# Targeted metabolite profiling of pigeon pea (*Cajanus cajan*) seeds and, toward product (falafel) development

by

**Carla de Beer**

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*Supervisor:* Dr. Shaun Peters
*Co-supervisor:* Dr. Bianke Loedolff

April 2022

# Declaration

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# Foreword from Supervisors

This work represents the first collaborative project between the Institute for Plant Biotechnology and the Department of Food Science at Stellenbosch University, where we sought to undertake research toward the production of pigeon pea-derived (Cajanus cajan) falafels, comparing sensory profiles against the common chickpea-derived falafel. This unusual project set out to explore the potential value of this nutritionally important pulse crop as part of a larger project toward the establishment of pigeon pea as a high value alternate crop in South Africa (NRF Grant No. 116312) and, considered the holistic nutritional value of the dry seed and, flouring ability of seed for product development. In nature, plants depend on their primary and secondary metabolites for growth and survival. In return, humans rely on these metabolites for health and wellbeing. Through understanding the accumulation of health-related metabolites in a diverse range of crops, we enable food systems to justify the incorporation thereof and ensure the transformation of our food systems to include nutrient-dense crops. The outcomes of this study (i) detail the accumulation of such health-relevant primary (total and resistant starches, total digestible carbohydrates) and secondary (phenolics, flavonoids, other) metabolites in pigeon pea seeds, (ii) sensory evaluation of pigeon pea flour-derived falafels comparing them to the common chickpea flour-derived falafels, (iii) presents a unique creative output in the form of a recipe book featuring pigeon pea as the showcase ingredient.

Bianke Loedolff | PhD

Shaun Peters | PhD

# Abstract

Global food systems are currently under pressure, not only to meet the demand for food production, but also toward diversifying our selection of crops based on their nutrient profile. Furthermore, the effects of global warming are key contributors in reducing agricultural food production. Food security issues have thus become an emergent future concern and the United Nations have included nutrition as part of their sustainable development goals, with SDG 2 speaking to equitable access of nutritious food. This has led to the belief that humanity needs to consider investigating alternativ, or future fit, crop models to increase food and nutrient production. Amongst a group of plants targeted are the pulses. They are harvested for their seeds and, are nutritionally superior to the common cereal crops (rice, maize, and wheat) which we currently rely on. Nutrients contributed by plants can be divided into primary and secondary metabolites. Primary metabolites, such as starch, proteins and lipids, are sources of energy for the human body, whereas secondary metabolites often display bioactive properties which can be utilised for their therapeutic potential. This study sought to uncover some of the basic aspects of primary and secondary metabolites present in pigeon pea seeds.

Apart from demonstrating the presence of resistant starch (a key driver in human gut microbiome health), we investigated the secondary metabolite profile of the hull from a pigeon pea line and, tentatively identified an array of therapeutically important metabolites using LC-MS/MS and metabolite database searches. Amongst them were luteolin and quercetin, also previously identified in pigeon pea. Furthermore, total antioxidant capacity and DNA protection assays demonstrated that the pigeon pea seed, especially the hull, could be considered as a functional health component of the crop, because of the presence of numerous bioactive metabolites (albeit tentatively identified) potentially providing such antioxidant capacity.

Despite the hull being removed during the processing of commercially available pigeon peas, we set out to determine whether we could develop a food product with pigeon pea flour, and to test for its quantitative and qualitative aspects using descriptive sensory analysis (DSA). Pigeon pea flour was compared to that of chickpea as a falafel base. The pigeon pea flour demonstrated stronger savoury-associated attributes (nutty, garlic, cumin, green, savoury, salty) and texture profile analysis indicated that coarsely ground flour produces a moister, less dense, and less hard falafel.

From the targeted metabolite analysis, there is motivation for further research and opportunity for product development based on the health benefits the crop poses. The DSA demonstrated that pigeon pea flour can be used to create a falafel, akin to that of chickpea. Depending on future consumer panels, such falafels could use the outcomes of the DSA to make necessary consumer-preferred flavour and aroma adjustments, and potentially successfully incorporate a new crop into our current food systems.

# Acknowledgements

Thank you to Bianke Loedolff for her constant support, advice, motivation and for being an inspiration throughout. To Shaun Peters for introducing me to the lab, the project, and a way of thinking. To Dr Jeannine Marais, Marbi, and her team, thank you for being such a big help during the second half of this study. Thank you to Emily Goodwin and Tessa Brooke for assisting me during my time at the food lab. To all the amazing people I have met across the department, I would not have been able to do this without you – with a special thank you to Alu and Nokwanda for always being there when I needed chemicals, protocols or just support. Thank you to my colleagues at FVZSI for always being understanding and supportive. Thank you for my wonderful family, who has supported me in all my endeavors, me and asking me what I study again. Thank you to all my people/partner/friends/housemates/cluster partner/family who have just been a constant support and inspiration. Lastly, I acknowledge the National Research Foundation, Stellenbosch University, and the Institute for Plant Biotechnology for funding this research.

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done using sensory techniques including descriptive sensory analysis, textural profile analysis and consumer panels.

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**Addendum A**



## CODE AND EQUATION

**Addendum A**

# Abbreviations and Symbols

| | |
|---|---|
| AMG | Amyloglucosidase |
| BMK | National Average Food Consumption or Benchmark Diet |
| C | Cotyledon |
| CAF | Central Analytical Facility |
| CH | Whole Seed |
| DNA | Deoxyribonucleic Acid |
| DSA | Descriptive Sensory Analysis |
| F1 | Factor 1 |
| F2 | Factor 2 |
| FC | Falafel Made with Chickpea Flour |
| FLX | Flexitarian |
| FP | Falafel Made with Pigeon Pea Flour |
| gDNA | Genomic Deoxyribonucleic Acid |
| GI | Glycaemic Index |
| GMB | Gut Micro-Biome |
| GT | Gastrointestinal Tract |
| H | Hull |
| LC-MS/MS | Liquid Chromatography–Mass Spectrometry |
| PCA | Principal Component Analysis |
| PSC | Pescatarian |
| ROS | Free-Radical Oxygen Species |
| SMs | Secondary Metabolites |
| TAC | Total Non-Enzymatic Antioxidant Capacity |
| TPA | Texture Profile Analysis |
| UN | United Nations |
| VEG | Vegetarian |
| VGN | Vegan |
| UTM | Universal Testing Machine |

| | |
|---|---|
| % | Percentage |
| °C | Degrees Celsius |
| x *g* | Relative Centrifugal Force, G-Force |
| h | Hours |
| J | Joule |
| min | Minutes |
| ml | Millilitre |
| N | Newton |
| s | Seconds |
| v/v | Volume/Volume |
| w/v | Weight/Volume |
| µl | Microliter |
| µg | Microgram |
| U | Enzyme Activity |
| SEM | Standard error of mean |
| m/z | Mass-to-Charge Ratio |

# Chapter 1

# Background

## 1.1 Important Crops for Food and Nutrition

Global food systems are currently undergoing a revolution regarding development and integration of more nutritious foods, as part of a global effort partitioned by the United Nations Sustainable Development Goals #2 (UN, SDG #2) to end global hunger by 2030 (Johnston, 2016). Consequently, researchers are investigating all aspects of 'orphan' crops (crops that are not internationally traded, usually bound to specific climate regions, and also known as underutilized or alternate crops), long believed to be more nutrient rich than the major staple crops that service the world currently. Staple crops (crops that are traded internationally, consumed all year round and form the main portion of standard diets) contribute to most of the population's energy needs and are produced in large quantities, worldwide. The integration of such orphan crops into our food systems could play an important role in managing the current malnutrition status around the world (Maphosa, Jideani et al., 2017; Succurro et al., 2019; Mabhaudhi et al., 2019; Mabhaudhi et al., 2017; McMullin et al., 2021). Generally, plants accumulate important primary and secondary metabolites that contribute to how we evaluate the nutritional status, and consequent dietary value addition, of a plant. Primary metabolites are the basic nutrients required in a healthy diet and include sugars, starch, proteins, and lipids, among others. Secondary metabolites represent nutritional characteristics beyond basic dietary requirements and include many bioactive compounds beneficial to human health (such as phenols, flavonoids and terpenoids). To benefit from such health-related compounds, a diverse and/or fortified diet is required. Unfortunately, for many low-income countries and households, following a diet that encapsulates all the required nutrition can become very expensive. Such cost-related problems could potentially be solved through the addition of orphan crops to our agricultural landscape and, the integration of derived products into current food systems. Current agricultural systems are largely driven by demand, and thus dietary patterns.

Global dietary patterns are currently posing challenges, to the general agricultural community, to produce more nutritious foods while still being aware of the environmental impact of farming practices, such as carbon emission, fertilizer application and land use (Sabaté, Soret, 2014; Driscol, 2019; FAO et al., 2020). One way this can be achieved is through conscientising the agricultural community and the consumer towardswhat an alternative diet refers to in terms of its nutrients and lower environmental impact. According to the EAT-Lancet Commission, when investigating alternative global dietary trends, diets can be classified into four groups namely flexitarian, pescatarian, vegetarian and vegan (**Table 1**). It was found that, by switching to one of these diets, the diet-related health costs could reduce up to 95% worldwide by 2030, when compared to the national average food consumption or benchmark diet (BMK; **Fig 1**; FAO, et al., 2020).Table 1: The types of food products encompassed within alternative diets.

| | |
|---|---|
| Flexitarian | A diet with moderate amounts of animal products. |
| Pescatarian | A diet with moderate amounts of fish but no other meat products. |
| Vegetarian | No meat products, but with moderate amounts of dairy, eggs. |
| Vegan | Completely plant-based diet with no animal products. |

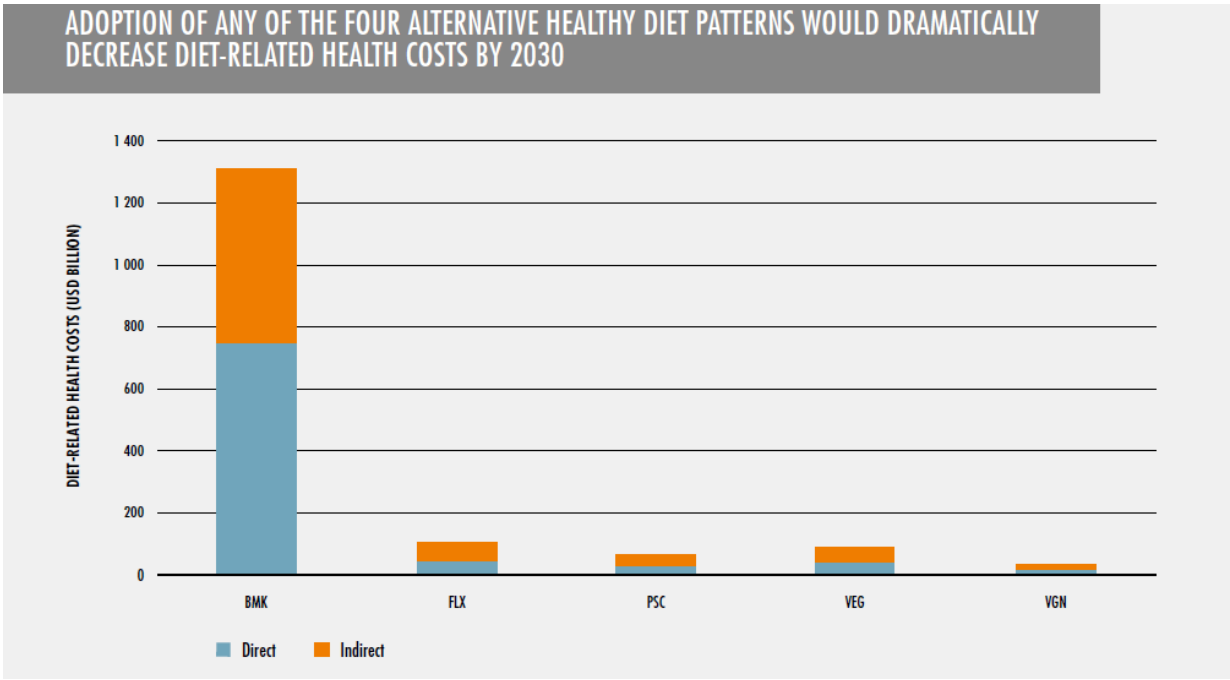


Figure 1: The graph (FAO, et al., 2020) shows that both direct and indirect diet related health costs is much more with current consumption patterns (BMK) than any of the alternative diets: Flexitarian (FLX), Pescatarian

(PSC), Vegetarian (VEG) and Vegan (VGN). Direct costs relate to medical support costs associated with diet-related diseases whereas indirect costs include the loss of productivity. The diet related diseases included in the study includes coronary heart disease, stroke, cancer, and type-2 diabetes mellitus.

Current diets (BMK) have both a direct and indirect negative impact on our cost-of-living and health related costs, because of its association with; diabetes type 2, coronary heart diseases, and more (**Fig. 1**). These costs are related to diseases that can largely be prevented through dietary interventions and alternative diets (**Table 1**) are proposed to potentially mitigate such negative impacts. Based on the current global consumption patterns, 77 % of agricultural land is used for livestock production which only contributes to 17 % of the global kilojoules consumption (Driscoll, 2019). Transitioning to one of the alternative diets would reduce (i) the amount of agricultural land used for livestock production (Aleksandrowicz et al., 2016; Driscoll, 2019) and (ii) agricultural water usage patterns (Harris et al., 2020). Alternative diets, and conventional (BMK) diets, could therefore consider or reconsider the holistic impact of its production, not only focused on the human health aspect but also the larger environmental aspect. Consequently, much interest in orphan crops has been generated over the last decade, as can be highlighted by the year of the pulses in 2016 (FAO, 2016).

### 1.2 Global Food Systems, Trends, and Globally Consumed Crops

Worldwide, consumers rely heavily on staple crops like wheat (*Triticum aestivum L.em. Thell.*), rice (*Oryza sativa L.*) and maize (*Zea mays L.*) for their daily energy requirements. These staple foods are not always nutrient rich and, as is the case in South Africa, they are fortified with vitamins and micronutrients such as vitamin A, thiamine, and folic acid (Steyn et al., 2008). There are however challenges coupled to fortification including ensuring effective distribution among the population, accessibility, and equity (Osendarp et al., 2018). To bypass the need for fortification producers, small or large scale, can grow a larger variety of food crops that contain the nutrients supplied through fortification.

Staples crops are heavily relied on , even though they are notnutrient-rich. Relying on a handful of crops as staples also makes countries more vulnerable to environmental effects, which is predicted to affect staple crop yields up to 27% by 2050 (Mabhaudhi et al., 2019). Having a variety of crops mitigates the risk effects of environmental variability and could

increase nutrient diversity. One way of introducing crop diversity is through incorporating orphan crops into our current agricultural practices. Orphan crops, neglected and underutilized crops, refer to crops that are not extensively traded with and do not have a lot of related research, compared to the staples (Varshney et al., 2012). Orphan crops can play an important role in developing a country's social economy, but some disadvantages can include low yields and the production of antinutritional substances like; phytates, oxalate and tannins (Varshney et al., 2012; Dawson et al., 2019; Gemede, Birhuanu, 2020; Jamnadass et al., 2020). The occurrence of these substances can be mitigated through crop improvement, but the historic lack of academic interest in orphan crops has led to breeding schemes being far behind that of the staples. Even though orphan crops might be regio-specific, in low-income countries some are produced on a large scale like; chickpeas (*Cicer arietinum*), pigeon peas, coconut (*Cocos nucifera*), and cassava (*Manihot esculenta;* Varshney et al., 2012). There is, however, potential in these crops, in terms of strengthening our food production systems and improved nutritional output.

### 1.3 Food Crops And Their Role In Healthy Diets

In an editorial piece on diet Van Horn wrote, “In our search for the silver bullet, we have overlooked the silver plate” (van Horn, Yancy, 2013). Human health is largely dependent on plant crops and thus diet. Crops are beneficial to human nutrition because of the array of vitamins, minerals, phenolics and other nutrients essential to human health, that the body can not produce itself (Moses, Goossens, 2017). Plants provide almost all the essential nutrients required in a balanced diet and have added health benefits (Tuso et al., 2013). In addition to water and oxygen, humans need a combination of lipids, proteins, carbohydrates, minerals, and vitamins (Grusak et al., 1999). The protein content of certain crops (soyabean, broad beans) match animal protein in quantity and outperforms in delivery efficiency (energy needed to produce a gram of protein), additionally plants can produce all the essential amino acids, making it a viable protein source (Sabaté, Siret, 2014; Tuso et al., 2013). Other nutrients, related to health including essential fatty acids (linoleic acid and alpha-linolenic acid) and iron can also be found in various plant crops, in various quantities, emphasizing the need for diverse diets with nutrient rich foods (Saunders et al., 2012; Tuso et al., 2013). Aside from providing basic nutrients, the increased consumption of plant crops also has various health benefits (Tuso et al., 2013).

Plant-based diets (some of which are mentioned in **Table 1**) have been recommended to prevent or ameliorate various conditions including diabetes, kidney diseases, neurological conditions, heart diseases, high blood pressure and heart failure (Tuso et al., 2013; McMacken, Shah, 2017; Kerley, 2018; Medawar et al., 2019; Joshi et al., 2021). It is often difficult to identify the exact link between diet, the pathway it influences and the subsequent health outcome. It is thus helpful to consider overall effects of multiple pathways, for example, the health benefits related to plant-based diets have been linked to pathways related to glycaemic control, reduced inflammatory activity and neurotransmitter metabolism (**Fig. 2**; Medawar et al., 2019)

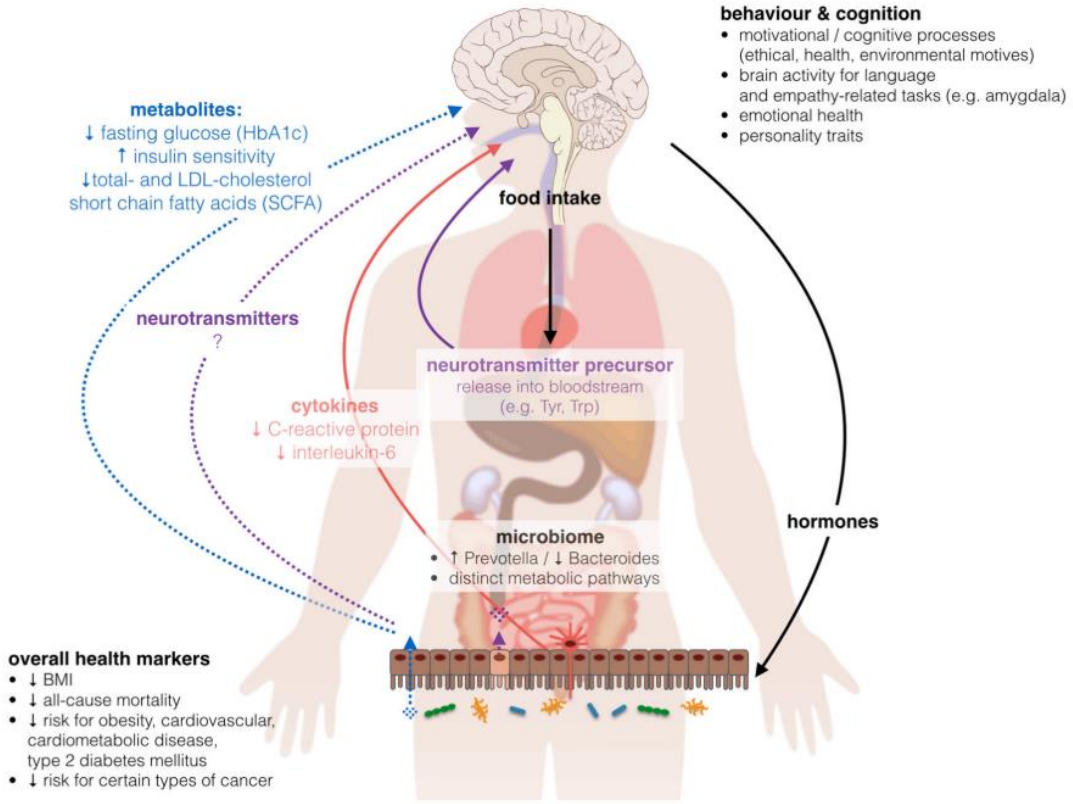


Figure 2: The effects of a plant-based diet on the microbiome−gut−brain axis. The image demonstrates the multiple pathways at play with a plant-based diet. The red arrow indicates the production of cytokines, the blue, metabolites and the purple neurotransmitters – produced by the GMB, all with related health effects. The image demonstrates how each GMB product has an individual role, but that it has a collective effect of; lowering body-mass-index (BMI), reducing the risk of obesity, cardiovascular diseases, type-2 diabetes, and certain types of cancer (Medawar et al., 2019).

## 1.4 The Role Of Nutritious Food In Our Bodies

Plant-based diets have been linked to health benefits including decreasing heart failure incidences and decreased risk for diabetes type-2 (McMacken, Shah, 2017; Kerley, 2018). Following a low-fat vegan diet has helped patients reduce their diabetes medication, subsequently reducing the side effects of the medication and cost of treatment (Tuso et al., 2013; McMacken, Shah, 2017). It is however important to understand the contributions of both primary and secondary metabolites (discussed later in this chapter) to human health to be able to refine treatments and further research identifying human metabolic pathways that it might have an impact on.

Therapeutics research demonstrates how the gut microbiome (GMB) could be linked to our genes, diet, how we absorb nutrients and has even been used to predict what diseases we might be susceptible to (**Fig. 3;** Bonder et al., 2016; Goodrich et al., 2017; Rothschild et al., 2018; Martin et al., 2018; Cani, 2018; Groot et al., 2020; Joshi et al., 2021; Frank et al., 2021). These links initiated a field of research where a change in the GMB can be used therapeutically (Durack, Lynch, 2018; Joshi et al., 2021). The GMB is the collective name for the colonies of microbes that live inside of our gastrointestinal tract (GT). The largest group of microbes lives in the large intestines in the lumen and on the mucosal surfaces. In the lumen the microbes facilitate metabolism, both primary and secondary, and on the surfaces, they interact with immune cells (Durack, Lynch, 2018). Some of the most prominent functions of the GMB includes the metabolism of carbohydrates, amino acids, fermenting dietary fibre and oxidative phosphorylation (Yatsunenko et al., 2012). The GMB has also been linked to the production of antibiotics, immunomodulatory compounds, and the tryptamine (Donia, Fischbach, 2015). Other microbial activities include producing essential vitamins (such as K and B) and hormones (such as, serotonin) (Durack, Lynch, 2018). With this wide range of metabolic influences, one can expect the GMB to be a key aspect of human health.

Dietary patterns, however, were shown to be the most influential, with the GMB even reacting to short term changes (Gentile, Weir, 2018; Zmora et al., 2019; Moszak et al., 2020;). Various macro and micronutrients (from our diets) play a role in the composition and activity of the GMB (Moszak et al., 2020). Carbohydrates play a critical role in moulding the GMB and while simple carbohydrates cause disruption, more complex carbohydrates, like resistant starches, are beneficial (Gentile, Weir., 2018).

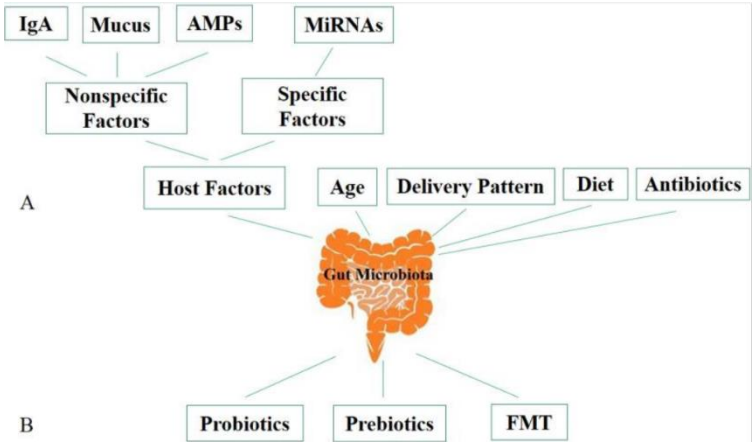


Figure 3: (A) Factors affecting gut microbiome (GMB). (B) Ways to modulate the GMB. AMPs, antimicrobial peptides; IgA, immunoglobulin A; miRNA, microRNA; FMT, fecal microbiota transplantation (Hasan, Yang., 2019).

Resistant starches are hydrolysed in the GMB to short-chain fatty acids, where it has been linked to the increase of *Bifidobacterium* spp., *Lactobacillis* spp and *Firmicutes* whose functions include carbohydrate fermentation and vitamin synthesis, and were shown to be more abundant in healthy individuals (Maier et al., 2017; So et al., 2018; Moszak et al., 2020). Even though the individual contributions of micro- and macro-nutrients influence the GMB, their interactions influence their effect. Different diets can cause variations in the pH, digestion time and differences in the number of substrates reaching the GMB (Zmora et al., 2019; Moszak et al., 2020). It is thus important to maintain a diet rich in dietary fibre, and predominantly plant-based proteins, since it is a good way to promote GMB diversity and activity (Moszak et al., 2020). Even though the GMB is largely dependent on diet, it is important to investigate contributing host factors because of the clinical potential of personalized nutrition (Zmore et al., 2019). Considering the influence of the GMB on health and the influence of diet on the GMB, it is essential to do research and conscientize consumers about the use of alternative crops, rich in dietary fibre, resistant starch and proteins as food products.

## 1.5 Deriving Health Benefits From Plants Also Requires Some Understanding Of The Complex Primary And Secondary Metabolite Production Pathways

F From time immemorial, plants have circulated water, created atmosphere, and built nutrients in its leaves all before humans invented fire. Plants have wondrous metabolic systems, they can break one of the most stable molecules known to man during the Calvin

cycle, create oxygen during photosynthesis all while building xylem structures to transport water up to 40 meters from the ground. Plants use the products from its various metabolic activities to grow, defend itself and to interact with their environment. These metabolic products can be divided into primary and secondary metabolites. Primary metabolites include organic compounds like carbohydrate, lipids, proteins, and nucleic acids. These metabolites are found in all plants and enable the plant to grow and develop and reproduce (Madani et al., 2021). Plants also have secondary metabolites which help the plant to interact successfully with its environment by enabling the plant to protect, defend, colour and flavour itself. These metabolites are classified according to their chemical structure, with six major categories; alkaloids, terpenoids, phenolics, saponins, lipids and carbohydrates (Hussein et al., 2017). Secondary metabolites are not universal and differ in presence and concentration among plants. Both primary and secondary plant metabolites can be utilized by humans for nutrition and health. Primary metabolites in plants, like starch and proteins are consumed by humans for their nutritional value while secondary metabolites are often utilized for their physiological effects on human health.

### 1.6 Primary Metabolites And Their Health Benefits

Amongst the primary metabolites the carbohydrates are of particular interest, because of the role they play in human health, especially the GMB as; probiotics, fermentable dietary fibres, and microbiota-accessible carbohydrates (Deehan et al., 2017). Carbohydrates include monosaccharides, disaccharides, sugar alcohols, oligosaccharides, and polysaccharides (Cole, Kramer, 2016). Starch, which is a major source of carbohydrates, is primarily made up of insoluble polymeric carbohydrates which consist of amylose and amylopectin (Tayade et al., 2019). Starch can further be subdivided into rapidly digestible, slowly digestible, and resistant starch (Raigond et al., 2014). The classification of these starches depends on their chemical properties and rate of digestion. Digestible starches are easily converted to glucose molecules in the body's GT. The starch is hydrolysed into monosaccharides which is easily absorbed in the bloodstream. Resistant starches are not fully hydrolysed by enzymes in the GT are labelled as resistant starch and plays a key role in maintaining the body's GMB (Zaragoza et al., 2010; Raigond et al., 2014). Resistant starch might not be digested fully in the GT because of its compact molecular structure, other starch granules prevent enzyme access,

starch granules form retrograded starch or starches have been chemically modified (Zaragoza et al., 2010).

### 1.7 Secondary Plant Metabolites And Their Health Benefits

Amongst the secondary metabolites there are many groups that are of interest with regards to human health, because of their role as antioxidants, vitamins, and pharmaceutical properties (Hussein, El-Anssary, 2018). Secondary metabolite sub-groups that play significant roles in human health include; polyphenols, isoflavones, anthocyanidins, carotenoids, phytosterols, saponins, isothiocyanates and dithiolthiones. Polyphenols can further be subdivided into classes, with flavonoids being the largest. Flavonoids can be identified by their distinct aromatic ring structure (**Fig. 4**) and are widely distributed in plant foods. Flavonoids and other phenolics, act as natural antioxidants, by protecting the plant against free-radical oxygen Species (ROS), by chelating metals (Bhaskarachary et al., 2015). The balance of ROS, pro-oxidants and antioxidants in cells are of interest to human health, where pro-oxidants induce oxidative stress by binding and depleting antioxidants or by generating ROS (Joubert et al. 2005)

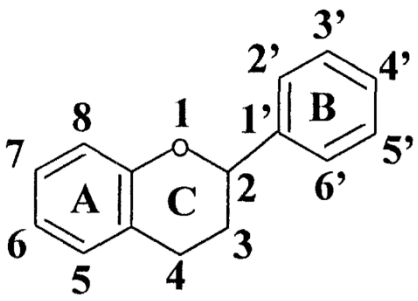


Figure 4 A generic structure of a flavonoid molecule (Cook & Samman 1996).

Cells generate ROS, like hydroxyl radicals, where an excess can be mitigated by exogenous antioxidants (Salganik 2001). Some artificial antioxidant supplements have recorded negative effects, making natural antioxidants from plant extracts a preferred source (Jiang et al. 2012; Tekale et al., 2016). Eating crops with a naturally high antioxidant levels is a cost-effective way of regulating the ROS levels in the body, because of the antioxidant variety, bioavailability and added energy source.

## 1.8 From Crop To Food

With an increased access to information and awareness of the effect of diet on human health, there is more interest to develop products from crops that are more nutritious. The increased exposure to food options and demand for sustainable food products haveresulted in a knowledge gap in market research regarding traditional or unique foods and potential for crop commercialization (Dawson et al., 2019; Yang, Lee., 2019; Fiorentini et al., 2020). Legumes play an important role in developing sustainable food options, like plant-based alternatives, since they generally have a high protein content and were shown to be more consumer acceptability than either insects or molecular meats (Cusworth et al., 2021). This combination of factors has led to the increased research interested in legumes, and not only for their potential consumer value, but also for their genetic diversity, creating opportunity for plant breeding. A variety of legumes are being researched for their commercial and agricultural value, with one of them being the pigeon pea (**Fig. 5**).

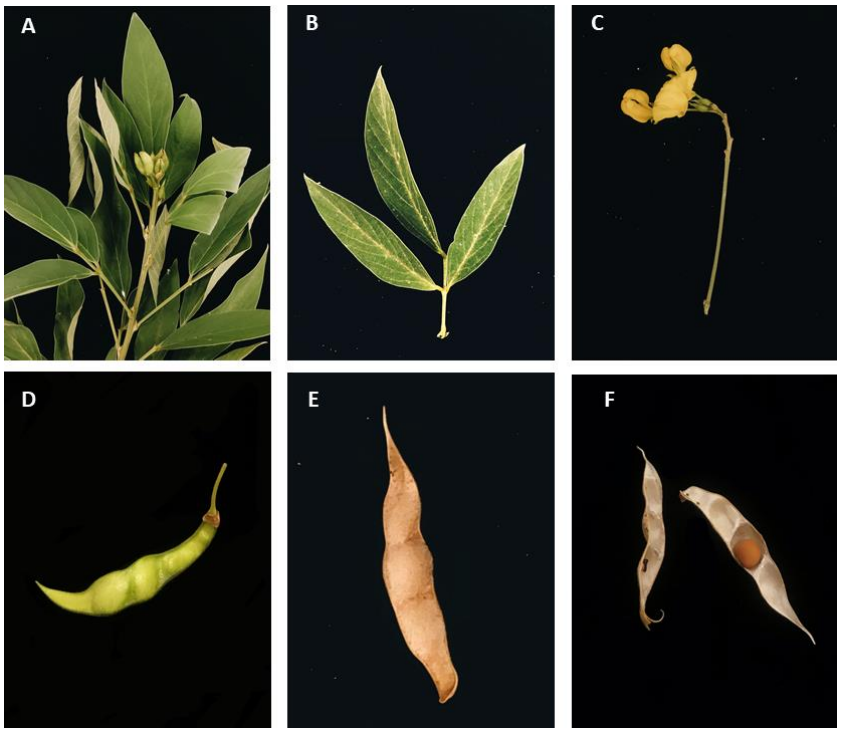


Figure 5: Images of different parts of the pea pigeon pea plant. (A) single shoot with a flower at the tip. (B) leaf cluster (C) the flower (D) green pod (E) dried pod (F) dried pod, opened with a seed inside.

The pigeon pea crop, also known as red gram or toor dhal, is an important food crop in South Asia, because of the seed's nutritional value and the plant’s drought resilience (Jeevarathinam,

Chelladuriai., 2020). Even though pigeon pea is considered an orphan crop, it is produced on a large scale, with India being the largest producer, followed by Malawi and Myanmar (FAOSTA; **Fig 6**).


Figure 6: A heat map to illustrating the average production quantity of pigeon pea around the world from 1994 till 2019 (FAOSTAT, 2021).

Pigeon pea is a legume crop within the family Fabaceae. It has known agricultural qualities (such as nitrogen fixing) and environmental adaptability, such as heat tolerance and drought tolerance where it outperforms crops like chickpeas and lentils in terms of adaptability (Khoury et al., 2015). There is an additional opportunity for plant improvement, where genes related to pod borer resistance, seed weight, high seed protein an increased number of seeds per pod, drought tolerance, heat tolerance, cold tolerance, and temperature variation tolerance have been identified in pigeon pea varieties (Saxena et al., 2010; Khoury et al., 2015). Along with potential plant breeding prospects pigeon peas are also a rich source of bioactive secondary metabolites (**Addendum A, Table 9**) making the crop of therapeutic value and interest. Various pigeon pea parts have been used as traditional remedies for: respiratory infections, headaches, inflammation and more (Saxena et al., 2010). Aside from the medicinal properties the pigeon pea has, it also is of nutritional value, especial with regard to the GMB.

The nutritional appeal of pigeon peas lies in their protein-, mineral- and starch content (**Table 2**; Miano et al., 2020) Pigeon pea seeds are a good source of lysine, methionine, and tryptophan and depending on, both internal and external, factors have 20-22% of protein, comparable to the lima bean and cow pea, and through breeding has been improved to 30% (Faris et al. 1987; Saxena et al., 2002 Saxena et al., 2010). In terms of starch digestibility,

pigeon peas have a much larger resistant starch content than other peas (mung beans, chickpeas, field peas, lentils, black gram peas) and subsequently the lowest glycaemic index (GI; Sandhu, Lim., 2007). The crop is however not free of antinutritional factors like oligosaccharides, polyphenols, phytolectins and enzyme inhibitors, luckily large variation has been found among genotypes (Saxena et al., 2002). The high amount of resistant starch and low GI indicate prebiotic potential, which is of importance for the GMB (Talari, Shakappa., 2018). This relates back to its importance in food products and the development thereof.

Table 2: The nutrient composition of the pigeon pea crop (Manickavasagan, Thirunathan., 2020).

| Composition | Nutritive value |
|---|---|
| Protein (%) | 22.3 |
| Fat (%) | 1.7 |
| Minerals (%) | 3.5 |
| Fiber (%) | 1.5 |
| Carbohydrate (%) | 57.6 |
| Calcium (mg/100 g) | 73 |
| Phosphorus (mg/100 g) | 304 |
| Iron (mg/100 g) | 5.8 |

Food items from this crop is generally prepared from the seeds in the form of whole seeds, de-hulled seeds, or flour (Manickavasagan, Thirunathan., 2020; Karri, Nirmala Nalluri et al., 2017). It is used in various traditional food products like *Mitti Handi Dal* and *toor sahl* where it serves as a staple in Gujarati homes (Bhagat, 2013). Even though there is an increased interest in the crop's agricultural potential there has been little research done on the consumer preferences regarding pigeon peas (Majili et al., 2020). An effective way for producers to develop or adjust a product is through descriptive sensory analysis (DSA), where flavour, aromatic and texture attributes are graded on a sliding scale. Pulse flour is commonly used in gluten-free baked goods and serves as a healthier alternative to wheat flour. When developing recipes with a pulse flour it is however important to be aware of the flour’s texture and flavour characteristics. Chickpea flour is a popular, commercially available pulse flour (Thakur et al., 2019). Development of new pulse flours could use the standards (texture and flavour) of chickpea flour as a reference point, which would also aid in product development.

## 1.9 Aims and Objective

The first aim of this study included understanding the potential health properties of the pigeon pea crop and determining its potential as a food product, in the form of a falafel. Characterizing the health benefits of the crop the contributions of both the primary and secondary metabolites were investigated. The first objective included tentatively identifying secondary metabolites in the pea hull through liquid chromatography–mass spectrometry (LC-MS/MS) analysis and metabolite identification, secondly the antioxidant activity for the pea parts were determined through; (i) measuring the total antioxidant capacity (TAC) and (ii) testing its ability and extent of the pea extracts to act as antioxidants in a dilution range, deoxyribonucleic acid (DNA) protection assay. For primary metabolites, carbohydrates, specifically resistant starch, were singled out as a contributor to human health. The fraction of starch, that would be considered as resistant was measured (g/100g) through a commercially available kit. The second ai mof the study is to determine the characteristics of the pigeon pea-based product, the falafel, the sensory profile was measured through (i) descriptive sensory analysis (DSA), where the; flavour, taste, aroma, and texture profile were determined using a trained panel and (ii) texture profile analysis (TPA) where texture related attributes were measured using an Universal testing Machine (UTM).

# Chapter 2
# Investigating Primary And Secondary Metabolites From A Targeted Health And Nutrition Perspective

## 2.1 Introduction

We eat plants as a source of energy, vitamins, minerals, proteins, lipids, essential amino acids, antioxidants, and other small molecules with health benefits. Besides the kilojoulescontribution, the additional nouritional benefits of plants to our diets have been overlooked. Consequently, an unprecedented increase in malnutrition and non-communicable diseases across the globe can be observed, all of which can be attributed to dietary patterns (FAO et al., 2020; FAO et al., 2021). The reason this problem persists is because of the undiversified kilojoules contributors, the lack of access to nutritious food and the lack of education with regard to nutrient benefits of food products. Considering these problems, agricultural biotechnology can play multiple roles in working towards diversifying crop selection, through breeding and crop improvement, and making nutritional information available through metabolite quantification and identification. Since staple crops experienced a significant increase in research and yield after the green revolution, they havebeen our main source of food security, largely contributed to our diets and are an economic driver through international trading. Crops that did not experience this surge in research can be termed orphan crops (lost crops, underutilized crops, neglected crops) and have also been referred to as crops for the future (CFF, 2019). Such ‘future crops’ are to demonstrate high nutrition benefits, as well as being adaptable to environmental stresses (biotic or abiotic) (Arora, 2019). Orphan crops can thus play a key role in diversifying the nutrient consumption profile, because of their nutrient diversity, richness, and environmental adaptability (Tadele, 2019; Mabhaudhi et al., 2019). Biotechnological efforts inform the nutritional value of crops, and crops that are generally prioritised include (i) crops with essential amino acids or lipids, (ii) crops with a high protein content, and (iii) crops that accumulate secondary metabolites (SMs) or bioactive compounds of health interest. Investigating the nutritional content and potential of a crop, with the aim of quantification and, or improvements, focus must be given to plant genetics, biochemistry and the relevant metabolic pathways involved (Succurro et al., 2018). Plant metabolites can be divided into two main groupings; primary metabolites, that contribute to human nutrition and, secondary metabolites that have pharmaceutical benefits.

In terms of primary metabolites, this study focused on the contributions of starch, specifically the contributions of soluble and resistant starch. The difference between soluble and resistant starch (a sliding scale) lies both in the chemistry of the molecules and the physiological effects. Soluble starches are hydrolysed by enzymes in the stomach and is thus readily taken up into the bloodstream as sugars. Resistant starches are either slower to hydrolyse or do not hydrolyse in the stomach at all and are transported to the small intestine where it is fermented and hydrolysed and utilized by gut microbiome (GMB). This differentiation in the rate of digestibility is important to note for the effect it has both on the blood glucose levels and its probiotic activity. A large fraction of resistant starch in a food product contributes to a low glycaemic index (GI) indicating a slower postprandial response to glucose and thus insulin (Singh, Lim, 2007). This is a very useful indicator for people with diabetes and cardiovascular diseases (Dávila et al., 2018; Viguiliouk et al., 2018; Brand-Miller et al., 2020). A high resistant starch content contributes to maintenance of the GMB, which in turn have been associated with influencing metabolic processes essential to human health (Cani, 2018). Other factors that influence human health are secondary metabolites (SMs) which is better known for their pharmaceutical and antioxidant activity.

Antioxidant activity in cells is regulated by various factors including the presence of antioxidants, reactive oxygen species (ROS) and pro-oxidant activity. Pro-oxidants can induce oxidative stress by depleting antioxidants or by generating ROS, where ROS plays a role in maintaining both human and plant health because it aids in various cell regulating processes (Joubert et al. 2005; Oomah et al., 2010). An imbalance in ROS has been linked to health risks related to damaged DNA, lipids, proteins or cell membranes, like cardiovascular diseases, cancer, diabetes, autoimmune and neurodegenerative disorders (Heim et al., 2002; Jiang et al., 2012). The antioxidant capacity of food has been linked to the secondary metabolites found in the product which, in legumes, have been attributed to phenolics, flavonoids, isoflavonoids, pterocarpans, catechin, anthocyanins and terpenes (Heim et al., 2002; Wink, 2013; Abourashed, 2013). A diet rich in antioxidants has proven to be beneficial in human health, making food products a viable option for antioxidant intake (Joubert et al. 2005; Salganik 2001; Talari, Shakappa 2018).

Being knowledgeable about the impact of diet on your health is not only empowering to the individual but also to communities. A negative feedback loop arises when healthier diets are inaccessible because of cost, hence creating larger disease related-costs down the line (FAO et al., 2020). This highlights the importance of characterisation and improvement of future

crops, to not only grow crops that is nutrient-rich and stress-resistant, but also for educational purposes. In nature, plants depend on their primary and secondary metabolites for growth and survival. In return, humans rely on these metabolites for health and wellbeing. This chapter aimed to characterize the accumulation of health-relevant primary (resistant starches) and secondary metabolites (phenolics, flavonoidsalkaloids, isoflavonoids)) in pigeon pea seeds.

## 2.2 Material And Methods

### 2.2.1 Seed Material And Preparation

Mature seeds from *Cajanus cajan* (pigeon pea origin: Malawi, Africa) and *Cicer arietinum* (chickpea, commercially available) were used in this study (**Fig. 7**). Seeds (16 g) were softened in water (24 h) and either retained as a whole seed (CH) or separated into two respective seed components namely (i) the hull (H) and (ii) the cotyledon (C) (Figure 1).


Figure 7: A representation of the different pea components that are being analysed, 1 - pigeon pea whole seed (CH), 2 - pigeon pea cotyledon (C), 3 - pigeon pea hull (H), 4 - chickpea whole seed (CH), 5 - chickpea cotyledon (C), 6 - chickpea hull (H).

Seed samples, respectively, were flash-frozen in liquid nitrogen and lyophilized (24 h), using a freeze dryer (Virtis sentry 2.0, model number: 2KBTES, United Scientific, Cape Town). Lyophilized samples were ground into a fine powder, using a tissuelyzer (Retsch, type: MM400, Retch-Allee, Germany), and stored (-20 °C) for downstream analyses.

### 2.2.2 Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Analysis Of Pea Hull

LC-MS/MS analyses were performed by the Central Analytical Facility (CAF) at Stellenbosch University, as previously described with a Waters Synapt G2 quadrupole time-of-flight mass spectrometer (Waters Corporation, Milford, MA, USA; Loedolff et al., 2017) equipped with a Waters Acquity UPLC. Samples were separated on a Waters UPLC BEH C18 column at a controlled flow rate and temperature (2.1 x 100 mm, 1.7 µm: 0.25 mL/min, 55 ºC). Solvent A consisted of 0.1% (v/v) formic acid in water and solvent B was 0.1% (v/v) formic acid in acetonitrile. The mobile phase gradient was initiated at 100% solvent A and linearly reduced to 28% solvent A (1 min at 100%; 22 min linear reduction). Subsequently, the mobile phase was changed to 40% solvent B followed by a wash step in 100% solvent B before the column was re-equilibrated to the initial conditions (8 min at 40%; 1 min wash; 4 min equilibrate). Electrospray ionization was applied, and samples were analysed in a negative mode run and a positive mode run. Data was acquired in MSE mode, which consists of a high collision energy scan range of m/z 125-1500 and a low collision energy scan from m/z 40-1500. The photo diode array detector was set to scan from 220-600 nm. The capillary voltage was set at 3.5 kV and the collision energy either at 6 V (low collision energy scan from) or 30-60 V (high collision energy scan). The cone voltage, source temperature, and desolvation temperature were set (15 V, 120 ºC, 275 ºC). The desolvation and cone gas (nitrogen) flows were similarly set and monitored (650 L/h and 50 L/h, respectively). Sodium formate was used for calibration and leucine encephalin was infused in the background as lock mass for accurate mass determinations. Metabolites were monitored using their deprotonated quasi-molecular ions.

### 2.2.3 Tentative Compound Identification

LC-MS/MS-derived chromatograms (measured at [M + H] +) were transposed to a BPI chromatogram and smoothed (window size +/-3, Number of smooths 2). The smoothing function allows the grouping of ions with similar retention times, considering isotopes and natural losses. The software identified various peaks of interest, based on peak intensity, which were recorded with their respective peak mass (m/z mass) and retention time. The

blank, solvent sample was analysed in a similar manner to identify peaks for elimination of solvent retention peak times and corresponding masses. The masses (with corresponding retention times) that occur in two or more of the pea samples were captured as common peaks (**Table 5**). A unique list of masses (with corresponding retention times) only identified in one of the pigeon pea samples was also captured (**Table 6**). Literature was surveyed for known metabolite masses in pigeon pea samples (**Table 7**) and was used to assist in the tentative identification of similar metabolites from the samples. Potential SM were first identified using Metabolite Workbench (MS search on RefMet) package for R (using the RStudio Version 1.2.5033 interface; **Addendum A, Code 1**), which in turn makes use of; Metabolomics Workbench Metabolite Database (https://www.metabolomicsworkbench.org/databases/metabolitedatabase.php) and PubChem (https://pubchem.ncbi.nlm.nih.gov/) database. The unidentified SMs were matched with compounds with known masses, at a threshold of 0.2 m/z (Barwick et al., 2016). The aim of this experiment was to identify SM, hence only metabolites that fell in the 'Flavonoid' or 'Other' category was included in the search. A set of possible metabolites was identified and referenced against the KEGG database for phytochemical compounds (https://www.genome.jp/brite/br08003), to verify whether the tentatively identified SM has been previously recorded in plants.

### 2.2.4 Tentative Metabolic Pathway And Drug Identification

The list of all the tentatively identified SMs was searched against the KEGG "Flavonoid biosynthesis - Reference pathway" repository. A list was constructed of the metabolites that had known involvement in plant pathways. The list of tentatively identified SMs was referenced against the Drug Bank online database (Hersey et al., 2015). Drugs containing the proposed SMs were listed and referenced against the Drug information Portal of the U.S National Library of Medicine (https://druginfo.nlm.nih.gov/drugportal/).

### 2.2.5 Seed Starch Staining

Mature pigeon pea and chickpea seeds were softened in water (24 h), prepared as described (**2.2.1**). All seed components were screened for the presence of starch by submerging (2 min) in Lugol's solution (1% $I_2$, 2% KI) and subsequently rinsed in cold water for visualization.

### 2.2.6 Total Starch Determination Assay

Total starch was extracted from lyophilized seed tissue (50 mg) using the Total Starch HK Assay kit (K-TSHK 01/20, Megazyme International Ireland Ltd.) following manufacturer's instructions.

Samples were incubated (85 ℃, 10 min) in ethanol (1 mL, 80%), to remove water-soluble carbohydrates. Samples were centrifuged (5 min, 1300 x *g*) and supernatants discarded. The remaining pellets were re-suspended in ethanol (2 mL, 80%), centrifuged (10 min, 1300 x *g*) and the supernatants discarded. Pellets were re-suspended in ethanol (20 µl, 80%) to aid with dispersion after which thermostable α-amylase (0.28 U) was added per sample and incubated (100 ℃, 12 min, with vigorous shaking), followed by addition of amyloglucosidase (AMG; 33 U) and incubated (50 ℃, 30 min, with vigorous shaking). The final volume was adjusted to volume (1 000 µl) using distilled water, mixed well and centrifuged (10 min, 1300 x *g*).

Components provided with the kit were combined as outlined in **Table 3** below. The reaction was initiated by the addition of hexokinase and glucose-6-phosphate dehydrogenase suspension and the increase in absorbance (340 nm) was measured. The amount of NADPH formed was measured as an indication of the amount of D-glucose in the sample and all samples were measured in triplicate.

Table 3: Plate contents for analysis of total starch, for taking the first reading.

| Solution | per microtiter-plate well (uL) |
|---|---|
| Deionised | 200 |
| Sample | 5 |
| Buffer | 10 |
| NADP+/ATP | 10 |
| *Take reading one (A1) at 340 nm after 5 minutes* | |
| HK/ G6P-DH | 2 |
| *Take reading two (A2) at 340 nm on a kinetic loop every 5 minutes.* | |

### 2.2.7 Resistant Starch

Total resistant starch was determined from lyophilized seed tissue (50 mg) using the Resistant Starch HK Assay kit (K-RSTAR 05/19, Megazyme International Ireland Ltd.) following manufacturer's instructions.

Ground up samples were weighed off (10 mg) and incubated with amyloglucosidase (AMG, 0.4 mL, 37 °C) with continuous shaking (16 h). Ethanol (0.4 mL, 99%) was added to the tubes and vortexed. Tubes were centrifuges (10 min, 1300 x *g*) and the supernatant was decanted. Pellets were resuspended in ethanol (0.4 mL, 50%), vortexed and centrifuged (10 min, 1300 x *g*), this step was then repeated. Excess liquid was drained from the tubes and the pellets were resuspended in KOH (0.2 mL, 2M) while on ice. Sodium acetate buffer (1.2 M, pH 3.8, 0.8ml) and AMG (0.01 mL) were added to the samples and incubated with intermitted vortexing (30 min, 50 °C). Samples were centrifuged (10 min, 1300 x *g*) and the supernatants were diluted with distilled water (fill up to 10 mL). Aliquots (0.05 mL) of the samples were pipetted into microtiter-plate well (96 well, F-bottom, Greiner) in triplicate, GOPOD (1.5 mL) was added, and the plate was incubated (20 min, 50 °C) before measuring the absorbance (510 nm). Samples were measured against a reagent blank, of sodium acetate buffer (0.05, pH 4.5) and GOPOD (1.5 mL), and a D-glucose standard.

### 2.2.8 Antioxidant Capacity And DNA Protection Assay

#### 2.2.8.1 Sample Preparation

Seeds (16 g) were soaked in water (20 h, 4 °C) where after they were separated into the varying seed components. Three separate components were investigated: H, C and C. Metabolites were extracted with acetonitrile from lyophilized seed constituents (100 mg), as previously described (Routaboul et al., 2006**;** Loedolff et al., 2017; Xonti et al., 2020). Each component was ground (5 min) in an Acetonitrile solution (1 mL, 75%) on ice. The mixtures were sonicated (20 min) and subsequently centrifuged (1300 x *g*, 5 min). The supernatant was transferred to a new tube (1.5 mL) and stored (4 °C, 24 h). Acetonitrile solution (75%, 1 mL) was added to the remaining pellet and stored (24 h, 4 °C). Tubes containing plant material ware centrifuged (1300 x *g*, 5 min) and the supernatant was extracted. The supernatants were pooled with the matching solution extracted the day before. The aqueous solutions were vacuum-dried (21 °C, GeneVac, model: EZ2.3, genevac LTD, England) to a powder. When all the liquid evaporated the extract was resuspended in deionized water (500 µL) and stored (16 h, 4 °C).

#### 2.2.8.2 DNA Damage Assay

The DNA damage assays were performed as previously described (Jiang et al., 2012; Xonti et al., 2020). The extracts (H, CH, C) were prepared using a dilution range using ddH20 (whole extract, 0.5 mL/mL; 0.25 mL/mL; 0.125 mL/mL; 0.0625 mL/mL; 0.03125 mL/mL). Human genomic DNA (gDNA) (0.5 µg/µL; cat. #11691112001, Roche, Sigma, South Africa) was diluted with a phosphate buffer (50 mM, pH 7.4), aliquoted and kept on ice. The gDNA was subsequently damaged using Fenton's reagent (1 mM FeSO4, 0.1 mM H2O2) both in the presence of pigeon pea extracts (C, CH, H) and Trolox (vitamin E derivative, 1 mM Trolox) and in the absence of any protectant. The samples were incubated and placed on ice to prevent subsequent DNA damage (45 min, 37 °C). Protection was determined using agarose gel electrophoresis (0.8 % agarose, 10 mg/mL ethidium bromide; 100 V, 10 min) and subsequently visualized using a gel documentation system (G: BOX, Syngene, United Kingdom). If a smear was observed instead of a clear band, the DNA was assessed as damaged.

Table 4: Aragrose gel well contents to assess gDNA damage, for gel electrophoresis.

| Component | Neutral (µL) | Damaged control (µL) | Protected control (µL) | Pigeon pea extract (µL) |
|---|---|---|---|---|
| Human gDNA (0.5mg) | 2.5 | 2.5 | 2.5 | 2.5 |
| Phosphate Buffer (50mM pH 7.4) | 20.5 | 13.5 | 3.5 | 3.5 |
| Protectant | 0 | 0 | 10 Trolox | 10 (Sample Extract) |
| $FeSO_4$ (1mM) | 0 | 3 | 3 | 3 |
| $H_2O_2$ (25%) | 0 | 4 | 4 | 4 |
| TOTAL | 23 | 23 | 23 | 23 |

### 2.2.8.3 Total Antioxidant Capacity

The total antioxidant capacity (TAC) was evaluated using a TAC Assay Kit (Sigma Aldrich, C# MAK187). Samples were diluted (X1, X10, X100) with ddH2O and directly added (100 µL) to a spectrophotometer (Thermoscientific, spectrophotometer, Multiskan Sky, type: 1530, ref# 51119500) plate (96 well, F-bottom, Greiner) in triplicate. A copper dilution (1:49, 100 µL) made up with Assay Diluent (Sigma Aldrich, C#MAK187B) was added to each microtiter-plate well containing sample. The plate was incubated in the spectrophotometer (90 min, 24°C) and absorbance were measured (570 nm). In addition to the samples being tested a Trolox (1 nmole/µL) standard curve was prepared, based on a dilution (ddH2O) series (0%, 4%, 8%, 12%, 16%, 20%) for colorimetric detection.

## 2.3 Results

### 2.3.1 LC-MS/MS Data Proposes Medicinal Secondary Metabolites found in Pigeon Pea Hull

To postulate which secondary metabolites are present in the hull of pigeon peas, a LCMS-MS analysis was performed on five pigeon pea varieties (SEFA, Nandolo, Dali, India, Lari). A total of 16 metabolites were identified among the common mass peaks (**Table 5**) and 23 metabolites were identified among the unique mass peaks (**Table 6**). Major chemical

groupings of the SMs consisted of; terpenoids (3), alkaloids (13), flavonoids (9), phenylpropanoids (3), polyketides (2) and shikimate (1). Two metabolites were verifiable in literature; luteolin (m/z: 286.06) and quercetin (m/z: 302.05) and both were found in the Nandolo variety. FDA-approved drugs (25) containing the tentatively identified SMs were identified alluding to the medicinal and market value of the plant (**Table 7**). Tentatively identified phytochemical SMs matching the chromatogram m/z mass peaks were identified, but verification of the metabolites in pigeon pea hulls will need to be done via metabolite extraction.

Table 5: Proposed phytochemical SMs for the common peaks. SMs were identified by matching masses (m/z) with chemical compounds using RefMet (PubChem database). The identified chemical compounds were further

| m/z (+) | Metabolite | PubChem | Secondary Metabolite | Super class | Main Class |
|---|---|---|---|---|---|
| 233.11 | Albine | 442936 | Alkaloids | Alkaloids derived from lysine | Quinolizidine alkaloids |
| | Melatonin | 896 | | Alkaloids derived from tryptophan and anthranilic acid | Indole alkaloids |
| 242.28 | Valeroidine | 443012 | Alkaloids | Alkaloids derived from ornithine | Tropane alkaloids |
| | Randainol | 5281866 | Phenyl-propanoids | Lignans | Neolignans |
| | Hymenoxon | 42295 | Terpenoids | Sesquiterpenoids (C15) | Pseudoguaianolide |
| | Pseudobaptigenin | 5281805 | Flavonoids | Isoflavonoids | Isoflavones |
| 295.13 | Cinchonidine | 101744 | Alkaloids | Alkaloids derived from tryptophan and anthranilic acid | Quinoline alkaloids |
| | Gingerol | 442793 | Shikimate | Others | Zingiber derived compounds |
| | Dehydrocyclo-guanandin | 5281625 | Polyketides | Pyrones | Xanthenes |
| | Tutin | 118701063 | Terpenoids | Sesquiterpenoids (C15) | Tutinolide |
| 362.16 | Anisotropine methylbromide | 657201 | Alkaloids | Alkaloids derived from tyrosine | Isoquinoline alkaloids |
| 577.13 | Proanthocyanidin \A 2 | 124025 | Flavonoids | Complex flavonoids | Proanthocyanidin |
| 579.15 | Deserpidine | 8550 | Alkaloids | Alkaloids derived from tryptophan and anthranilic acid | Indole alkaloids |
| | Podorhizol beta-D-glucoside | 443015 | Phenyl-propanoids | Lignans | Lignan glycosides |
| | Procyanidin B4 | 147299 | Flavonoids | Complex flavonoids | Proanthocyanidins |
| | Violanthin | 442665 | | Flavonoids | Flavones |

filtered out based on the KEGG phytochemical compounds database.

Table 6: Proposed phytochemical SMs for the unique peaks. SMs were identified by matching masses (m/z) with chemical compounds using RefMet (PubChem database). The identified chemical compounds were further filtered out based on the KEGG Phytochemical compounds database. The SMs marked with a star (*) has been verified in literature.

| m/z (+) | variety | metabolite | PubChem | Secondary Metbolite | super class | main class |
|---|---|---|---|---|---|---|
| 166.09 | India | Anatoxin A | 431734 | Alkaloids | Alkaloids derived from ornithine | Tropane alkaloids |
| | | Hordenine | 68313 | Alkaloids | Alkaloids derived from tyrosine | Tyramine derivatives |
| | | Ephedrine | 9294 | Alkaloids | Alkaloids derived by amination reactions | Phenylalanine derived alkaloids |
| | | Pseudoephedrine | 7028 | Alkaloids | | Phenylalanine derived alkaloids |
| 175.02 | India | Juglone | 3806 | Others | Naphthoquinones | alpha-Naphthoquinones |
| | | N-Methyltryptamine | 6088 | Alkaloids | Alkaloids derived from tryptophan and anthranilic acid | Indole alkaloids |
| 263.13 | India | Argyrolobine | 118701430 | | Alkaloids derived from lysine | Quinolizidine alkaloids |
| 287.06 | Nandolo | Kaempferol | 5280863 | Flavonoids | Flavonoids | Flavonols |
| | | Luteolin * | 5280445 | | | |
| | | Vestitone | 439310 | | Isoflavonoids | Isoflavanones |
| 303.05 | Nandolo | Hesperetin | 72281 | Flavonoids | | Flavanones |
| | | Quercetin * | 5280343 | | | Flavonols |
| | | Ellagic acid | 5281855 | Others | Tannins and galloyl derivatives | Galloyl derivatives |
| 311.12 | SEFA | 6-Deoxyjacareubin | 5281629 | Polyketides | Pyrones | Xanthenes |
| | | Picrotin | 442291 | Terpenoids | Sesquiterpenoids (C15) | Others |
| | | Sinapine | 5280385 | Phenylpropanoids | Monolignols | Sinapate derivatives |
| 325.11 | SEFA | Quinidine | 441074 | Alkaloids | Alkaloids derived from tryptophan and anthranilic acid | Quinoline alkaloids |
| | | Glabranin | 124049 | Flavonoids | Flavonoids | Flavanones |
| | | Phaseollidin | 119268 | Flavonoids | Isoflavonoids | Pterocarpans |
| 535.14 | India | Dalpanin | 442769 | Flavonoids | Isoflavonoids | Isoflavanones |
| | | Luteolin 7-O-(6''-malonylglucoside) | 44258060 | Flavonoids | Flavonoids | Flavanones |
| 543.13 | SEFA | Belladonnine | 442995 | Alkaloids | Alkaloids derived from ornithine | Tropane alkaloids |
| 727.21 | SEFA | Adiantifoline | 167937 | Alkaloids | Alkaloids derived from tyrosine | Isoquinoline alkaloids |

Table 7: Proposed metabolites were searched against the Drugbank online (https://go.drugbank.com/) NIH, FDA approved (https://druginfo.nlm.nih.gov/drugportal/) drug databases for their therapeutic potential. A selected group of metabolites had verification on either of the repositories. Metabolites marked with a star (*) has been verified in literature.

| m/z | metabolite | Drugbank Online | FDA Approved drug name |
|---|---|---|---|
| 233.11 | Melatonin | DB01065 | Melatonin |
| 242.28 | Pseudobaptigenin | | Psi-baptigenin |
| 295.13 | Cinchonidine | | Cinchonidine |
| | Gingerol | | Gingerol |
| | Tutin | | Tutin |
| 362.16 | Anisotropine methylbromide | DB00517 | Anisotropine methylbromide [USAN:INN:JAN] |
| 577.13 | Proanthocyanidin A2 | | Proanthocyanidin A2 |
| 579.15 | Deserpidine | DB01089 | Deserpidine [INN:BAN] |
| | Procyanidin B4 | | Procyanidin B4 |
| | Violanthin | DB03460 | |
| 166.09 | Anatoxin A | | Anatoxin A |
| | Hordenine | | Hordenine |
| | Ephedrine | DB01364 | Ephedrine [USAN:BAN] |
| | Pseudoephedrine | DB00852 | Pseudoephedrine [INN:BAN] |
| 175.02 | Juglone | | Juglone |
| | N-Methyltryptamine | | N-Methyltryptamine |
| 287.06 | Kaempherol | DB01852 | Kaempferol |
| | Luteolin * | DB15584 | Luteolin |
| 303.05 | Hesperetin | DB01094 | Hesperetin |
| | Quercetin * | DB04216 | Quercetin |
| | Ellagic acid | DB08846 | Ellagic acid [INN:DCF] |
| 311.12 | Picrotin | | Picrotin |
| | Sinapine | | Sinapine |
| 325.11 | Quinidine | DB00908 | Quinidine [BAN:NF] |
| 543.13 | Belladonnine | | Belladonnine |

### 2.3.2 Extracts From Pigeon Pea Seeds Indicates A Higher Total Antioxidant Capacity Than Extracts From Chickpea Seeds

To determine antioxidant levels, compared to that of chickpeas, of pigeon peas, pea parts (**Fig. 7**) were tested for total non-enzymatic total antioxidant capacity (TAC). The TAC kit used in the study is based on the copper-reducing antioxidant capacity method. The total non-enzymatic antioxidant capacity (TAC) of a plant is indicative of the amount of oxidative stress it can counteract. The TAC of pigeon pea and chickpea extracts was determined and expressed as a Trolox (vitamin E) equivalent (**Fig. 8)**. The TAC extracts from the pea hulls (H) for both pigeon pea and chickpea were higher than extracts from either the full seed (CH) or the cotyledon (C). Pigeon pea extracts were all significantly different (two tailed t-test) from their chickpea counterparts. Pigeon Pea H displayed the highest TAC value (1.94 ± SEM 0.01 nmole/µL) among all the samples. Pigeon pea CH and C ranged from 0.98 nmole/µL – 0.93 nmole/µL respectively which is still higher than chickpea extracts. Chickpea extracts were in in a similar range (0.74 nmole/µL - 0.46 nmole/µL).

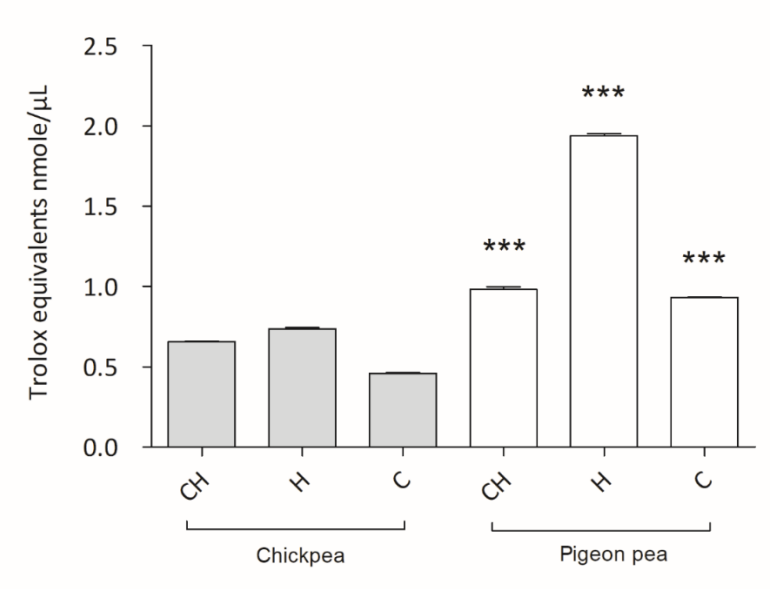


Figure 8: Total antioxidant capacity (TAC) was compared between extracts from respective seed components from pigeon pea and chickpea seeds. TAC in lyophilized tissue was determined from three independent experiments, using pooled samples of seeds (approximately 20 seeds per replicate) and expressed as Trolox equivalents. Statistical significance is indicated by stars as determined by a two tailed t-test, using chickpea as the comparison control for each seed component (HC, ***p = 0.00005; H, ***p < 0.00001; C, ***p < 0.00001). HC, whole seeds; H, hull; C, cotyledon.

### 2.3.3 Extracts From Pigeon Pea Seeds Display The Ability To Protect Human Genomic DNA From Oxidative Damage

To assess whether extracts from pigeon pea components (**Fig. 7**) would be efficient as oxidative-stress protective molecules, DNA damage/protection assays were done on human genomic DNA using Fenton's reagent ($H_2O_2$). Damage was based on the level of smearing observed on the gel (**Fig. 9**). All of the pea parts protected the gDNA sufficiently up until X8 dilution. Thereafter the H was able to protect sufficiently up to X32 dilution, where the CH and C indicated smearing, thus not sufficient protection.

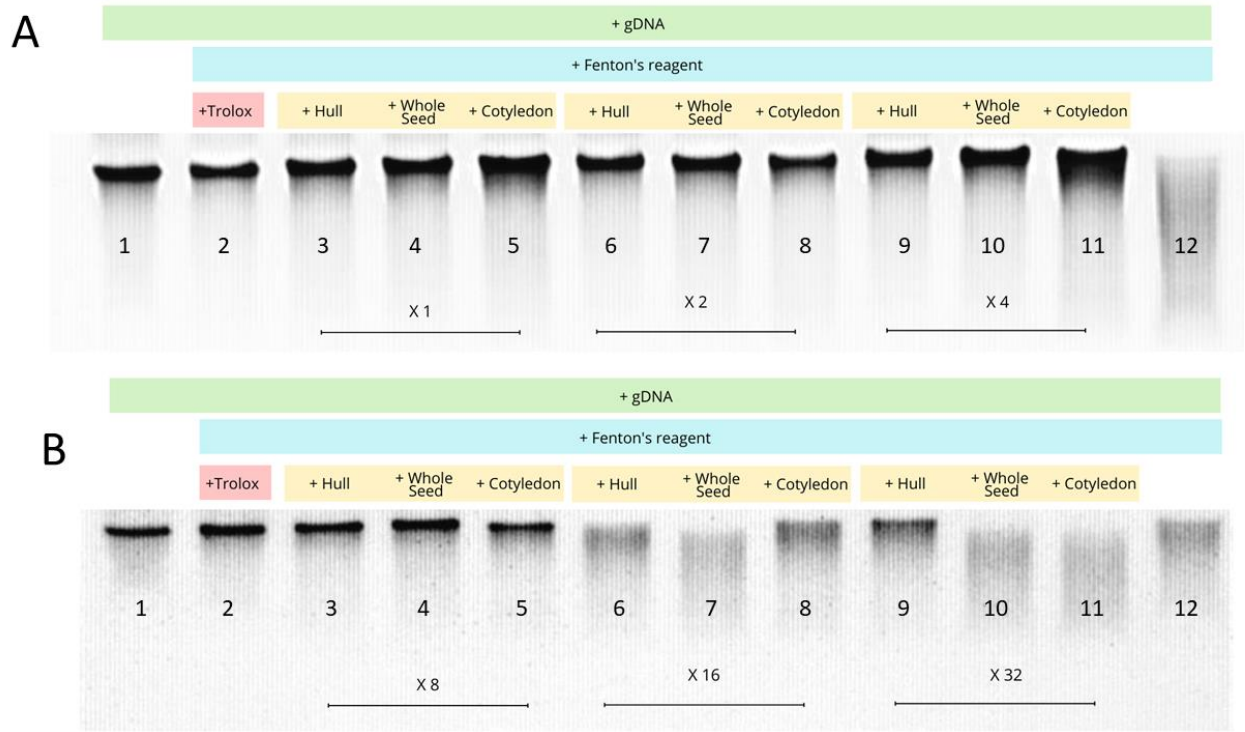


Figure 9: DNA protection assay demonstrated the ability of serum from varying pea parts (C, CH, H) at different dilutions to protect gDNA. This assay is indicative of the ability of pea parts to act as antioxidants, where Trolox was used as a control. 1 – DNA without protectant or Fenton's reagent, 2 - Positive control using Trolox as protectant, 3-11 - using pigeon pea H, CH, and C as protectant at various dilutions. (A) 3-5 - X1 dilution, 6-8 - X2 dilution, 9-11 - X4 dilution. (B) 3-5 - X8 dilution, 6-8 -X16 dilution, 9-11 X32 dilution.

### 2.3.4 Starch Accumulates In Both Peas, But Resistant Starch Mainly Occurs In Pigeon Peas

To establish how the starch is spread out throughout the pea components, pea components (HC, C, H) were stained using Lugol stain (5%). The peas were soaked in water overnight, dehulled and stained. Pea components with a high starch content turned black. Components with a high amount of starch showed a higher intensity of black discoloration (**Fig 10**).


Figure 10: Chickpeas and pigeon pea components after Lugol staining. 1 - pigeon pea HC, 2 - pigeon pea C, 3 - pigeon pea H, 4 - chickpea HC, 5 - chickpea C, 6 - chickpea H.

For both pigeon peas and chickpeas, the main source of starch lay in the C and not in the embryo or H. No colour change was observed in the H of either chickpea to pigeon pea indicating low levels of starch.

To determine how the starch is distributed in the pea components the total starch was measured. Total starch was measured in gram starch per 100 g dry sample. As observed in the initial staining, most of the pea's starch is situated in the cotyledon. This was confirmed in the results where the cotyledon of both peas indicated to have the highest concentration of starch. The highest amount of starch was observed in pigeon pea C (25.5 g ± SEM 1.67) followed by pigeon pea CH (23.55 g ± SEM 5.51). Both the H of chickpeas and pigeon peas contained very low starch concentrations (0.47 g and 0.28 g respectively). A significant difference in starch content was determined using a two tailed t-test ($p < 0.05$). Both the C and H indicated a significant difference between the starch content of pigeon pea and chickpeas (**Fig. 11**).

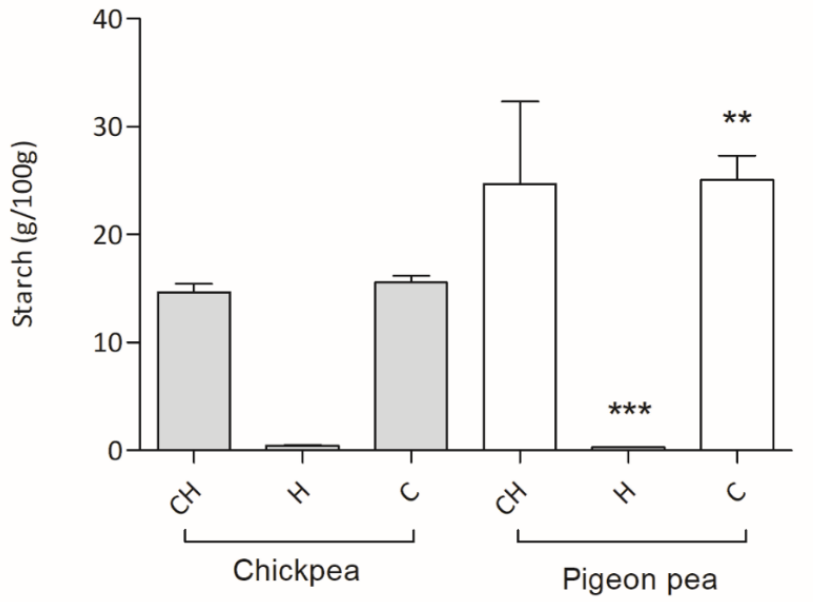


Figure 11: Total starch was compared between extracts from respective seed components from pigeon pea and chickpea seeds. Total starch was determined from three independent experiments, using pooled samples of seeds (approximately 15 seeds per replicate). Statistical significance is indicated by stars as determined by a two tailed t-test, using chickpea as the comparison control for each seed component (HC, $p = 0.26$; H, ***$p < .001$; pigeon pea C, *$p = .016$). HC, hull, and cotyledon representing whole seeds; H, hull; C, cotyledon.

Resistant starch was determined for each seed component comparing pigeon peas and pigeon peas. A statistically significant difference was seen between each pea part when compared to chickpea ($p < 0.05$). The largest difference in resistant starch content was seen in the CH where pigeon pea (79.83g ± SEM 0.774) had a much larger amount than chickpea (0.886g ±

SEM 0.066). All pigeon pea components measured higher resistant starch levels than their chickpea counterparts (**Fig 12**).

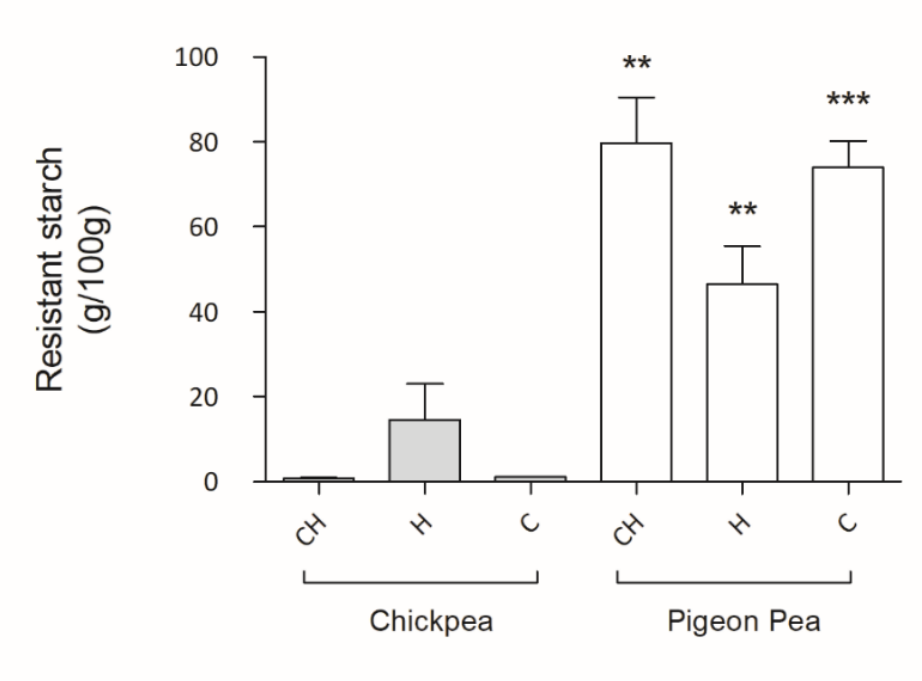


Figure 12: Resistant starch was compared between extracts from respective seed components from pigeon pea and chickpea seeds. Resistant starch was determined from four independent experiments, using pooled samples of seeds (approximately 15 seeds per replicate). Statistical significance is indicated by stars as determined by a two tailed t-test, using chickpea as the comparison control for each seed component (HC, **p = 0.003; H, **p < .04; pigeon pea C, ***p < .0001). HC, hull, and cotyledon representing whole seeds; H, hull; C, cotyledon.

## 2.4 Discussion

Consumers are moving towards more sustainable food options creating an opportunity for innovation of healthy plant-based products, like pulses, because of their numerous health benefits (Aschenmann-Witzel et al., 2021). These leguminous crops are planted for their seeds, ability to put nitrogen back into the soil (through a process termed nitrogen fixation) and, are generally considered to be nutritionally superior to the common cereal crops, which we currently rely on for most of our food production (Cusworth et al., 2019; Khoury et al., 2014). While we already consume numerous pulse crops (e.g., the common bean, *Phaseolus vulgaris*; the common pea, *Pisum sativum*; Chickpea ,*Cicer aritineum*) there are a number of pulses that are categorized as orphan crops and represent those that are only of regio-specific importance (e.g., Bambara groundnut, *Vigna subterranea*; Pigeon pea, *Cajanus cajan*; mungo bean, *Vigna mungo*). These orphan crops can play a major role in future cropping systems through the diversification of crops and nutrients. With numerous orphan crops available, metabolite characterization can identify crops to prioritise for their health benefits. This study aimed to investigate the secondary metabolite (SM) profile and starch profile of pigeon pea to evaluate its potential for human health, apart from its known agricultural and crop-related characteristics (Talari, Shakappa, 2018; Tayande et al., 2019; Heuzé et al., 2019). Such investigations are necessary to establish the foundation for further research into alternative crops and their value-addition, beyond food.

Pigeon peas are traditionally de-hulled prior to being packaged and sold commercially. However, the dense SM profile of the hull (H), also identified in this study, could motivate its inclusion in future food products, purely based on the value addition to health and well-being. The SM profile of legumes generally includes chemical groups such as flavanols, phenolic acids and anthocyanins, which are in turn related to health benefits (**Table 8**). One of the identified health benefits related to SMs, is an antioxidant capacity, which protects, flavours, and colours the plant (Erb, Kliebenstein, 2020). Antioxidant activity of legumes have been linked to the colour intensity of the hull, where a darker colour is postulated to have a higher TAC (Maphosa, Ideani, 2017). From the latter analysis, various masses of interest (m/z) were recorded as unidentified SMs, for the respective pea samples, with India having the most (17) followed by Lari (13) and Dali (13), Nandolo (12) and SEFA (10). Based on the masses (m/z) SM were tentatively identified using Metabolite Workbench and the KEGG phytochemical compound repository.

Among the five pea samples' hulls that were tested, 39 tentative SM were identified for 16 masses (m/z). The main chemical classes that were identified among the SM were Flavonoids (7), Alkaloids derived from tryptophan and anthranilic acid (5), isoflavonoids (4), alkaloids derived from ornithine (3), alkaloids derived from tyrosine (3) and sesquiterpenoids (3) (**Table 6, 7**). The most abundant among the groupings, the flavonoids, and alkaloids, which respectively has multiple pharmaceutical uses. Flavonoids are known for their anti-inflammatory, antiallergic effects, antithrombotic and tumour inhibition properties whereas alkaloids are associated with local anaesthesia, cardiac stimulation, and anti-inflammatory activity (Hussein, El-Anssary, 2018; Heinrich et al., 2021). The tentatively identified SMs and health related benefits is a good starting point for further, targeted SM identification. Because the H is traditionally not included in the final food product, there is a drive towards finding alternative uses. With the identified SM's and corresponding drugs (**Table 8**) the hulls could possibly be used for either extraction of these SM's or as a holistic supplement. SM's that were identified and are supported in literature, was found in the Nandolo sample, and includes luteolin and quercetin.

Table 8: A collection of pharmacological activities that has previously been identified in the pigeon pea plant along with the corresponding SM related to the activity (Pal et al., 2011).

| Pharmacological activity | Identified SM |
|---|---|
| antimicrobial activity | Pinostrobin, vitexin, cajaninstilbene acid |
| antioxidant activity | Genistein, genistin, Pinostrobin, cajaninstilbene acid, orientin, vitexin |
| antibacterial activity | Cajanuslactone |
| Hypocholesterolemic | Cajanin, longistylin C, longistylin A |
| Neuroactive Properties | Pinostrobin |
| Anticancer Activity | Cajanol |
| Anthelmintic Activity | Phenolics (flavanoids, tannin |
| Antiplasmodial | Betulinic acid, longistylin A and longistylin C, Pinostrobin |
| Antiinflamatory | Pinostrobin |

This study indicated that luteolin and quercetin could potentially be extracted from Nandolo, pigeon pea hull. Both these SMs have proven medical uses. Luteolin, a flavonoid, is a known antioxidant, strong ROS inhibitor, prevents tumour development and neuroprotectant (Choi et

al., 2013; Nabavi et al., 2015; Tuorkey, 2015). Quercetin also a flavonoid, is also a known for its antioxidant, anti-inflammatory, and anti-fibrosis activities (Sakanashi et al., 2008; Zhao, Liu, 2012). These SMs have known pathways in the plant (KEGG) which can aid in increasing the production of the metabolites for enhancing medicinal properties or extraction purposes.

Based on the array of tentatively identified SM, literature support and the therapeutic potential of luteolin and quercetin, the Nandolo line was selected for further research on antioxidant activity and starch composition. The Nandolo line was obtained from Malawi, the second largest producer of pigeon peas (FAOSTAT, 2019). The distribution of the Nandolo seed hulls were investigated as part of an observational quest. The hulls are varied in colour (**Fig. 13**) with the majority of seeds being brown and spotted (**Fig. 14**). Even though inquiry was out of curiosity, the effect of phenolic compounds on hull colour is well documented where a more intense colouration generally implies a higher concentration of phenolics, which in turn positively influences the antioxidant capacity (Rocha-Guzmán et al., 2006). This large ratio of dark coloured seeds in the Nandolo sample could thus indicate antioxidant activity.

Figure 13: Representation of the seed hull colour of the Nandolo pigeon pea (Nandolo Pigeon Peas, 2021).

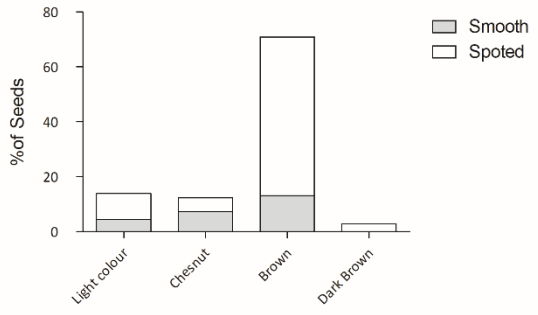


Figure 14: A small survey of 150 Nandolo seeds indicating the distribution of seed hull colour.

The significantly larger total antioxidant capacity (TAC) of pigeon pea, when compared to the chickpea samples which do not have any dark colouration, alludes to the link between hull colour and TAC. This capacity was put to the test through a DNA protection assay, to

indicate whether the extract could protect DNA against ROS species. Pigeon pea hull had the largest capacity for protecting DNA, by having the highest TAC measurements, and by protecting DNA even at very low concentrations. Other pea parts: C and CH was also able to protect DNA, at low concentrations, but not as low as the H (exclusively). Other studies on legumes also found the that the H has a larger TAC than the CH and C and their TAC were correlated to the amount of SMs found in the pea components (Boudjou et al., 2012; Attree et al., 2014; Singh et al., 2017). Antioxidants are generally divided up into two groups: enzymatic and non-enzymatic antioxidants. The high TAC in the hull could be attributed to theSMs tentatively identified in the hull, acting as non-enzymatic antioxidants. In a non-representative study it was observed that the H only contributes to 12% of the CH (**Fig.15**) and the hence the SMs, contribute by H, will be less in the CH than in the H. This reduction in SM concentration could have led to the reduced TAC observed in the CH. The CH does indicate a higher TAC than C showing that the SMs contributed by the H are not neutralised in the CH state. Pigeon pea hull's TAC level and the ability to protect DNA at low concentrations warrants further research into product development of the hull. The hull of the pigeon pea is currently seen as a by-product, but the significant TAC could motivate studies into SM identification, product development and isolation of antioxidant species from the hull. In terms of food production, the TAC could motivate producers to either not de-hull the seed or valorize the seed for the extraction of potential therapeutics. Being able to utilise a product that is currently being used for animal feed could add value to the crop (Karri, Nalluri, 2017).

Figure 15: A small survey of 150 Nandolo seeds indicating the contribution of full seed (CH) weight. Where the full circle represents the weight of the CH (100%), the green indicates the weight of the cotyledon (C; 88%) and the grey indicates the weight of the hull (H; 12%).

Studies have shown that consumers are willing to pay more for food if the label mentions antioxidants, compared to their counterparts, but there is an aversion towards products enriched with antioxidants (Markosyan et al., 2009; Marette et al., 2021). Pigeon pea seeds,

specifically the H, have the benefit of naturally being rich in antioxidants, making it a more desirable product. The combination of TAC and healthrelated SMs makes pigeon peas a health crop with potential market value. Other factors that affect the health, aside from medicinal properties, are the primary metabolite composition. There have been numerous studies on the protein content of pigeon peas, but few that looked at the starch, specifically resistant starch content, which directly affects the GMB.

There is a lack of research with regard to the quantification of starch content for different pigeon pea varieties, which could be attributed to its orphan crop status and the lack of registered cultivars. When comparing the Nandolo variety with similar studies, investigating the starch content of whole ground dry seeds, the starch content was comparable to reported values (ranging from 19.85g – 28g / 100g; Narina et al., 2012; Narina et al., 2013). The Nandolo seed indicated an average starch content, when compared to other pigeon peas, and the largest amount of starch was found in the cotyledon of the seed, as verified by the Lugol stain (**Fig. 11**). When compared to the chickpea sample, the pigeon pea had a higher starch content, but when comparing it is important to compare the same type of starch.

Starch can be broken up into categories based on their digestion rate, with the extremes being resistant and soluble starch. Soluble starch is readily absorbed in the bloodstream, where resistant starch is mostly fermented in the GMB. Pigeon peas not only tested higher for total starch, but also for the concentration of resistant starch, when compared to chickpeas, which has previously been demonstrated (Sandhu at al., 2007). The amount of resistant starch contributes to a lower (44.2) GI, compared to chickpeas (49.8), which contributes to positive health outcomes (Sandhu, et al., 2007; Dávila et al., 2018; Viguiliouk et al., 2018; Brand-Miller et al., 2020). Low-GI diets have been robustly associated with the reduction in incidence of type-2 diabetes and pre-diabetes (Brand-Miller et al., 2020). Type-2 diabetes in turn has been identified as a major source of mortality in South Africa, impelled by unhealthy lifestyles, which the GI Foundation of South Africa aims to improve through supporting the labelling of food , with a clear indication of the GI status of the product (Mshunqane et al., 2012; Pheiffer et al., 2018; Barclay et al., 2021). With the South African consumer market, predisposed to diabetes type-2, it is important to develop and promote products that is of low cost and has a low GI.

Pigeon peas have the potential to play a vital role in being a crop that has proven antioxidant properties, potential pharmaceutical benefits, energy rich, has a low GI and feeds the GMB

(**Fig. 16**). The tentatively identified SMs for the pigeon pea hulls indicated potential pharmaceutical importance and is good motivation to either include or repurpose the hull is food products. Luteolin and quercetin, known pigeon pea SMs identified in the Nandolo sample, have verified antioxidant activity and motivated the further research of the variety. Such foundational knowledge of Nandolo variety motivates further research into the single variety. However, this is notwithstanding the important properties of other varieties, and such can be investigated as part of the larger research program.

The H of the Nandolo sample measured a high TAC, when compared to chickpea and the ability to protect DNA at low concentrations. Furthermore, the large fraction of resistant starch measured in pigeon pea indicates application to the GMB and low GI, both of which is linked to various diseases that can be prevented or ameliorated through diet. Future work could include chemical analysis of the SMs to verify identification and the subsequent health effect they have. Pigeon peas have clear potential for human health but is unknown to many consumers outside of the main growing areas (India, Malawi). Efforts should thus not only be directed towards metabolite identification and application, but also product development. Combined, such efforts to understand and introduce nutrient-rich orphan crops, and derived products, could play a role in mitigating the negative effects imparted by malnutrition (stunting, wasting, micronutrient deficiencies and obesity) as set out by the UN's #2 sustainable development goal.

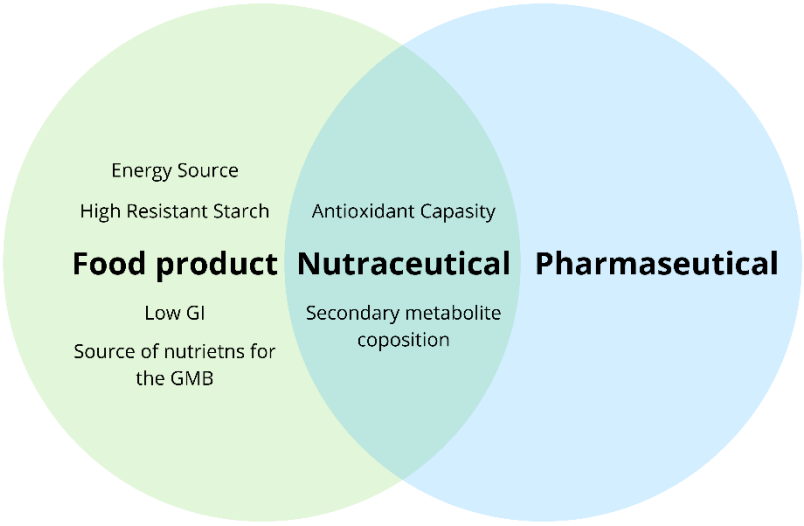


Figure 16: Indicating the pigeon pea potential covered in this study. The green circle indicates its potential as a food product and the blue indicates its potential as a pharmaceutical crop.

# Chapter 3

# Comparative Sensory Analysis

## 3.1. Introduction

Orphan crops are generally grown, and consumed, in specific climatic regions, with the result being that only people form that area are familiar with it. Any attempt at commercialising orphan crops, not only needs to go hand in hand with breeding and improvement, but also education and product development. With a surge in demand for functional food (a food product containing bio-active compounds, with documented health benefits), advances in biotechnology that can verify functionality have led to the increase of functional food product development (Martirosyan, Singh, 2015; Guiné et al., 2020). Product development often relies on established food products, or improvement thereof. The basic step of developing a new product involves; consultation, a concept, formula, technical development, evaluation, quality testing and market response (Stewart-Knox et al., 2003). Recent product developments of note include the development of the plant-based KFC menu and the plant-based Burger king whopper ® (Rabb, 2021). With the progression of globalisation, consumers are exposed to and demand a wider variety of food options (Yang, Lee, 2019). A food product, that is being consumed in many forms (cooked, dry-mix, frozen, fast food) and is traditionally made with peas (chickpea or fava beans) is the falafel.

The falafel (**Addendum A, Fig. 31 A)** is a traditionally a Middle Eastern dish, where it is a very popular fast food (Al-Asmara et al., 2019). The falafel has since been introduced in South Africa in various formats ranging from locally produced pre-mixes (Outcast Falafel), pre-made frozen falafels (Fry's, Woolworths, Simple Truth, Lexi's) and various restaurant options (Kauai, Ocean basket, Knead, Annat, Mugg&Bean, Bootlegger, Lexi's). Falafels are characterized by a crisp exterior and light, airy interior and the flavour is mainly derived from the additional ingredients, since the pulse base, chickpeas or fava beans, do not have prominent flavours of their own (Jaron, 2021). Additions that give the falafel its distinct flavour includes onion, parsley, coriander, cayenne pepper, cumin seeds, coriander seeds and cardamom seeds (Ottolenghi Tamimi, 2012).

Two strategies, consumer panels and descriptive sensory analysis (DSA), are available for product development, focusing on technical development and product evaluation. DSA employes trained assessors and are used to evaluate sensory quality, whereas a consumer panel determines preference or acceptability on a hedonic scale. One method of sensory evaluation is descriptive sensory analysis (DSA). DSA enables researchers measre attributes

both qualitatively, by identifying attributes, and quantitatively by scoring attributes (Lawless & Heyann, 2010). Sensory science allows researchers to interpret product properties, not limited to consumer preference (Fiorentini et al., 2020). In the decision to purchase a plant-based alternative food product, consumers are influenced by many factors including ethics and politics, but the main deciding factor is the sensory attributes of the product (Hoek et al., 2011). This makes sensory evaluation an important part of product development, if standards for consumer preference are available, it can also be used to predict consumer acceptability.

Another tool that can be used to evaluate the product is texture profile analysis (TPA). Through double compression, TPA aims to imitate a jaw motion where a texturometer is used to calculate characteristics like gumminess, chewiness, hardness, cohesiveness, and springiness (Trinh, 2012; **Addendum A, Table 15**). These are objectively measurable factors that can be done in a short amount of time, when compared to DSA, making it an efficient tool. TPA can also be included, along with DSA ormulae optimization, and was shown to be of significance in falafels (Janhager, 2020). Generally, sensory quality is first determined, thereafter a consumer panel is employed to assess the liking of the product, whereafter the two datasets can be used to statistically link attributes that influence consumer acceptability. Falafels are not a well-research product, with limited peer-revied publications on the consumer preferences, indicating the importance of sensory testing before product release.

Throughout the study, pigeon pea was been compared to chickpea, a well-established legume, and the main ingredient of the falafel. In this section the sensory and texture attributes are compared with the aim to inform future product developers on how these pulse flours compare.

## 3.2. Materials And Methods

### 3.2.1. Sample Preparation

The aroma profile of a traditional falafel was recreated using a traditional recipe (Ottolenghi, Tamimi., 2012). Commercially available, pre-milled and microbiologically tested chickpea flour (Pick n Pay house brand, Pick n Pay, South Africa) was used as control throughout the falafel product- and sensory design. Commercially available pigeon peas (Cape Spice Emporium, Claremont, Cape Town, South Africa) were milled (200g, Nutribullet 600W Personal Blender, 17 s) and passed through a household sieve to create a grainy flour. The

pigeon pea flour was further tested by an external laboratory (Microchem Lab Services, Cape Town) for any colony forming species. Tests that were performed included total plate count, *Enterobacteriaceae* Count (Presumptive), *Coliforms* Count, *Escherichia* coli Count, *Staphylococcus aureus* Count, *Pseudomonas* species Count, Lactic Acid Bacteria Count, Yeast Count, Mould Count, *Salmonella* species Detection and *Bacillus cereus* Count (presumptive). All samples were quality assured and, deemed safe to continue. To establish a base line, one recipe was developed where all ingredients and quantaties stayed constant for the two treatments (pigeon pea and chickpea flour). The dry spices (**Table 10**) were blended (Nutribullet 600W Personal Blender, 10 s), per replicate, to ensure a homogenous distribution and added to the pea flour (175 g). These dry mixes were stored (4°C) in resealable plastic bags until use.

### 3.2.2. Physical measurement

Texture profile analysis (TPA) of the cooked falafels (two treatments) were done using an UTM (Instron force transductor, model: 2519-107, Advanced Lab Solution, Johannesburg). Falafels were stored (4◦C, 24 h) prior to TPA. All TPA was executed at room temperature (21°C). Falafel (1-3) from each replicate (**Addendum A, Table 16**) and Bluehill® software (version 3. 65.3916) was used to record the data. Falafels were compressed with a flat cylindrical probe (20 mm) on a double bite setting (**Fig. 18**) and the cut of force (0.02 N) and test speed (2 mm/s) was set accordingly.

Figure 18: QR code for a slow-motion video (4:07) of the UTM machine taking a double compression measurement. The UTM measured; Max. Force 1st Cycle (F1) [N], Energy to Max Load 1st Cycle (A1) [J], Max Force 2nd Cycle (F2) [N] and Energy to Max Load 2nd Cycle (A2) [J].

Measurements that were taken using this method included Max. Force 1st Cycle (N), Energy to Max Load 1st Cycle (J), Max Force 2nd Cycle (N) and Energy to Max Load 2nd Cycle (J)

From the raw data the Hardness (N), Cohesiveness, Springiness (mm), Gumminess and Chewiness was calculated (**Addendum A, Table 16**, **Equation 1**).

### 3.2.3. Descriptive Sensory Analysis (DSA)

This part of the study obtained necessary ethical approval from the Research Ethics Committee: Social, Behavioral and Education Research, Stellenbosch University (Project number: 22484). Due to a lack of research with regard to sensory attributes related to falafels, attributes to be tested for was determined anecdotally. The attributes were divided into three categories based on how they are measured: flavour and taste, texture and mouthfeel, and aroma attributes. These attributes were further specialised (**Addendum A, Table 14**) and evaluated using a trained panel.

Descriptive Sensory Analysis (DSA) was conducted to determine whether there is a significant difference in aroma, taste, texture, and mouthfeel between falafels made from pigeon pea flour (FP) and chickpea flour (FC). For each treatment (pigeon pea or chickpea flour) nine random replicates and two batches per replicate was made (**Table 10; Table 11**). Boiling water (200g) was added to the dry pre-mixes (stored at 4 °C) prior (90 min) to sensory analysis. The pre-mixes were left at ambient temperature, uncovered (30 min) whereafter it was shaped into balls (34 mm diameter, using meatball tongs manufactured by Ibili). The inside of the tongs was sprayed with canola oil (Woolworths, South Africa) before scooping the falafel balls. The balls were air fried (Philips XL Rapid Air Tech Essential Airfryer, 10 min, 180 °C) and served in a glass ramakin (covered with a petri dish). The Falafels were kept warm before serving by placing it in a mug (half filled with water) in a water bath (60 °C). A trained descriptive panel (10 individuals, 1 male, 9 females), was trained over three days (six interactive sessions; **Table 12**) to familiarize them with the treatments and to identify and score the aroma, flavour, texture, and mouthfeel characteristics associated with the product.

The panellists started off with evaluating one (batch 1) for both treatments to familiarize themselves with the product before calibrating the assessors to conduct the sensory analysis with the reference standards. Reference standards (**Table 13**) were used to assist panellists in calibrating their sensory perceptions and further training with the product, gave them a chance to score all the characteristics tested in the two treatments. The questionnaire (**Table**

**14**) was refined in terms of attributes and definitions and tested during the training phase. An unstructured line scale ranging from 0 – 100 was used to the attributes , but attribute-specific ranges were noted as guidance during the testing phase. These ranges were established in the training phase and is subjective to this study since the scale for each attribute was determined through consensus by the panellists.

During the testing phase panellists sat in individual booths fitted with Compusense® software (Compusense®, Guelph, ON, Canada) with temperature (20 °C) and light control (equivalent to daylight). The characteristics were rated on a 100-point line scale line with indicated parameters established in the training sessions. An individual sample (one whole falafel, 34 mm) of each treatment was served to the panellists in a randomized order in nine replicate testing sessions (Table 3). Each sample was coded with a three-digit blinding code to equalize the serving order. Palete cleansers (Pink Lady apple slices, 1% pectin solution, mineral water) was served to reduce carryover form one sample to the other.

### 3.2.4. Statistical Analysis Of The Sensory Data

The project was designed in a factorial array (1 by 2 factorial design, one cooking method, two treatments). Firstly, the panel reliability was tested using a model that includes panellist, sample, and replicate effects. Subsequently the normality of the standardized residuals for the model were evaluated using the Shapiro-Wilk test. Outliers were then removed if the standardized residual of an observation deviated more than three standard deviations from the model value. After confirming panel reliability and normality, statistical analyses on the DSA data were conducted on means over judges. DSA and TPA data were subjected to ANOVA according to the (completely random) experimental design to determine treatment differences. Fisher's LSD (5%) was used to compare treatment means. A probability level of 5% was considered significant. Univariate analyses were performed using SAS software (Version 9.4, SAS Institute Inc, Cary, USA). Principal component analysis (PCA), employing the correlation matrix, was performed using XLStat (Version 2016, Addinsoft; New York, USA) to determine the association of DSA and TAC variables and the falafel samples. When analysing the data, the sensory attributes evaluated for was divided into three categories based on how they are measured; flavour and taste related attributes, texture and mouthfeel attributes, and aroma attributes.

## 3.3. Results

### 3.3.1. Texture Profile Analysis Indicates Significant Differences

To assess the texture profile of a cooked falafel after 24h, the FP and FC (N = 22) were tested for factors such as hardness, cohesiveness, springiness (mm), gumminess and chewiness (F**ig. 18**). There was a statistically significant ($p < 0.0001$) difference seen between pigeon pea-based falafels and chickpea-based falafels for all the texture attributes.

The Hardness of the FC (37.22 ± SEM 2.71) was larger than that of FP (12.23 ± SEM 0.78) indicating a larger force (N) needed to break through the falafel on first bite. The cohesiveness, the area of the FC (1.50 ± SEM 0.09) was larger than that of the FP (2.32 ± SEM 0.16) indicating that the FP crumbles more easily. The springiness, determined by the ratio of the height of the falafel during second compression and fist compression of the FC (6.49 ± SEM 0.17) was larger than that of the FP (3.1 ± SEM 0.2). The gumminess is determined by the product of the hardness and the cohesiveness and was larger for the FC (53.64 ± SEM 3.69) than the FP (28.23 ± SEM 2.48). The chewiness, which is the product of the gumminess and springiness, of the FC (354.82 ± SEM 30.3) was larger than that of the FP (87.28 ± SEM 9.37).

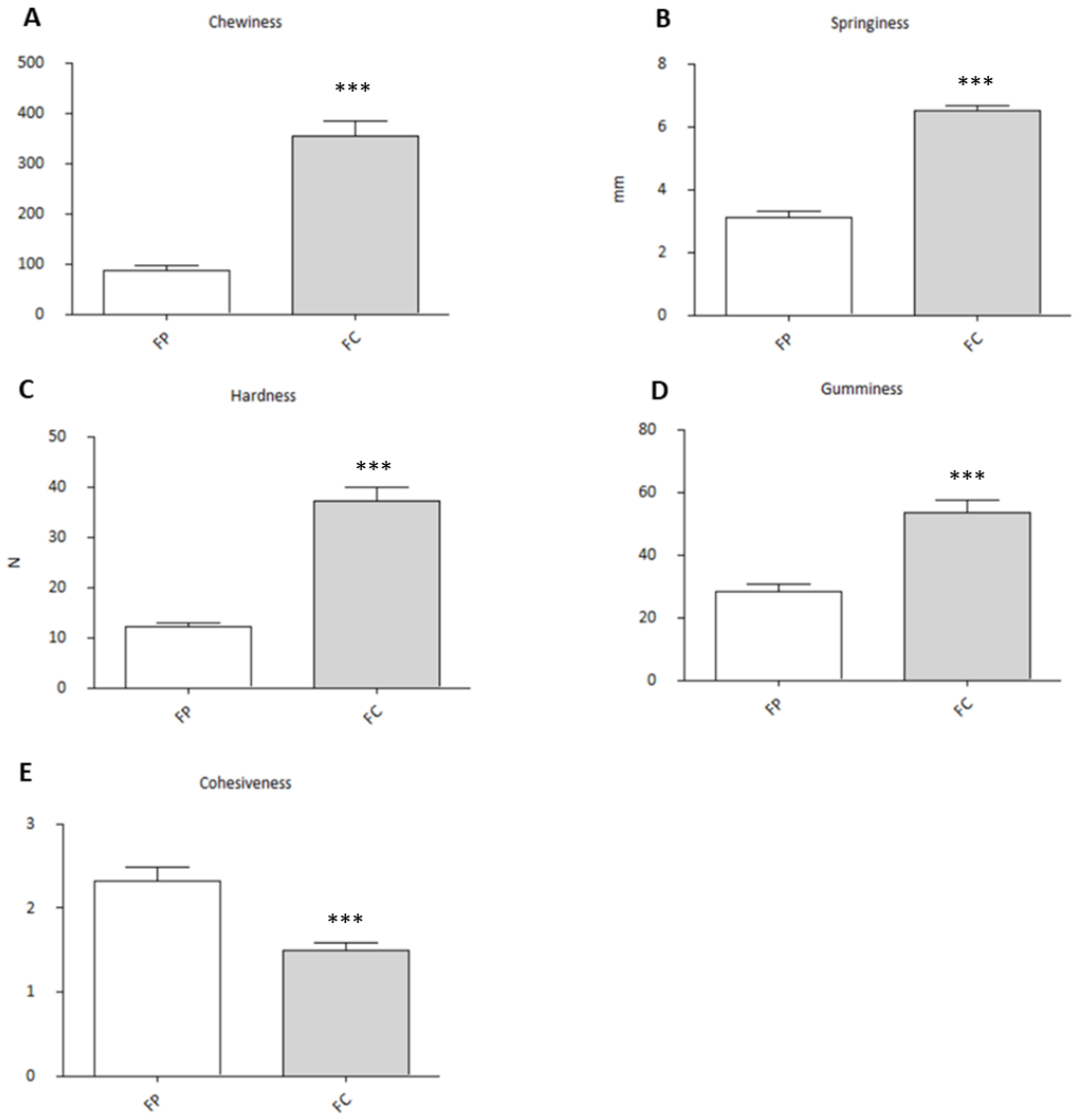


Figure 18: The texture profile compared between falafels made from pigeon pea (FP) flour and chickpea flour (CF). The attributes were simultaneously measured, with 22 independent falafels from 9 individual batches. Statistical significance is indicated by stars as determined by a two tailed t-test, using chickpea as the comparison control. A statistically significant difference (***p < .0001) was observed for all of the attributes. (A) Chewiness, (B) Springiness, (C) Hardness, (D) Gumminess, (E) Cohesiveness.

PCA is used to assess multivariate data where data sets with multiple variables and dimensions are transformed into a graphical representation with two principal componenets (PC) , with the aim of capturing most of the variation observed in the data. The linear vector factor 1 (PC1), indicates the maximum variation observed in the data and is used. The factor 2 (P2), which is perpendicular to P1. The influence of the variables (measured through DSA or TPA) on the data points are indicated on the PCA plot with each variable forming an eigenvector. The eigenvectors have both magnitude and direction. The magnitude of the

vector indicates the influence the variable has on the variation observed in the data. The direction indicates the relationship between variables. A variable with a large eigenvalue would indicate that a lot of the variance observed in the PCA graph can be attributed to that variable.

The PCA bi-plot (**Fig 19**) indicates the relationship between falafel flour-type and textural attributes observed. On the right of PC1 hardness, springiness and chewiness associate with FC and on the left the attribute cohesiveness associates with FP. Gumminess is mutually exclusive with chewiness (**Addendum A, Equation 1**) where the gumminess measurement is used when evaluating semi-solid products (Texture Profile Analysis, 2021). Gumminess was hence omitted from further statistical analysis. There is strong positive correlation between hardness and springiness (r=…; $p < 0.05$), and a negative correlation between cohesiveness and hardness (r = XX; $p < 0.05$)

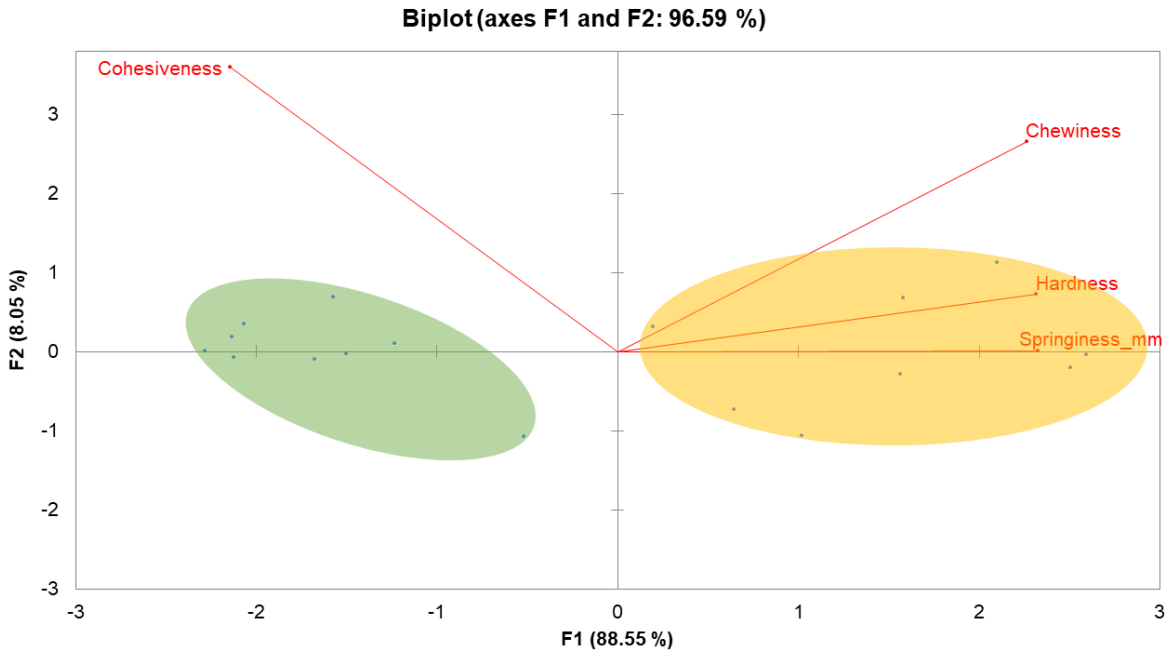


Figure 19: PCA bi-plot of physicochemical analyses (cohesiveness, chewiness, hardness, springiness) in red, and falafels coloured depending on type of flour used; FP (green) and FC (yellow). Factor 1 (PC1) explained 88.55% of the variation and Factor 2 explained 8.05% of the variation observed in the model, thus a total of 96.59% of the variation could be explained by the first two components.

### 3.3.2. Descriptive Sensory Analysis Shows Significant Difference Between Pigeon Pea And Chickpea-Based Falafel

The PCA bi-plot demonstrated how the flour the falafel was made with, and the sensory attributes that waere tested, related to each other (**Fig. 20**). The first factor is equally influenced by all of the variables, ranging in absolute eigenvalues from 0.19 - 0.21. The direction of the eigenvector, whether it is positive or negative, indicates its relationship to sensory attributes were subsequently split into; aroma, flavour and taste, texture and mouthfeel attributes.

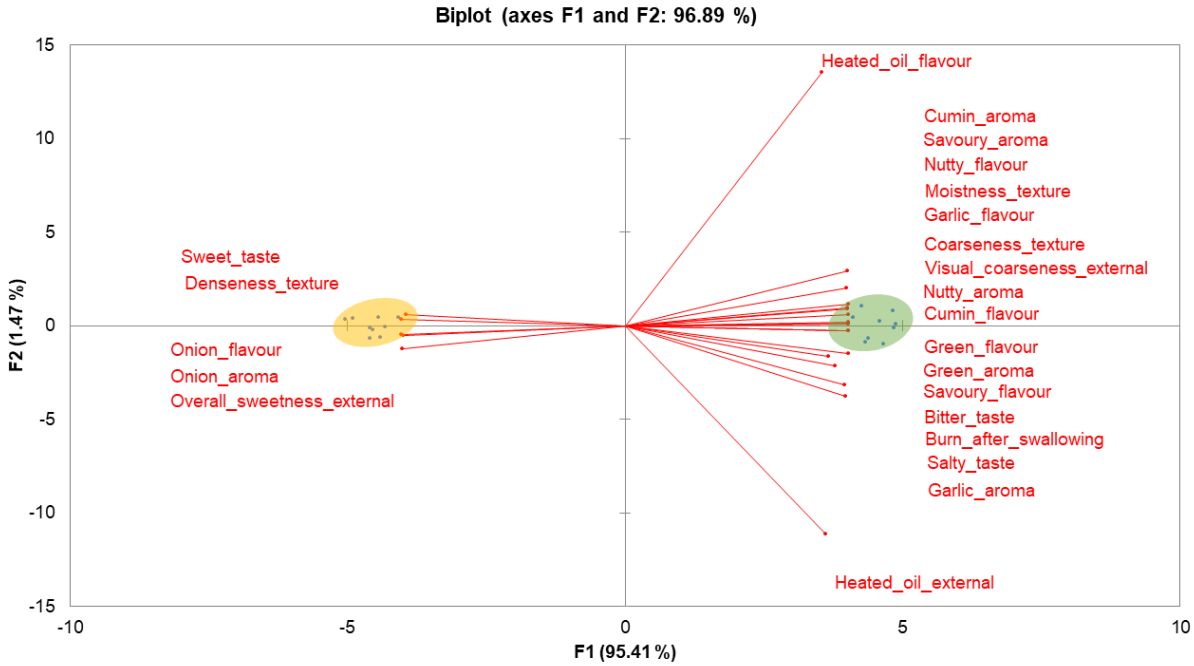


Figure 20: PCA bi-plot of the sensory attributes that was tested for in red, and falafels coloured depending on type of legume flour used; FP (green) and FC (yellow). Factor 1 (PC1) explained 95.4 % of the variation and Factor 2 (PC 2) explained 1.47 % of the variation observed in the model, thus a total of 96.89% of the variation could be explained by the first two factors.

### 3.3.2.1 Aroma Attributes

The PCA bi-plot for aroma attributes indicated how they are related to each other and how it differed between pea flours (**Fig 21**).

On the left side of PC1 an onion- and overall sweet aroma associates with FC, whereas a green, nutty, cumin, savoury, garlic and heated oil aroma is associated with FP. There is a strong positive correlation between the overall sweet aroma and the onion aroma ( r = 0.99 ; p <0.05). a strong negative correlation is observed between the onion aroma and the nutty aroma (r = - 0.992; p<0.05).

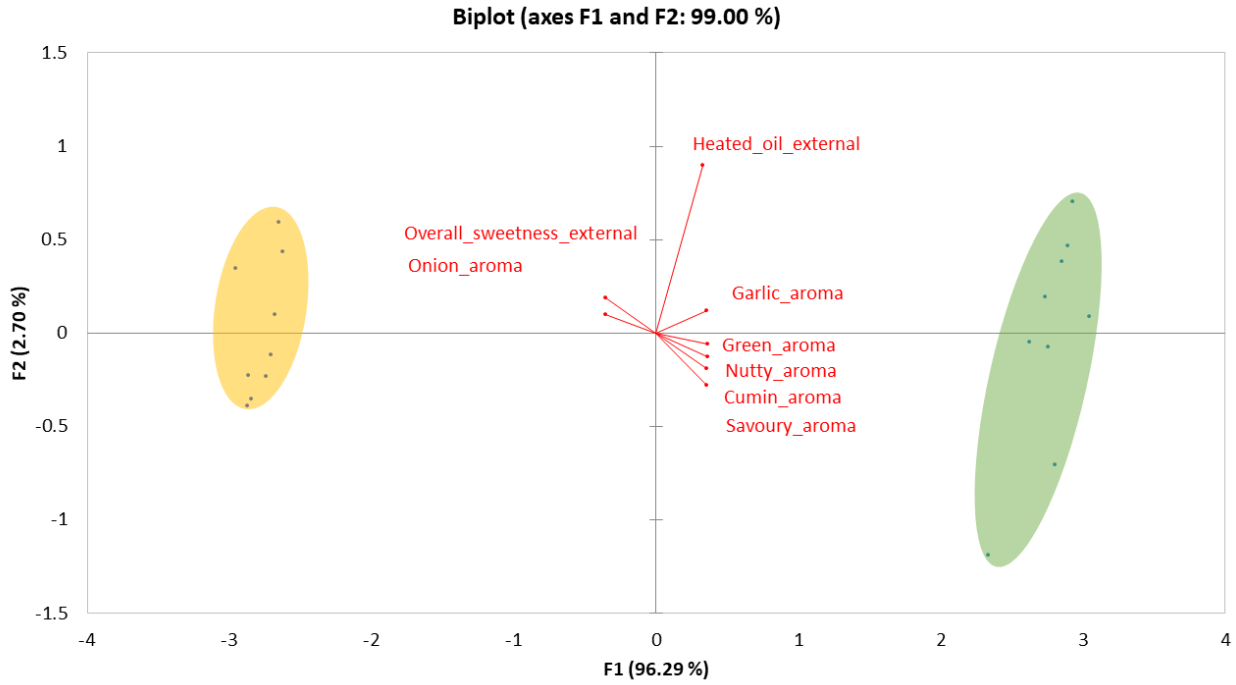


Figure 21: PCA bi-plot of the aroma attributes that was tested for (red) depending on type of legume flour used; FP (green) and FC (yellow). Factor 1 (PC1) explained 96.29 % of the variation and Factor 2 (PC 2) explained 2.7 % of the variation observed in the model, thus a total of 99 % of the variation could be explained by the first two components.

### 3.3.2.2 Flavour And Taste Attributes

The PCA bi-plot for flavour attributes indicated how they are related to each other and how it differed between pea flours (**Fig 22**). On the left side of PC1 an onion- and sweet taste associates with FC, whereas to the right of PC1, a green, nutty, cumin, savoury, garlic and flavour is associated with FP. There is a strong positive correlation between the sweet taste

and the onion aroma ( r = 0.95 ; p < 0.05) a strong negative correlation is observed between the onion aroma and the green flavour (r= - 0.992 ; p < 0.05).

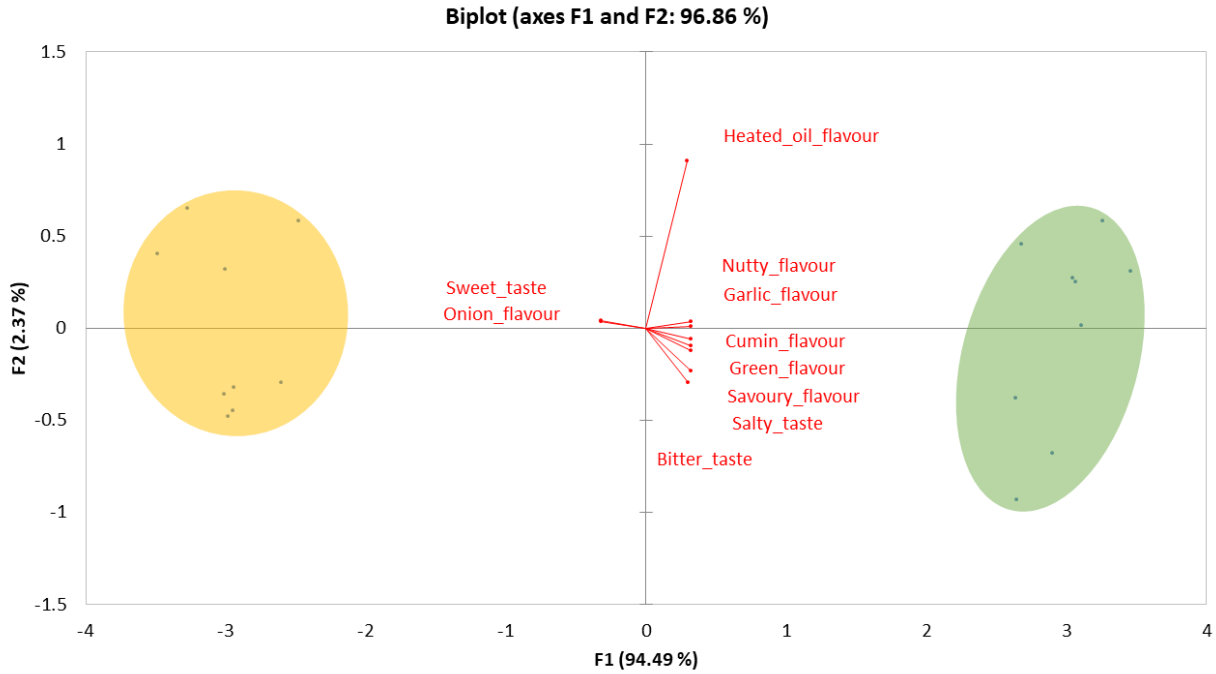


Figure 22: PCA of the flavour and taste attributes that was tested for (red) depending on type of legume flour used; FP (green) and FC (yellow). Factor 1 (F1) explained 94.49% of the variation and Factor 2 explained 2.37% of the variation observed in the model, thus a total of 96.86% of the variation could be explained by the first two components.

### 3.3.2.3 Texture and mouth-feel attributes

The PCA bi-plot for texture and mouthfeel attributes indicated how they are related to each other and how it differed between pea flours (**Fig 23**). On the right side of PC 1 a dense texture is associated with FC, whereas to the left, a moist, course, burn taste is associated with FP. There is a strong negative correlation between the denseness and ( r = -0.998 ; p < 0.05) a

strong positve correlation is observed between the coarseness and moistness (r= 0.996 ; p < 0.05).

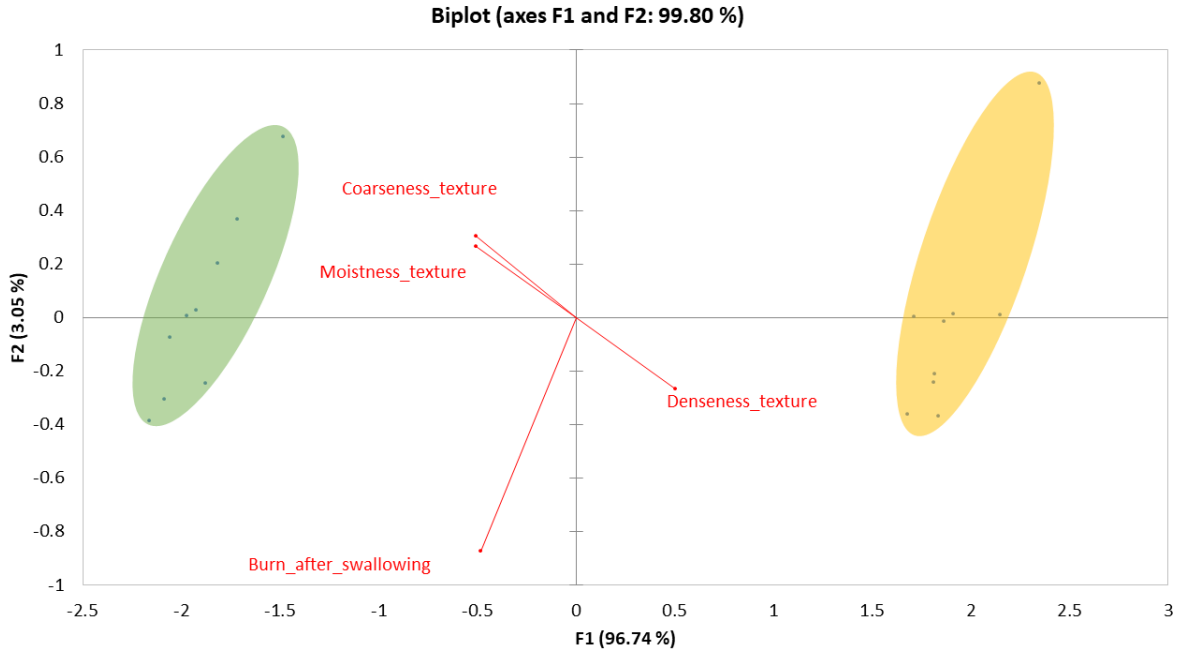


Figure 23: PCA of the texture and mouthfeel attributes that was tested for (red) depending on type of legume flour used; FP (green) and FC (yellow). Factor 1 (F1) explained 96.74 % of the variation and Factor 2 explained 3.05 % of the variation observed in the model, thus a total of 99.8% of the variation could be explained by the first two components.

### 3.4. Discussion

There has been an increased demand for more sustainable, healthier food options, with an emphasis on making it affordable, thus more accessible (Borelli et al., 2020; Cusworth et al., 2021). Combined with the consumer preference for healthier food options there has also been a drive towards plant-based protein options (Tuso et al., 2013; Graçaa et al., 2019; Aschemann-Witzel et al., 2021; Pohlmann, 2021). Concurrent with consumer pressure for more nutritious products is the need to diversify cropping systems, and orphan crops are a suitable way to achieve this (Modi, Mabhaudhi, 2016; Mabhaudhi et al., 2017; Tadele, 2019; Mabhaudh et al., 2019; Succurro et al., 2019). Using orphan crops comes with challenges as it is unknown to many consumers and there is little to no product development related to it. The introduction of new or alternative food crops and related products requires careful consideration of the movement from production to consumption. Such considerations include,

among others, (i) establishing evidence on the benefits of the crop, (ii) linking research to policy and markets to increase the availability of the crop and (iii) to raise awareness regarding its nutrition (Borelli et al., 2020). Raising awareness about the benefits of a crop however needs to go hand in hand with product options to introduce the food crop to the consumer. And one way to do this is to link it to product that is already known to the consumer (Linneman et al., 2005). A product that is already established in the South African market is the falafel, this traditional middle eastern dish is traditionally made with chickpeas or fava bean, leading us to consider the use of another pea for replacing the base ingredient.

Chapter 2 of the study focused on characterizing the potential health benefits of the crop. However, successful integration of pigeon peas into a commercial market could benefit from a focus on product development. Since the product (the falafel) is well established in the South African market, this chapter aimed to develop a pigeon pea-based falafel and characterize its sensory attributes through descriptive sensory analysis (DSA). It was important to establish the differences in sensory attributes when switching between peas, as a base for the falafel, for recipe optimization and future product development.

Although the falafel recipe design was based on traditional ingredients, traditional fresh ingredients were purposely left out to enable the development of a ready to use, dry-mix product (**Addendum A, Table 10**), with the end user/consumer in mind. Cooking options for the falafel traditionally includes frying in oil and sometimes baking. More recent advances in cooking technologies include air-frying, a food preparation technique where hot air is circulated in a contained space (ranging from 2 – 10L capacity). Interestingly, recent investigations toward falafel development produced a scientific output on air-frying conditions for falafels (Fikry et al., 2021). Our descriptive sensory analyses relied on air frying as the cooking method, for its (i) uniformity in cooking and health benefits compared to frying, (ii) time efficiency compared to baking and (iii) even cooking abilities to create homogenous sensory profiles (Fikry et al., 2021).

Aroma, flavour, and taste are all perceived differently by the human sensory system (**Fig. 26**). Both aroma and flavour is perceived through the nose, but aroma is measured through the orthonal and flavour the retronasal. Aroma is perceived through the nose whereas flavour and taste are evaluated after the first bite, using taste buds on the tongue (Redondo et al., 2014). The texture and mouthfeel were also evaluated after biting into the falafel and is based on the oral sensations of the product in the mouth.

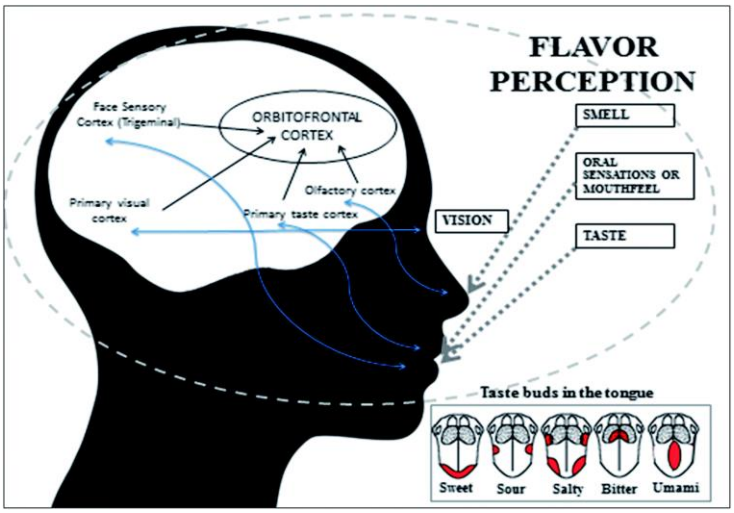


Figure 24: Illustration indicating how varying sensory attributes are perceived by the consumer. The different measurement techniques are indicated with dotted lines, differentiating between smell, oral sensation or mouthfeel and taste (Redondo, et al., 2014).

The sensory attributes (**Addendum A, Table 14, 17**) were significantly influenced by the falafel flour for all of the sensory groupings. In terms of aroma and flavour, the FP indicated had a more intense attributeaside from the sweet and onion associated attributes. For texture, the FP had a coarser and less dense texture, or mouthfeel, which could be attributed to the grain texture of the flour that was used. The clear distinction between the two treatments indicates that pigeon pea flour cannot be used to replace chickpea flour in products without making flavour and aroma adjustments. This also highlights the need for sensory profiling of the pigeon pea for future product development ventures. A known sensory profile for pigeon peas will allow product developers to incorporate pigeon peas into existing and new products while being able to create a flavour profile that is acceptable to consumers.

Strongly associated aromas, such as the savoury-associated attributes (green, nutty, cumin, savoury, garlic, and heated oil aroma), could overpower each other and should be considered as an adjustment factor during product development. The savoury-associated aromas could either have been masked by the chickpea flour or the pigeon pea flour naturally contains some of these aromas. Because of the correlation and possible influence between the savoury-associated aroma's a higher measurement of one could influence the associated - indicating the need for further research mapping the aroma profile inherent to pigeon pea flour. Since

not all the attributes, e.g., onion aroma, were more pronounced in the FP, it diminishes the possible effect of granule size on the aroma carrying capacity of the falafels.

Like aroma, there was a strong, positive correlation between savoury-associated flavours (nutty, garlic, cumin, green, savoury) with the addition of salty and bitter taste. Onion flavour and sweet taste was however more pronounced in the FC – as observed with the aromas. The savoury-associated flavours were accentuated in the FP which could indicate that chickpea flour masks these flavours or that pigeon pea flour intrinsically has some of these flavours. Attributes less desirable to consumers like; bitterness and green flavour, have previously been linked to peas (Roland et al., 2017). These less desirable attributes have been linked to the presence of saponins, phenolic compounds and alkaloids, all of which were tentatively identified in the pigeon pea hull (**Chapter 2**). In product development, the less desirable flavour attributes should either be minimized or masked. These flavour attributes could be avoided by excluding the hull from the product, but that would also mean excluding a part of the pigeon pea with medicinal properties. Different flavours are all perceived simultaneously in the mouth, some flavours affect each other, either by reducing, enhancing, or balancing the perception thereof (Coucquyt, et al., 2020). When adjusting flavours of a product one of these three routes can be followed to alter the flavour profile. A way to manage bitterness in a product is either to balance it with a sour flavour or reduce its prevalence with either sweet or salty flavours (**Fig. 27**). The higher onion and sweet taste mimic the linked aromas attributes, in that it is more pronounced in the FC. When creating pigeon pea-based products adjacent to chickpea product the influence of sweetness should be considered. Sweetness reduces pungent, salty, sour, and bitter flavours (**Fig. 27**). This could confirm why there was a large discrepancy between treatments in terms of aroma and flavour. If chickpea flour naturally has more sweet-associated attributes, than pigeon pea flour, it would reduce the flavour intensity of the savoury-associated attributes hence polarising how the treatments are perceived.

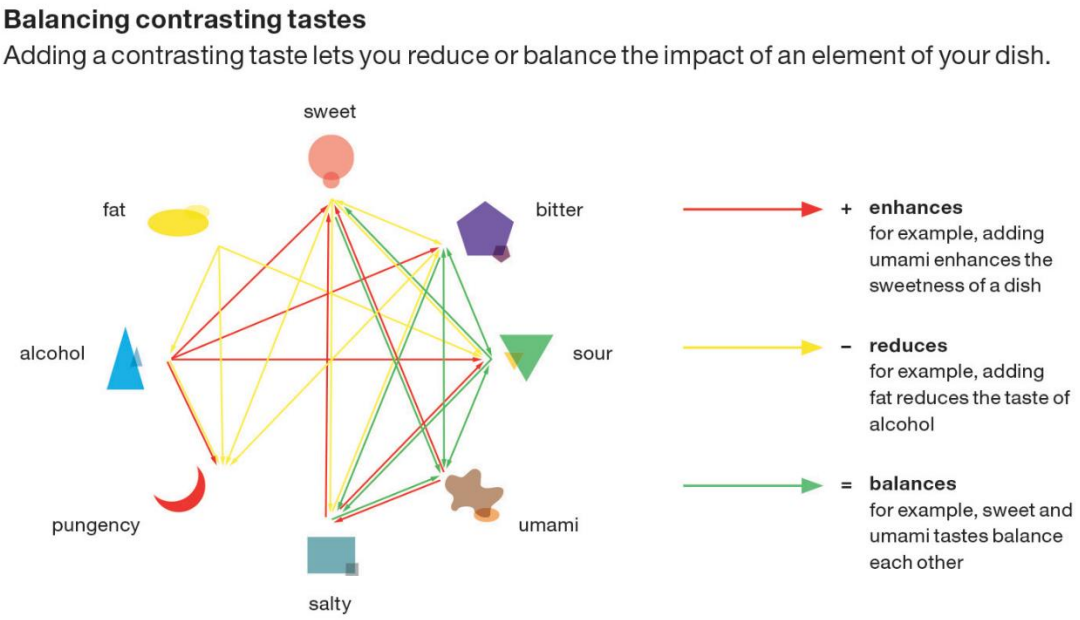


Figure 25: A basic illustration of how basic tastes can be manipulated (Coucquyt, et al., 2020). The yellow arrows indicate what flavours reduce each other, the red enhance and the green balance. The direction in which the arrow is pointing indicates which other flavour it effects, with not all interactions being bi-directional.

The differences observed in texture (**Fig. 19, Fig. 24**) can largely be ascribed to the effect of granule size of the flour. A smaller granule size has increased amounts of damaged starch and subsequently reduced water holding capacity (Thirunathan et al., 2020; Pang et al., 2021). A strong positive correlation was observed between course and moist texture attributes confirming other pulse flour studies on the relationship between particle size and water holding capacity (Thirunathan et al., 2020). The burn-after-swallowing attribute was grouped together with the texture attributes, because it relates more to mouthfeel than it does flavour and showed a higher prevalence in the FP. Capsaicin, the compound that causes a, burn-after-swallow mouthfeel, has never been identified in the pigeon pea, implicating it is not inherently a pigeon pea metabolite and that the increased burn-after-swallow effect could be related to the sweet taste of the chickpea (Smutzer et al., 2018).

It can be deduced that the particle size and thus water holding capacity of the dough influences the hardness of the product inversely and the cohesiveness directly, as previously observed in falafels (Janhager, 2020). One study showed that water uptake and hardness influence the consumer acceptability of falafels and should thus be considered in further falafel optimisation with pigeon pea flour (Janhager, 2020).

Pigeon pea flour cannot be handled exactly like chickpea flour, with differences in all sensory and texture attributes, further research should include creating a lexicon specifically for pigeon pea flour. The increased perception of savoury-associated attributes in the FP could either be attributed to the flour itself or interactions between the flour and added ingredients. Despite the absence of a consumer panel, this DSA study can assist recipe designers/product with regard to the incorporation of pigeon pea flour in products as a viable pulse flour alternative. This comparative study indicates how the flavour and aroma of pigeon pea flour reacts to the addition of certain ingredients, indicating how future product developers could adjust a recipe towards a certain flavour profile and how pigeon pea flour compares as a replacement for chickpea flour. Having known sensory attributes, the flour can be incorporated in recipes with predictable product outcomes.

Despite the differences in flour granule size (finer chickpea flour and coarser pigeon pea flour) our TPA and DSA analyses indicated that pigeon pea flour could be used to make falafels, with flavour adjustments when compared to chickpea-based recipes. The DSA and TPA results indicated a higher cohesiveness and moistness associated with more coarsely ground flour. This indicates that home-ground pigeon pea flour can be used successfully to create a falafel product. The success of the study also lies in the development of a dry mix with sensory attributes comparable to industry standards, with DSA attributes values similar to that of commercially available products (**Addendum A, Table 13**). Further analysis could look at the holistic nutritional properties, of the dry mix, for informed product development purposes.

Future work on this project, and analyses of newly developed products from orphan crops, can benefit from consumer panel results. Consumer panels are generally a good prediction of consumer acceptance of the product in the market (Stewart-Knox, Mitchell, 2003). By correlating consumer panel data with DSA and TPA, it will allow for recipe optimisation and identification of significant variables (flavour, aroma, or texture attributes specific to the product) when it comes to consumer preferences in a product. If these variables are identified it will create a base for further product development with other orphan crops, guiding the recipe development in terms of consumer preference.

# Chapter 4

# Concluding Remarks

The aim of the study was to characterise pigeon pea, an orphan crop, in terms of its potential for human health, by looking at secondary metabolites (SMs), the ability to protect DNA and measure of resistant starch. The main focus of the project centred around the application of crops to human health. Functional attributes of food crops are informed by biotechnological efforts, which in turn motivates product development **(Fig. 27).** Orphan crops have been identified as 'crops for the future', because of their potential for human health and adaptability, but because of their regio-specificity, consumers will need to be guided on its use through food product. The second aim for the study was thus profiling the sensory and texture attributes of a proposed pigeon pea product, the falafel, as part of a product development project. Anecdotally, to aid in the use of the pigeon pea crop a small collection of recipes were made (**Fig. 31**).

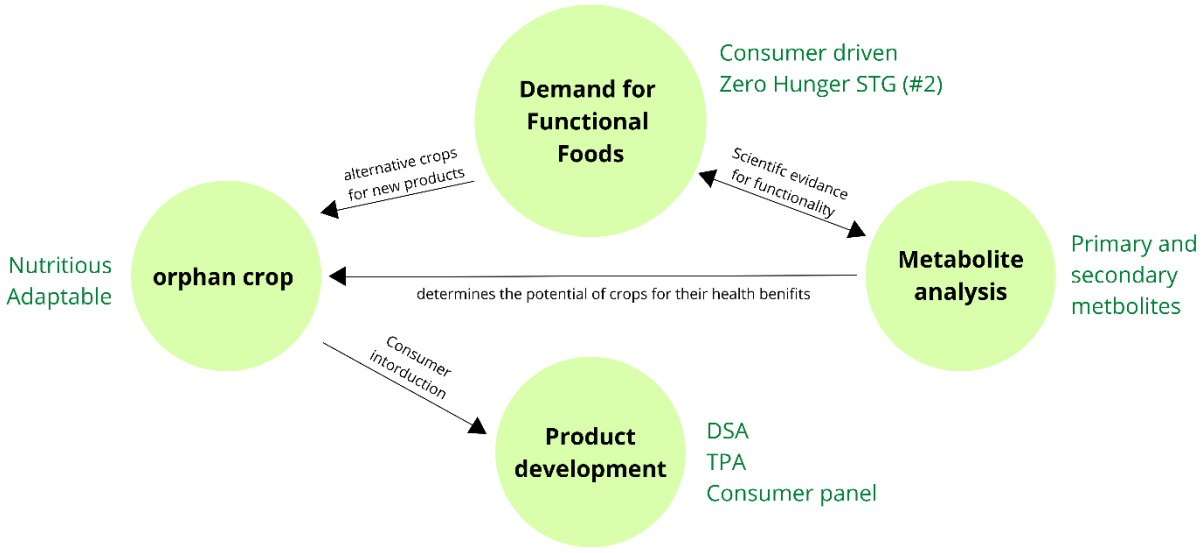


Figure 26: A graphical representation of the co-dependent relationship between the demand for functional foods, metabolite analysis, orphan crops and product development. There is an increased demand for the development of functional foods, driven by consumers and the global pursuit to alleviate malnutrition and hunger. For the development of functional foods, developers can look to orphan crops, because of their nutrient profiles and environmental adaptability, making them more attractive to producers. Metabolite analysis informs producers on nutritional benefits for the orphan crops and in turn informs product developers of the functionality

of the food product. With most orphan crops not being well known, crop introduction through product development is essential for consumer success. Product development can be done using sensory techniques including descriptive sensory analysis, textural profile analysis and consumer panels.

The analysis identified health benefits related to pigeon pea, based on the SM profile, ability to protect DNA and resistant starch content. An array of SMs were tentatively identified, with main groupings including Flavonoids, Alkaloids, Isoflavonoids and Sesquiterpenoids, all of which has known health benefits. Additionally, two metabolites verified by literature, luteolin and quercetin was found in the Nandolo pigeon pea sample variety. The high total antioxidant capacity (TAC) and ability to protect DNA against hydroxyl groups, when compared to other pea parts, alludes to its importance to human health. Further studies should thus focus on capitalising on the health benefits of the H, where it can be used in food and pharmaceutical products, like supplements, bio-fortification and used for extracting SMs.

In terms of primary metabolites, pigeon pea was identified as and good source of resistant starch, compared to chickpeas. Resistant starch acts as substrate for the GMB, where maintaining a diverse and well populated GMB has been linked to various health benefits. The ratio of resistant to soluble starch also informs the glycaemic index (GI) of food product, where low GI food products are high in resistant starch and does not elicit a pronounced increase in blood glucose levels, as observed in food product with a high GI. Low GI diets have health benefits related to diabetes type-2 which is a major cause of mortality among South Africans. Even with the health benefits related to pigeon pea, crop introduction through product development is essential for gaining consumer uptake.

With the aim of product development and pea characterisation in mind, falafels made form pigeon pea flour (FP) were compared to falafels made from chickpea flour (FC) regarding their sensory and texture attributes using descriptive sensory analysis (DSA) and texture profile analysis (TPA). A dry-mix falafel recipe was developed that enables the consumer to just add water and cook, while maintaining traditional falafel flavours and aromas through the addition of dried spices. During the DSA, trained panellists (11) evaluated the sensory attributes (aroma, flavour, texture, mouthfeel) of the two falafels (FC, FP) resulting in significant differences for all tested attributes. In terms of aroma and flavour the savoury-associated aromas and flavours was more pronounced in the FP, whereas the sweet-associated aromas and flavours were more pronounced in the FC. The pea flours used (chickpea and pigeon pea) underwent different milling methods and thus, intrinsically had different textures.

Subsequently the disparities seen in the textural differences of the falafels could largely be attributed to the flour coarseness. While the texture attributes were not comparable between peas, we can deduce how texture attributes are influenced by flour fineness. The finer chickpea flour produced a falafel that was perceived as; more dense, harder, and chewier. This data was compared with texture attributes measured in DSA, it was seen that courses, pigeon pea flour, resulted in a falafel with a coarser and moister interior. This information can be used to predict pulse flour attributes, with regard to milling, where a courses flour will have a larger water holding capacity and result in a less dense product. Product developers are now able to make predications regarding pigeon pea flour, and its texture attributes and can be guided in terms of flavour profiling. With regard to flavour and aroma characterisation, it can be deduced that the two flours (pigeon pea and chickpea) are not interchangeable, and that research should be directed at creating a lexicon specifically for pigeon pea, or pigeon pea flour, to be able to make flavour adjustments in predictions of future products. Future studies can focus on consumer panels to identify sensory attributes associated to consumer preference. Additionally, both the cooked and pre-cooked falafels used in this study was stored (-80 °C) with the option of doing further research on the metabolite change in the product through cooking. The Nutritional Information Table on the label, based on food labelling regulations and industry standards (**Addendum A, Fig. 30**) could be used as a starting point for nutrient improvement, on a recipe basis, but future studies could include developing a nutritional index for the falafel product and assessing the interactions between ingredients and cooking method in nutritional value, when using the nutritious pigeon pea.

Virginia Woolf said, “One cannot think well, love well, sleep well, if one has not dined well” (Woolf, 1929). The increased conscientization of the link between diet and health created a demand for functional food products, creating the opportunity for product development. The demand for nutritious food products has also led producers to investigate alternative crops, that has a broad range of benefits. Pigeon peas indicted that it has the potential for both nutritional and pharmaceutical/therapeutic purposes, and with known environmental adaptability it is a viable crop for the use of crop- and nutrient diversification in various climatic regions.

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# Addendum A

Table 9: Secondary metabolites that have been identified in the pigeon pea crop (Nahar et al., 1990; Singh et al., 2017; Lui et al., 2010; He et al., 2018; Gai et al., 2020; Jiao et al., 2020; Tungmunnithum. Hano. 2020).

| Compound | Molecular Mass | Compound | Molecular Mass |
|---|---|---|---|
| Glycerol | 92.09 | Isoprenylated-Genistein | 270.24 |
| Etythritol | 122.12 | Pinostrobin | 270.28 |
| Threitol | 122.12 | Epiafzelechin | 274.26 |
| Salicyclic Acid | 138.12 | Biochanin A | 284.26 |
| Arabinose | 150.13 | Luteolin | 286.24 |
| Xylose | 150.13 | Catechin | 290.26 |
| Arabinitol | 152.14 | Epicatechin | 290.26 |
| Xylitol | 152.15 | Longistylin | 294.16 |
| Dihydroxybenzoic Acid | 154.12 | Cajanin | 300.26 |
| Gentisic Acid | 154.12 | Quercetin | 302.24 |
| Protocatechuic Acid | 154.12 | Gallocatechin | 306.27 |
| P-Coumaric Acid | 164.05 | Isorhamnetin | 316.26 |
| Rhamnose | 164.16 | Quercetin-3-Omethylether | 316.26 |
| Vanillic Acid | 168.14 | Cajanol | 316.30 |
| 2.3.4-Trihydroxybenzoic Acid | 170.12 | Sucrose | 342.30 |
| Gallic Acid | 170.12 | Chlorogenic Acid | 354.31 |
| Ascorbic Acid | 176.12 | Genistin | 432.40 |
| Galactose | 180.16 | Isovitexin | 432.40 |
| Glucose | 180.16 | Vitexin | 432.40 |
| Mannose | 180.16 | Pelargonidin-3-O-Glucoside | 433.40 |
| Caffeic Acid | 180.16 | Orientin | 448.40 |
| Fructose | 180.16 | Catechin Gallate | 458.37 |
| Myo-Inositol | 180.16 | Epigallocatechin | 458.37 |
| Glucitol | 182.17 | Epigallocatechin Gallate | 458.37 |
| Galact Itol | 182.17 | Peonidin-3-O-Glucoside | 463.40 |
| Mannitol | 182.17 | Quercetin-4-O-Glucoside | 464.10 |
| Uronic Acid | 194.14 | Delphinidin-3-O-Glucoside | 465.40 |
| Ferulic Acid | 194.18 | Petunidin-3-O-Glucoside | 479.40 |
| Terulic Acid | 194.18 | Cyanidin-3-O-Glucoside | 484.80 |
| Syringic Acid | 198.17 | Malvidin-3-O-Glucoside | 493.43 |
| Sinapic Acid | 224.21 | Procyanidin B2 | 578.52 |
| 3-Methoxy-5-(2-Phenylethenyl)-Phenol | 226.09 | Procyanidin B3 | 578.52 |
| 2-Hydroxy-4-Methoxy-6-(2-Phenylvinyl)-Benzoic Acid | 270.08 | Peonidin-3-O-Rutinoside | 625.60 |
| Pinostrobin Chalcone | 270.08 | Procyanidin C1 | 866.77 |
| Apigenin | 270.24 | Longistylin C | 294.40 |
| Genistein | 270.24 | Cajaninstilbene Acid | 338.40 |

Code 1 R-script code for MS search on RefMet

```
library(shiny)
library(RSQLite)
library(DT)
library(data.table)
runUrl('https://www.metabolomicsworkbench.org/data/RefMet_MS_search.zip')
```

Equation 1, indicating how the various texture attributes were calculated and how they are related to each other

$Hardness$ = The maximum force of the 1st compression.
Cohesiveness = Area 2/Area 1.
Springiness = Distance 2 / Distance 1.
Gumminess = Hardness x Cohesiveness
Chewiness = Hardness x Cohesiveness x Springiness.

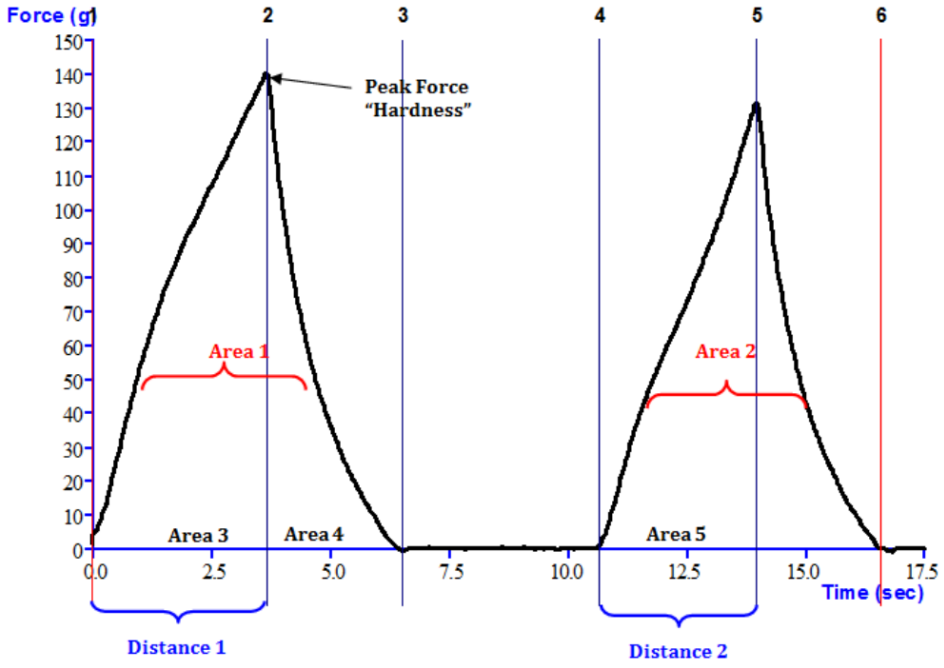


Fig 27: A TPA plot indicating measurements; area, that is used in calculating texture attributes namely, hardness, springiness, cohesiveness, gumminess, and chewiness.

Table 10. Falafel ingredient list (makes +/- 15, 34 mm falafels).

| Ingredients (Cape Spice Emporium) | g |
|---|---|
| Chickpea (Pick and Pay) | 175 |
| Pigeon Pea flour | 175 |
| Cumin seeds | 10.00 |
| Coriander seeds | 10.00 |
| Dried mixed hebs | 10.00 |
| Chili powder | 1.25 |
| Onion flakes | 8.75 |
| Garlic flakes | 8.75 |
| Salt (fine) | 3.00 |
| Bicarb | 3.00 |
| Sesme seeds | 35.00 |
| Total mixture | 264.75 |
| Add Boiling water | 200.00 |

Table 11: Information regarding the batches and replicates of the respective pea flours.

| | Batch 1 (used for training) | Batch 2 (used for testing) | | Batch 1 (used for training) | Batch 2 (used for testing) | Batch Number (indicated on packaging) |
|---|---|---|---|---|---|---|
| Pigeon pea flour | Replicate 1 | Replicate 1 | Chickpea flour | Replicate 1 | Replicate 1 | MO4599/32 |
| | Replicate 2 | Replicate 2 | | Replicate 2 | Replicate 2 | MO4599/32 |
| | Replicate 3 | Replicate 3 | | Replicate 3 | Replicate 3 | MO4599/32 |
| | Replicate 4 | Replicate 4 | | Replicate 4 | Replicate 4 | MO3005/18 |
| | Replicate 5 | Replicate 5 | | Replicate 5 | Replicate 5 | MO4599/32 |
| | Replicate 6 | Replicate 6 | | Replicate 6 | Replicate 6 | MO4599/32 |
| | Replicate 7 | Replicate 7 | | Replicate 7 | Replicate 7 | MO3005/18 |
| | Replicate 8 | Replicate 8 | | Replicate 8 | Replicate 8 | MO4599/32 |
| | Replicate 9 | Replicate 9 | | Replicate 9 | Replicate 9 | MO4599/32 |

Table 12: Daily schedule for the DSA.

| DSA phase | Day | Session | Time | Samples served | Session objective |
|---|---|---|---|---|---|
| Training | 1 | 1 | 10:15 – 11:05 | Reps 1 & 2 | Qualitative assessment of replications.<br>Rep 1 = Aroma & Flavour<br>Rep 2 = Aroma & Texture |
| Training | 1 | 2 | 11:20 – 12:10 | Referance standards | Establishing intensities for reference standards |
| Training | 2 | 1 | 10:15 – 11:05 | Referance standards & Rep 3 | Additional references from Day 1<br>Rep 3 = Aroma |
| Training | 2 | 2 | 11:20 – 12:10 | Reps 4 & 5 | Rep 4 = Flavour<br>Rep 5 = Texture |
| Training | 3 | 1 | 10:15 – 11:05 | Reps 6 & 7 | Rep 6 = Aroma<br>Rep 7 = Targeted Flavour & Texture |
| Training | 3 | 2 | 11:20 – 12:10 | Reps 8 & 9 | Rep 8 = Targeted Aroma<br>Rep 9 = Palate & Texture |
| Testing | 1 | 1 | 10:15 – 10:45 | Rep 1 | |
| Testing | 1 | 2 | 10:55 – 11:25 | Rep 2 | Testing |
| Testing | 1 | 3 | 11:35 – 11:55 | Rep 3 | |
| Testing | 2 | 1 | 10:15 – 10:45 | Rep 4 | |
| Testing | 2 | 2 | 10:55 – 11:25 | Rep 5 | Testing |
| Testing | 2 | 3 | 11:35 – 11:55 | Rep 6 | |
| Testing | 3 | 1 | 10:15 – 10:45 | Rep 7 | |
| Testing | 3 | 2 | 10:55 – 11:25 | Rep 8 | Testing |
| Testing | 3 | 3 | 11:35 – 11:55 | Rep 9 | |

Table 13: Reference standard, to calibrate the DSA panellist for the falafel attributes to be tested for.

| Code | Description of reference standard | Relevance to sensory attribute(s) | Scores decided on by the tasting panel |
|---|---|---|---|
| Oil (A) 1 | Microwave heated canola oil (3 min) | Rancid (aroma) | Rancid aroma = 5 |
| Oil (A) 2 | Microwave heated canola oil (5 min) | Rancid (aroma) | Rancid aroma = 40 |
| Chips 1 | Lay's lightly salted chips | Heated oil (aroma) | Heated oil aroma = 40 |
| | | Rancid (aroma) | Rancid aroma = 0 |
| | | Salty taste | Salty taste = 30 |
| Chips 2 | Kettle fried chips | Heated oil (aroma) | Heated oil aroma = 60 |
| | | Rancid (aroma) | Rancid aroma = 0 |
| | | Salty taste | Salty taste = 40 |
| Cumin (A) | Cumin solution (25%) | Cumin (aroma) | Cumin aroma = 100 |
| Garlic 1 | Fresh garlic slices in water | Garlic (aroma) | Garlic aroma = 100 |
| Green | Fresh parsley in water (11.5%) | Green (aroma) | Green aroma = 80 |
| Salt (T) | Sodium chloride solution (2%) | Salty taste | Salty taste = 80 |
| Umami (T) | Aromat solution (25%) | Savoury (aroma) | Savoury aroma = 80 |
| | | Savoury (flavour) | Savoury flavour = 100 |
| Sweet (T) | Sucrose solution (2%) | Sweet taste | Sweet taste = 20 |
| Peas 1 | Canned peas (without brine) | Cooked pea (aroma & flavour) | Cooked pea aroma = 50 |
| Peas 2 | Canned peas brine | Cooked pea (aroma & flavour) | Cooked pea aroma = 70 |
| Tahini | Tahini paste | Earthy (aroma & flavour) | Nutty aroma = 60<br>Nutty flavour = 70 |
| | | Bitter taste | Bitter taste = 50 |
| Hummus | WW Caramelised onion hummus | Sweet (aroma & taste) | Overall sweet aroma = 10<br>Sweet taste = 30 |
| | | Onion (aroma & flavour) | Onion aroma = 20<br>Onion flavour = 30 |
| Coarse 1 | White roll (inside) | Coarseness (texture) | Coarseness (texture) = 0 |
| Coarse 2 | Donut (inside) | Coarseness (visual & texture) | Coarseness (visual) = 0<br>Coarseness (texture) = 0 |
| Coarse 3 | WW seeded rolls (inside) | Coarseness (visual & texture) | Coarseness (visual) = 20<br>Coarseness (texture) = 10 |
| Falafel 1 | WW falafel (RTE) | Coarseness (visual & texture) | Coarseness (visual) = 30<br>Coarseness (texture) = 40 |
| | | Cumin (aroma & flavour) | Cumin aroma = 40<br>Cumin flavour = 30 |
| | | Garlic (aroma & flavour) | Garlic aroma = 10<br>Garlic flavour = 15 |
| | | Hardness (texture) | Hardness (texture) = 20 |
| Falafel 2 | Orgran falafel mix | Coarseness (visual & texture) | Coarseness (visual) = 50<br>Coarseness (texture) = 30 |
| | | Cumin (aroma & flavour) | Cumin aroma = 20<br>Cumin flavour = 20 |
| | | Garlic (aroma & flavour) | Garlic aroma = 30<br>Garlic flavour = 40 |
| | | Onion (aroma) | Onion aroma = 30 |
| | | Hardness (texture) | Hardness (texture) = 10 |

Table 14: Sensory attributes that were tested for in DSA. The attributes were divided into; aroma, flavour and taste, texture and mouthfeel and visual attributes. The value ranges linked to the attributes were determined by the panel members on the basis of consensus. FP refers to falafels made from pigeon pea flour and FC; falafels made form chickpea flour. Statistical significance is indicated by stars as determined by a F test, (***p < 0.001) For treatment A pigeon pigeon pea flour was used, and chickpea for treatment B.

| | ATTRIBUTE<br>0 = none; 100 = prominent | DESCRIPTION | REFERENCE STANDARD | Treatment A | Treatment B |
|---|---|---|---|---|---|
| VISUAL | | *External/surface evaluation (evaluate BEFORE cutting falafel)* | | | |
| | **Visual coarseness*** ** | The visual perception of texture, i.e. size and shape of the particles. | White roll [Coarse 1]=0<br>Donut [Coarse 2]=0<br>Seeded roll [Coarse 3]=20<br>Falafel 1=40<br>Falafel 2=50 | 50 | 20 |
| AROMA | **Overall sweetness*** ** | The aromatics associated with the impression of sweet. | Caramelised onion [Hummus]=20 | 20 | 30 |
| | **Heated oil*** ** | The aromatics associated with oil heated to a high temperature. | Lay's potato chips [Chips 1]=40<br>Kettle fried chips [Chips 2]=60 | 10 | 5-10 |
| | | *Internal evaluation (CUT falafel in half & evaluate the interior of the falafel)* | | | |
| | **Garlic*** ** | Characteristic aroma of garlic (*Allium sativum L.*). | Falafel 1=10<br>Falafel 2=30<br>Fresh garlic solution [Garlic 1]=100 | 40 | 10-20 |
| | **Onion*** ** | Characteristic aroma of onion (*Allium cepa*). | Caramelised onion [Hummus]=20<br>Falafel 2=30 | 10 | 20-30 |
| | **Cumin *** ** | The aromatics commonly associated with cumin and characterized as dry, pungent, woody and slightly floral. | Falafel 2=20<br>Falafel 1=30<br>Ground cumin solution (25%) [Cumin]=100 | 50 | 20-30 |
| | **Green*** ** | Sharp, slightly pungent aromatics associated with green/plant/vegetable matters such as parsley, spinach, pea pod, fresh cut grass, etc. | Fresh parsley water [Green]=80 | 30-40 | 15-20 |
| | **Nutty*** ** | Nutty characteristics are: sweet, oily, slightly musty and/or buttery, earthy, woody, astringent, bitter, etc. | Sesame seed paste [Tahini]=60 | 30 | 15-20 |
| | **Savoury*** ** | Aromatics associated with salty, meaty and brothy characteristics | Aromat solution [Umami]=80 | 40-50 | 30 |
| | **Rancid** | The aromatics commonly associated with oxidised fat and oils. These aromatics may include cardboard, painty, varnish and fishy. | Lay's potato chips [Chips 1]=0<br>Kettle fried chips [Chips 2]=0<br>Microwave heated oil 3 min [Oil 1]=5<br>Microwave heated oil 5 min [Oil 2]=40 | 0 | 0 |
| PALATE (FLAVOUR & TASTE)& MOUTH FEEL | | (*CUT falafel in quarters & evaluate the interior of the falafel)* | | | |
| | **Nutty*** ** | Nutty characteristics are: sweet, oily, slightly musty and/or buttery, earthy, woody, astringent, bitter, etc. | Sesame seed paste [Tahini]=70 | 40 | 20 |
| | **Garlic*** ** | Characteristic flavour of products containing garlic (*Allium sativum L.*). | Falafel 1=20<br>Falafel 2=40 | 40 | 20-30 |
| | **Onion*** ** | Characteristic flavour of products containing onion (Allium cepa). | Caramelised onion [Hummus]=30 | 10 | 20-40 |
| | **Green*** ** | Sharp, slightly pungent aromatics associated with green/plant/vegetable matters such as parsley, spinach, pea pod, fresh cut grass, etc. | | 40 | 10-20 |
| | **Cumin*** ** | The aromatics commonly associated with cumin and characterized as dry, pungent, woody and slightly floral. | Falafel 1=40<br>Falafel 2=20 | 50-60 | 30-40 |
| | **Heated oil*** ** | The flavour associated with oil heated to a high temperature. | | 0-5 | 0-5 |
| | **Rancid** | The flavour commonly associated with oxidized fat and oils. These aromatics may include cardboard, painty, varnish and fishy. | | 0 | 0 |
| | **Savoury*** ** | Flavour associated with salty, meaty and brothy characteristics | Aromat solution [Umami]=100 | 50-60 | 30-40 |
| | **Salty*** ** | The fundamental taste factor of which sodium chloride is typical. | Lay's potato chips [Chips 1]=30<br>Kettle fried chips [Chips 2]=40<br>Sodium chloride solution (2%)=80 | 20 | 10-20 |
| | **Sweet*** ** | The fundamental taste factor associated with sucrose. | Sucrose solution (2%) [Sweet]=20<br>Caramelised onion [Hummus]=30 | 5-10 | 10-20 |
| | **Bitter*** ** | The fundamental taste factor associated with a caffeine solution. | Sesame seed paste [Tahini]=50 | 0-20 | 0-5 |
| | **Burn*** (after swallowing)** | The magnitude of the perceived hot sensation. | | 40-60 | 30-50 |
| TEXTURE | **Hardness*** ** (0 = not resistant; 100 = very resistant) | The force needed to reach a particular deformation. Force required to bite completely through sample placed between molars. | Falafel 2=10<br>Falafel 1=20 | 15-20 | 70-80 |
| | **Moistness*** ** | Amount of moisture perceived on the surface of the product, when in contact with the oral cavity | | 40 | 10 |
| | **Coarseness*** ** (0 = very fine; 100 = very coarse) | The size and shape of the particles as perceived in the mouth. | White roll (inside) [Coarse 1]=0<br>Donut [Coarse 2]=0<br>Seeded roll [Coarse 3]=10<br>Falafel 1=40<br>Falafel 2=30 | 50-60 | 20-30 |

Table 15. The sample codes for the replicates tested on each day. There are 9 replicates for each treatment with a corresponding sample code. The colours indicate the number of samples from each replicate was used for TPA testing, with orange being one sample, yellow being two and blue being three.

| Day | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| Treatment | PP | CP | PP | CP | PP | CP |
| Sample code | 134 | 436 | 137 | 116 | 184 | 347 |
| | 824 | 397 | 935 | 325 | 226 | 780 |
| | 906 | 639 | 536 | 206 | 739 | 902 |

Table 16: Definitions and units for the attributes measured by the UTM on double bite setting.

| Attribute | Definition and Calculation | Unit |
|---|---|---|
| Hardness | Force required to achieve a given deformation or penetration of the product. It is the maximum peak force that occurs during the first compression | N |
| Springiness | Rate at which a deformed sample returns to its original size and shape. It is measured by the distance of the detected height during the second compression divided by the original compression distance | (mm) |
| Gumminess | Energy required to disintegrate the product to the state ready for swallowing, | (N) |
| Chewiness | It can be interpreted as the energy required to chew solid food. | |

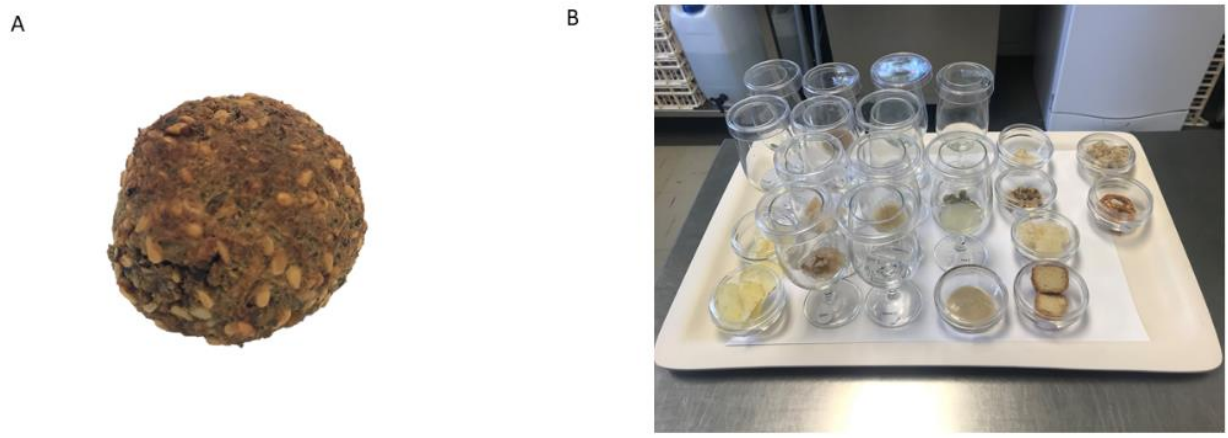


Figure 28: (A) A photo of a falafel made form pigeon pea flour (FP) that was used for DSA and (B) a photo of the presentation of the reference standards used in calibrating the panellist.

Figure 29: A QR code directed to the small recipe book for the introduction of pigeon peas.

| | kcal | |
|---|---|---|
| ENERGY | 368 | 100% |
| | | |
| PROTEIN | 79 | 21% |
| FAT | 98 | 27% |
| of which SATURATES | 17 | 5% |
| MONOUNSATURATES | 37 | 10% |
| POLYUNSATURATES | 39 | 11% |
| TRANS | 0 | 0% |
| AVAILABLE CARBOHYDRATE | 167 | 45% |
| of which STARCH | 147 | 40% |
| SUGARS | 13 | 4% |
| DIETARY FIBRE (AOAC) | 23 | 6% |

Figure 30 : The energy contributions of the dry-mix pigeon pea falafels as calculated by NutriCalc® (https://app.nutricalc.co.uk/).

Table 17: DSA measured attributes with the corresponding treatment average and the statistical significance as determined by an ANOVA.

| | | Chickpea based falafel | Pigeon Pea based Falafel | Statistical significance (Anova) |
|---|---|---|---|---|
| Aroma | Overall_sweetness_external | 27.62 | 20.62 | <.0001 |
| | Heated_oil_external | 5.14 | 7.11 | <.0001 |
| | Garlic_aroma | 20.40 | 33.93 | <.0001 |
| | Onion_aroma | 25.43 | 12.94 | <.0001 |

| | | | | |
|---|---|---|---|---|
| | Cumin_aroma | 33.76 | 46.63 | <.0001 |
| | Rancid_aroma | 0.00 | 0.00 | . |
| | Green_aroma | 21.28 | 37.37 | <.0001 |
| | Nutty_aroma | 18.11 | 29.05 | <.0001 |
| | Savoury_aroma | 33.47 | 46.59 | <.0001 |
| Flavour | Nutty_flavour | 21.56 | 38.20 | <.0001 |
| | Garlic_flavour | 24.02 | 37.30 | <.0001 |
| | Onion_flavour | 29.74 | 15.14 | <.0001 |
| | Green_flavour | 21.73 | 38.87 | <.0001 |
| | Cumin_flavour | 36.77 | 55.98 | <.0001 |
| | Heated_oil_flavour | 0.98 | 2.55 | <.0001 |
| | Savoury_flavour | 33.46 | 51.98 | <.0001 |
| | Salty_taste | 16.20 | 19.30 | <.0001 |
| | Rancid_flavour | 0.00 | 0.00 | . |
| | Sweet_taste | 14.89 | 8.70 | <.0001 |
| | Bitter_taste | 3.07 | 7.29 | <.0001 |
| Texture and mouthfeel | Burn_after_swallowing | 34.52 | 48.39 | <.0001 |
| | Denseness_texture | 65.02 | 22.84 | <.0001 |
| | Moistness_texture | 12.45 | 36.58 | <.0001 |
| | Coarseness_texture | 25.06 | 57.55 | <.0001 |