract

The demand for protein is projected to double by 2050. Most of the protein sources consumed come from that of mainstream unsustainable sources. In order to combat this, globally there are numerous lesser-known proteins which have the potential to act as alternatives to both animal and traditional plant-based proteins, however the quality of these proteins is unknown. Here we have scoped existing literature to identify what these lesser-known protein sources are, and to identify the quality of protein by focussing on the levels of branched chain amino acid (BCAA) (isoleucine, leucine, and valine) in comparison to traditional animal and plant sources. BCAA are essential amino acids, which play an important role in the building and repairing of muscle. Therefore a higher quantity of amino acid will generally mean a higher quality of protein. After a process of iterative database searching, we identified 29 relevant articles. Studies were categories into three themes (1) mainstream meat alternatives (MMA), (2) neglected underutilised crop species (NUCS) and (3) Insects. Alternative protein sources included Lupin, Gram, Flower, Seeds, Bean, Yam, Cricket, Beetle, Fly and Moth. Further assessment identified that very few of the alternative proteins had similar or higher BCAA levels when compared to traditional animal meat sources. However when compared to animal product and vegan protein

a number of sources have significantly higher levels of BCAA. In conclusion, this scoping review identified that there are no alternative proteins to rival the quality of animal meat sources, however there are at least four sources (Gram, Bean, Fly and Beetle) which could have the potential to act as alternatives to the traditional animal product and plant-based proteins. Particularly Bean which were comparable to animal products and the mainstream plant-based proteins. However understanding the bioavailability of these proteins is unknown and should be the focus of future work.

Introduction

Dietary protein plays a critical role in numerous physiological processes in the body (Williams, 2005, Cintineo et al., 2018). Recently, socioeconomic change such as increased incomes, urbanisation and longer lifespan (where consumption of protein is linked to healthy aging), has led to increased protein intake (Delgado, 2003, Popkin et al., 2012).

Due to current recommendations, the increase in dietary protein consumption has been especially noted within the recreational athlete market (Cintineo et al., 2018). The international society of Sport Nutrition has stated that exercising individuals should ingest between 1.4 2.0g/kg/day. Dietary protein is composed of 20 different amino acids, of which nine are essential, meaning that the body cannot produce them (Williams, 2005, Cintineo et al., 2018), these include and of these isoleucine, leucine and valine and often termed branched chained amino acids (BCAA). BCAAs have been shown to help prolong aerobic exercise by reducing ratings of perceived exertion as well as increasing muscular hypertrophy (Brosnan and Brosnan, 2006).

To obtain the additional dietary protein, many often ingest protein powders as they are convenient and can be cost-efficient depending on the product (animal vs. plant based) (Jäger et al., 2017). The global market for protein supplements was valued at $20.5 billion in 2021 with a projected annual growth rate of 8.5% from 2021 to 2030 (2022b). However, these current sources of dietary protein have been met with sustainability and environmental issues (Sanchez-Sabate and Sabaté, 2019). The increased reliance on animal proteins has led to an increase in greenhouse gas emissions and the need for more land and water (Henchion et al., 2017) and for this reason many have started to consume alternatives, predominantly such as soy and pea (2020, Young and Pellett, 1994, Siegrist and Hartmann, 2019). However the production of these proteins on a global scale has led to natural land being converted into plantations causing wide-scale deforestation, loss of biodiversity, soil erosion and water contamination, furthermore as vast amounts of soy is exported resulting in an increase in carbon emission (Olsen and Bishop, 2009). Thus are there alternatives which could be more sustainable? Neglected underutilised crops (NUCS) are protein sources that are cultivated throughout the world but have been universally ignored (Dansi et al., 2012). These sources are able to grow in environments that would be inhospitable for many mainstream alternatives, thus allowing people in these conditions to have a sustainable supply of produce (Bhag, 2007, Ingweye et al., 2010a).

Moreover some wild plants are a strong source of protein, therefore are able to reduce levels of protein deficiency in deprived communities (Bvenura and Sivakumar, 2017).

Alongside NUCS more intriguing sources are also gaining interest these include insects of which there are thousands fit for human consumption. Insects have a high quantity of digestible protein, which is enhanced by removing the exoskeleton of insects (Ramos-Elorduy et al., 1997, Huis, 2018). Consumption of insect protein reduces environmental damage and has an extremely high protein uptake efficiency (van Huis, 2013, Oonincx et al., 2010, Müller et al., 2017). Recent studies have shown that consumption of insect protein leads to greater muscle protein synthesis in young men who perform resistance exercise (Vangsoe et al., 2018).

The aim of this review was to scope the literature to identify alternate protein sources and compare the protein and amino acid composition focusing on the levels of BCAAs to animal and plant protein sources.

Methods

The overall idea behind a scoping review is to categorise and record information on databases, thus enabling gaps in research to be filled (Arksey and O'Malley, 2005, Munn et al., 2018, Anderson et al., 2008). A scoping review can also provide a broader aspect compared to that of a systematic review which has more precise and specific characteristics (Armstrong et al., 2011). Thus while we specified our objectives and methods in advance, we adapted the search terms and inclusion criteria during the process as the scope of the literature was identified.

Research Questions

This review seeks to answer the following questions:

1) What are the variety of alternative protein sources available?

2) Do alternative protein sources have similar BCAA content to animal proteins?

3) Do alternative protein sources have similar BCAA content to mainstream plant-based proteins?

Identifying relevant studies

An initial broad search of the literature was conducted to define key concepts. Initial search terms included underutilised protein, neglect protein, pea protein, soy protein, lupin, insect protein, vegan, sustainable, leucine, isoleucine, valine and United Kingdom, these were then mapped together to give an indication to the depth of research from each area (Table S1). Searches were performed by A.D. using three electronic databases (NUsearch, Scopus and Web of Science) using identical search terms. Following title and abstract screening, the reference lists of relevant articles were searched to identify all studies.

Study selection

Preliminary literature searching revealed a wide range of studies investigating underutilised crops. While some of these studies investigated nutritional assessment, some were related to cultivation, biotechnology of genetically modified crops, genetic breeding, political and social issues; sensory evaluations, investigating the microbiomes and nutritional information not related to BCAA. This study focuses on identifying the levels of BCAA in these proteins. The process is summarised in Figure 1.

Charting the data

The included articles were mined for the type of protein and amino acid content. The data was transferred to Microsoft Excel where a methodical collection could be made. When transferring the data to excel, each category (mainstream meat alternatives (MMA), NUCS, and Insect protein) had two separate spreadsheets, one including the study characteristics and the other including the full proximate analysis of amino acid content.

Collating, Summarising and Reporting the Results

Study characteristics (including author, protein analysed year, location and extraction method of protein) were tabulated. In addition full proximate analysis of the essential and non-essential amino acids were mined, with a standardised measurement value of g/100g. The data was organised to fit into the specific subcategories of the type of protein as this organisational method allowed for themes to be drawn and concluded (Table S2). Statistical analysis (Graphpad Prism 9.2.1) was conducted to assess the levels of BCAA (isoleucine, leucine and valine) in the alternative protein sources against animal meat, animal product and vegan product protein. The term ‘Animal meat’ included BCAA content from chicken meat, Herring, White fish and Palaemon graver, the term ‘Animal product’ included BCAA content from whey protein, milk, protein and egg and the term ‘Vegan product’ included BCAA content from soy and pea products. The results are shown as a percentage comparison away from the threshold of the three comparative subgroups.

Results

The outcomes of the literature searching, and article screening are summarised in Figure 1. Using three databases (Scopus, NUSearch and WoS) we identified 1160 articles published between 2010 and March 2021 (Table S1). Following the removal of duplicates, titles and abstract were assessed against an post-hoc exclusion criteria to leave 111 articles to be assessed for full amino acid analysis of the protein source. A total of 29 articles from 2013-2020 were included in this review, of these 6 articles were related mainstream meat alternatives, 9 articles were related to NUCS and 14 articles were related to insect protein source.

Study characteristics

The characteristics of the 29 studies are summarized in Table 1. The selected studies were located across the world ranging from Europe (11) United States (4), Asia (6) Africa (4) not specified (4). All 29 articles measured BCAA levels from the proteins investigated, however there was a variety of methods used for extraction. Overall the most popular method was high performance liquid chromatography (HPLC) and was used in six articles (Taufek et al., 2018, Rodríguez-Miranda et al., 2019, Sayed et al., 2019, Stone et al., 2019, de Carvalho et al., 2020, Parker et al., 2020) with varying machines (Shimadzu fluorescence detector (Parker et al., 2020) and pico tag amino acid analysis system (Stone et al., 2019) D spectrophotometry using the standard procedures with slight modifications (Paul and Dey, 2014), and Kjeldahl Foss Automatic 16210 (A/S N. Foss Electric, Denmark) analyser (Józefiak et al., 2019)). Other extraction methods included hydrolysing the protein in hydrochloric acid 6N for 24 hours followed by amino acid separation by the Dionex BioLC Chromatographic System (Glew et al., 2010), use of multi-sample amino acid analyser (Ingweye et al., 2010b), detoxifying and defattening (Gulzar and Minnaar, 2017), total protein extraction by the Basha method (Doss et al., 2019); AccQtag Ultra chemistry (Shelat et al., 2019) and AOAC method using an amino acid analyzer (Adeyeye et al., 2020) (Sykam-S7130) (Babarinde et al., 2020); fraunhofer process (van de Noort, 2016) and diafiltration procedure (Thrane et al., 2017). There were 9 articles (Venkidasamy et al., 2019, Coelho et al., 2020, Gulzar and Minnaar, 2017, Belluco et al., 2013, Stamer, 2015, Müller et al., 2017, Józefiak et al., 2019, Kewuyemi et al., 2020, Mastoraki et al., 2020) that failed to include a methodology. When examining the amino acid content, only 8 of the articles (Glew et al., 2010, Ingweye et al., 2010b, Senger et al., 2017, Doss et al., 2019, Adeyeye et al., 2020, Babarinde et al., 2020, Thrane et al., 2017, Stone et al., 2019) provided full amino acid content for all its protein sources.

What are the variety of alternative protein sources available?

We identified a number of proteins, from the 29 articles, which we grouped into mainstream meat alternative (MMA; lupin (van de Noort, 2016), Gram (Venkidasamy et al., 2019), other MMA termed (Lentils (Venkidasamy et al., 2019), peanuts (Venkidasamy et al., 2019), mycoprotein (Coelho et al., 2020), rice and wheat (Lu et al., 2020)), NUCs (flower (Glew et al., 2010, Shelat et al., 2019), seeds (Ingweye et al., 2010b, Adeyeye et al., 2020), beans (Gulzar and Minnaar, 2017), yam (Doss et al., 2019), other NUCS (fonio flour (Babarinde et al., 2020), Solanum macrocarpon (Glew et al., 2010), Vigna unguiculatus (Glew et al., 2010), Chuata (Senger et al., 2017) and Prosopis pods (Al-Harthi et al., 2019)), and insects (cricket (Józefiak et al., 2016, Taufek et al., 2018, Rodríguez-Miranda et al., 2019, Stone et al., 2019, Kewuyemi et al., 2020), beetle (Józefiak et al., 2016, Stone et al., 2019, de Carvalho et al., 2020, Kewuyemi et al., 2020, Mastoraki et al., 2020, Parker et al., 2020), fly (Stamer, 2015, Józefiak et al., 2016, Müller et al., 2017, Józefiak et al., 2019, Sayed et al., 2019, Mastoraki et al., 2020), moth (Paul and Dey, 2014, Sayed et al., 2019, Kewuyemi et al., 2020) and other insects (Encosternum Delegorguei (Kewuyemi et al., 2020), Macrotermes Bellicosus (Kewuyemi et al., 2020), Silkworm pupae (Belluco et al., 2013)).

We found that the average amount of isoleucine was comparable between the MMA, NUCs and insect proteins (isoleucine (g/100g): MMA 3.82 ± 0.6; NUCS 5.10 ± 0.5; Insect 3.91 ± 0.4 p= n.s.). While the amount of leucine and valine was comparable between the NUCs and insect group, there was significantly lower amounts the amino acids in the MMA group compared to NUCs protein group (Leucine (g/100g): MMA 5.57 ± 0.5; NUCs 8.06 ± 1.0*; Insect 6.64 ± 0.8; Valine (g/100g): MMA 3.05 ± 0.4; NUCs 6.23 ± 0.8*; Insect 5.08 ± 0.7; *$P<0.05$ vs. MMA).

BCAA levels in underutilised proteins are not comparable to animal meat proteins but comparable to animal product.

To determine which of these underused proteins could be a suitable alternative we assessed the percentage difference of isoleucine, leucine and valine to that contained in animal meat protein. We observed that majority of the proteins (10/13) contained a significantly lower percentage of all three amino acids (Figure 2A-C). The only protein with comparable levels of all amino acid was bean (percentage difference to animal meat: Isoleucine -6.10% ± 4.31; leucine -3.50% ± 0.26, valine -4.60% ± 13.2, p = n.s to animal meat Figure 2A-C). However when comparing to animal meat product protein there was completely different results. Majority of the alternative proteins had higher levels of isoleucine (9/13) and valine (8/13) in comparison to animal product protein, however the levels of leucine were very variable ranging from +22.51% to -57.44% (Figure 3A-C). Interestingly the only proteins which had higher or comparable in all three amino acids were lupin, gram, bean, cricket, beetle and fly.

Do alternative protein sources have similar BCAA content to mainstream plant-based proteins?

To determine which of these underused proteins could be a suitable plant protein alternative we assessed the percentage difference of isoleucine, leucine and valine to that contained in mainstream plant-based products, namely soy and pea. Unlike animal meat we observed that

that a third of the underused proteins had higher levels, a third had lower levels and a third had comparable levels of isoleucine, leucine and valine when compared to mainstream plant-based proteins (Figure 4A-C). Isoleucine was higher in 4 proteins (gram, bean, beetle and fly) levels ranged from 15.54-27.73%. As for leucine only 2/13 proteins (seed and bean) were found to be significant higher with +17.74 ± 5.22 and +22.51 ± 0.58, respectfully, while 6/13 were lower and 5/13 were comparable (Figure 4B). This was similar to the valine content, as 5/13 protein sources (gram, bean, cricket, beetle, fly) were significantly higher, however there was a much larger range within these results ranging from +66.57 to -46.74 (Figure 4C).

Discussion

Demand for protein is ever increasing and whether the demand is associated with animal protein or plant-based proteins there are numerous negative environmental impacts, therefore identifying other sustainable protein sources is of importance.

Here we identified a number of underutilised protein sources across the globe (including Africa, Europe, Asia and North America), these included MMA (Lupin, Gram, Other MMA), NUCS (Flower, Seeds, Bean, Yam, Other NUCS,) and insects (Cricket, Beetle, Fly, Moth, and other insects). We then assessed whether these proteins met nutritional requirements by focussing on the BCAAs isoleucine, leucine and valine content and whether they were suitable alternatives to animal proteins and/or common plant-based proteins. Overall we found that none of the alternative's proteins were comparable for all three amino acids when compared to animal meat, this was similar to Berrazaga *et al* (Berrazaga et al., 2019) who also observed that animal products had higher levels of BCAAs when compared to plant based proteins. There were at least four sources which could act as potential alternatives to the current plant-based protein these included Bean, Gram, Fly and Beetle. Interestingly Bean and Gram have a number of non-nutritive benefits include the ability to thrive in a wide variety of geographical locations even in those areas that have short growing seasons, thrive in poor quality soil such as those with heavy metal, high salinity (Reddy et al., 2005, Sreenivasulu Reddy et al., 1998, Bhardwaj and Yadav, 2012), and thrive under the harshest of climate (Cullis et al., 2018). This may be due to the ability of these protein to improve nitrogen fixation and crop residue incorporation to restore soil nutrient content (Reager et al., 2020). While consumption of insects (Beetle and Fly) not only has the ability to produce large quantities of protein due to rapid growth cycle, it also reduces the pressure on the environment leading to lower greenhouse emissions and decreased use of land and water but more importantly, similar to plant based legumes, insects have the potential to manage chronic diseases such as diabetes and cardiovascular disease (Aguilar-Miranda et al., 2002) (Appleby and Key, 2016, Marsh et al., 2012).

Some researcher's question insect consumption from a food safety perspective due to the fact they are phylogenetically far removed from regularly consumed mammals, making them risky (Henchion et al., 2017). While many consumers, particularly in Europe and USA, are not willing to consume insects due to the aversion they evoke, as many associate insects with decaying matter and faeces (Berger and Wyss, 2020). On the other hand, the adoption of a plant-based diet is increasing due to the presence on social media and via the promotion of the potential health benefits including reduced risk of heart disease, cholesterol, blood pressure, type II diabetes and cancer (Appleby and Key, 2016, Marsh et al., 2012). Furthermore, as a number of high-profile athletes are now adopting this lifestyle many recreational athletes have turned towards plants-based supplementation. Although the overall prevalence is unknown the plant-based protein supplement market is expected to reach at $7 billion by 2026 (Vitale and Hueglin, 2021). They often turn to soy or pea protein, which accounts for 60% of all the plant sourced protein in the world (2020, Young and Pellett, 1994), as they have comparable levels of BCAAs to whey protein (Fuhrman and Ferreri, 2010). In addition, studies report no difference in muscular size and strength in participants consuming soy and/or pea protein supplementation when compared to whey protein (Babault et al., 2015, Messina et al., 2018).

Interestingly of the four potential proteins identified, we observed that the level of all three BCAAs in beans (Marama bean, Fava bean and Black bean) were comparable to animal products and the mainstream plant-based proteins. This is not surprising as the Marama bean, native in Africa, has been showed to have a similar nutritional compositional profile to that of soybean and peanuts (Venkatachalam and Sathe, 2006, Mujoo et al., 2003). Furthermore the roots of the Marama bean plant produces a high protein tuber which is more nutritious that potato, yam and sugar beets and most important the amino acid profile of Marama bean was similar to milk or casein (Biesele, 1983). Similarly, Fava bean, native to the Mediterranean and southwest Asia, and the black bean, native to South America, a high protein content (approximately 22.5g and 7.6g per 90g portion). Interestingly fava bean is often used as an alternative for soy (Crépon et al., 2010), indeed Fava bean is used as an alternative to whey in infant formulas without altering the nutritional content (Le Roux et al., 2020). On top of this, it is found that fava bean is grown in the UK, however many get exported as chickpea is imported (2022a). As for marama bean, it can grow range of conditions, including in the UK, as long as it was supplemented was light (Mitchell et al., 2005). However the only plant not suited for UK climate was black bean, as it generally grows in warmer climates where soil temperatures can range from 18-21 degrees (Finley, 2021).

Numerous professional and recreational athletes consume protein supplements as research has shown that BCAA supplementation has beneficial effects on muscle protein synthesis and muscle recovery (Negro et al., 2008), however these benefits are dependent on the rate of digestion (Boirie et al., 1997). Typically the Digestible Indispensable Amino Acid Score (DIAAS) of beans, and other plant crops, tend to have a lower DIAAS score than that of animal proteins (FAO, 2013), possibly due to the high amount of β-sheet compared to that of α-helix in the secondary structure of the protein (this is different in animal secondary structure) (Carbonaro et al., 2012). β-sheet are found to be unaffected by the gastral enzymes which would result in proteolysis and therefore reducing protein digestibility. This lower DIAAS may be the reason why muscle protein synthesis does not increase at the same rate following the consumption of plant-based protein when compared to animal protein/products (Yang et al., 2012). Another reason might be due to the lower levels of leucine which is important for muscle synthesis and for the inhibition of muscle degradation (Zanchi et al., 2008) indeed we have shown that all the underutilised proteins identified lower levels of leucine, in some cases we have seen reductions of 70% and 57% when compared to animal protein / product respectively. Therefore improving the digestibility of these proteins is important to increase the availability of the BCAAs. A common method used to increase amino acid digestibility is fermentation, this has shown to improve the amino acid content in soybean by 3-10 fold (Wang et al., 1968), while heat treatment has been shown to increase protein digestibility by 18%, possibly due to the reduction in the levels of trypsin activity (Rutherfurd et al., 2015) however more recently (Vogelsang-O'Dwyer et al., 2020) have shown that wet fractionation of the fava bean increases its protein content and making the bean more digestible. Similarly the digestibility levels of insects can be improved by 75-99% by removing the exoskeleton, which contains a large proportion of chitin which is hard to digest (van Huis, 2016, Schlüter et al., 2017, SG, 1997, DeFoliart, 1992, Rumpold and Schlüter, 2013).

Limitations

The study was conducted in order to assess the BCAA content of underutilised protein sources to see whether it was comparable to that of the mainstream products. Therefore limitations occurred when a protein source did not fully include an amino acid profile as they were rejected, meaning that they could have had high nutritional potentials elsewhere. This was found to be common as generally the underutilised protein source nutritional content has not been covered at depth therefore only few papers cited BCAA content meaning it was more difficult to find a range of results and increase accuracy. It was found that most of the underutilised protein sources were grown abroad and therefore would be considered unsustainable to either import them over, together with grow them in greenhouses in order to suit the growing conditions of the product, therefore leading to environmental problems. Another limitation with this study is that most of the products researched are grown outside of the UK and therefore have no confidence whether they can grow in UK or not. Therefore if they could not grow in the UK and had to be imported in, then this would lose its credibility of being a sustainable source.

Conclusion

In conclusion, there was potentials found within each three categories: Gram for MMA, Beans for NUCS and Fly and Beetle for Insect. For future research it would be of interest to perform a dietary experiment of these three sources compared to that of conventional protein sources. As actually practically testing in a controlled setting will allow us to understand the protein synthetic characteristics.

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Figures

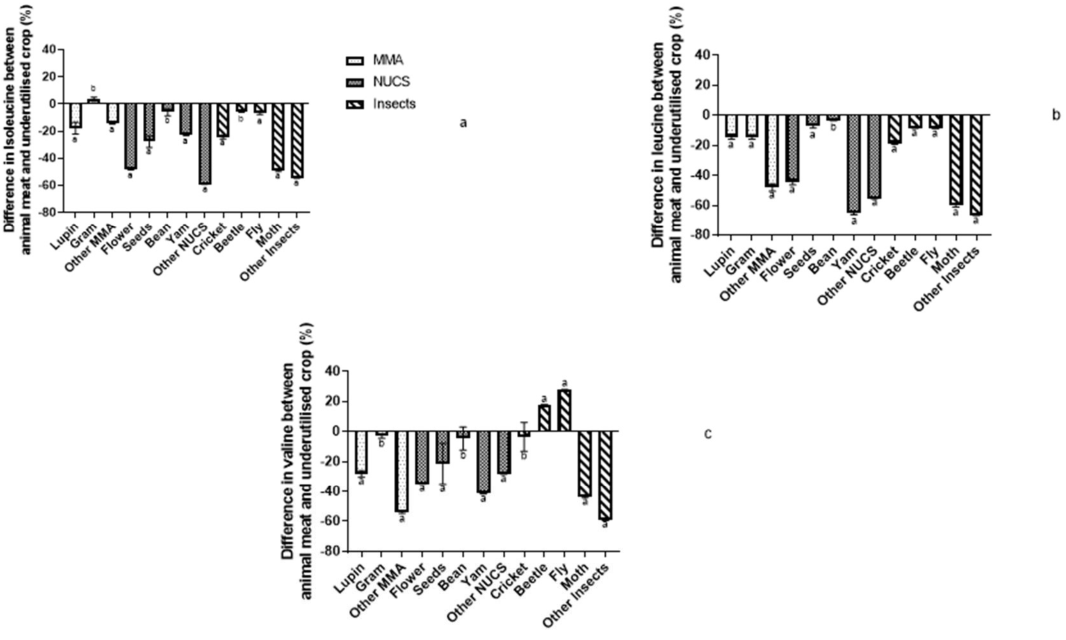

Fig 2. The animal meat percentage content of (a) isoleucine (4.7g/100g), (b) leucine (8.25g/100g), and (c) valine (5.15g/100g), to that of alternative protein sources. With "a" showing significance and "b" showing no significance.

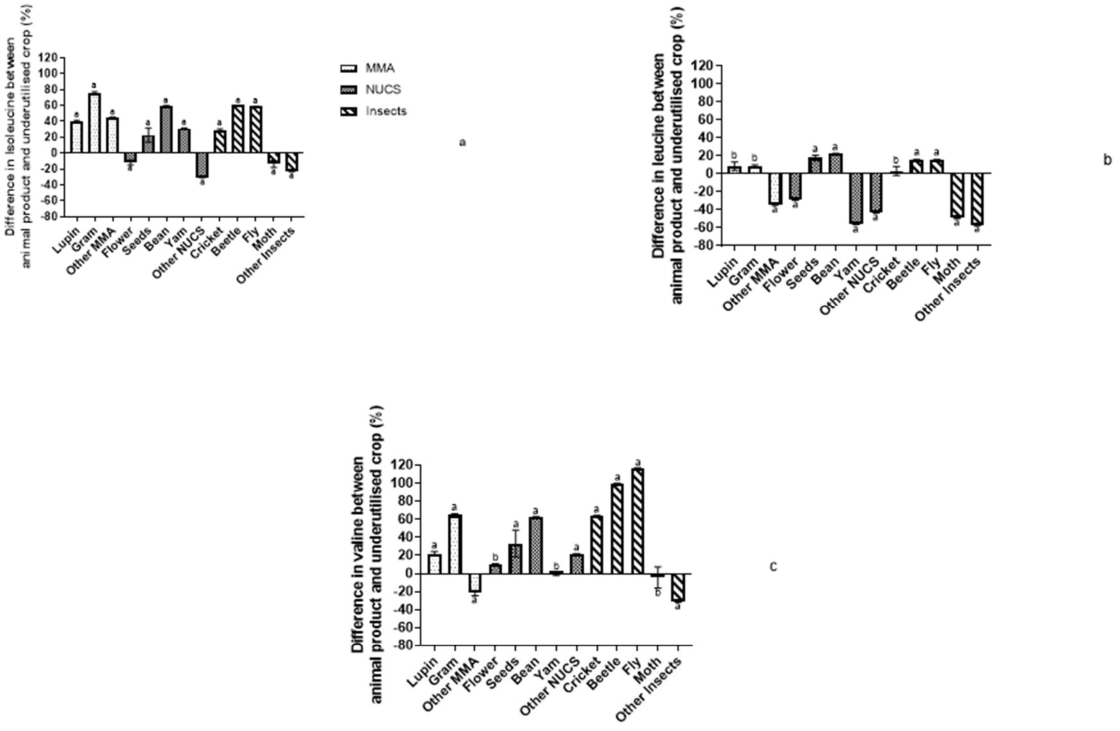

Fig 3. The animal product percentage content of (a) isoleucine (2.77g/100g), (b) leucine (6.5/100g), and (c) valine (3.03g/100g), to that of alternative protein sources. With "a" showing significance and "b" showing no significance.

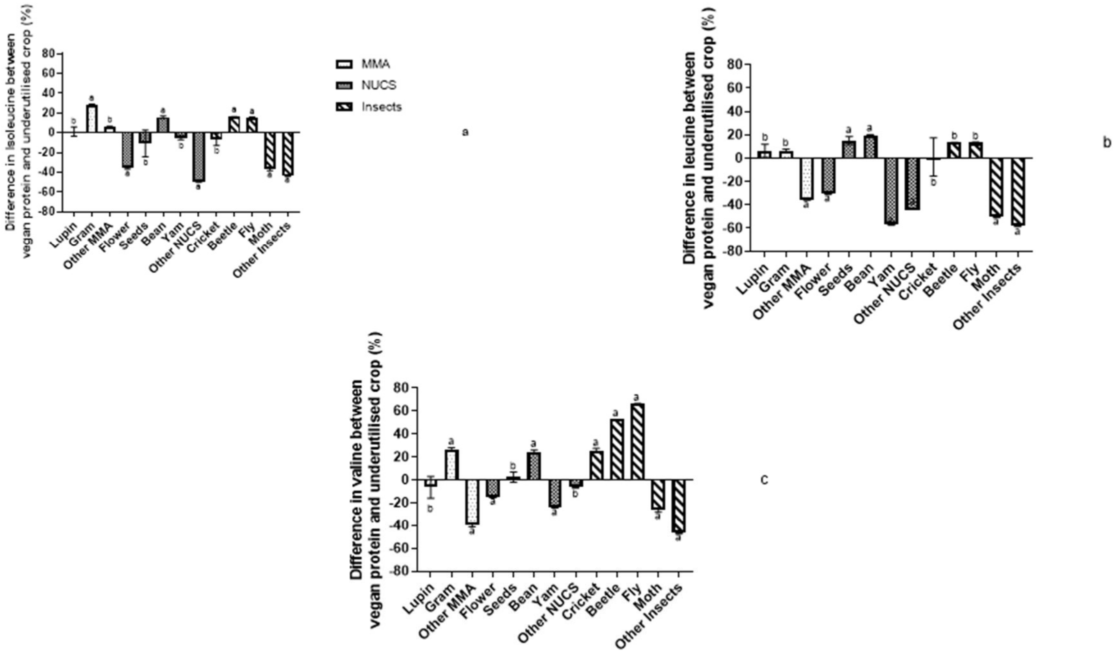

Fig 4. The vegan product percentage content of (a) isoleucine (3.81g/100g), (b) leucine (6.65/100g), and (c) valine (3.95g/100g), to that of alternative protein sources. With "a" showing significance and "b" showing no significance.