Revalorization, Characterization and Application of Tofu Industry By-product ‘Okara’ as a Food Ingredient

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Abstract

The potential of revalorising okara into a powder for use in various food products has been researched in the literature, but no universal consensus has been achieved. Some studies have focused on drying small quantities of okara under different conditions, while others have attempted to substitute wheat flour with okara powder, often with limited success or by combining it with other grains to form composite flours. This project aimed to characterize powders obtained under varying drying conditions to identify the optimal method in terms of nutritional value, functionality and efficiency. Okara powder was then used in bread, cookies and as bread crumbs to maximize the substitution of wheat flour. Two drying methods were tested: freeze-drying and convection oven drying. For convection oven drying, six different temperatures (50, 60, 70, 80, 90 and 100°C) were evaluated to determine their effects on the physicochemical characteristics of okara powder. A total of 580 g of fresh okara was dried per trial to simulate industrial conditions where much larger quantities are processed. Proximate analysis was conducted for each drying treatment. The results showed no significant differences in ash, protein, or fat content across drying conditions. The physical properties of the powders, such as colour, particle size distribution as a powder and as suspension, water activity, water-holding capacity, solubility, bulk density, tapped density, Carr Index and flowability, were also assessed, as these influence food formulation and development. The best drying conditions were determined based on drying time, water activity, water-holding capacity, particle size, and flowability. Convection oven drying at 90 °C and 70 °C was identified as optimal due to the quick drying time, and low water activity (e.g. improved stability against microbial growth and lipid oxidation). Although freeze-drying provided superior water-holding capacity, it required significantly more time and resulted in less stable powders in terms of moisture and water activity. Okara powder was then incorporated into its use in bread and cookies at varying concentrations. Okara powder and okara bread crumb were also tested as a coating agent for frying vegetables.

For bread, preliminary trials with okara substitution levels (10, 25 and 50 %) of wheat flour showed that a maximum substitution level of 20% (okara-to wheat flour ratio of 20:80) was feasible. Hydration levels of 80%, 90% and 100% were tested, with 100% hydration yielding the best results in terms of height, colour, and textural properties due to complete protein hydration and minimal interference from fibre.

For cookies, okara powder was used as the sole flour. The formulation of cookies avoided allergens apart from soy by excluding eggs and dairy, substituting with chia seed gel and coconut shortening. Three formulations were compared (V1, V2, V3) were developed, varying corn flour and fat content to address surface cracks caused by poor binding. The best formulation, V2, contained higher fat and less okara powder., resulting in improved binding and lower hardness, a desirable trait for biscuits.

Although informal testing was conducted, formal sensory evaluation was not performed, which would have provided valuable insights into flavour, texture and overall acceptability. Future studies should include sensory evaluations to assess flavour, odour, soybean aftertaste, texture, and consumer acceptance. Additionally, determining the nutritional composition of the okara-containing bread and cookies compared to wheat flour-based products would provide further justification for okara revalorization.

This project highlights the impact of drying treatments on okara powder functionality, new application levels in breads and cookies, and the critical role of water and fat in formulations using okara.

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Chapter 1. Introduction

1.1 Background

Soy-based products have become more popular among the population due to their health benefits (Feng et al., 2021) and their role as alternatives to animal-based products. Soy milk, one of the soy-based products, is a popular milk alternative around the world, especially for those who are lactose intolerant. Isoflavones, known for their antioxidant and anticancer effects, have been noted to reduce the risk of coronary heart disease, osteoporosis, and various cancers, including prostate, breast, and colon cancers, and these beneficial compounds are present in soybean products (Canaan et al., 2022).

Another soy-based product is tofu, which has been a part of Asian cuisine since the Han Dynasty, meaning it has been included in the Asian diet for about 2000 years (Ali et al., 2021). Tofu is a soybean curd rich in lipids and proteins obtained through the acid precipitation of soy milk (Serrazanetti et al., 2013). While its appearance is similar to firm yoghurt or soft cheese; its flavour is completely different and it is used often as a meat substitute through methods like deep-frying or grilling (Serrazanetti et al., 2013). In addition to being a source of protein, tofu is also a good source of minerals and vitamins, including calcium, manganese, selenium, B vitamins and vitamins A, C, D, E and K (Pal et al., 2019). Like soymilk, tofu can help prevent cardiovascular diseases, cancer, osteoporosis, and oxidative and inflammatory damage to blood vessels, thanks to the presence of soy peptides and omega-3-fatty acids (Pal et al., 2019).

On a global scale, the tofu market size was estimated at approximately $2.31 billion in 2018 (Ali et al., 2021). In New Zealand, the retail value of tofu and its derivates is 14.8 million NZD and it is projected to grow to 19.6 million NZD by 2028 (Euromonitor International, 2023). However, managing the by-products generated during production poses a significant challenge. Okara, also known as soybean pulp, is a by-product of soy milk and tofu production, and is obtained after filtering the soy slurry to produce soy milk (Liu, 2008). For every 1000 litres of soy milk produced, approximately 250 kg of okara is generated (Li et al., 2013). Additionally, for every 1 kg of soybeans used in tofu production, 1.2 kg of fresh okara is produced (Guimarães et al., 2018).

Okara is rich in dietary fibre, protein, and phytochemical compounds such as isoflavones and saponins (Fuentes et al., 2022; Yoshida & Prudencio, 2020). Despite its nutritional value, the common practice for dealing with okara is to use it as animal feed, fertilizer, or to burn it, or it is simply dumped in landfills (Li et al., 2013). Disposing of okara in landfills can cause environmental issues as this by-product is high in water and nutrients, promoting rapid microbial growth and lipid oxidation when not treated. This can result in quick putrefaction, potentially contaminating surrounding lands and water systems, especially during the rainy season (Davy & Vuong., 2022). Moreover, the disposal of okara causes economic costs. Taking Japan which has a large tofu production, as an example, the annual amount of okara disposed is approximately 800,000 tons, corresponding to a cost of about 173 million NZD (Li et al., 2013).

One of the solutions to waste management in the food industry is the revalorization of by-products. In the case of okara, its revalorization has presented difficulties due to its high water content, polyunsaturated fatty acids, aldehydes, and insoluble dietary fibre, which leads to its short shelf life, off-odours, digestion problems and grainy mouthfeel (Feng et al., 2021). The most common approach has been the drying of okara which reduces high water content and lengthens the shelf life (Voss et al., 2018). Then, dried okara can be ground to obtain a powder similar to flour which can be used as an ingredient in food products.

Dried okara hasn't been used as a 100% substitute for wheat flour in food products, and most projects have focused on blending a mixture of okara and wheat flour for cookies (Hawa et al., 2018; Davy & Vuong, 2022), bread (Li et al., 2012), and noodles (Davy & Vuong, 2022). Currently, okara flour is sold in the market as a gluten-free alternative and is also used as a meat substitute along with chickpeas.

The goal of the project was to explore the possibility of revalorising okara as an ingredient for bakery products and other food products. The specific objectives of the project were to determine the best methods and conditions for drying okara by evaluating its physicochemical properties, characterization of okara flour, and assessing the feasibility of using it as a 100% substitute for wheat flour in bakery products. Some factors investigated were the optimal temperature for drying okara, the amount of okara flour that could be incorporated into bakery products, and the relationship between okara and water, as well as its effect on the texture properties of the final products.

1.2 Overview of the Thesis

This thesis is composed of five chapters.

Chapter 1 introduces the concept of revalorizing by-products in the food industry, discusses the growing demand for soy-based products, and provides background information on okara. It also touches on the research opportunity, the project’s goal, and specific objectives.

Chapter 2 is a literature review focusing on providing essential information to understand the opportunity for revalorizing okara. It covers the tofu manufacturing process, key concepts related to tofu production, okara obtention, challenges associated with revalorizing okara, antinutritional properties of soybeans, and examples of how other studies have used okara in food products.

Chapter 3 is an experimental chapter focused on the drying and characterization of okara. It describes different drying methods and conditions and their effects on the physicochemical properties of okara powder to determine the best treatment to obtain okara powder for further applications.

Chapter 4 is the second experimental chapter, focused on the use of okara powder as a food ingredient and its effectiveness as a replacement for wheat flour. It assesses the use of okara powder in bread, cookies and as a coating agent for frying by comparing their physical characteristics.

Chapter 5 presents the results of the project and offers recommendations for further studies and potential opportunities in the subject area.

Chapter 2. Literature Review

2.1 Introduction

There is a necessity to revalorize by-products to ensure food security for future generations. It is predicted that the global population will reach 9.1 billion by 2050 with a projected increase in demand for food and feed by 70% (FAO, 2009). This implies a need for the additional production of 1 billion tonnes of cereals and 200 million tonnes of meat. To accomplish these goals, economic growth and higher productivity are required (FAO, 2009). Therefore, developing sustainable food systems should be a priority in the food industry. The majority of food systems are considered linear meaning resources are used for the production of food products while by-products and excess food are considered waste (Burey et al., 2022).

The waste management portion of the food system provides an opportunity to ensure food security by adapting features of the circular economy, where the main goal is to design ways to achieve zero waste. The favoured approach is to reduce waste from the beginning of the production process. For already generated by-products, instead of incineration or use for animal feed, revalorization into a nutritional and useful product is a preferred strategy (Burey et al., 2022). Additionally, the use of by-products in the food industry has been increasing due to societal demand for less waste-generating processes. Therefore, okara should be utilized to create new food products. It has a high content of protein, soluble and insoluble fibres, isoflavones and soyasaponins, making it a high-value bioproduct (Canaan et al., 2022). Since a large amount is produced, converting it into a potential ingredient for new foods at low cost is attractive for consumers seeking plant-based protein or those suffering from celiac disease (Canaan et al., 2022).

Another reason why the revalorization of okara is crucial is the growing interest in plant proteins from consumers and the environmental impact of animal products. Soybean protein is highly digestible, contains all essential amino acids, is low-cost and is ideal for vegetarians and lactose-intolerant populations (Stanojevic et al., 2013; Tripathi & Shrivastava, 2017). Currently, 48% of consumers are seeking plant-based food and drinks in the market, 25% of adults have increased their plant protein consumption. 7 of 10 adults have started trying new plant-based alternatives (Sloan, 2021). Furthermore, 65% of the world's nitrous oxide emissions and 15% of greenhouse gas emissions are generated by animal agriculture, making a principal source of environmental decline (Conzachi, 2022).

2.2 Tofu

Tofu is produced by coagulating soybean proteins and oil from soymilk (Yang & James, 2016). It can be classified based on the manufacturing process into two main types: pressed or packed. Pressed tofu undergoes a pressing process after coagulation to stabilize the gel, while packed tofu is not pressed; its coagulation occurs within its container, followed by a water bath at 85-90°C (Yang & James, 2016; Evans et al., 1997). Packed tofu, also known as silken tofu or Japanese style tofu, is creamy and soft, making it suitable for use in dips, desserts, smoothies, and puddings. The coagulant used in this type of tofu is GLD (Glucono-δ-lactone), which transforms into gluconic acid after being in the water bath for 35 to 60 minutes (Pal et al., 2019; McHugh, 2016). Pressed tofu is further subclassified into soft, hard, and dry varieties based on their water content and firmness (Liu et al., 2004; Cai et al., 1997).

Table 2.1 shows the differences in water content, firmness, and applications of the three types of pressed tofu. Soft tofu is used in liquid food products such as salad dressings and sauces due to its higher water content. During the pressing stage of tofu production, soft tofu is not pressed enough to break the bean curd which results in less water loss and a tofu too soft to be able to cut into (Tsai et al., 1981; Shih et al., 1997).

Table 2.1 Characteristics of the different types of tofu (Cai & Chang, 1997; Tsai et al., 1981).

Pressed tofu	Water content (%)	Firmness	Use in food
Dry/ Extra firm	<76	Firmest variety	Baking, stir-frying, and grilling
Hard / Firm	76-81	Firmer than soft tofu	Baking, stir-frying, and grilling
Soft	87-90	Very soft	Salad dressings, desserts, and sauces

2.2.1 Tofu manufacturing process and okara byproduct generation

A flowchart of the tofu manufacturing process is depicted in Figure 2.1. The flowchart illustrates how the two main types of tofu are obtained, including the water to soybeans ratio, the temperatures used, and okara as a by-product of both soymilk and tofu manufacturing. Okara can be separated from soymilk either before or after the heat treatment, depending on the manufacturer’s preference. In the case of Tonzu, a tofu manufacturer based in Auckland, the filtration is done after the heat treatment. The main difference between the two types of tofu in terms of the manufacturing process is that packed tofu skips the pressing stage; instead, the curd is coagulated within its package and subjected to a water bath. From the pressed tofu process, three additional types of tofu can be produced depending on the desired firmness and water content, as explained in the previous section.

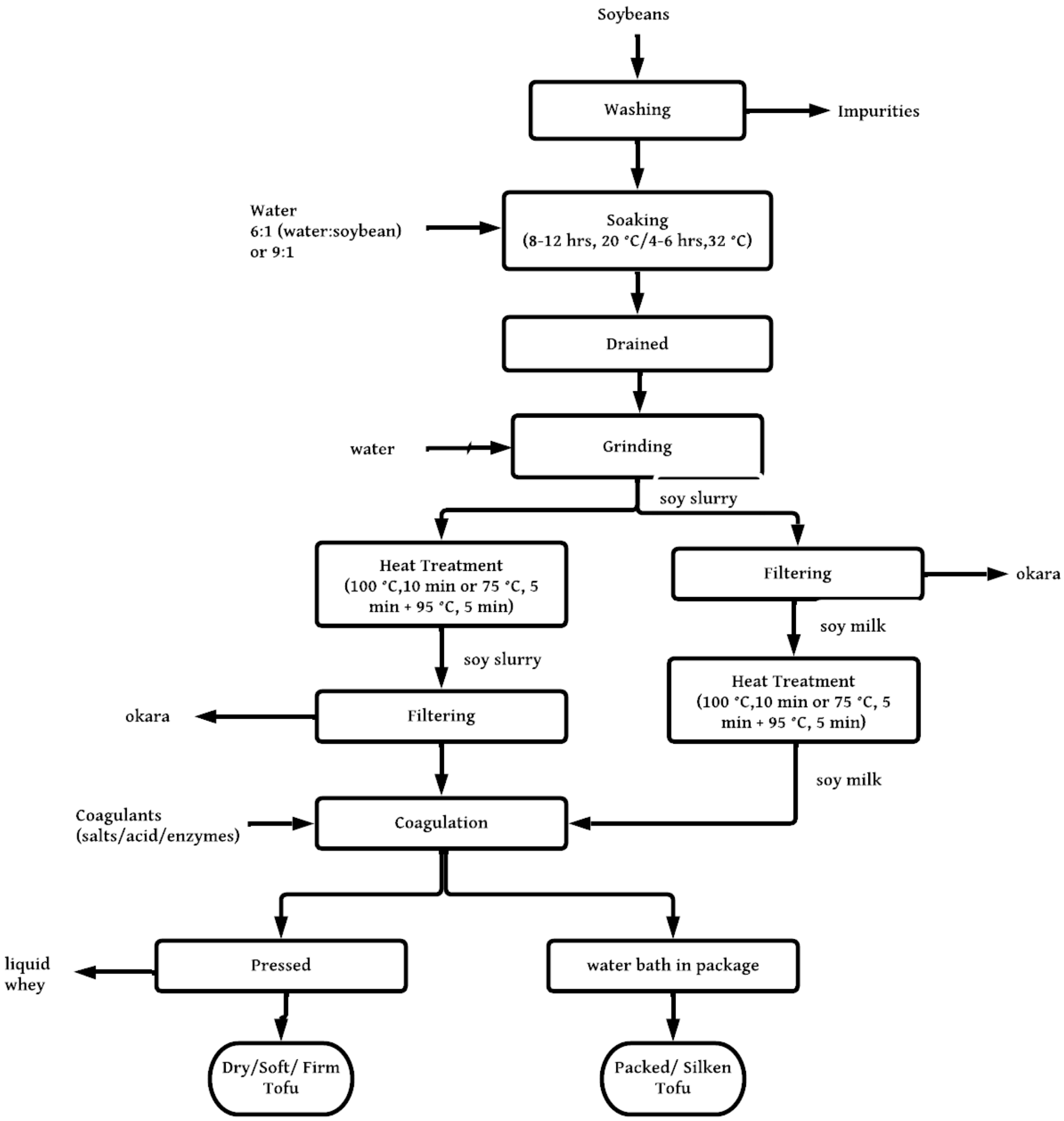


Figure 2.1 Flow diagram for the manufacture of tofu (Budiyono & Syaichurrozi, 2020; Colletti et al., 2020; Liu et al., 2004; McHugh, 2016; Obatolu, 2008; Zhang et al., 2018).

As illustrated in Figure 2.1, there are several steps in the process of obtaining tofu from soybeans, such as soaking, grinding, heating, filtering, coagulation and pressing, which are explained below.

Soaking the beans is a crucial step in the production of tofu and soymilk. The primary purpose of soaking the soybeans is to achieve a greater extraction of protein, which results in a higher protein content in both tofu and soymilk. In the case of tofu, a greater amount of extracted protein leads to tofu with higher water-holding capacity due to a greater gel strength (Guan et al., 2021). This extraction of protein is achieved because by soaking the soybeans at an appropriate water ratio and time, their structure is modified and as mentioned before, it contributes to a higher quality tofu (Zhang et al., 2018). The soybeans-to-water range ratio that is recommended is 1:8 to 1.3:10 (Ostermann-Porcel et al., 2017a). According to Zhang et al. (2018), the best soybeans-to-water ratio to obtain tofu with the best textural properties is 1:9. Additionally, fully grinding the beans with water extracts more protein; the equipment that is usually used in this step is a hammer mill (McHugh, 2016).

The key aspect of heat treatment is to denature soybean proteins to facilitate the formation of curd when the coagulant is added later in the process, but it also reduces the activity of the anti-nutritional components, such as trypsin inhibitor and lipoxygenase the enzyme responsible for unpleasant taste (Ostermann-Porcel et al., 2017a). The two main proteins found in soybeans are glycinin and β-conglycinin, also known as 11S and 7S, respectively. The result of heat denaturation is the unfolding of these proteins by exposing their amino acid side chains, which leads to protein aggregation when the coagulant is added and the formation of the gel structure known as tofu (Liu et al., 2004). It is important to note that these two proteins have different denaturation temperature ranges; glycinin is 85-95°C while β-conglycinin is 65-75°C. Hence, the most common strategy is to denature both simultaneously by applying 100°C for 3-10 minutes to the slurry obtained from soaking and grinding. The other option is a two-step heating process where the slurry is first heated to 75°C and maintained for 5 minutes to denature 7S, followed by raising the temperature to 95°C for the remaining 5 minutes to denature 11S (Guan et al., 2021; Liu et al., 2004; McHugh, 2016).

Before obtaining tofu through the addition of coagulant, soymilk must be separated from the solid pulp known as okara, which is the focus of this project. The separation of soymilk and okara can be done before or after the heating step (McHugh, 2016). In the case of Tonzu, the company that provided the okara for this research project, they separate it after heating. This separation is achieved through filtration or centrifugation, resulting in soymilk and okara being separated from each other.

The coagulation step involves adding coagulants to soymilk to form a gel structure similar to a honeycomb due to the aggregation of proteins (Guan et al., 2021). Heat treatment prepares the proteins for the coagulant by breaking down their secondary structure which increases their hydrophobicity. When the coagulant is added, the pH changes until it reaches the soybeans' isoelectric point, at which the net charge becomes zero, causing instability that leads to protein aggregation and gel formation (Wang et al., 2023). Although different types of coagulants have specific coagulation mechanisms, they are generally added at a concentration of 1.5 to 5 g per kilogram of soymilk at 60 to 90°C while stirring the mixture (McHugh, 2016). The gel formed due to coagulation is not only composed of aggregated proteins, but it also contains trapped water and soy lipids dispersed throughout the protein gel (McHugh, 2016; Obatolu, 2008).

In the case of soft tofu, there is no compression step, the tofu production ends at the coagulation stage done directly in its final package (McHugh, 2016). Firm tofu, however, requires this last step to stabilize the gel network. The compression of tofu involves applying pressure either with a hydraulic press or centrifuge or using a cheese cloth to release excess yellow water, also known as whey, resulting in less syneresis during storage. The pressure applied is crucial as it directly influences the final structure. If the pressure is too low, excess water cannot be fully released, leading to an irregular shape and more syneresis. Conversely, if the pressure is too high, excessive whey is lost, damaging the gel structure (McHugh, 2016; Guan et al., 2021).

2.2.2 Coagulants in tofu

There are three types of coagulants used in tofu production: salt, acid, and enzymes. The key is understanding that the chosen coagulant must help reach a pH of 4-5, which is the isoelectric point of soybean proteins, because coagulation is a pH-dependent process (Qin et al., 2022; Wang et al., 2023).

The most commonly used coagulants for tofu production are salt-based coagulants, such as calcium chloride, calcium sulfate, magnesium sulfate, magnesium chloride, and calcium acetate (Guan et al., 2021). The key aspect when using a salt coagulant is the amount used; if too much salt is added, the final product will be too firm, while if too little salt is added, inadequate protein precipitation occurs (Lu et al., 1980). Many have tried to explain the mechanism of salt coagulants, but there is no clear consensus. Three possibilities include the cation bridge theory, salting-out and isoelectric point theory (Guan et al., 2021).

However, for acid coagulants, the aggregation of proteins is achieved by reaching the proteins' isoelectric point. As the name suggests, acid coagulants when added lower the pH of soymilk from 6.4-7 to 4-5, which is the isoelectric point of proteins, resulting in their precipitation and aggregation through hydrophobic interactions (Bai et al., 1998; Guan et al., 2021). Examples of acid coagulants include lactic acid, succinic acid, malic acid, citric acid, and glucono-delta-lactone (GDL) which is the most commonly used of all the acids. Although the gelation process achieved using salt and acid coagulants is similar, GDL takes more time to form a gel because its coagulation rate is slower (Guan et al., 2021).

The possibility of using coagulant enzymes in tofu products has been explored as a way to improve the gel strength in tofu. The most researched enzymes are transglutaminase, papain, bromelain, and pepsin (Guan et al., 2021). According to Rizkaprilisa & Setiadi (2018), using papain instead of calcium sulfate in tofu production is successful, resulting in a product with more coagulated protein and less water than tofu made with calcium sulfate, though overall yield is lower.

2.3 Revalorization of okara

Okara hasn't been fully utilized in the industry. Firstly, immediate processing is necessary due to its high-water content (76-80%), which makes it highly susceptible to rapid fermentation. However, dehydration methods used to preserve it add to the cost for manufacturers (Orts et al., 2019). Secondly, despite being rich in fibre containing 12.6–14.6% soluble fibre and 40.2 -43.6 % insoluble fibre on a dry basis (van der Riet et al., 1989), dietary fibres in okara can impart a grainy and coarse texture to final food products and reduce their volume (Yoshida & Prudencio, 2020; Tripathi & Shrivastava, 2017). One strategy to counteract these effects is to apply fibre modification techniques such as fermentation and hydrolysis (Yoshida & Prudencio, 2020).

As mentioned before, okara is defined as a by-product. In the food industry, this term refers to the desired product's specific waste that is generated in high amounts during food processing, which consists mostly of organic residue (Socas-Rodríguez et al., 2021). This waste is difficult to use or dispose of due to its biological instability, potential pathogenic environment, high water content, autooxidation and high enzymatic activity (Tokuşoğlu, 2018). Okara meets these characteristics as the specific waste of soymilk production and is difficult to dispose due its 80% moisture content, oil content and fibre, which can together create an environment conductive to microorganism growth.

2.3.1 Antinutritional properties of soybeans

One of the potential drawbacks for utilizing okara as an ingredient may be the presence of anti-nutritional factors in soybeans, such as saponins, trypsin inhibitors, lectins, estrogens, goitrogens, phytic acid, anti-vitamin factors and cyanogens (Colletti et al., 2020; Hymowitz, 2022). These components can be divided into heat-labile and heat-stable categories. Trypsin inhibitors, lectins, goitrogens and anti-vitamins factors are considered heat-labile, meaning that their activity in soybeans can be significantly reduced through heat treatment. On the other hand, saponins, estrogens, cyanogens and phytic acid are heat-stable, which poses a problem (Bueno et al., 2018; Liener,1994).

Saponins are glucosides found in a variety of legumes and are responsible for destroying red blood cells (Hymowitz, 2022). Although soybeans, containing 42 g/kg of saponins, are the main source of saponins, and they can also be found in okara, soybean saponins have a weaker effect on the organism (Lásztity et al., 1998). Liener (1994) explains that, unlike other legumes, soybean saponins have little to no effect on active nutrient transport and may even have positive effects, such as lowering high blood sugar and cholesterol levels in rats.

Trypsin inhibitors, however, poses the main threat because they affect pancreas activity, leading to poor protein digestion and can't be completely inactivated (Vagadia et al., 2017; Voss et al., 2018). It is important to understand that trypsin is one of the enzymes responsible for digestion along with chymotrypsin and elastase. When trypsin inhibitors are present, as its name suggests, they inhibit these digestive enzymes' activities which causes the pancreas to overproduce them, leading to a trypsin enzyme-trypsin inhibitor complex with a surplus of digestion enzymes that can't aid with digestion (Vagadia et al., 2017). The problem with the inactivation of trypsin inhibitors is that complete inactivation hasn't been able to reach, the reachable goal is a major reduction in activity. Heat treatment is a preferred method of inactivation as trypsin inhibitor is heat labile. It has been demonstrated that a reduction of 80% in activity can be achieved by heating at 100°C for 2 hours, with the time reduced by 70% by soaking the soybeans in water (Liener, 1994; Vagadia et al., 2017). For those reasons, the trypsin inhibitor activity in okara is likely to be low since okara is derived from soaked and heat-treated soybeans. Following this line of thought, drying okara into flour, may further reduce trypsin inhibitor activity.

Tannins, similar to trypsin inhibitors, affect digestion, but instead of inhibiting enzymes activity, they directly decrease the digestibility of carbohydrates and proteins. Although tannins are heat-stable, they aren't an issue in soybeans because they contain only 45 mg/100 g, compared to 2000 mg/100 g in other legumes such as faba beans, meaning no nutritional concern in okara. Also, soaking soybeans in water and discarding the water reduces their already low concentration in soybeans (Liener, 1994).

As for heat-labile factors, lectins are glycoproteins responsible for the agglutination of red blood cells, which can lead to blockages in blood vessels. The agglutination occurs as a result of lectins that can bind specifically to the sugar unit of carbohydrate molecules. If heat treatment is not applied to soybean products, apart from agglutination, lectins also interfere with the absorption of iron and lipids, lower insulin levels and affect the activity of disaccharides and proteases because approximately 60% of ingested lectins can survive digestion and bind to the intestinal epithelium (Hymowitz, 2022; Liener,1994).

Goitrogens affect the thyroid gland directly by enlarging it, but this effect can be prevented through heat treatment and the addition of iodide to soybean products (Hymowitz, 2022). Goitrogens, however, are not a concern in okara because they are concentrated in tofu, meaning that when soymilk is separated from okara, goitrogens remain in the soymilk (Liener, 1994). Similarly, cyanogens aren't a threat in okara because their concentration in soybeans is minimal. It has been reported that per 1 gram of soybean meal, 0.26 ug of hydrocyanic acid is released and those levels have no significance (Hymowitz, 2022).

Estrogens are heat-stable but similar to goitrogens and cyanogens, the type and amount encountered in okara are not considered problematic. Firstly, daizin and genitsin which are the main estrogens in okara have a weak estrogenic activity and secondly, their concentration in okara is about 0.10%, resulting in a level too low to stimulate growth in female reproductive organs (Hymowitz, 2022; Liener, 1994).

In summary, the anti-nutritional components present in raw soybeans aren't a significant problem in okara because most of them are removed during the processing of soybeans into soymilk and tofu (Guan et al., 2021).

2.3.2 Dehydration methods used for okara

Drying okara to convert it into flour is the most useful way to revalorize this tofu by-product. One of the most recurrent options to stabilize this insoluble, high-moisture component is to dry it taking into account that the temperatures and time chosen will affect nutritional components and functional properties (Voss et al., 2018). Converting okara into dehydrated flour not only provides a range of possibilities to use it as a food ingredient but is also the easiest way to stabilize it. Although okara flour (OF) is deemed as having less nutrient value than fresh okara due to the loss of qualities, such as colour, odour, texture, bulk properties and retention of nutrients and volatile compounds, during the drying processes, it results in a longer shelf life because of the removal of moisture and less space needed to store the product (Santos et al., 2019; Bhatta et al., 2020).

There are several drying methods used in the food industry; choosing one depends on the characteristics desired for the final product and the food matrix that is being applied. In the case of Okara, the methods commonly used are freeze drying, air oven drying with or without forced air, and vacuum oven drying. Table 2.2 shows the mechanism, advantages and disadvantages of each dehydration method used for okara.

Table 2.2 Dehydration methods used for okara

Drying method	Mechanism	Advantages	Disadvantages	References
Freeze-drying	Use of sublimation to remove water of a frozen product High vacuum and low temperatures	Whitest colour Preferred for thermally sensitive compounds	Expensive equipment High energy cost Takes longer time to achieve desired moisture level	Sandhya & Disha, 2020 Inyang et al., 2017 Santos et al., 2019 Bhatta et al., 2020 Ostermann-Porcel et al., 2017a
Oven drying	Application of heat at a constant temperature for a specific period of time	Low cost Shorter drying time Decrease in anti-nutritional factors	Obtain a lower luminosity colour in flour Unpleasant odour sometimes Protein denaturation leading to low solubility	Ozyalcin & Kipcak, 2022 Inyang et al., 2017 Voss et al., 2018
Vacuum drying	Indirect-heat dryer Drying under a low-pressure environment	Reduces risk of oxidation Preserves colour of the product Shorter drying time	More expensive than regular oven drying	Ozyalcin & Kipcak, 2022 Davy & Vuong, 2021 Parikh, 2015

		Less energy needed Avoids heat damage to the product		Inyang et al., 2017

2.3.2.1 Freeze drying (lyophilization)

Freeze drying or lyophilization is a technique used to obtain high-quality food powders that have thermolabile compounds and are susceptible to oxidation (Bhatta et al., 2020). Its mechanism is based on the sublimation phenomenon in which water is removed in its solid state under temperatures and water vapour pressure below 0.01°C and 0.619 kPa. First, the sample must be completely frozen for the primary drying where ice is sublimated at the specified pressure, followed by the secondary drying, where the remaining water is removed from the food matrix (Bhatta et al., 2020). The equipment and conditions used for the production of okara flour are a freeze dryer with pre-frozen stainless-steel trays programmed to 50 µm Hg for 48 hours or a Terroni lyophilizer for 73 hours (Guimarães et al., 2020; Ostermann-Porcel et al., 2017a). Although okara flour obtained by this method presents the lowest final moisture and whitest colour (Santos et al., 2019), the process can take up to 3 days which depends on the amount of product to dry and availability of equipment, resulting in a high energy cost (Ostermann-Porcel et al., 2017a; Santos et al., 2019).

2.3.2.2 Oven drying

Oven drying consists of applying heat at a constant temperature for a specific period (Ozyalcin, & Kipcak, 2022). In an air-circulation oven, okara has been dried at 50 °C or 60 °C for 24 hours or with a two-stage process to reduce drying time to 6 hours by applying 200 °C for 1 hr and 80 °C for the other 5 hours. However, this reduced time process approach could damage the final product due to non-enzymatic browning and protein denaturation (Santos et al., 2019; Moraes Filho et al., 2016; Voss et al., 2018; Hawa et al., 2018). On the other hand, when using temperatures lower than 70 °C, okara flours with lower luminosity and unpleasant odour are obtained (Guimarães et al., 2020) (Yoshida & Prudencio, 2020). The key is to determine the optimal temperature and time combination that has a lesser negative effect on the functional properties of okara.

2.3.2.3 Vacuum oven drying

A vacuum oven has been used to dry okara as it has been more effective to maintain nutritional values, taste, texture, and colour and reduce the risk of oxidation (as cited in Ozyalcin & Kipcak, 2022). For okara flour, the final moisture content of the product varies depending on the temperature and time of process. Under the vacuum pressure at 41.3 kPa; when subjected to 40 °C for 11.6 hours, a moisture content of 7.73% was obtained, for 60°C and 4.4 hours the final moisture was 7%, for 80 °C and 3 hours moisture ended up being 7.6%, and when drying at 100 °C for 2.2 hrs, the moisture content was 6.98%

(Davy & Vuong, 2021). This confirms that the most efficient temperature for drying okara in a vacuum oven or a normal convection oven is 60 °C to obtain a lower moisture content and avoid degradation of nutrients.

Before analysing powder characteristics, milling is required to obtain a uniform average particle size and throughout the product. Milling is used in the cereal industry to grind grains into powders, it reduces the size of particles and usually includes sifting and purification where the typical average particle diameter obtained for wheat flour is 60 µm with a yield of 75 to 80% (Loïc et al., 2023; Thakur et al., 2019). The first milling operation is grinding, where grains are broken into large particles, either in a stone mill or a roller mill, to produce semolina (Loïc et al., 2023). Since okara is already dried, roller mills should be used at the industry level to obtain higher yields, as they apply compressive stress and shear to disintegrate particles. In contrast, hammer mills use impact and is typically used for harder materials (Thakur et al., 2019). In the lab, a Perten 3100 Laboratory mill, which is a hammer mill, has been used to grind okara; this type is an impact mill where a collision between wall and powder particles occurs (Thakur et al., 2019). After grinding, the product obtained is known as semolina which is directed into the sifting operation that consists of separating the particles according to size with different sieves to obtain flour; in the industry is usually done in a plansifter where agitation accelerates the process but in laboratories, meshes are used (Loïc et al., 2023). According to FDA (2024) guidelines, 98% of powder particles have to pass through a 212 µm sieve (US Standard mesh No.70) to be considered flour. When using a laboratory hammer mill, an 800 µm sieve was used, but in another experiment, the size of sieve 125 µm was used; this second powder could be considered more as flour than the one where the hammer mill was used (Thakur et al., 2019; Santos et al., 2019).

2.3.3 Proximate composition of okara flour

Proximate analysis provides information about the components in a food product and allows to compare the amount of nutrients lost after drying. The proximate composition of okara varies depending on the amount of water extracted from the ground beans, the production method, the soybean variety used and its agroclimatic conditions (Li et al., 2012; Stanojevic et al., 2013; Hawa et al., 2018). Table 2.3 shows the chemical composition of okara flours prepared using different dehydration techniques.

Several authors base their proximate analysis on the AOAC methods. Moisture can be determined with the vacuum oven method or hot air oven; ash according to AOAC method 923.03, lipids with Soxhlet extraction, proteins with the Kjeldahl method where first the total nitrogen is determined and then multiplied by a factor of 6.25, carbohydrates by difference and dietary fibre with the enzyme-gravimetric method (Santos et al., 2019; Hawa et al., 2018; Voss et al., 2018). Dry okara is reported to contain 50% fibre, 25% protein and 10% lipid; its protein contains all essential amino acids and has a protein efficiency index of 2.71, which is higher than the 2.11 of soymilk but the proteins have low water solubility (Li et al., 2012; Colletti et al., 2020).

As for the fat content, it can be seen in Table 2.3 that the fat content of okara flour is inconsistent in the literature. Stanojevic et al. (2013) reported a fat content of only a maximum of 2.3%, while Santos et al. (2019) reported 6.3%, which is lower than the commonly reported range of 8-10%. The reason for this wide range of fat content is hypothesized to depend on the genotype of soybean used and the amount of fat retained in soymilk (Stanojevic et a., 2013). The mineral content can be determined by using an atomic absorption spectrophotometer, and ash content typically varies between 3 and 4% (Hawa et al., 2018; Santos et al., 2019)

Table 2.3 Chemical composition (%) of okara flours from different dehydration techniques

Drying treatment	**Moisture**	**Ash**	**Protein**	**Fat**	**Total fibre**	**Insoluble fibre**	**Reference**
AO 80 °C for 5 h	48.19 ± 0.23	1.82 ± 0.22	14.58 ± 0.21	5.66 ± 0.15	17.69 ± 0.88	10.67 ± 0.64	Voss et al., 2018
AO 200 °C for 5 h	44.55 ± 0.45	1.89 ± 0.52	15.31 ± 0.26	6.02 ± 0.11	18.55 ± 1.63	12.75 ± 1.21	Voss et al., 2018
AO 60 °C for 11.5 h	6.46 ± 0.49	2.73 ± 0.68	24.74 ± 0.06	15.96 ± 07	58.27 ± 2.08	44.56 ±1.70	Santos et al., 2019
FRD 48 hrs, 25 at 50 um Hg	3.62 ± 0.49	2.24 ± 2.03	33.39 ± 3.20	18.98 ± 1.23	23.14 ± 9.6	---	Ostermann-Porcel et al., 2017a
Rotary dryer at 150 °C	17.68 ± 2.85	1.80 ± 2.74	30.78 ± 22	14.74 ± 4.04	22.45 ± 1.64	--	Ostermann-Porcel et al., 2017a
FAD 70 C 12 hr	3.32 ± 0.07	3.71 ± 0.11	3.32 ± 0.07	9.12 ± 0.19	60.16 ± 2.15	46.01 ± 1.75	Guimarães et al., 2020

AO represents air oven, FAD represents Forced-air oven, FRD and MWD represents microwave drying

2.3.4 Characterization of okara flour

The global generation of okara in the soy industry has been estimated to be of 14 million tons (Nguyen et al., 2013) with Asian countries leading production. It has been reported that tofu production generates approximately 2,800,000 tons in China, 800,000 tons in Japan, 310,000 tons in Korea and 10,000 tons in Singapore (Vong et al., 2016; Li et al., 2013; Tian et al., 2020).

2.3.4.1 Particle size

The particles of okara flour vary in size and shape. To determine particle size distribution, different-sized sieves are often used as they are simple to use and cheaper than other equipment. The sieves have a wide range of mesh pore sizes from 2 to 125,000 µm and are easy to use by stacking together according to size. First, flour is placed in the first sieve which is the one with a wider opening, usually 1.88 mm, and shaken until the flour stops passing through the sieves. The weight of the sample in each sieve is divided by the total weight to obtain the percentage of the full sample retained per sieve, thus determining particle size distribution (Sonaye et al., 2012).

The size distribution can also be measured with a laser particle analyser where the results are represented as cumulative volume percentage, or with a Mastersizer to obtain the volume mean diameter and surface area (Agrahar-Murugkar et al., 2015; Pang et al., 2021; Jan et al., 2017b). One of the factors that influences the particle size of the flour is the grinding method used. In the case of okara flour, Vishwanathan et al. (2011) used a hammer mill to grind dry okara flakes and reported that the particle sizes were distributed in the range of 37 to 710 µm. On the other hand, Santos et al. (2019), obtained okara flour with particle size no larger than 125 µm by macerating dry okara.

2.3.4.2 Microstructure

Scanning electron microscopy (SEM) has been used for characterizing the microstructure of okara flour. By observing their geometric structure and composition in the micrographs, one could reach certain conclusions on okara's relationship with water, which can later be confirmed in more detail by obtaining the water absorption index. Santos et al (2019) noticed some gaps in the flour's geometric structure, which translate to permeable pores responsible for water absorption; they demonstrated this relationship with water using water absorption index.

2.3.4.3 Water absorption index

It is important to understand water absorption index (WAI) because it helps us hypothesise the functionality of flour as a food ingredient, as it depends on its interaction with water when used in food products. The WAI can be used to analyse the flour's functional differences depending on the dehydration technique used. Flours with a good WAI are ideal for use in bakery products and viscous food. In the case of bakery products, more water added to the dough results in better texture, crumb, and freshness of bread, whereas in viscous foods such as soups and custards, it develops the body and thickening without affecting its proteins (Sreerama et al., 2012).

2.3.4.4 Colour

The colour of a product can influence the decision of a consumer. In bakery products, a uniform light yellow is attractive for consumers. Therefore, if okara flour is used as a substitute for wheat flour, the colour must be emulated, starting by choosing the right drying technique to avoid significant disparities in colour and the Maillard reaction in flour. The instrument used to measure colour in flour is a colour spectrophotometer (Guimarães et al., 2020; Davy & Vuong, 2021; Santos et al., 2019).

2.3.4.5 Water activity and density

Water activity is usually measured, as it provides information for preservation techniques, while the bulk density, tapped bulk density and Carr's compressibility index provide information on the tendency to compress and the flowability of flour (Davy et al., 2022).

Bulk density is defined as the amount of mass that can be packed in a specific volume (Abdullah & Geldart, 1999; Awuchi et al., 2019). This functional property is often measured in powders like flours to decide the packaging needs for the food product (Iwe et al., 2016). Even though the bulk density is helpful to select the design, size and even material of the packaging needed, the information is incomplete. Powders can behave differently when pressure is applied, this is the reason why tapped density is also obtained (Sengar, 2022).

Tapped density is the density obtained after the powder has been subjected to a compression process consisting of constant tapping which results in the rearrangement of the powder particles (Awuchi et al., 2019; Amidon et al., 2009). The bulk density is larger than the tapped density as it doesn't consider compression effects.

These 2 densities are used to determine the Carr's compressibility index, used to indicate the flowability of the powder (Hao, 2015; Davy et al., 2022). By understanding the flowability and how the particles are packed, the processing and manufacturing stages can be set up successfully. For example, if the chosen powder has a poor flowability, it will slow down the conveying lines due to clogging or if it its tightly packed, the amount of packaging needed is smaller (Bian et al., 2015; Hao, 2015).

2.3.5 Application of okara as food ingredients

Okara hasn't been used as a 100% substitute for wheat flour in food products, most projects have focused on a mixture of okara and wheat flour for cookies (Hawa et al., 2018; Ostermann-Porcel et al., 2017b), bread (Ostermann-Porcel et al., 2017c), and noodles (Kang et al., 2018). However, the drying technique hasn't been standardized. Table 2.4 shows the food products in which okara has been utilized as a food ingredient in different studies, which ingredient is partially substituting, and the amount of okara used.

Table 2.4 Application of okara as an ingredient in various food products, including amounts and purposes.

Food product	% okara	Purpose	Reference
Cookies	40,43,44,45,46,47,49 & 50	Partial substitute of wheat flour, in combination with teff flour	Hawa et al., 2018
Cookies	50,30 & 15	Partial substitute of wheat flour	Ostermann-Porcel et al., 2017b
Noodles	0,5,10 & 20	Partial substitute of rice flour	Kang et al., 2018
Steamed bread	0,5,10,15,20,25,30,35 & 40	Partial substitute of wheat flour	Lu et al., 2013a
Cake	0,25,50,75,100	Wheat flour substitution	Lee et al., 2020
Frankfurt sausages	1.5 & 4	Partial substitution of meat protein	Grizotto et al., 2012
Pork meat gels	5.5,16.5, 27.6,38.7 & 50	Substitution of pork fat	Chang et al., 2014
Peanut butter	5,15,20 & 25	Substitution of peanuts	Nasution et al., 2012
Beef patties	7.5, 15, 22.5, 30 & 37.5	Substitution of beef meat	Turhan et al., 2007
Wheat Bread	0,5,7.5 & 10	Partial substitute of wheat flour	Ostermann-Porcel et al., 2017c

The "% of okara used" refers to the proportion of the ingredients that okara is substituting, rather than the percentage of overall product composition. In the case of beef patties, wet okara was used instead of okara flour as in the other products.

Bread made with a 10% substitution with okara flour had the same sensory and physicochemical characteristics as regular bread with a higher protein and fat content. When substituting 25%, it needs fortification with gluten (Li et al., 2012; Davy & Vuong, 2022).

In contrast, a combination of 50% okara, 40% red teff and 10% wheat flour was not successful in cookies as the moisture content was increased due to okara's ability to hold more moisture. Biscuits also showed negative responses in volume and texture, which is hypothesized to be due to the absence of gluten in okara (Davy & Vuong, 2022). However, in the case of muffins and cakes, the volume and sensory results were better than those of commercial gluten-free flour (Davy & Vuong, 2022) (Hawa et al., 2018).

For rice noodles, a 15% substitution of okara has been used, along with alginate before cooking. This combination helped to avoid negative textural effects in the final product (Kang et al., 2018). In pasta production, okara hasn't been used while other vegetable powders have been utilized. This presents another area of research opportunity.

Okara could be used to provide more fibre to bakery products. Some bakeries that are connected to tofu shops revalorize wet okara by adding it to bread, muffins, and brownies. This approach enhances the fibre content of their products without the need to purchase bran, but it can result in a crumbly texture (Tripathi & Shrivastava, 2017).

Okara has also been used in meat products as a fat and meat replacement. Grizotto et al (2012) investigated the effectiveness of substituting 1.5 and 4% of meat okara flour in frankfurter sausages because soy protein has been previously successful in this type of food product. The sausages containing okara flour presented no negative effects on quality. Their emulsion stability, firmness, colour, odour, taste and texture presented no significative difference to sausages without okara flour. Another example is the use of okara flour as a fat replacer in pork sausages. Chang et al. (2014) decided to substitute pork fat al levels from 5.5 to 50% with okara flour to improve the dietary fibre content of sausages. The substitution was favourable because the sausages' springiness and resilience wasn't affected by the okara flour addition. Also, their water holding capacity improved due to okara flour helping retain more moisture. The effect okara flour had on water holding capacity was also noticed by Turhan et al. (2007) in beef patties when substituting beef meat from 7.5 to 37.5%. However, the substitution had a negative effect on the colour and flavour of beef patties when high amounts of okara were used. To sum up, okara flour can not only be used successfully as a partial substitution of wheat flour in bakery products and noodles but also as a meat and fat replacement in meat products

2.4 Conclusions

The purpose of this literature review was to understand okara as a by-product of tofu production, how it is obtained, and the importance of revalorizing by-products for food security. It highlights the opportunities that okara brings to develop new products and its composition after being dried into flour. Currently, okara is often discarded as landfill or used as livestock feed, rather than revalorizing it and taking advantage of its nutritional benefits, including protein, fibre, and vitamins.

It is important to revalorize okara as a novel food ingredient to ensure food security for future generations, minimizing nutrient wastages and providing more plant protein options. However, its high-water content has limited its applications. Hence, the best route to stabilize okara has been drying, which allows the production of a gluten-free flour alternative that is high in fibre.

While freeze-drying and convection oven methods are the most popular, the optimal drying technique has yet to be determined. One of the focuses of this study was to determine which drying method is actually the most effective by comparing drying time and physicochemical properties of the okara flours. The physicochemical properties evaluated were proximal analysis, particle size, microstructure, water activity, solubility, bulk density, tapped density, flowability and colour.

The okara flour can be considered a gluten-free flour and has many promising applications as a food ingredient, specifically in bakery products. However, the incorporation of okara flour as a replacement of wheat flour in the development of bakery products can be tricky. Firstly, gluten-flours flours cannot fully replicate the functionality of gluten in bakery products which results in products with less volume and texture problems. Secondly, okara flour is high fibre which results in an increased level of moisture needed during formulation. Therefore, modifications to standard formulations are necessary, including adjustments to hydration levels and the incorporation of hydrocolloids.

Although some researchers have successfully incorporated okara flour as a partial substitution of wheat flour in cookies, biscuits and breads, there is no report yet on the use of okara flour as a complete replacement of wheat flour in bakery products. The maxim substitution of other flours with okara flour in cookies has been of 50% and 10% for bread. Thus, the objective of this project was to explore the possibility of revalorising okara as an ingredient and use it as a complete replacement in bakery products and other food products.

Chapter 3. Physicochemical Evaluation of Okara powders Obtained Under Different Drying conditions.

3.1 Abstract

The popularity of gluten-free products has increased the demand for a wider variety of gluten-free flours that are not only cheaper but also, functional and nutritionally superior to those currently available on the market. The aim of this study was to determine which drying methods and conditions are optimal to produce functional and nutrient-rich okara powders from fresh okara by comparing drying temperatures and times and physicochemical characteristics of okara powders obtained through freeze-drying and convection drying at different temperatures (50, 60, 70, 80, 90 and 100 °C). The physicochemical characteristics evaluated included proximate compositional analysis (moisture, ash, fat, protein, fibre, and carbohydrates), particle size distribution, microstructure, water activity, water holding capacity, water solubility, bulk density, flowability and colour. Drying curves were constructed for convection drying to compare the time taken to reach moisture equilibrium at each temperature. The fastest drying conditions were observed through convection drying at 90 °C and 100 °C with almost half the drying time required at 50 °C. This was due to increased heat and mass transfer at higher temperatures, leading to a greater drying rate. All samples successfully, achieved a moisture content of less than 14% and a water activity of less than 0.62, which are recommended levels for flour stability. The different drying treatments had minimal impact on the nutritional composition as all samples exhibited similar protein and ash content with variations in fat and fibre content. Okara powder proved to be a good alternative to wheat flour in terms of nutrient content as it contains more protein and fibre, as well as essential amino acids and fatty acids. Its high fibre content was reflected in its microstructure, where fibre structures dominated. Particle size distribution varied among the drying treatments, which was also reflected in the bulk density results. Smaller particle sizes correlated with higher bulk density, particularly for the samples treated at 80 °C and 90 °C. Although okara flour has good water holding capacity, the freeze-drying sample presented the best results due to its porous and capillary structure. In terms of colour, all samples were quite similar, with the best condition for achieving a bright powder being convection oven at 70 °C. Overall, taking into account all physicochemical factors and drying time, the best drying conditions for obtention of okara powder are freeze drying and convection oven at 90 °C and 70 °C.

3.2 Introduction

Gluten-free products have been gaining popularity in the market as part of the healthy foods trend, but their origin can be due to the rise in celiac disease diagnoses in the population (Woomer & Adedeji, 2021). Celiac disease currently affects approximately 1% of the global population. It is defined as an immune-related disorder that affects the absorption of nutrients and is triggered as a response to the ingestion of gluten (Green & Cellier, 2007; Fasano & Catassi, 2012; Ostermann-Porcel et al., 2017a). The common symptoms of celiac disease in the body are chronic diarrhoea, frequent abdominal pain, and chronic fatigue. Gluten is a protein complex rich in glutamine and proline amino acids; and it is found in wheat, rye, and barley (Fasano & Catassi, 2012; Green & Cellier, 2007). As mentioned, celiac disease is triggered by gluten ingestion, so the only effective treatment is a strict gluten-free diet, which has led to a greater demand for gluten-free food products in the market (Ostermann-Porcel et al., 2017a; Green & Cellier, 2007; Caio et al., 2019).

The production of gluten-free flours has been one of the main approaches in response to the increasing demand for gluten-free products because wheat flour is used in the production of many food products such as pasta, breakfast cereals, puffed snacks and bakery products (Gasparre & Rosell, 2023; Woomer & Adedeji, 2021). This provides consumers who suffer from celiac disease an opportunity to consume the food products that normally contain wheat flour (Davy & Vuong, 2022). Although wheat flour alternatives from grains like rice, manioc and maize are available, gluten-free products lack the nutritional characteristics of those containing gluten. These alternatives have a lower content of protein, fibre, minerals, and vitamins. Additionally, other challenges with following a gluten-free diet include high cost of alternative products, restricted variability, limited availability, and product quality defects. For these reasons, the use of legumes and pseudocereals as gluten free flours is increasing (Demirkesen & Ozkaya, 2022; Ostermann-Porcel et al., 2017a). Pseudocereals such as quinoa, amaranth, buckwheat, and millet can be used to address fibre deficiency in gluten-free diets. On the other hand, legumes are a better nutritional alternative as they are considered a source of micronutrients, complex carbohydrates, fibre, antioxidants, and protein. In terms of protein quality, legumes have higher concentrations of lysine, arginine, leucine, aspartic and glutamic acid than cereal proteins (Demirkesen & Ozkaya, 2022; El Khoury et al., 2018). Soybean has been one of the legume choices due to their foaming, emulsifying and gelation properties, high protein quality by containing all essential amino acids, and ability to reduce cholesterol and triglycerides (Gasparre & Rosell, 2023; Ostermann-Porcel et al., 2017a). For these reasons, soy-based foods have gained the interest and awareness of consumers. Additionally, food by-products are underutilized, along with the high cost of producing gluten-free products and limited availability (Feng et al., 2021; Demirkesen & Ozkaya,2022), okara can be considered a good alternative to wheat flour.

Okara is a by-product of soymilk and tofu production. Although the chemical composition of okara varies on the soybean variety and processing conditions used, fresh okara is composed on average of 80% water, 2.6 to 4% protein and other solids such as fibre (Colletti et al., 2020; Liu, 2008). Once dried, fibre is the main component of the dry matter, specifically insoluble fibre (Colletti et al., 2020; Feng et al., 2021). In terms of protein, okara has better protein quality than soymilk as shown by the protein efficiency index, 2.71 and 2.11, respectively. Okara contains all essential amino acids, and its main proteins are globulin 7S and globulin 11S (Colletti et al., 2020; Privatti et al., 2023). The fat content in okara consist primarily of unsaturated fatty acids specifically linoleic acid, linolenic acid and oleic acid (Privatti et al., 2023; Feng et al., 2021; Colletti et al., 2020). The main limitation in trying to revalorize okara as a food ingredient is its prone to rapid deterioration and oxidation due to its high moisture content. One solution is to dry the okara. Drying is one of the most used preservation methods because it reduces the moisture content of food while retaining most of its original nutritional content (Ostermann-Porcel et al., 2017a; Davy et al., 2022; Guimarães et al., 2018). There are several drying methods and conditions, and it is necessary to study which one is better in terms of nutritional content, powder functionality, and energy demand.

Nevertheless, the best method for dehydrating okara hasn't been established universally. Some studies have focused on comparing freeze drying with forced air oven or normal convection oven at a single temperature setting, rather than a full range of temperatures. Hence, this study was carried out to determine the optimal dehydration technique and conditions for producing okara powder. This was conducted by comparing the effects of freeze drying and convection oven drying at temperatures of 50, 60, 70, 80, 90 and 100 °C on the physicochemical properties of the resulting okara powder. The specific objectives were to compare drying time, proximate composition, colour, solubility, flowability, bulk density, microstructure, and particle size distribution for each treatment.

3.3 Materials and Methods

3.3.1 Materials

The fresh okara used in this project was provided by the Tonzu company located in Auckland, New Zealand. After being collected from the factory, the okara was divided into aluminium trays (Confoil, New Zealand), covered with aluminium foil, labelled and frozen at -20°C until further use.

3.3.2 Drying of okara

The frozen okara was dried using two different methods: freeze drying and oven drying. For freeze drying, the freeze dryer (Labconco FreeZone® Freeze Dry System) was set in an auto program for 7 days where the vacuum pressure was 0.1 mBar and the temperature -52 °C. The vacuum pressure, collector temperature and tray temperature were monitored and recorded of 3 times per day. The samples were prepared by poking holes in the aluminium foil covering the already frozen okara samples and placed in stainless steel trays that were collocated in the freeze dryer when it achieved a high vacuum pressure and -50 °C. For oven drying, three trays of frozen okara without the aluminium foil were collocated inside the oven (ESCO Isotherm Forced Convection Laboratory Oven, equipped with air circulation) once it reached the designated temperature. Every 2 hours, the trays were weighted in an analytical balance (KERN KB Precision Balance) to monitor the weight loss. The end point of the drying time was determined when the trays didn't lose any more weight. The six designated temperatures selected were 50, 60, 70, 80, 90 and 100 °C. The samples obtained at the end of drying were labelled as: O50, O60, O70, O80, O90, and O100, respectively. The okara dried by freeze drying was labelled as FD. The moisture content of okara after drying was <10%. After drying, samples were milled to obtain okara flour.

3.3.3 Milling of okara

The milling of dried okara was done in two stages. First, the dried okara was ground in a food processor (Breville Kitchen Wizz 11 Plus Food Processor) using the quad blade and the 'ON' setting for 15 minutes to reduce the size of the samples. Then, it was ground into a fine powder using a coffee grinder (Bistro Electric coffee grinder) on the fine grinding setting. The okara flour obtained was placed in Mylar bags and stored in the fridge at 2-4 °C.

3.3.4 Proximate composition

All dried okara powder samples were analysed to determine moisture, ash, fat, protein, dietary fibre and carbohydrate content through proximate analysis. All samples were analysed in duplicate.

3.3.4.1 Moisture

The moisture content was determined using the air-oven method based on AOAC Method 925.09 for flour (AOAC, 2023).

3.3.4.2 Ash

Ash content was determined using the dry ashing method, following AOAC method 923.03, with a muffle furnace (NEY A-550 Vulcan Muffle Furnace). The sample size used was 3 grams (AOAC, 2023).

3.3.4.3 Fat

Fat content was determined using the Mojonnier method, with diethyl ether and petroleum ether as solvents for fat extraction. Two extractions were conducted accordingly. After decanting the extracted fat, the solvents were evaporated at 38 °C. Finally, the extracted fat was dried in the air oven (Thermo Scientific HERATHERM oven) at 100 °C for 10 minutes.

3.3.4.4 Protein

The protein content was determined according to AOAC Official Method 954.0. First, the nitrogen content was determined using the Kjeldahl method with the Kjeltec™ 8100 distillation unit, Digestor™ 2520 and 2501 Scrubber Unit.

The nitrogen content, when H_2SO_4 is used for digestion, was calculated using Equation 1

$$\%N = \frac{[(mL\ standard\ acid * 2 * molarity\ of\ acid) - (mL\ standard\ NaOH * molarity\ NaOH)] * 1.4007}{g\ sample} \qquad \text{Equation 1}$$

After determining nitrogen content, the conversion factor of 5.8 was applied. This conversion factor corresponds to soybean as a source of plant protein (Orts et al., 2019).

3.3.4.5 Dietary fibre

The dietary fibre content was determined using the Megazyme, AOAC 991.43 method, which was performed at the Nutrition Laboratory of Massey University in Palmerston North, with the samples sent to the laboratory for analysis.

3.3.4.6 Carbohydrates

The carbohydrate content was determined by difference (Santos et al., 2019; Hawa et al., 2018). Once the fat, protein, moisture, ash, and dietary fibre contents were determined and expressed as percentages, they were subtracted from 100 to calculate the percentage of carbohydrates in the product (FSANZ, 2020).

3.3.5 Fatty acids, minerals, sugars and amino acids content

Two batches of freeze-dried okara were sent to the Nutrition Laboratory at Massey University in Palmerston North for analysis of sugars, starch, amino acids, fatty acids, and mineral composition.

3.3.5.1 Sugar content

Sugar content extracted using 80% boiling ethanol. The extracted sugars in the ethanolic solution were then quantified using the phenol sulphuric acid method which can detect monosaccharides, disaccharides and a bit of oligosaccharides (Hall et al., 1999). The wavelength of absorption used was 490 nm.

3.3.5.2 Mineral composition

The mineral composition was determined using inductively coupled plasma-mass spectrometry (ICP-MS).

3.3.5.3 Starch content

Starch was determined using the α-amylase Megazyme kit, following AOAC method 996.11, with diluted thermostable α-amylase' and performing two dilutions. The sample sizes used were 0.0791grams and 0.0941 grams for batch 1 and 2, respectively.

3.3.5.4 Amino acids

Amino acids content was determined with the Performic Acid Oxidation with Acid Hydrolysis-Sodium Metabisulfite method, following AOAC 994.12. It stated with the performic acid oxidation, followed by the addition of sodium metabisulfite and hydrolysis with 6 M HCl. For the first batch of freeze-dried okara powder, it was done by duplicate with 2 sample sizes (0.00583 and 0.00931grams), while for the second batch was done in a single rep (0.00758 grams).

3.3.5.5 Fatty acids

Fatty acids content was determined using an in-house method based on GCL Sukhija & Palmquist (1988) and closely following AOAC 996.01, 996.06. First, fat was extracted from okara powder samples by acidic hydrolysis with HCl into ether, followed by methylation to obtain fatty acid methyl esters (FAMEs). Fatty acids were measured using a gas chromatograph (GC) with capillary split/splitless injection system and flame ionization detector. The operating conditions of the GC were as follows: injector temperature, 250°C; flame ionization detector temperature, 275°C; inlet temperature, 250 °C; H2 flow, 40 mL/min; air flow, 400 mL/min; split ratio, 1:25 ; carrier gas, hydrogen; make up gas, nitrogen; oven gradient, 140 – 240 °C and linear velocity, 15.9 cm/s. The sample sizes used were 0.20236 grams and 0.20787 grams for batch 1 and 2, respectively.

3.3.6 Characterisation of physicochemical properties of okara flour

3.3.6.1 Particle size and size distribution

The particle size of okara flour samples was characterized using the Malvern Mastersizer 3000. This instrument provided information on the average size in terms of $D_{3,2}, D_{4,3}$ as well as cumulative volumes that indicate particle distribution, such as D_{10}, D_{50} and D_{90} (Pang et al., 2021; Agrahar-Murugkar et al., 2015). The instrument was used for two determinations: the particle size of okara powder in its dried state and the particle size of okara in solution (okara suspension). For the powder measurements, the powder was placed in the hopper of the powder unit (Aero S). The refraction index used for okara flour was 1.45 as this is the refraction index of solid soy products (Syll et al., 2013), and 0.1 was used for the absorption coefficient, which is the value assigned to slightly coloured powders (Malvern Panalytical, 2017). For the okara suspension, 0.5 g of okara powder were mixed with 10 mL of water and kept at 25 °C for 30 min, the unit used was the Hydro SM with a manual stirrer speed of 1,500 rpm and an obscuration range of 8 to 12. Each of the samples was measured in both powder and suspension modes.

3.3.6.2 SEM Microstructure

A scanning electron microscope (SEM) (Hitachi TM3030 Plus) was used to observe the microstructure of the okara powder samples. The samples for SEM were prepared by placing them on a specimen stub with double sided carbon tape and putting the powder on it, making sure it sticks to the tape. Once this was done, the samples were transferred to the chamber, where the vacuum was activated, and a 15 kV voltage was selected for the beam. SEM imagining was then performed at various magnifications to analyse the shape, size and surface morphology of the samples.

3.3.6.3 Water activity

Water activity was measured using the AQUALAB 4TEV. The instrument was warmed up for 15 minutes before use. Once ready, a cell was filled to half capacity with okara powder. The water activity was measured in triplicate for each sample.

3.3.6.4 Water Holding capacity (WHC)

Water Holding Capacity was determined following the method proposed by Umaña et al (2013). One gram of okara powder was mixed with 30 mL of water using a magnetic stir bar, transferred into a centrifuge tube and left to hydrate for 18 hours at 20°C. Then, the tubes were placed in a refrigerated benchtop centrifuge (Sigma 6-16KS) at 2000 rpm, 25 °C for 30 minutes. The humid residue was collected and dried at 100 °C for 24 hours in an oven (ESCO Isotherm Forced Convection Laboratory Oven). Water holding capacity was calculated using Equation 2.

$$WHC = \frac{weight\ of\ humid\ residue - weight\ of\ dry\ residue}{weight\ of\ dry\ residue} \qquad \text{Equation 2}$$

3.3.6.5 Solubility

Solubility was determined following the method proposed by Umaña et al (2013). One gram of okara powder was mixed with 30 mL of water using a magnetic stir bar, transferred into a centrifuge tube and left to hydrate for 18 hours at 20°C. Then, the tubes were placed in a refrigerated benchtop centrifuge (Sigma 6-16KS) at 2000 rpm, 25 °C for 30 minutes. The dry residue was collected and dried at 100 °C for 24 hours in an oven (ESCO Isotherm Forced Convection Laboratory Oven). Solubility was calculated using Equation 3.

$$solubility = \frac{weight\ of\ sample - weight\ of\ dry\ residue}{weight\ of\ sample} \qquad \text{Equation 3}$$

3.3.6.6 Bulk density and tapped density

The bulk density of okara flour was determined following the method used by Koç et al (2014).

Twenty grams of okara flour were transferred into a 100 mL graduated cylinder. The sample weight was then divided by the volume it occupied in the cylinder (Equation 4).

$$Bulk\ density = \frac{Mass\ of\ powder}{Volume\ of\ powder} \qquad \text{Equation 4}$$

The tapped density was determined by tapping the cylinder containing twenty grams of okara flour 100 times by hand on a flat surface (Koç et al. 2014., Bala et al. 2020). The sample weight was then divided by the volume obtained after tapping (Equation 5).

$$Tapped\ density = \frac{Mass\ of\ powder}{Volume\ of\ tapped\ powder} \qquad \text{Equation 5}$$

3.3.6.7 Flowability

The flowability was determined based on the Carr Index and flow character table shown in Appendix 4. The Carr Index was calculated using the equation 6.

$$CI = \frac{Bulk\ density - tapped\ density}{tapped\ density} * 100 \qquad \text{Equation 6}$$

3.3.6.8 Colour

The colour of okara powders was determined using a colorimeter (Konica Minolta CR-200 Chroma Meter) based on the CIELAB colour space coordinates. The colour measurement was conducted using illuminant C as the light source, including spectacular reflection. Before starting the measurements, the colorimeter was calibrated using the CR-A47 white calibration plate. For the measurement, the sample was placed in a cell, which was then positioned above the measuring head of the CR-200. Each measurement was repeated three times per cell, with three cells measured per okara powder sample, resulting in with a total of nine readings per sample. The values obtained were expressed in terms of L*, a*, b*, C* and h. Each coordinate helps determine the colour, L* refers to lightness ranging from 0 (black) to 100 (white), a* represents the spectrum from green (-a) to red (+a), b* indicates the spectrum from blue (-b) to yellow (+b), C* is chroma, which indicated colour intensity, and h represents the hue angle. The total colour difference (ΔE) was calculated using equation 7.

$$\Delta E_{ab} = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2} \qquad \text{Equation 7}$$

3.3.7 Data analysis

The data obtained for each physical property (water activity, solubility, bulk and tapped density, flowability and colour) and the proximate analysis were analysed using Minitab 18 Software. The means were compared using one-way analysis of variance (ANOVA) and Tukey's range test to determine if they were significantly different with an α=0.05 ($p < 0.05$).

3.4 Results and Discussion

3.4.1 Drying

Drying is one of the most widely used preservation techniques in the food industry. By removing moisture from the sample, it reduces moisture-related deterioration reactions, such as microbial growth. In the case of oven drying, moisture removal occurs through mass transfer in which heat is transferred to the interior of the food product and the internal moisture is released to the surface (Inyang et al., 2017).

The average moisture content of fresh okara before drying was 78.01 ± 1.25. The moisture content achieved after drying treatments was <10% depending on the heat treatment methods and conditions used. Figure 3.1 presents the drying curves illustrating the rate of moisture removal using a convection oven at temperatures of 50, 60, 70, 80, 90 and 100 °C. It was observed that the samples followed a similar pattern with a rapid weight loss within the first 5 hours. Subsequently, the rate of weight loss slowed down, varying depending on the drying temperature, before plateauing with no further significant weight loss. This marked the end point of the drying process. This endpoint was reached when the sample's weight remained constant. This behaviour can be explained through drying kinetics. The first stage of drying can be perceived as the warm-up period where the drying rate is the highest followed by the second stage, the fall period, where the rate slows down due to insufficient water movement to maintain the initial rapid evaporation rate. Finally, the last stage is when the moisture content reaches equilibrium and remains constant, meaning that the drying has stopped (Lazarin et al., 2020).

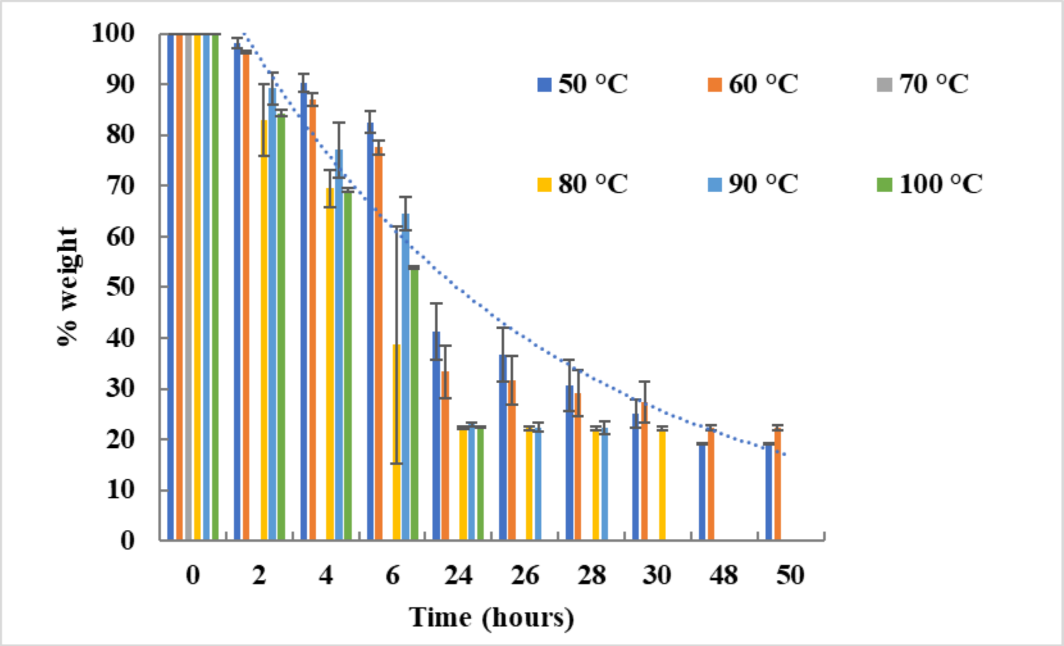


Figure 3.1 Drying curves showing the weight loss of fresh okara during convection oven drying at 6 different temperatures (60 °C, 70 °C, 80 °C, 90 °C and 100 °C). n=4 for all temperatures. Error bars represent +/- SD.

The differences in drying rates during the drying period can be attributed to the type of water being removed. Plant-based foods, such as okara, are hygroscopic and porous, and their water can be classified into free and bound water. Free water, located near the surface, is more easily removed than bound water because it evaporates readily. Once the surface moisture is removed, the drying rate decreases because the critical moisture content is reached. At this stage, the remaining water, known as bound water, is more tightly bound to the material. Bound water is strongly bonded to proteins and polysaccharides due to its greater affinity for polar groups, requiring more energy to remove compared to free water (Khan et al., 2017; Kerr, 2019).

The drying (weight loss) rate also appears to depend on the temperature used. Table 3.1 presents the time taken for each temperature to lose 50% of weight and to reach the plateau or endpoint. At the higher temperatures, such as 90 °C and 100 °C, the end point was reached in less than 30 hours, while drying at 50 °C required up to 50 hours. Guiné et al. (2020) reported similar findings regarding the pattern of drying times when drying the petals of *Cynara cardunculus* at different temperatures: using 35°C required more 10 more hours to achieve ideal moisture content compared to 65°C. Drying kinetics are largely influenced by drying temperature and material thickness. In this study, the thickness of samples used was constant, making the temperature the most significant factor. When drying temperatures are increased, the drying rate increases due to more rapid heat and mass transfer, resulting in faster water loss and reduced drying time to reach equilibrium moisture (Krokida et al., 2003; Doymaz, 2005, Shi et al., 2021; Inyang et al., 2018, Gumus & Ketebe, 2013). In this study, the sample size used was 580 grams per tray with a thickness of 34 mm. If the drying time needs to be reduced further, smaller sample sizes could be used, as drying time is also dependent on material's thickness.

Table 3.1 Time taken to lose 50% of weight and to reach the plateau phase using different convection oven drying temperatures.

Sample	Approximate time taken to lose 50% of weight (hours)	Time taken to reach plateau phase (hours)
O50	16	48
O60	12	48
O70	10	50
O80	5	30
O90	8	28
O100	7	24

Table 3.2 shows the total drying time for each drying treatment and initial and final moisture. Apart from 90 °C and 100 °C taking the least time to lose water, these temperatures also achieved the lowest moisture content at 1.75%. Observing the results shown in Figure 3.1, the three lowest temperatures (50, 60, 70°C) exhibited a similar drying rate, however, drying at 50 °C had the greatest difficulty in moisture loss (i.e. weight reduction), taking almost 10 hours to lose just 10% of the initial weight. It appears as though the drying process was initially hindered before reactivating to a more constant rate. This could be attributed to 50 °C being too low for efficient water evaporation and the increased difficulty of removing bound water at lower temperatures compared to higher ones.

Table 3.2 Initial and final moisture and drying time of okara powders prepared using different drying conditions

Sample	Moisture before drying (%)	Moisture after drying (%)	Drying time (hours)
FD	77.08 ± 0.11	8.46±0.06	170
O50	80.76 ± 0.15	5.47±0.04	50
O60	77.67 ± 0.55	5.49±0.06	50
O70	77.23 ± 0.17	2.50±0.02	48
O80	77.85 ± 0.31	4.70±0.32	30
O90	77.92 ± 1.37	1.75±0.35	28
O100	77.54 ± 0.11	1.75±0.35	24

3.4.2 Proximate Analysis

Okara powders prepared by two different methods, such as air oven drying at various temperatures and freeze drying, were analysed for proximate composition, as shown in Table 3.3. The moisture content of freeze dried okara sample was 8.46%, while it ranged from 1.75% to 5.49% for the samples dried using the air oven method at different temperatures. The okara powder samples with the lowest moisture content after drying were O70, O90 and O100, indicating higher moisture loss as the drying temperature increased. The ash and protein contents were consistent across all okara powders ($p > 0.05$), averaging approximately 4% and 31%, respectively. The fat content of all samples was around 17%, except for O60 that has a significantly lower fat content of 13.5%. The carbohydrate content ranged from 40 to 46%, primarily composed of dietary fibre (37 -43%), with minimum amounts of starch (0.25%) and sugars (2.6%).

The moisture content in okara powders varies with the drying conditions applied, as shown in Table 3.3. A moisture content of less than 10% is recommended to ensure the shelf stability of flour (Guimarães et al., 2020). In this study, all okara samples met this criterion, with the most effective drying treatment achieved using a convection oven at 90 °C or 100 °C. In comparison to other studies on the revalorization of fresh okara into powder, Davy et al. (2022) reported a similar moisture value (8.6%) to the freeze-drying sample (FD) in this study when using an oven at 100 °C. In studies using freeze-drying, Ostermann-Porcel et al. (2017a), Guimarães et al. (2020) and Lu et al.(2013b) reported moisture contents in the range of 2.97% to 6.7%, which were lower than the moisture content of the freeze-dried sample (8.6%) in this study. Extending the freeze-drying treatment in this study might have further reduced the moisture content. Santos et al. (2019) and Grizotto et al. (2010) obtained similar results (6.46%, 6.88% and 6.51%) comparable to O60 and O70 using convective drying at 60 °C and flash drying, respectively.

It appears that ash and protein contents were not affected by the drying conditions. The results of this study are similar to those obtained by Davy et al. (2022), Voss et al. (2018), Ostermann-Porcel et al. (2017a) and Guimarães et al. (2020).

Regarding fat content, several studies reported by Davy et al. (2022), Voss et al. (2018), Guimarães et al. (2020) and Lu et al. (2013b) showed lower fat content in dried okara (13.8%, 11.14%, 9.49% and 5.90%), while studies conducted by Ostermann-Porcel et al. (2017a), Santos et al. (2019) and Grizotto et al. (2010) reported fat content similar to that in this study. Among several factors, the variation in fat content could be attributed to differences in the soybean cultivars used to obtain the okara and the method used for fat determination. The Mojonnier method was used in this study, while the other authors used Soxhlet extraction (Privatti & Rodrigues, 2023).

Table 3.3 Proximate composition of okara powders prepared using different drying conditions.

Sample	CHOs (%)	TDF (%)	Protein (%)	Fat (%)	Ash (%)	Moisture (%)
FD	40.17±0.00^{c}	37.85±0.92^{c}	31.09±0.04^{b}	16.56±0.27^{a}	3.72±0.36^{a}	8.46±0.06^{a}
O50	42.36±0.47^{b}	39.46±0.47^{b}	31.68±0.30^{ab}	16.71±0.01^{a}	3.79±0.23^{a}	5.47±0.04^{b}
O60	45.49±0.81^{a}	42.59±0.82^{a}	31.68±0.30^{ab}	13.55±0.26^{b}	3.79±0.20^{a}	5.49±0.06^{b}
O70	45.13±0.34^{a}	42.23±0.34^{a}	31.84±0.02^{ab}	16.90±0.13^{a}	3.80±0.21^{a}	2.50±0.01^{c}
O80	42.34±0.35^{b}	39.44±0.35^{b}	31.84±0.21^{a}	16.93±0.50^{a}	4.18±0.26^{a}	4.70±0.32^{b}
O90	46.12±0.02^{a}	43.22±0.02^{a}	31.96±0.05^{a}	16.57±0.36^{a}	3.60±0.08^{a}	1.75±0.35^{c}
O100	45.14±0.59^{a}	42.24±0.60^{a}	31.95±0.06^{a}	17.17±0.26^{a}	3.99±0.44^{a}	1.75±0.36^{c}

- Values are presented as mean ± standard deviation; for moisture, ash, fat, and protein (n=2); carbohydrates were obtained via difference. TDF for FD (n=2), for the rest of the samples was calculated based on FD.
- TDF: Total dietary fibre. CHOs: Carbohydrates
- Superscripts with the same letter within the same column indicate no significant differences. ($p < 0.05$).
- FD, okara dried by freeze-drying; O50, O60, O70, O80, O90 and O100 are okara dried in a convection air oven at 50, 60, 70, 80, 90, and 100 °C, respectively.

Similarly, the fibre content differed from those reported in other studies. The okara powders prepared and analysed by Ostermann-Porcel et al. (2017a) and Grizzoto et al. (2010) contained about half of the total fibre reported in this study even though different drying conditions were used, such as freeze-drying, forced air oven, microwave, and flash drying. In contrast, Santos et al. (2019) reported 58.27% fibre but had lower protein content, while Guimarães et al. (2020) reported 60% and Lu et al. (2013b) reported 58%, but both had about half the protein and fat content reported in this study. Although it is not certain, the difference in fibre content is likely due to the processing of the soybeans during soymilk preparation; using non-decorticated soybeans results in higher fibre content (Grizotto et al., 2010). The decortication of soybeans involves removing the hull or pericarp from the cotyledon. The hull, composed of 86% complex carbohydrates, contains insoluble fractions like pectin, hemicellulose and cellulose, making it a significant source of dietary fibre. In fact, soy bran, a product of soybeans hulls, has a higher fibre content, so using decorticated soybeans for okara production results in lower fibre content (Johnson, 2008; Peng & Sun, 2010; Riaz, 2016). Additionally, in all studies where a distinction was made between insoluble and soluble fibre, the content of insoluble fibre was much higher, as okara is the insoluble residue obtained in the production of soymilk and tofu (Guimarães et al., 2020).

Since okara powder is intended to be used as a wheat flour substitute and it has been stated that gluten-free flours such as maize, rice and manioc are nutritionally poorer than wheat flour as they have lower protein, dietary fibre, iron, calcium and folate contents (El Khoury et al., 2018; Fasano & Catassi, 2012; Ostermann-Porcel et al., 2017a), it is essential to compare their nutrient content. Wheat flour, as analysed by David et al. (2015), contained only 10.23% protein, a moisture content similar to O80 (4.70%) as observed in this study, less ash (1%) and fat (1.33%), and almost double the amount of carbohydrates (83.60%) which are primarily composed of starch, compared to fibre in okara powder. This means that okara powder provides more protein and fibre that wheat flour. When comparing with other gluten-free flours (rice, brown rice, maize, oat, millet, teff, amaranth, buckwheat, quinoa, chickpea, gram, tiger nut and plantain) studied by Culetu et al. (2021a), the only flour with similarly high protein and fibre content was tiger nut flour, while other flours, such as rice and maize, have half the protein content and minimal fibre content. The only components where these flours are similar are fat and ash content.

3.4.3 Amino Acid Composition

Protein content is important for consumers, but its quality is also significant for nutritional purposes. Protein quality is associated with the type of amino acids present and their digestibility. There are 20 amino acids, which are classifies into 3 groups: essential, nonessential, and semi-essential. When discussing food products, the essential amino acids are particularly important because they cannot be produced by the human body and must be obtained through the diet. The essential amino acids include histidine, lysine, isoleucine, leucine, methionine, threonine, tryptophan, valine, and phenylalanine (Millward et al., 2008; Lopez & Mohiuddin, 2023). Okara, a soy-based by-product, is considered an excellent source of protein because its amino acid composition is similar to that of animal proteins, particularly in its content of essential amino acids (Wolfe et al., 2016). Table 3.4 shows the amino acid composition of freeze-dried okara powders prepared from two different batches, which contains eight of the essential amino acids. The amino acids found in greater amounts in okara powder are aspartic acid, glutamic acid, leucine, lysine, and arginine. Table 3.4 also presents the amino acid composition of casein studied by Lauer & Baker (1977), egg by Attia et al. (2020) and whey protein, pea protein and wheat flour by Gorissen et al. (2018). In comparison with animal- based proteins, whey and casein had greater amounts of almost all amino acids, meanwhile egg protein had lesser amounts of all amino acids with the exception of tryptophan. Okara flour had greater amounts of methionine, aspartic acid and cysteine than pea protein. Compared to wheat flour, okara powder has higher levels of lysine, cysteine and proline.

In comparison with other underutilised legume flours studied by Aremu et al. (2006), okara powder contains greater amounts of all amino acids than Bambara groundnut, Kersting groundnut and cowpea flour, and is most similar to cranberry bean flour in lysine, arginine, proline, and valine content. Although, cranberry bean flour has higher amounts of protein and other amino acids, okara powder has greater amounts of glutamic acid, cysteine, and aspartic acid. In summary, okara powder has good protein quality due to its complete amino acids profile, offering a similar quality to pea protein and better than egg.

In terms of bioavailability, okara protein exhibits resistance to complete digestion. Jiménez-Escrig et al. (2010) reported the resistance of pepsin and pancreatic digestion by okara protein when analysed via *in vitro* gastrointestinal digestion. Ambawat & Khetarpaul (2018) reported an *in vitro* protein digestibility of only 68.26%, further indicating limited digestibility. Similarly, Kamble et al. (2019) observed a decrease in protein digestibility when okara was incorporated into pasta formulations: the *in vitro* digestability decreased from 94.71% to 75.68% with a 50:50 ratio of okara to durum wheat semolina. One of the reasons for the reduced amino acid bioavailability from okara is the presence of trypsin inhibitors (Ambawat & Khetarpaul, 2018). Although their activity may be reduced due to heat treatment, residual trypsin inhibitors could still negatively impact protein digestibility.

Table 3.4 Amino acid composition of freeze-dried okara powder, casein, whey, pea protein, wheat flour and egg (g/100 g product) (Lauer & Baker, 1977; Attia et al., 2020; Gorissen et al., 2018).

	Amino Acid	**FD Okara**	**Milk protein (casein)**	**Milk protein (whey)**	**Pea protein**	**Wheat flour**	**Egg**
Essential	Histidine	0.80 ± 0.03	3.08	1.4	1.6	1.4	0.31 ± 0.02
	Isoleucine	1.37 ± 0.08	6.97	3.8	2.3	2	0.51 ± 0.29
	Leucine	2.37 ± 0.11	13.5	8.6	5.7	5	1.09 ± 0.03
	Lysine	1.90 ± 0.11	8.73	7.1	4.7	1.1	0.96 ± 0.07
	Methionine	0.59 ± 0.01	3.26	1.8	0.3	0.7	0.35 ± 0.07
	Phenylalanine	1.63 ± 0.11	5.94	2.5	3.7	3.7	0.65 ± 0.09
	Threonine	1.30 ± 0.05	5.17	5.4	2.5	1.8	0.64 ± 0.06
	Tryptophan	-	1.45				0.14 ± 0.01
	Valine	1.62 ± 0.07	8.89	3.5	2.7	2.3	0.74 ± 0.07
Non-Essential	Alanine	1.24 ± 0.04	3.79	4.2	3.2	1.8	0.66 ± 0.08
	Arginine	2.30 ± 0.18	4.25	1.4	5.9	2.4	0.81 ± 0.10
	Asparagine	-					-
	Aspartic acid	3.25 ± 0.16	8.89				1.27 ± 0.04
	Cysteine	0.47 ± 0.04	0	0.8	0.2	0.7	0.19 ± 0.02
	Glutamine	-					-
	Glutamic acid	4.70 ± 0.22	26.7	15.5	12.9	26.9	1.65 ± 0.07
	Glycine	1.41 ± 0.18	2.52	1.5	2.8	2.4	0.43 ± 0.04
	Proline	1.71 ± 0.13	15.4	4.8	3.1	8.8	0.51 ± 0.02
	Serine	1.40 ± 0.07	10.1	4	3.6	3.5	1.00 ± 0.10
	Tyrosine	1.37 ± 0.08	4.42	2.4	2.6	2.4	0.52 ± 0.03

Values are presented as mean ± standard deviation; for freeze-dried okara (From 2 different batches) and egg protein (4 different batches).

One of the disadvantages mentioned of a gluten-free diet is the reduced amount of minerals compared to gluten-containing food products. The mineral content of freeze-dried okara analyzed from two different batches is shown in Table 3.5 as well as the recommended daily allowance for each mineral. The minerals present in the highest concentrations in the okara samples were in the following order: phosphorous > calcium > sulphur > magnesium, with sodium, iron, zinc and manganese also present. Comparing these results with okara powder analysed by Santos et al. (2019), the sulphur, zinc, iron, and manganese contents are similar, being 236, 3.14, 4.45 and 2.36 mg/100g, respectively. However, the okara powders had higher levels of calcium, magnesium and phosphorous. Rybicka & Gliszczyńska-Świgło (2017) studied other gluten-free flours (buckwheat, corn, oat, rice, amaranth, chickpea, chestnut, millet, teff and acorn flour) and found that okara powder has a higher mineral content than corn and rice flour. Furthermore, okara powder can be considered a good source of iron, containing 5.27 mg/100g, compared to wheat flour, which only contains 1 mg/100 g (Santos et al., 2019). This is comparable to teff and buckwheat, which contain 9.8 and 8.2 mg/100 g, respectively, and are considered good sources of iron. Additionally, Rybicka & Gliszczyńska-Świgło (2017) identified amaranth flour as a good source of zinc, with a content of 2.5 to 3.1 mg/100 g, which is similar to okara powder zinc content (2.85 mg/100 g) found in this study. Based on this analysis, okara powder appears to be a good source of minerals, helping to address one of the nutritional issues associated with gluten-free flours.

Comparing the mineral content of freeze-dried okara with the RDA established by the joint initiative between the Australian and New Zealand Health Departments, the consumption of products with freeze-dried okara can help with achieving the RDAs established. By consuming 100 g of freeze-dried okara, an adult woman between 19-50 years old would be consuming 25% of the recommended calcium intake, 50% of magnesium, 3% of sodium, 40% phosphorous, 29% iron, 69% copper, 47% manganese, 20% selenium and 36% zinc.

Table 3.5 Mineral composition of freeze-dried okara

Minerals	mg/100g	RDA (mg/day)	%RDA
Calcium	250 ± 14.14	19-50 yr 1000^{ab}	19-50 yr 25^{ab}
Magnesium	195.50 ± 0.71	19-30 yr 400^{a} & 310^{b} 31-50 yr 420^{a} & 320^{b}	19-30 yr 40^{a} & 51^{b} 31-50 yr 38^{a} & 50^{b}
Sodium	12.65 ± 5.30	19-50 yr $460\text{-}920^{ab}$	19-50 yr 3^{ab}
Phosphorous	475 ± 7.07	19-50 yr 1000^{ab}	19-50 yr 48^{ab}
Sulphur	250 ± 14.14	-	-
Iron	5.25 ± 0.07	19-50 yr 8^{a} & 18^{b}	19-50 yr 66^{a} & 29^{b}
Copper	0.83 ± 0.08	19-50 yr 1.7^{a} & 1.2^{b}	19-50 yr 49^{a} & 69^{b}
Manganese	2.35 ± 0.07	19-50 yr 5.5^{a} & 5^{b}	19-50 yr 43^{a} & 47^{b}
Selenium	0.01 ± 0.00	19-50 yr 0.07^{a} & 0.06^{b}	19-50 yr 17^{a} & 20^{b}
Zinc	2.85 ± 0.21	19-50 yr 14^{a} & 8^{b}	19-50 yr 20^{a} & 36^{b}

Values are presented as mean ± standard deviation. n=2. RDA represents Recommended Daily Allowance. a is men and b women. Information on RDA values obtained from Nutrient Reference Values for Australia and New Zealand.

3.4.4 Fatty Acid Composition

Fatty acids are part of the lipid classification and are essential for good health, as they play a key role in the structural, functional, and biological activities of the human body. Similar to amino acids, some fatty acids are considered essential which refers to polyunsaturated fatty acids (PUFAs) that the body can’t synthetize and must therefore be obtained directly through the diet, including linoleic acid, alpha-linoleic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Nagy & Tiuca, 2017; Spector, 1999).

Soybeans contain several essential fatty acids (Kudełka et al., 2021). The fatty acid content of freeze-dried okara is shown in Table 3.6, with the presence of linoleic, alpha-linolenic and eicosapentaenoic acids. Linoleic acid, an omega-6 fatty acid, is the most prominent fatty acid (1022 mg/100g) in the okara samples acids with the presence of a small amount of α-linolenic acid, an omega-3 fatty acid. Both of these fatty acids have positive effects on brain cognitive functions. Additionally, omega-3 fatty acids are beneficial for cardiovascular health because they help prevent heart attacks due to their anti-inflammatory, anti-arrhythmic, anti-thrombotic and lipid-reducing properties (Nagy & Tiuca, 2017; Spector, 1999). Therefore, the consumption of okara powder is beneficial for both cardiovascular and neuronal health. The contents of omega 3, omega 6 and omega 9 fatty acids in freeze-dried okara samples were 1630, 1023 and 3750 mg/100g, respectively (Table 3.6). Comparing these results with those obtained by Nikolić et al. (2008) for wheat flour, wheat-white rice flour and wheat-brown rice flour, okara flour had higher omega 3 and omega 9 content, meanwhile wheat flour (1095.73 mg/100 g) and wheat-brown rice flour (1047.27 mg/100g) had higher contents of omega 6. Vivar-Quintana et al. (2023) studied other gluten-free flours (chickpea, pea, soybean, hemp and rice) and okara flour had higher contents of omega 3 and omega 9 than all of these flours. On the other hand, soybean flour was the best source of omega 6, with a content of 7562.58 mg/100 g, followed by hemp flour (5109.58 mg/100 g). The high content of omega 6 in soybean could explain why okara flour has a good amount of these fatty acids since okara is the by-product of soybean products such as soymilk and tofu.

Table 3.6 Fatty acids composition of freeze-dried okara

Fatty acids	mg/100 g
C8:0 Caprylic	10 ± 7.07
C12:0 Lauric	10 ± 0.00
C14:0 Myristic	15 ± 7.07
C15:1n5-cis-10-Pentadecenoic	10 ± 0.00
C16:0 Palmitic	2060 ± 127.28
C16:1n7-cis-9-Palmitoleic	10 ± 0.00
C17:0 Margaric	20 ± 0.00
C18:0 Stearic	20 ± 0.00
C18:1n9t Elaidic	10 ± 0.00
C18:1n9c Oleic	3710 ± 282.84
C18:1n7c Vaccenic	210 ± 0.00
C18:2n6c Linoleic	10220 ± 1032.38
C20:0 Arachidic	60 ± 14.14
20:1n9 - cis-11-Eicosenoic	30 ± 0.00
C18:3n3 - cis-9,12,15-Alpha linolenic	1585 ± 346.48
C21:0 Heneicosanoic	10 ± 0.00
C20:2n6 - cis-11,14-Eicosadienoic	10 ± 0.00
C22:0 Behenic	65 ± 7.07
C22:1n9 - cis-13-Erucic	10 ± 0.00
C23:0 Tricosanoic	10 ± 0.00
C24:0 Lignoceric	30 ± 0.00
C20:5n3 - cis-5,8,11,14,17-Epa	45 ± 7.07
C24:1n9 - cis-15- Nervonic	10 ± 0.00
Omega-3	1630 ± 339.41
Omega-6	1023 ± 1032.38
Omega 9	3750 ± 282.84

Values are presented as mean ± standard deviation. n=2

3.4.5 Physical characteristics of okara powders

3.4.5.1 Particle size

The particle size of flour depends on the milling equipment and the intensity of milling used (Pang et al., 2021). In this study, freeze-dried and air oven-dried okara samples were ground into powders using a coffee grinder, and their mean particle size and particle size distribution were measured using diffraction technology. Table 3.7 presents particle size attributes obtained from this analysis.

Table 3.7 Particle sizes (µm) in diameter of okara powders.

Sample	D3.2 (µm)	D4.3 (µm)	SSA (m^2/g)	Dx10 (µm)	Dx50 (µm)	Dx90 (µm)
FD	93.81±0.95^{b}	298.82±3.84^{b}	63.97±0.63^{d}	34.06±0.35^{b}	241.18±3.22^{b}	650.27±8.08^{b}
O50	87.70±3.11^{c}	303±12.50^{b}	68.43±2.39^{c}	30.80±0.95^{c}	252±10.41^{b}	656±22.85^{b}
O60	111±5.57^{a}	394±23.07^{a}	54.14±2.77^{e}	39.8±2.07^{a}	318±16.50^{a}	855±48.00^{a}
O70	111±5.57^{a}	394±23.07^{a}	54.14±2.77^{e}	39.8±2.07^{a}	318±16.50^{a}	855±48.00^{a}
O80	57.5±0.25^{d}	193±2.65^{d}	104.3±0.45^{a}	21.6±0.06^{d}	142±2.31^{c}	447±5.69^{d}
O90	62.1±0.81^{d}	204±8.19^{cd}	69.66±1.31^{b}	23.2±0.21^{d}	151±2.52^{c}	463±12.06^{d}
O100	63.6±0.61^{d}	224±3.21^{c}	94.36±0.93^{b}	23.4±0.15^{d}	162±3.51^{c}	524±6.93^{c}

Values are presented as mean ± standard deviation. n=4 for all samples. Different superscript letters in the same column indicate significant differences ($p < 0.05$). The SSA stands for specific surface area as (m^2/g).

The mean particle size (D3.2) of okara powder obtained from freeze-drying was 93 µm, while the corresponding D4.3 value was 299 µm. In contrast, the mean particle size of okara powders produced through oven drying varied based on the drying temperature, ranging from 58 µm to 112 µm for D3.2 (surface mean diameter) and from 193 µm to 394 µm for D4.3 (volume mean diameter). The Dx90 values presented in Table 3.7 indicate the particle size below which 90% of the particles fall. Among the okara samples, O80 and O90 had the smallest particle sizes, with 90% of their particle sizes being below 447 µm and 463 µm, respectively. Conversely, O60 and O70 exhibited the largest particle sizes, with 90% of their particles below 855 µm.

Compared to wheat flour particles, which Pang et al. (2021) reported as having 90% of their sizes below 142 µm on average, okara powder particles were significantly larger. This difference may be due to different milling equipment used. In this study, grinding was performed using a coffee grinder rather than specialized milling equipment designed to produce finer powders. Regarding the volume mean diameter (D4.3), the results indicate that O60 and O70 were statistically similar and larger than the other samples. The samples with the smallest D4.3 and D3.2 were O80, O90 and O100. Additionally, the D3.2 and D4.3 values for okara powder were larger than those reported for composite flours (31 µm and 81-82 µm, respectively) containing corn, wheat, sorghum, finger millet, green gram, soy protein isolate, and papaya studied by Agrahar-Murugkar et al. (2015).

Nevertheless, a larger particle size in okara powder isn't necessarily negative, as it can positively influence its properties. Ullah et al. (2017) explained that the swelling power, apparent viscosity, and water solubility of okara powder decrease as particle size decreases. The specific surface area (SSA) of okara powders, shown in Table 3.7, reflects properties like solubility, reactivity, and interaction with other substances when added water (Jan et al., 2017b). The SSA value for ground, freeze-dried okara powder was 64 m^2/g, while the SSA values for other okara powders produced through air oven drying ranged from 54 to 104 m^2/g. Larger SSA values generally indicate finer and more reactive particles.

Additionally, composite flours with coarser particles are often preferred for producing higher-quality biscuits, as finer flours tend to result in higher density and underdeveloped biscuits (Agrahar-Murugkar et al., 2015). Therefore, the use of okara powder in the production of hard biscuits should be effective with O60 and O70 likely having better water solubility.

The particle size of okara powders in solution was also measured by first dispersing the powder in water, followed by thorough mixing and allowing the suspension to stabilize at 25 °C for 30 min. This analysis aimed to assess changes in particle in suspension compared to their initial size in the dry state. The results are presented in Table 3.8. Among the okara powders suspensions, the smallest particles sizes were observed in samples O100, O90 and O80, with 90% of their particle sizes (D4.3) below 435 µm, 555 µm and 633 µm, respectively. These findings align with expectations, as these samples exhibited the smallest particle size in their original dry powder form. Overall, the surface mean diameter (D3.2) of okara powders dispersed in water was significantly smaller than the corresponding dry particle sizes. Consequently, the specific surface area of the dispersed powders was much larger compared to dry powders.

Table 3.8 Particle size analysis of okara suspensions

Sample	D3.2 (µm)	D4.3 (µm)	SSA (m^2/g)	Dx10 (µm)	Dx50 (µm)	Dx90 (µm)
FD	55.90 ± 3.61^{a}	313.2 ± 46.8^{bc}	109 ± 7.05^{e}	26.50 ± 1.63^{a}	273 ± 43.20^{ab}	676 ± 122.05^{b}
O50	32.5 ± 5.79^{c}	357 ± 50.09^{ab}	190.7 ± 37.39^{cd}	22.5 ± 5.83^{a}	300 ± 63.54^{a}	783 ± 73.52^{ab}
O60	45.7 ± 9.81^{b}	401 ± 34.71^{a}	136.9 ± 29.25^{de}	25.4 ± 4.89^{a}	330 ± 37.70^{a}	906 ± 64.28^{a}
O70	29.7 ± 2.28^{cd}	291 ± 28.20^{bc}	203.2 ± 16.42^{bcd}	15.0 ± 1.73^{b}	222 ± 31.16^{bc}	684 ± 65.02^{b}
O80	27.7 ± 2.62^{cd}	261.2 ± 31.24^{c}	218.2 ± 20.10^{bc}	14.2 ± 1.75^{b}	159 ± 29.08^{cd}	662 ± 74.41^{b}
O90	27.9 ± 4.05^{cd}	266.7 ± 20.31^{d}	219.3 ± 32.0^{ab}	14.2 ± 2.89^{b}	153.8 ± 30.74^{d}	554.5 ± 40.97^{c}
O100	24.8 ± 10.74^{d}	186 ± 103.65^{d}	277.4 ± 105.27^{a}	12.3 ± 6.31^{b}	138.3 ± 110.18^{d}	434.8 ± 199.3^{c}

Values are presented as mean ± standard deviation. For all samples n=6. Different superscript letters in the same column indicate significant differences ($p < 0.05$).

The particle size distribution of okara samples, both in their dry state and in suspensions, is illustrated in Figure 3.2 as well as Appendices 1 and 2, showing either a predominantly unimodal or bimodal pattern. The distribution for the okara suspension samples ranged from 1 to 1800 µm, with the highest volume of particles concentrated around 500 µm, as shown in Figure 3.2A. The FD sample and the O50 sample exhibited similar particle size distributions while the O100 sample differed significantly from the FD sample, showing a more noticeably bimodal distribution with the highest particle volumes around 50 µm and 500 µm.

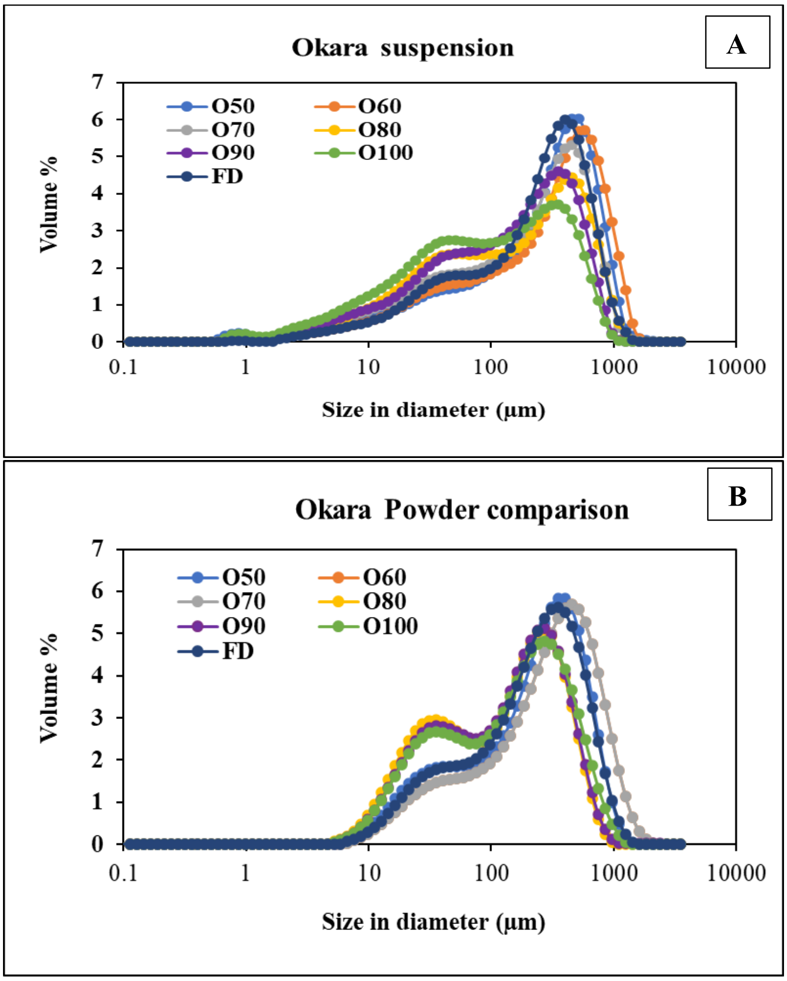


Figure 3.2 Particle size distribution of okara suspensions (A) and okara powders (B).

The particle size distribution for the dry powder samples is shown in Figure 3.2B. Overall, their particle size distribution patterns were similar to those of the suspensions, ranging from 10 to 2000 µm, with the highest volume of particles around 352 µm. The distributions for the O50 and FD samples were the most similar, while the O80, O90 and O100 samples exhibited more pronounces two distinct peaks, with small peaks at around 30 µm and larger peaks at 272 µm. When comparing these distributions with those reported by Agrahar-Murugkar et al. (2015) for composite flours, which are mixture of different plant-based materials (corn, wheat, sorghum, finger millet, green gram, soy protein isolate, and papaya), okara powder had a wider particle size range. In contrast, the composite flours ranged from 0.1 to 100 µm, with the highest volume found at 140 µm.

Comparing the powder and suspension samples, the suspension samples had a wider range of particle sizes, with a small volume of particles smaller than 10 µm. Appendix 3 shows a comparison between each powder and suspension sample under the same drying conditions.

3.4.3.2 SEM microstructure of okara powder

The microstructural features of okara powder samples were analysed using SEM, and the resulting micrographs are shown in Figures 3.3 and 3.4. Figure 3.3 illustrates the microstructure of okara powder dried at different temperatures and times through convection drying in an air oven, while Figure 3.4 shows the freeze-dried okara powder at three magnifications: 80x, 100x and 300x.

There is minimal structural difference between the samples, all of which appeared irregular in shape and size, with both small and large agglomerated fragments and fibre bundles. Although not immediately clear, the microstructure seems to be predominantly fibrous. The fibrous appearance is consistent with okara's high fibre content. The presence of fibre layered bundles is more pronounced in the image of the freeze-dried okara powder sample at 300x magnification. Gaps (a) between the structures, which represents pores, were particularly noticeable in O50 and O60 samples. The pores were less pronounced in the samples produced at higher temperatures, likely due to the structural shrinkage and contraction during moisture evaporation at higher temperatures. These pores contribute to the powder's highwater absorption, oil holding and swelling capacity (Kamble et al., 2020). The dark-coloured areas (b) observed are believed to result from water loss during the drying process. The structures observed in this study are similar to those obtained by Li et al. (2019) and Santos et al. (2019), where fibre bundles are arranged in an ordered structure with permeable pores. However, the honeycomb structure reported by Voss et al. (2018) is not present in this study, and smooth surfaces are barely observed. It is possible that some artifacts in the freeze-dried okara samples were created during the freezing stage due to the alteration of the sample matrix. This may have occurred due to the slow formation of ice crystals or potential recrystallization of the water content, both of which are recognized risks associated with freeze-drying (Kalab, 1984; Oyinloye & Yoon, 2020).

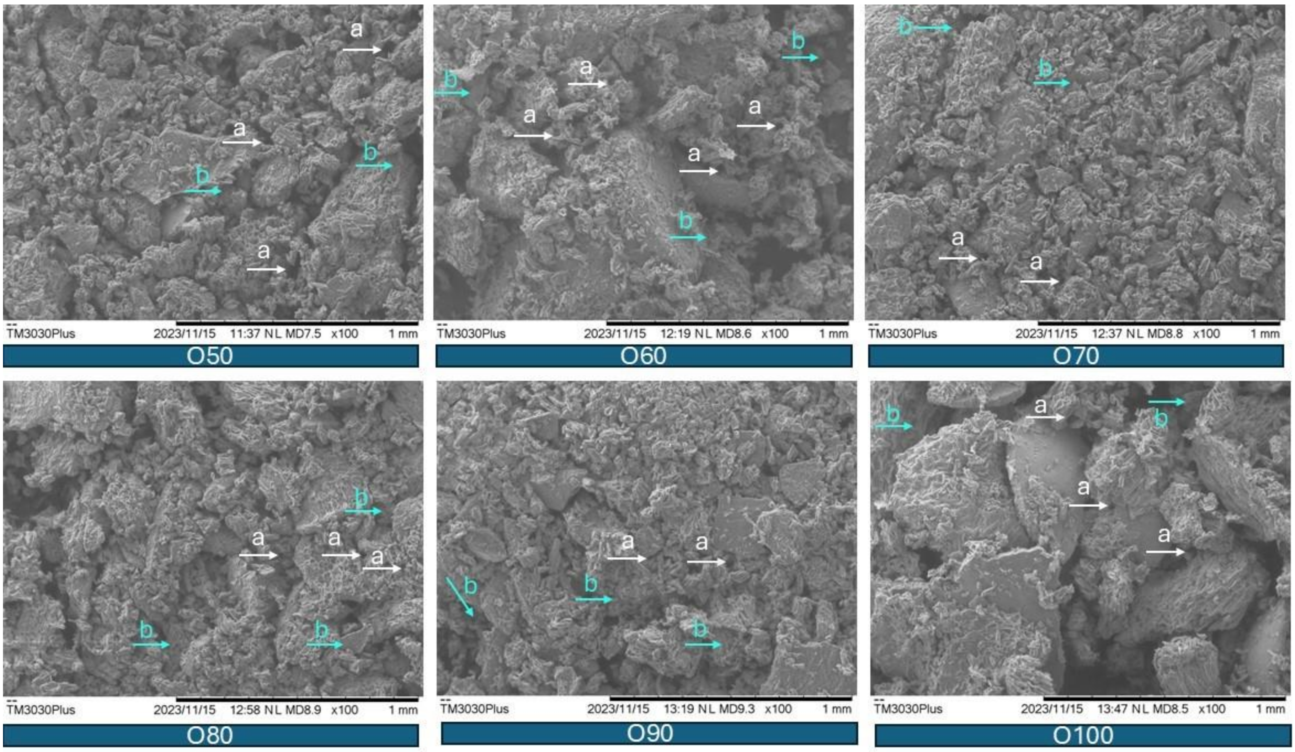


Figure 3.3 Micrographs (100x magnification) of okara powder dried by convection at different temperatures. Pores (a) and dark-coloured areas (b) are indicated with arrows.

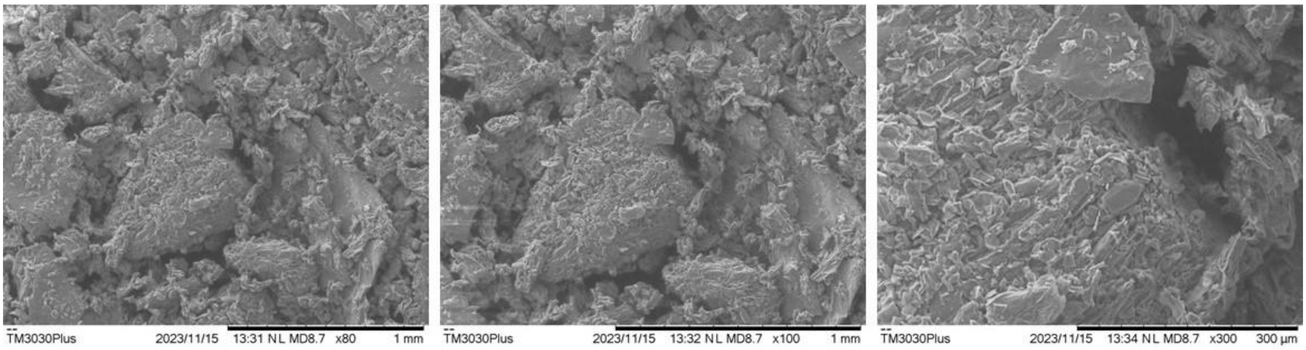


Figure 3.4 Micrographs of freeze-dried okara powder at 80x, 100x and 300x magnifications.

3.4.3.3 Technical properties of okara powder

The physical characterization of okara powder is important as it provides valuable insight into its behaviour and helps predict how the powder will act when interacting with other ingredients during food product development. In this study, various physical properties of okara powder were analysed, including water activity, water holding capacity, solubility, bulk density, Carr index and flowability. The results are shown in Table 3.9.

Table 3.9 Physical characteristics of okara powders obtained using different drying treatments.

Drying treatment	Aw	WHC (g water/g sample)	solubility (g soluble portion/g sample)	Bulk density (g/mL)	Carr index (%)	Flowability
FD	0.24 ± 0.00^{c}	8.01 ± 0.13^{a}	0.28 ± 0.01^{ab}	0.28 ± 0.01^{d}	33.34 ± 3.15^{a}	Very poor
O50	0.43 ± 0.14^{a}	6.93 ± 0.81^{ab}	0.31 ± 0.06^{a}	0.37 ± 0.01^{a}	31.05 ± 3.47^{a}	Poor
O60	0.35 ± 0.35^{b}	5.98 ± 0.29^{bc}	0.21 ± 0.01^{abc}	0.37 ± 0.01^{abc}	30.26 ± 1.51^{a}	Poor
O70	0.19 ± 0.00^{c}	5.23 ± 0.13^{c}	0.25 ± 0.01^{abc}	0.37 ± 0.02^{ab}	29.28 ± 6.03^{a}	Poor
O80	0.21 ± 0.03^{c}	4.80 ± 0.52^{c}	0.20 ± 0.01^{bc}	0.34 ± 0.01^{bc}	28.84 ± 4.45^{a}	Poor
O90	0.12 ± 0.00^{d}	5.73 ± 0.43^{bc}	0.28 ± 0.36^{ab}	0.33 ± 0.01^{c}	29.46 ± 3.95^{a}	Poor
O100	0.14 ± 0.02^{d}	4.82 ± 0.13^{c}	0.16 ± 0.01^{c}	0.29 ± 0.01^{d}	35.62 ± 4.62^{a}	Very poor

- Values are presented as mean ($p < 0.05$) ± standard deviation (n=4) for all properties.
- Different lower-case letters in the same column indicate significant difference ($p < 0.05$).

Water activity is a crucial indicator of food product stability, with values ranging from 0 to 1. It significantly influences microbial growth, enzymatic activity, and chemical reactions in food products (Voss et al., 2018). While, moisture content also relates to shelf life, A_w specifically indicates the availability of water participating in these in reactions. Lipid oxidation is a concern at low A_w (<0.3), microbial spoilage occurs at higher A_w ranges (0.75-0.98 for bacteria and 0.62 -0.90 for yeast), and the Maillard reaction is optimal at $A_w = 0.65$ (Doblado-Maldonado et al., 2012, Marynin et al., 2021; Mathlouthi, 2001; Carter et al., 2015).

In this study, all okara powder samples had the A_w levels lower than the recommended threshold (≤ 0.62) for flours (Carter et al., 2015), indicating good stability, as higher levels increase risks of microbial spoilage and chemical reactions, leading to caking, colour loss, rancidity, and nutrient loss. The A_w ranged from 0.12 to 0.43 for air-oven dried okara powders and was 0.24 for the freeze-dried sample. Among the air-oven dried samples, O50 and O60 exhibited relatively higher A_w values of 0.43 and 0.35, respectively, which was due to their lower drying temperatures used.

The drying conditions used in this study were more effective in reducing A_w, compared to other studies reported by Davy et al. (2022) and Voss et al. (2018) where the A_w values were 0.55 and 0.95, respectively. The higher A_w levels in these studies suggest that the drying times were insufficient. For instance, in Voss et al. (2018), one okara sample was dried for 5 hours at 80 °C and another for 1 hour at 200 °C, which was inadequate to remove the necessary water content. This is reflected in their sample's high water content of 88%, significantly higher than the <10% water content of okara powders in this study. While, Davy et al. (2022) achieved a more stable $A_{w,}$ their value (0.55) still differed markedly from O100 (A_w 0.14) in this study as drying time they used was shorter (i.e. 2.3 hours), despite both being prepared using a convection oven at 100 °C.

Water holding capacity (WHC)

WHC determined a flour's ability to absorb and retain water, a key property for applications like bread making. FD (freeze-dried) okara powder exhibited the highest WHC (8.01 g water /g sample) while oven-dried okara powder samples had relatively lower WHC ranging from 4.28 to 7.74 g water/g samples being mostly no significant difference. The WHC values obtained in this study are similar to those reported by Ostermann-Porcel et al. (2017a) despite their use of different drying conditions, including freeze-drying, microwave drying, and rotatory evaporator drying. A good WHC is essential for flour because, in food applications, such as bread, the flour must absorb and retain water to achieve desirable textural properties. The only sample with a good enough WHC is FD meaning that it's the best sample to for bread production because its fibre content is high which has the capacity to hold water, but it is also not as high as the oven-dried samples which can lead to fibre stealing too much water (Davy et al., 2022). By adding a higher amount of moisture to bread formulation, this problem can be managed.

Solubility

Table 3.9 shows that some samples are significantly different ($p < 0.05$). The least soluble in water is O100 and O50 the most soluble. The behaviour observed in O100 was similar to the one by Davy et al. (2022). This may mean that okara powder has poor solubility in water, but it can be improved depending on drying conditions used.

Bulk density and flowability

Understanding the flow properties is essential when developing a powder product since they have irregular shapes and are affected by friction and cohesive forces (Thalberg et al., 2004). The flowability of a powder provides information on handling, processing and manufacturing. One of the conventional methods used to provide fundamental information on flowability is Carr Index, which depends on the bulk and tapped density (Suhag et al., 2024). Both densities were determined in this study to gather information on the flowability of okara powder.

The bulk density is used to determine the Carr Index and Flowability with tapped density. FD and O100 are statistically the same ($p > 0.05$) with the lowest values and different from all the other okara powder samples. Bulk density is influenced by the particle size of the powder; this explains the difference between bulk densities between okara powders. The smaller the particle size, the bigger the bulk density of the powder (David et al., 2015). In the case of O80 and O90 which had the smallest particle sizes, their bulk density values are one of the highest. The bulk density results for this study are a bit higher than those obtained by Davy et al. (2022), but lower than wheat flour and other gluten-free flours' bulk densities reported by Patil et al. (2017) that were in a range of 0.45 to 0.57 g/mL.

The values obtained by Carr Index are used to determine the flowability of a powder based on the Appendix 4. The flowability of powders gives information on the most effective ways to handle powders during transportation, processing, and storage. Food powders with poor flowability like flours are more prone to caking, this phenomenon consists of the formation of lumps and agglomerated solids in the powder which results in lower quality of the product (Juliano & Barbosa-Cánovas, 2010; Nkurikiye et al., 2023; Düsenberg et al., 2023). Based on Carr Index results, the flowability of Okara powder samples is poor and very poor for FD and O100. Davy et al. (2022) obtained higher Carr Index results in their okara powders meaning a poorer flowability. Patil et al. (2017) noted even higher Carr Index for other gluten-free flours such as 73%, 50% and 64% for sorghum, rice, and moong flour, respectively; these values are more similar to the reported wheat flour Carr Index of 65% by the same authors. The okara powders samples of this study will tend to cake during storage but less than wheat flour and other gluten-free flours because they have better flowability.

The flowability is also affected by the particle size. The smaller the particle size, the less flowability the powder will have because when particle size is smaller, the surface area is increased. An increase in surface area means more area that could be affected by cohesive forces specifically frictional forces which restrict the ability to flow (Jan et al., 2017a). In this study, the flowability wasn't affected by the particle size because FD and O100 had the worst flowability and weren't the samples with lower particle size. Another factor that has been discovered to affect flowability is fat content. Nkurikiye et al., (2021) noted that higher fat content in chickpea flour resulted in greater compressibility thus greater Carr Index and poorer flowability. In this study the fat contents are statistically the same but FD and O100 did have some of the highest fat contents and as a result their Carr Indexes are the highest.

3.4.3.4 Colour

The colour of okara flour samples was measured using a tristimulus colorimeter and is expressed in CIELAB coordinates: L* for lightness, a* for redness (+ve) or greenness (-) and b* for yellowness (+ve) or blueness (-ve). The values of C* (chroma, representing colour intensity/saturation) and h° (hue angle, representing colour perception were calculated from the a* and b* values. The results of L, a*, b*, C* and h° are presented in Table 3.10.

Table 3.10 Colour (CIE L, a, b, C and h° values) of okara powders produced by different drying conditions

Drying treatment	L*	a*	b*	C*	h°
FD	84.46 ± 0.06^{a}	-1.08 ± 0.28^{e}	18.76 ± 0.12^{b}	18.79 ± 0.11^{b}	93.22 ± 0.87^{a}
O50	79.63 ± 0.24^{d}	1.84 ± 0.05^{a}	19.27 ± 0.03^{a}	19.35 ± 0.04^{a}	84.56 ± 0.14^{e}
O60	78.97 ± 0.36^{e}	0.38 ± 0.09^{c}	18.90 ± 0.15^{ab}	18.90 ± 0.15^{b}	88.91 ± 0.26^{c}
O70	83.89 ± 0.20^{b}	0.43 ± 0.01^{bc}	18.06 ± 0.04^{c}	18.06 ± 0.04^{c}	88.70 ± 0.00^{cd}
O80	83.52 ± 0.21^{b}	0.56 ± 0.09^{b}	18.22 ± 0.50^{c}	18.22 ± 0.50^{c}	88.29 ± 0.25^{d}
O90	83.67 ± 0.49^{b}	-0.21 ± 0.06^{d}	18.18 ± 0.25^{c}	18.18 ± 0.25^{c}	90.63 ± 0.18^{b}
O100	82.32 ± 0.22^{c}	-0.15 ± 0.03^{d}	18.63 ± 0.47^{b}	18.64 ± 0.49^{b}	90.39 ± 0.11^{b}

Values are presented as mean ± standard deviation. Different lower-case letters in the same column are significantly different ($p < 0.05$).

Figure 3.5 shows the actual pictures of okara powder samples with different drying methods (FD, O50, O60, O70, O80, O90 and O100). Based on visual observation, there were noticeable colour differences between the samples. O50 and O60 appeared to be darker than the other powders while O90 and FD were the brightest. O50 appeared to have a more yellowish tone than the other samples.

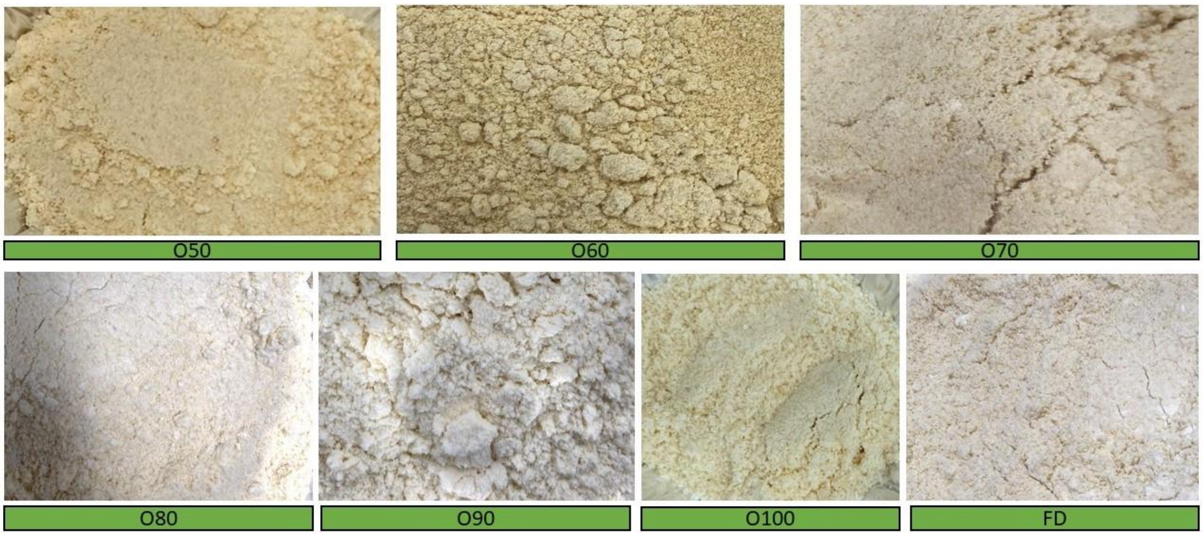


Figure 3.5 Okara powders produced by different drying conditions

The data indicated that okara powder is a relatively bright flour with a mild yellowish tone, as reflected by the relatively high positive b*values around +18. In contrast. In contrast, the a* values were very low, ranging between 0 and ±1, indicating minimal red or green tones. By looking at each of the colour space coordinates shown in table 3.10, there is no significant difference between O70, O80 and O90 for L* which determines the brightness of the product. The results also indicate that the brightness was influenced by the drying temperatures. The samples dried at relatively lower temperatures, such as freeze-dried (FD) or oven dried at 50 °C (O50) and 60 °C (O60), were slightly darker compared to those dried at higher temperatures (70 -100 °C). However, in a study reported by Perussello et al. (2009), pellets of extruded okara pre dried in a spray dryer at 130, 150 and 170 °C followed by drying in a rotational drum at 50, 60 and 70 °C; the use of low temperatures (50, 60 and 70 °C) lead to less darker tones which was suggested to be due to the low rates of Maillard reaction at these temperatures. The differences in the lightness or darkness of samples observed between this study and the study by Perussello et al. (2009) could be attributed to physical factors, such as variations in particle size, both within and between the studies.

In terms of the a* coordinate, the okara powders exhibited either a barely greenish or reddish tone, as previously mentioned, with all samples having minimal a* values close to 0. However, all the samples displayed a yellowish tone with no significant differences, as indicated by their relatively high b* coordinates, which were around +18.

In terms of C* representing colour saturation, its values appeared to be similar between all samples as their no significance difference in both a* and b* values, although O50 appeared to be slightly higher than the other samples.

The hue angle (h°) value for the FD sample was 93, whereas the other samples (O50-O100) exhibited slightly lower hue angle values in the range of 85-90, tending to increase with higher drying temperature. The hue angles value indicate that all samples had a yellow tone with minimal red or green hues.

When compared with okara powders analysed in other studies, the L*, b* and C* values are quite similar to those obtained by Santos et al. (2019) and Guimarães et al. (2020). In contrast, Davy et al. (2022) reported a brighter okara powder with similar h ° and C* values, while Ostermann-Porcel et al. (2017a) presented darker powders with higher a* and b* values. More importantly, based on the results reported by Patil et al. (2017), the okara powders in this study have similar values of L* and a* to wheat, rice, and sorghum flours, however, these flours have a less yellowish tone than the okara samples. Guimarães et al. (2020) confirmed that freeze-drying treatments result in higher h° values compared to convective drying; they obtained a value of 81.92 for freeze-drying while the h° values for convective drying were 80.8 (40 °C, 41 hours), 79.60 (50 °C, 25 hours), 78.95 (60 °C, 20 hours) and 78.95 (70 °C, 12 hours).

3.5 Conclusions

Fresh okara was dried using different drying conditions and then ground to obtain a powder that could be used as a gluten-free alternative to wheat flour. The use of gluten-free flours as substitutes for wheat flour has posed nutritional and technological challenges. This study focused on determining whether okara powder could be a successful substitute and identifying the best drying conditions based on the flours' physicochemical characteristics. The drying conditions compared were freeze-drying and convection oven drying at six different temperatures: 50, 60, 70, 80, 90 and 100 °C.

Based on drying time and flour stability, the best conditions were convection oven drying at 90 °C and 100 °C. These samples required the least time to reach moisture equilibrium, had the lowest moisture values (1.75%) and the lowest water activity (<0.14), making them less prone to deterioration. In terms of nutritional composition, okara is a good substitute for wheat flour because it has a higher protein content, contains essential amino acids and fatty acids, and has higher fibre content. The samples with the best composition in terms of higher fibre content and less moisture are O70, O90 and O100.

For particle size, okara powder samples had larger sizes than wheat flour, likely due to the equipment used. The finer samples were O80 and O90, while FD was the only sample with a sufficient water-holding capacity, which is essential for food applications where water is used. The most soluble in water were O50, followed by O90, O60 and O70. The okara samples exhibited better flowability than wheat flour, making them less likely to cake during storage, though they still had poor solubility, especially FD and O100.

Finally, regarding colour, the okara samples had acceptable colour coordinates and were similar to wheat flour with O70, O80 and O90 being the brightest out of all the samples. Among the samples, the colour was not significantly different, although there were some minor differences in colour between a few of the samples. Although FD had the best WHC, it is the most expensive method and takes the longest time.

For further studies, it would be recommended to combine okara powder with tiger nut or cranberry bean flour due to their good nutritional content and explore their functional properties for use in food applications. Additionally, it would be valuable to investigate the oil-holding capacity of okara powder as an additive for emulsion stability in salad dressings.

Chapter 4. Use of Okara Powder as a Substitution for Wheat Flour in Food Applications: Bread, Cookies, and Coating Agent

4.1 Abstract

The use of gluten-free flours in food products has been increasing, but a complete substitution of wheat flour hasn't been achieved due to the lack of gluten. This has led an increase in tests of wheat flour substitution with different composite flours in combination with hydrocolloids. The aim of this study was to determine if okara powder could be used as a wheat flour substitute in bread and cookies by evaluating their textural properties, colour, spread factor (for cookies), and crumb structure and height (for bread). For bread, a 100% substitution of wheat flour wasn't possible because the bread structure wouldn't form. However, a maximum substitution of 20% was successfully achieved. Three different hydration levels were tested to determine the best formulation for obtaining bread similar to that made with 100% wheat flour. Comparisons were made based on height, crumb and crust colour, crust appearance and textural properties. The optimum hydration level when substituting 20% wheat flour with okara powder was 100%, as it allowed for the complete hydration of protein particles and fibre. As a result, the crumb structure was less compact, and the height and textural properties were most similar to control bread. Although, okara powder has higher protein content than wheat flour, the hardness of bread was not significantly affected due to the addition of more water, which resulted in a softer texture. In the case of cookies, gluten doesn't play a critical structural role as it does in bread. Thus, wheat flour was successfully substituted. Additionally, to address allergen concerns related to egg and dairy products, the cookies were formulated with chia and coconut shortening instead. The best formulation when using okara powder was V3 (okara flour, coconut shortening, brown sugar, chia gel, HMPC, vanilla and chai extracts, salt, water and corn flour), which has a higher fat content and a lower amount of okara powder. This combination resolved the poor binding of ingredients observed in the other two formulations with less fat. The higher fat content resulted in a softer and more cohesive cookie which is desirable. Finally, the possibility of using coarse okara powder as a deep-frying coating instead of breadcrumbs was explored by comparing batter pick up and taste. The use of okara as a deep-frying coating was successful, as it demonstrated good oil-holding capacity and satisfactory sensory attributes.

4.2 Introduction

Bakery products, such as bread and cookies are widely popular among consumers due to their flavour, availability, affordability, and convenience (Agrahar-Murugkar et al., 2015). Bread, in particular, has been a staple food since the days of Mesopotamia. Its enduring popularity and versatility have led to the development of numerous types and shapes of bread, with an average per capita consumption of 70 kg per year globally (Carocho et al., 2020; De Boni et al., 2019). However, the consumption trends and consumer perception of bakery products have shifted with increasing demand for higher quality products that offer health benefits and align with healthier lifestyle. The shift has created a need to develop new products. One prominent solution has been the use of non-wheat cereals to produce gluten-free bakery products (Carocho et al., 2020). Among these alternatives, the use of okara flour, a by-product of soybean processing, presents a promising gluten-free option for enhancing the nutritional profile of bread and cookies while meeting consumer expectations for healthier bakery products.

The demand for gluten-free products has increased for two primary reasons: the prevalence of celiac disease and the growing perception that gluten-free products contribute to a healthier diet (Ostermann-Porcel et al., 2017b). However, developing gluten-free bakery products, particularly bread, poses significant challenges (Gallagher et al., 2004). Gluten is a protein essential for bread-making and provides structural integrity in bread production by imparting elasticity, extensibility, and gas-retention capacity. These properties result in bread with a well-defined crumb structure and appealing overall appearance. Without gluten, bread dough resembles a liquid batter, lacking elasticity and resulting in low-quality bread (Bender & Schönlechner, 2020; Gallagher et al., 2004). Gluten-free dough can't effectively trap gases incorporated during mixing or those produced by yeast fermentation because its structure is not strong enough to support them since in the absence of gluten protein. The lack of strength prevents the dough from fully rising. While some gas may initially be trapped in the dough structure, it is released prematurely, with only a small amount remaining trapped. The result is bread with an irregular texture and a fragile crumb structure (Baldino et al., 2018). Thus, developing entirely gluten-free bread is challenging, and one strategy to mimic gluten's role is the use of hydrocolloids. The use of hydrocolloids in gluten-free bread is based on their ability to strengthen the protein network, enabling it to retain carbon dioxide and increase the binding between starch granules, which results reduced mobility and a more cohesive dough (Torbica et al., 2012; Onyango et al., 2009). Hydroxypropyl methylcellulose (HMPC) has been the preferred hydrocolloid for gluten-free products due to its ability to form a thermo-reversible gel when heated. This improves dough viscosity, stabilizes gas cells, and prevents moisture and volume loss (Bender & Schönlechner, 2020; Belorio & Gómez, 2020; Baldino et al., 2018).

On the other hand, gluten is less essential in biscuits and cookies. The flour typically used in cookie production is soft wheat, which has a lower protein content, resulting in less gluten, as a larger volume is not required for this product. Although the role of gluten is smaller in cookies, it still influences the product's quality. Gluten helps to develop the structure of the cookie by holding the ingredients together, thus without it, the cookie lacks structural stability, leading to cracks or an overly crumbly texture. This is why hydrocolloids are often introduced into gluten-free cookie formulation to improve structure, texture and appearance (Devisetti et al., 2015, Di Cairano et al., 2018).

Despite this, no ingredient has been found that can fully replace the role of gluten. As a result, bread and cookies developed in previous studies typically contain only partial substitutes of wheat flour. For bread, the maximum amount of okara flour used as a substitute for wheat flour was 12.5% while for cookies, it was 50% (Ostermann-Porcel et al., 2017c; Philip et al., 2022; Lee et al., 2020; Ostermann-Porcel et al., 2017b).

This study aimed to evaluate the potential of okara flour as a complete substitute for wheat flour in bread, cookies and as a coating agent for frying vegetables. This study also sought to determine the optimum hydration level for bread made with okara and the best formulation for gluten-, dairy- and egg-free cookies. By comparing breads with identical wheat-okara ratios but varying water content, the effects of okara and water content on the textural properties, colour and height of bread were assessed. For the cookies, the best formulation was identified by comparing textural properties and spread factor. The specific objectives of this study were to determine the effects of okara flour on the colour, height, spread ratio, and textural properties of bread, cookies and frying products. This study aims to fill existing gaps regarding the use of okara flour in cookies, the feasibility of utilizing okara without prior drying, and the potential of using partially ground dry okara as breadcrumbs for frying vegetables.

4.3 Materials and Methods

4.3.1 Materials

The following ingredients were used to produce bread, cookies, and batter: wheat flour (Pams Pure Plain Flour, Foodstuffs), active dried yeast (Tasti), vegetable oil (blend of 95% canola oil and 5% sunflower oil, Woolworths), soy bean oil (Pams, Foodstuffs), white sugar (Chelsea, New Zealand Sugar Company Limited), brown sugar (soft brown sugar, Chelsea, New Zealand Sugar Company Limited), table salt (Woolworths), chia seeds (Pams, Foodstuffs), vanilla extract (Hansells), coconut shortening (99% coconut oil and <1 % soy lecithin, Kremelta, Kellogs Company), cornflour (Edmonds, Goodman Fielder) and panko breadcrumbs (Nisshin Seifun Group), all purchased from the local supermarkets (PAK'nSAVE and Woolworths, New Zealand). Hydroxoyproply methylcelluose (HMPC) (WELLENCE GF 47129 Food Additive) was provided by Hawkins Watts (Auckland, New Zealand). The okara flour containing 8.46% moisture, 3.72% ash, 16.56% fat, 31.09% protein, 37.85% total dietary fibre and 40.17% carbohydrates was obtained using freeze-drying as described in Chapter 3. The coarse okara powder was prepared by grinding the powder in a food processor (Breville kitchen wizz 11 plus food processor) while the fine okara powder was grinded in the food processor followed by grinding in a coffee grinder (Bistro Electric coffee grinder).

4.3.2 Preparation of chia gel

When chia seeds are mixed with water, a gel is formed known as chia gel or mucilage. This gel can be used as an egg replacer in bakery products because it can preserve the protein net and can be used as a gelling agent, thickener, stabilizer, emulsifier, bulking agent and texture modifier when added to food

(Borneo et al., 2010; Fernandes & Mellado, 2018; Gallo et al., 2020). To prepare the chia gel, chia seeds were mixed with water at 45 °C. The chia seeds-water ratio used was 1:6.

4.4.3 Preparation of coatings for deep-frying Zucchini

For the deep-frying test, two coatings were used. The first coating was made with wheat flour and bread crumbs. In the second coating, fine okara powder was used as a substitute for wheat flour, and okara crumbs (coarse okara powder obtained by grinding dry okara in a food processor (Breville kitchen wizz 11 plus food processor) replaced the bread crumbs. Both coatings used egg.

Zucchini was cut into 0.5 cm thick slices, and each slice was first dipped in egg that has been pre-stirred, then in wheat flour or okara powder, followed by egg again, and finally dipped in breadcrumbs or okara crumbs. Soybean oil was heated using an induction cooker (Living & Co Induction cooker Model SRO8321), with the temperature monitored using a thermocouple thermometer until it reached 110°C. This temperature was selected based on the onset of bubbling, indicating it was sufficiently heated and safe for frying. The coated zucchini slices were fried in the oil for 2 min on each side.

4.3.4 Formulation of Bread

Initially, preliminary trials were conducted to select the appropriate amount of okara powder to add to the bread by varying the substitution level of wheat flour (10, 50 and 25 %). Table 4.1 shows the formulations used for the preliminary trials; the proportions are flour-based. These four breads formulations were compared based on their physical appearance and flavour. The results are shown in Appendix 5. After evaluation, it was decided to use 20% okara powder as the maximum substitution for wheat flour. This decision was based on the fact that 25% substitution resulted in an undesirable of soybean aftertaste, while 50% caused the bread structure to collapse. The 10% substitution did not impart any noticeable soybean aftertaste.

Table 4.1 Formulations for okara: wheat flour bread for preliminary trials

Ingredients (%)	**Bread Test**			
	1	**2**	**3**	**4**
Wheat Flour	90	50	75	75
Okara flour	10	50	25	25
Water	64	128	85	100
Yeast	1	1	1	1
Oil	3	3	3	3
Sugar	2	2	2	2
Salt	1.5	1.5	1.5	1.5
HMPC	-	1.5	1.5	1.5

4.3.5 Preparation of Bread

Figure 4.1 illustrates the process for the production of bread using okara and wheat flour based on methods described by Davy et al. (2022), Philip et al. (2024) and Serna-Saldivar (2020). First, the yeast needs to be hydrated before mixing all bread dough ingredients. The yeast was mixed with sugar and water, then incubated at 35 °C for 20 minutes (Thermo Scientific IMP180 Heratherm Refrigerated Incubators, 178 L, 5-70 °C) to allow for yeast proofing. The proofed yeast was then mixed with wheat flour, okara flour, oil, salt, HMPC and the remaining water for 3 minutes at low speed using a standard mixer (KitchenAid K5SS Heavy Duty Series 5qt Stand Mixer), followed by mixing at high speed for 13 minutes. In the case of the control bread, okara flour, wet okara and HMPC were not used. The bread dough was placed in the incubator for the first or intermediate proofing at 40 °C for 90 minutes. Afterward, the dough was punched down, shaped and placed in a bread tin (Prestige Loaf Pan Non Stick with inner dimensions: 230.5 x 131 x 68mm, Briscoes) for the final proofing at 35 °C for 50 minutes. Once the final proofing was complete, the dough should have risen, after which it was baked in the oven (MOFFAT BARKBAR turbofan 32max) at 200 °C for 32 minutes. The amount of water added depended on the desired level of hydration used, which varied according to the specific formulation (14.78, 64, 80, 90, or 100%) (Belorio & Gómez, 2020; Baldino et al., 2018; De La Hera et al., 2014; Davy et al. 2022). Formulations with gluten-free flours for bread production require up to 150% water content to ensure that the weak dough structure does not collapse and a bread with high volume is obtained (Belorio & Gómez, 2020; Baldino et al., 2018). Several studies have used 70, 80, 90 and 100% as hydration levels in gluten-free breads made with rice flour and noticed a positive effect on volume for 90 and 100% when HPMC is incorporated in the formulation (De La Hera et al., 2014; Belorio & Gómez, 2020) while Davy et al. (2022) designated a 64% hydration level for bread made with 12.5% okara flour.

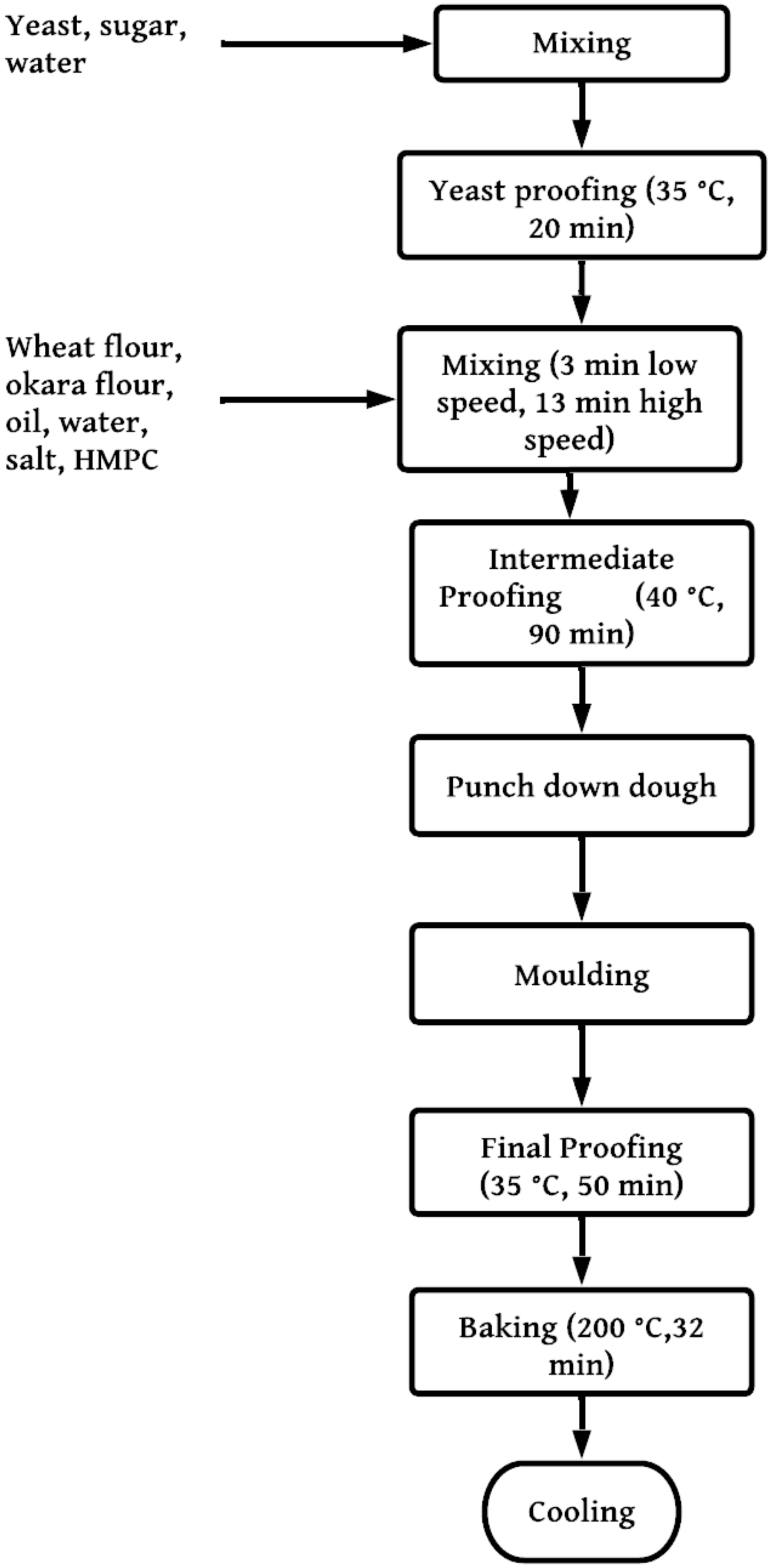


Figure 4.1 Bread-making process adapted from Davy et al. (2022), Philip et al. (2024) and Serna-Saldivar (2020).

Table 4.2 shows the formulations used to obtain the breads, with the initial amount of water varying according to the hydration level, which represents the percentage of hydration in the dough (Equation 4.1). The control bread refers to the bread made with 100% wheat flour. For the breads containing okara flour, the wheat-okara flour ratio for all three is 80:20, as determined from the results of preliminary trials when deciding the amount of okara to use, with the maxim substitution level set at 20%. When formulating bread recipes, the proportions are flour-based, meaning that the quantities used of other ingredients depend on the amount of flour used. The flour is considered as 100%, and the quantities of the other ingredients are calculated based on this. For example, if 250 grams of flour are used in a bread formulation with a hydration level of 64%, the amount of water added would be 160 grams, and so on.

$$Hydration\ level\ (\%) = \frac{Amount\ of\ water * 100}{Amount\ of\ flour} \qquad \text{Equation 4.1}$$

Table 4.2 Formulations of control and okara bread with flour content as the basis.

Ingredient (%)	**Bread Variation**				
	Control (64% hydration)	**100% hydration**	**90% hydration**	**80% hydration**	**Wet okara bread (14.78% hydration)**
Wheat Flour	100	80	80	80	80
Okara flour	-	20	20	20	-
Fresh okara	-	-	-	-	76.92
Water	64	100	90	80	23.2
Yeast	1	1	1	1	1
Oil	3	3	3	3	3
Sugar	2	2	2	2	2
Salt	1.5	1.5	1.5	1.5	1.5
HMPC	-	1.5	1.5	1.5	1.5

Table 4.3 shows the same formulations to obtain the breads, instead of proportion being flour-based, they are on a total basis. The control bread formulation was obtained from Philip et al. (2022) and serve as the basis for the okara breads, with modifications to the water content and the addition of HMPC. The amount of water, or hydration level, used for the bread samples containing 20% of okara flour was higher than that of the control bread because bread made with gluten-free flours requires up to 150% water to achieve a bread with a quality closer to that of wheat flour bread (Baldino et al., 2018). For wheat-okara bread, the hydration level is 80% taking into account that fresh okara contains 74% water and 26% solids.

Table 4.3 Formulations of control and okara bread based on total basis

Ingredient (%)	Bread Variation				
	Control (64% hydration)	**100% hydration**	**90% hydration**	**80% hydration**	**Wet okara bread (14.78% hydration)**
Wheat Flour	58.31	38.28	40.20	42.33	42.30
Okara flour	-	9.57	10.05	10.58	-
Fresh okara	-	-	-	-	40.67
Water	37.32	47.85	45.23	42.33	12.27
Yeast	0.58	0.48	0.50	0.53	0.53
Oil	1.75	1.44	1.51	1.59	1.59
Sugar	1.17	0.96	1.01	1.06	1.06
Salt	0.87	0.72	0.75	0.79	0.79
HMPC	-	0.72	0.75	0.79	0.79
Total	100	100	100	100	100

4.3.6 Preparation of Cookies

Figure 4.2 illustrates the process for the production of okara flour cookies, adapted from cookie-making process by Sykes & Davidson (2020). Coconut shortening, chia seeds gel, brown sugar, vanilla, and tea chai extract were mixed using a standard mixer (KitchenAid K5SS Heavy Duty Series 5qt Stand Mixer) for about 8 minutes until a creamy consistency was achieved. In the second mixing stage, okara flour, HMPC, salt and corn flour were added. While incorporating the dry ingredients, water was slowly added to ensure proper hydration of the dough. Once mixed, the dough exhibited a well-hydrated appearance. The dough was then rolled to a thickness of 6 mm, and cookies were cut using a cookie cutter. The formed cookies were baked in an oven (MOFFAT BARKBAR turbofan 32max) at 170 °C for 15 minutes.

The formulations for okara cookies are presented in Table 4.4.

Table 4.4 Formulation of okara powder cookies.

Ingredient (%)	**V1**	**V2**	**V3**
Okara Flour	31.62	29.6	26.85
Shortening	11.72	10.96	15.41
Brown sugar	11.72	10.96	11.68
Chia gel	5.85	5.48	5.84
HMPC	0.4	0.30	0.32
Vanilla extract	0.5	0.48	0.51
Tea chai extract	0.16	0.15	0.16
Salt	1.33	1.25	1.33
Water	36.7	39.67	36.66
Corn flour	-	1.17	1.25
Total	100	100	100

The percentage (%) is based on the total basis.

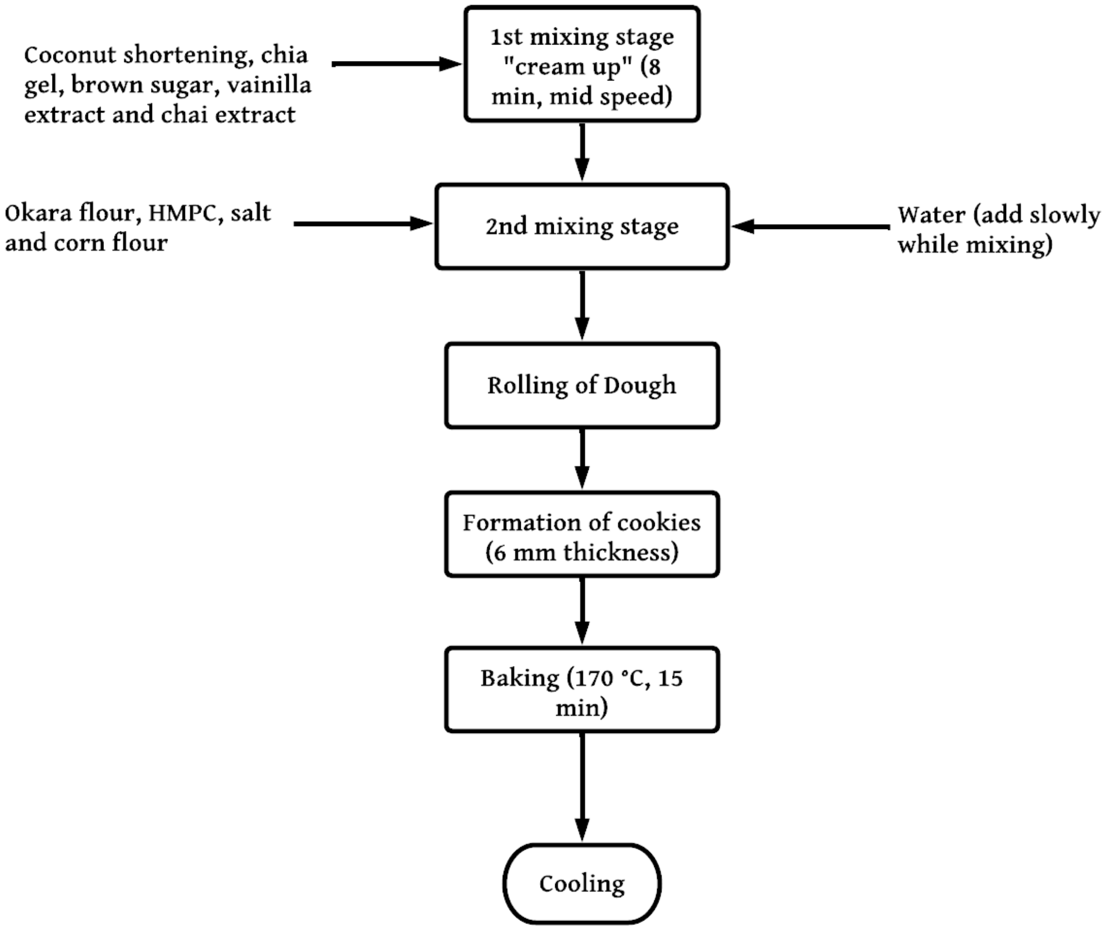


Figure 4.2 Flow diagram for the production of okara cookies adapted from Sykes & Davidson (2020).

4.3.7 Analysis of samples

4.3.7.1 Colour Analysis

The colour of cookies and bread was determined using a colorimeter (Konica Minolta CR-200 chroma meter), as described in Chapter 3, based on the CIELAB colour space coordinates. Before starting the measurements, the colorimeter was calibrated using the CR-A47 white calibration plate. All samples were wrapped in plastic wrap for the measurements, with the measuring head of the CR-200 placed directly on top of the plastic.

Bread samples were cut into 15 mm slices, and both the crumb and crust were measured at two different spots per slice. Colour measurements were performed on four slices per sample, resulting in eight readings (four crumb and four crust) per bread variation. For the control bread, two batches were made while the 80:20 wheat: okara breads (80, 90 and 100% hydration levels) had three batches each. Similarly, for cookies, three random spots per cookie were measured, and each sample was tested in triplicate, resulting in nine measurements for each sample. For each cookie variation (labelled as V1, V2 and V3), three batches were prepared.

The values of colour measurement obtained included L*, a*, b*, C*, and h. These coordinates provide comprehensive colour information: L* refers to lightness ranging from 0 (black) to 100 (white), a* represents the green (-a) to red (+a) hue, b* indicates the blue (-b) to yellow (+b) hue, C* is chroma, indicating colour intensity and h represents hue angle, representing the colour's specific tone.

4.3.7.2 Bread Height Measurement

The height of bread was measured using a vernier caliper (Mitutoyo 150mm Vernier Caliper 0.02 mm Resolution, SERIES 530). The measurement was taken in triplicate per sample, and each bread variation had 3 batches.

4.3.7.3 Spread Factor of Cookies

The spread factor is considered an indicator of cookie quality. It was determined by measuring the diameter and thickness of the baked cookie based on the method described by Park et al. (2015). The diameter and thickness of the cookies were measured using the same vernier caliper as used for the bread height measurement. The spread factor was calculated using the formula shown in Equation 4.2.

$$Spread\ Factor = \frac{Diameter}{Height} \qquad \text{Equation 4.2}$$

4.3.7.4 Texture Analysis of Bread

The textural properties of bread samples were measured using a texture analyser (Stable Micro Systems TA-XT plus Texture Analyser) based on the methods from Ammar et al. (2016) and Carocho et al. (2020). From each sample, three slices with a thickness of 15 mm were cut for analysis. The probe used was a 35 mm diameter aluminium cylinder. The type of test conducted was compression, specifically a cycle until count; the test parameters were set with a pre-test speed of 60 mm/min, a test speed of 120 mm/min, and a post-test speed of 120 mm/min with a trigger force of 5 g. The number of compressions carried out was five per slice tested. The Exponent software was used to obtain the results on hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience. This analysis was done in triplicate per batch and three batches were analysed per sample.

4.3.7.5 Texture Analysis of Cookies

The textural properties of cookie samples were measured using a texture analyser (Stable Micro Systems TA-XT plus Texture Analyser) based on the methods from Kaur et al. (2019) and Pareyt et al.

(2008). For each variation, seven cookies per batch were tested. Three batches of cookies were prepared per formulation. The probe used was the three-point bend ring. The type of test used was compression, specifically a cycle until count; the test parameters were set at a pre-test speed of 5 mm/sec, test speed of 3 mm/sec, post-test speed of 5 mm/sec and a trigger force of 5 g. Two compressions were carried out per cookie. The sample parameters were a depth of 6.5 mm, a width of 58 mm, a support gap of 22 mm, and a strain height of 12.41 mm. The Exponent software was used to obtain the results on hardness, fracturability, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience.

4.3.7.6 Evaluation of coating agent

The coating agent was evaluated visually and through taste testing. The visual aspects evaluated were golden colour, uniformity and adhesion of coating and dry appearance. The taste was tested to be desirable specifically the crispiness and dryness of the crust and the soft texture of the vegetable (Mellema, 2003; Voong et al., 2018; Wang et al., 2023).

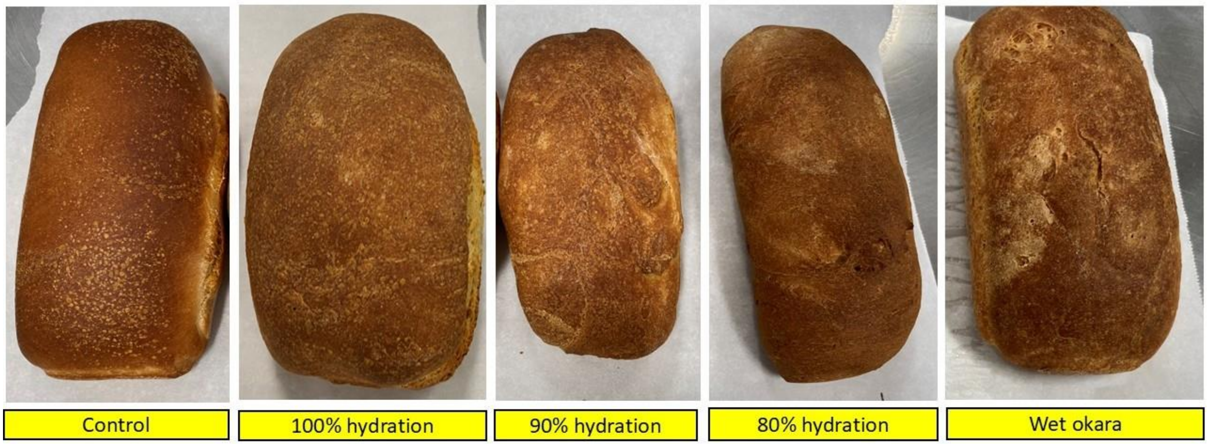
4.3.8 Data Analysis

The data obtained for each physical property (colour, heigh, diameter, spread factor and textural properties) were analysed using Minitab 18 Software. The means were compared using one-way analysis of variance (ANOVA) and Tukey’s range test to determine if they were any significant differences with a significance level set at $\alpha=0.05$.

4.4 Results and Discussion

4.4.1 Evaluation of Bread

Five bread samples were prepared (control, 100% hydration, 90% hydration, 80% hydration and wet okara). The control sample consisted of 100% wheat flour as the flour component in the formulation and followed a typical bread formulation including sugar, yeast, salt and water (64%). The following three samples were prepared with a ratio of 80:20 of okara: wheat flour, different hydration levels (100%, 90% and 80%) and hydroxypropyl methylcellulose (HMPC) was added to the formulation. An additional bread sample was prepared using 76.92% wet okara (okara that was not dried) and 80% wheat flour as the flour components, 23.2% water, HMPC and the rest of the ingredients. For the wet okara bread only the texture and height was analysed. The bread samples are shown in Figure 4.3. The crust colour of bread samples appeared to be similar while the height seemed to vary, 80% hydration seemed to have the shortest height out of all samples. While 100% hydration sample has the largest width. Even though, okara bread samples had lower gluten content, it seems the dough developed correctly.



Control 100% hydration 90% hydration 80% hydration Wet okara

Figure 4.3 Bread samples: control (wheat flour), okara-enriched with HMPC at different hydration levels (80%, 90% and 100%) and wet okara.

4.4.1.1 Crust and crumb colour

The colour development in bread takes place during the baking process through biochemical reactions such as the Maillard reaction and caramelisation. Maillard reaction occurs at temperatures below 150 °C and requires the presence of amino acids and reducing sugars. In contrast, caramelization takes place at temperatures above 150 °C and does not require amino acids. (Castro et al., 2017, Dessev et al., 2020).

The crust and crumb colours of the bread samples were assessed and expressed in the *CIE LAB* coordinates, indicating L* (lightness), a* (redness/greenness), b* (yellowness/blueness), C* (chroma or saturation) and h° (hue angle). The results of crust and crumb colour are shown in Tables 4.5 and 4.6, respectively. Significant differences were observed among the samples for some parameters, reflecting the impact of hydration levels on crust colour development. Based on the results, the crust colour of the bread samples can be defined as fairly light, with a red-yellowish tone for the control, 90% hydration and 80% hydration samples. In contrast, the 100% hydration sample is perceived as more pronounced yellow tone due to its hue angle being is greater than 90 °.

Examining the colour coordinates of the bread crust samples in Table 4.5, the L* values for the control was 56.51, while the other samples exhibited higher L* values, ranging from 61 and 66. The a* values for the okara samples were significantly similar ranging from 10 to 11, meanwhile the control sample had a lower a* value (7.64). The samples with the lowest b* values were 80% hydration and 100% hydration with 6.78 and 8.78, respectively. From all the okara bread samples, 90% hydration was the most similar to the control samples for this colour coordinate. The C value for the 90% hydration was 17.45, while the other samples exhibited lower C values, ranging from 13 to 16. For h°, the sample with the highest value was 100% hydration (107.50), while the other samples ranged from 41 to 53.

Table 4.5 Crust colour of bread samples

Bread	L*	a*	b*	C*	h °
Control	56.51 ± 7.52^{b}	7.64 ± 3.25^{b}	13.74 ± 3.96^{a}	16.29 ± 2.63^{ab}	59.39±15.73^{ab}
100% hydration	61.02 ± 8.29^{ab}	10.15 ± 1.38^{a}	8.78 ± 7.78^{bc}	14.85 ± 4.47^{ab}	107.50±83.91^{a}
90% hydration	61.76 ± 8.41^{ab}	10.62 ± 3.11^{a}	12.12 ± 6.75^{ab}	17.45 ± 4.43^{a}	41.94±19.09^{b}
80% hydration	66.07 ± 12.09^{a}	11.29 ± 1.53^{a}	6.78 ± 7.77^{c}	13.71 ± 2.22^{b}	43.60±10.67^{b}

Values are presented as mean ± standard deviation for all bread colour coordinates. Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

Looking at each of the colour coordinates presented in Table 4.5, the L* values for the control sample are significantly different from the 80% hydration sample. The a* values for the control sample are significantly different from those of the other samples. The b* and C* values differ between some samples, while the h° values of 100% hydration differ from the other okara based breads. The a* coordinate indicates the redness/greenness of the bread. In all the bread samples, the values are positive, meaning the bread has red tones, but the control sample value has a smaller a* value compared to the okara breads, indicating that the okara breads have a stronger red tone than the bread made solely with wheat flour. The reason for this could be that okara flour has more pronounced red tones than wheat flour. This is supported by the fact that the three samples containing the same amount of okara flour in their formulation showed no significant differences, despite varying water content. The inclusion of okara flour in the bread formulation results in more reddish colours due to an increased production of melanoidins, which form through interactions between protein and reducing sugars during the baking process (Palermo et al., 2012; Ostermann-Porcel et al., 2017c; Dessev et al., 2020). The rate of this reaction is increased by factors such as temperature, water activity, and the amounts of sugars and proteins present. In the case of okara flour, its high fibre content and higher levels of reducing sugar influence the intensified colour development. Fibre has a high water-holding capacity, which leads to a decrease in the water activity of bread dough resulting in quicker crust colour development because the Maillard reaction is sped up when less water is available (Palermo et al., 2012; Ahrné et al., 2007). Another factor responsible for higher colour development is the content of reducing sugar and okara flour contains more reducing sugars than wheat flour, 3.38g/100 g and 1.7 g/100 g, respectively (Palermo et al., 2012; Ostermann-Porcel et al., 2017c; Dessev et al., 2020).

The results for L* and b* are similar to those obtained by Ostermann-Porcel et al. (2017c), Baldino et al. (2018) and Philip et al. (2022), whereas the C* and h ° values in this study were lower. This could be because the maximum level of okara flour used by Ostermann-Porcel et al. (2017c) and Philip et al.

(2022) was 10 and 12.5%, respectively, while Baldino et al. (2018) used rice and wholemeal buckwheat flour as a complete substitution for wheat flour.

The total colour difference (ΔE) is used to compare the colour between two samples and identify the Euclidean distance between them (Mokrzycki & Tatol, 2011). The total colour difference for the okara bread samples when compared to the control sample was 6.20, 4.97 and 17.32 for 100%, 90% and 80% hydration, respectively. Based on these results, 80% hydration appeared to be the most different to the control samples in terms of total colour while 90% hydration is the most similar to control. When ΔE is larger than 5, the observer can notice two different colours between samples (Mokrzycki & Tatol, 2011). Thus, a consumer theoretically could notice that the control sample and 100% and 80% hydration samples have different colours, while with 90% hydration samples, an observer would notice a difference in colour but not two completely different colours.

The colour of bread crust is mainly affected by the temperature used during baking, while the crumb colour is determined by amount of moisture retained because moisture retention leads to a delay in the change of colour during baking (Philip et al., 2022).

Figure 4.4 shows the picture of the bread crumb samples, including the control and the breads made with partial replacement of wheat flour with okara flour. The results of colour measurements are shown in Table 4.6. The L* value for the control was 71.95 while the other samples exhibited other L* values, ranging from 84 to 93. The a* value for the control was -0.11 while the other samples exhibited other a* values ranging from 0.65 to 0.71. The b* values for all samples are significantly similar ranging from 8.03 to 12.20. The sample with the lowest C* value and that differ from the others was 80% hydration with 7.99, while the other C* values ranged from 11.45 to 12. For h° value, okara bread samples were significantly similar ranging from 85.93 to 86.89, meanwhile the control sample had a higher h° value of 90.54.

Table 4.6 Crumb colour of bread samples.

Bread	L*	a*	b*	C*	h °
Control	71.95 ± 2.47^{b}	-0.11 ± 0.22^{b}	11.68 ± 0.83^{a}	11.66 ± 0.82^{a}	90.54 ± 1.03^{a}
100% hydration	85.24 ± 12.79^{a}	0.71 ± 0.43^{a}	11.42 ± 4.34^{a}	11.45 ± 4.31^{a}	85.93 ± 3.40^{b}
90% hydration	84.75 ± 14.19^{a}	0.65 ± 0.44^{a}	12.20 ± 5.15^{a}	12.00 ± 4.38^{a}	86.89 ± 1.78^{b}
80% hydration	93.25 ± 12.21^{a}	0.67 ± 0.66^{a}	8.03 ± 4.47^{a}	7.99 ± 4.36^{b}	86.07 ± 4.24^{b}

Values are presented as mean ± standard deviation. Different lowercase letters in the same column indicate significant differenence ($p < 0.05$).

The crumb colour of all bread samples is quite lighter compared to the crust, with a perceived yellow tone. The differences in formulation are more noticeable in the crumb colour, with b* being the only factor unaffected by the inclusion of okara flour or variation in water content. As a result, all the bread samples have a yellow tone. The control bread sample is not as light as okara bread samples. Regarding chroma (C*), the only bread significantly different from the others is the 80% hydration bread with lower colour saturation compared to the other samples. The crumb colour in bread depends mostly on the colour of flour (Popov-Raljić et al., 2009), but the 80% hydration bread contained the same flours as the other okara breads samples, meaning difference in chroma was not affected by flour. One possible explanation for lower C* value for 80% hydration could be the lower b* values since C* depends on a* and b* values.

In comparison with other studies, the L* values are higher than those obtained by Philip et al. (2022) and Ostermann-Porcel et al. (2017c). It appears that when okara flour content is increased, the lightness of the crumb also increases. Phillip et al., (2022) observed this in their study, where the 5% okara bread sample had a lower L* value than the 10% and 12.5% samples. This helps explain why, when comparing the bread sample in this study to those made with less than 20% okara flour, the lightness is greater. The values of h° and C* obtained in this study are similar to those reported by Philip et al. (2022), while and the a* and b* values are similar to those reported by Baldino et al. (2018), where rice flour, among the gluten-free flours used substituted for wheat flour, showed results most similar to those obtained with okara flour.

The ΔE values representing the total colour difference between the control and the okara bread samples were 22.5, 14.76 and 14.30 for 80% hydration, 90% hydration and 100% hydration, respectively. Out of the three okara breads, 100% hydration is the most similar to control bread in terms of crumb colour.

The differences in colour measurements between bread samples could have been influenced by some of the physical factors of the bread such as crust thickness, crumb structure and texture. Bread crust is considered a limiting factor in bread quality because once formed, it restricts dough expansion which affects the cell structure, density and volume of the final product (Soleimani Pour-Damanab et al., 2014). In terms of colour coordinates, thicker crusts have lower L* values because a thicker crust presents a darker tone, meanwhile the a* and b* values are higher as the redness and yellowness increases with thicker crust (Jusoh et al., 2008; Jusoh et al., 2009). The crumb structure of bread is non-uniform meaning the cell distribution varies from regions with large amounts of small cells to regions with fewer cells or regions with bigger cells. The regions that contain smaller cells will result in higher L* values because with finer pores, the light reflection is greater while coarser regions reflect less light (Scanlon & Zghal, 2001; Rathnayake et al., 2018; Pang et al., 2021). In terms of texture, Matos & Rosell (2012) observed that the luminosity of crumb increases when cohesiveness and resilience of bread increases.

4.4.1.2 Crumb structure

In addition to the colour of the crumb being altered, the addition of okara flour also impacts its structure. Bread crumb structure forms during baking as proteins denature due to the high temperature. As the dough is heated, wheat proteins denature and cause gas cells to expand, which results in gluten forming a network that creates the bread's crumb structure (Rosell, 2011). In case of gluten-free flours, the dough is more viscous and resembles a batter. Without gluten, the structural network formed during baking is weaker, leading to a crumbling texture (Ngemakwe et al., 2015). A strategy to improve the texture of gluten-free bread, is the use of hydrocolloids to mimic the viscoelastic properties of gluten (Ziobro et al., 2016). One of the most effective hydrocolloids in gluten-free bread formulations is HMPC because it strengthens the dough's network by improving its ability to retain gas and water during baking which leads to a better bread volume and a less crumbly crumb structure (Ngemakwe et al., 2015). In this study, HMPC was used, and as shown in Figure 4.4, the addition of okara led to a more compact crumb structure than a fragile one. Ostermann-Porcel et al.(2017c) explained that the presence of okara in bread increases the number of alveoli, contributing to a denser and more compact crumb.

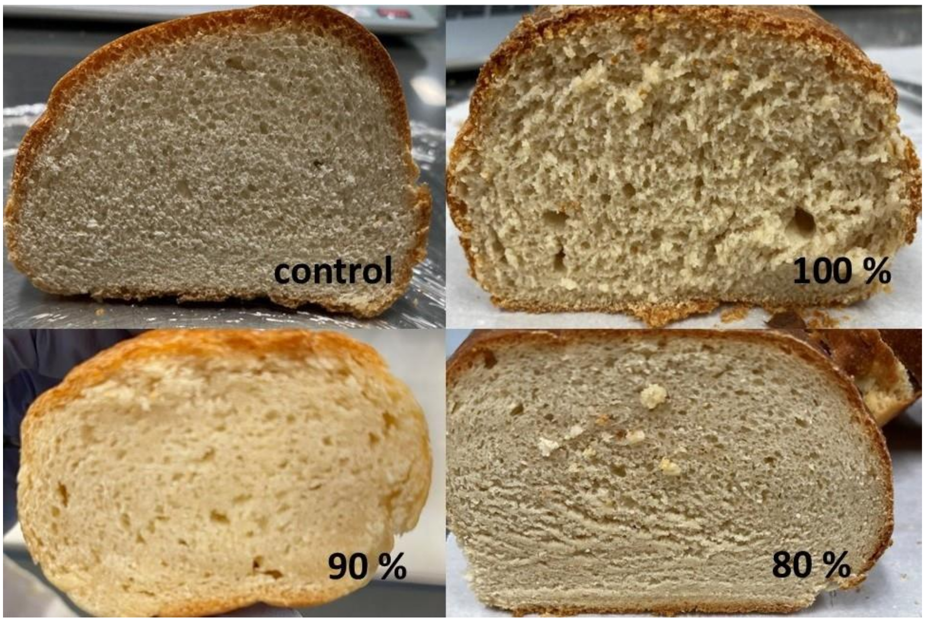


Figure 4.4 Comparison of bread crumb structure: okara-enriched with HMPC at different hydration levels (80%, 90% and 100%) vs. control bread.

4.4.1.3 Height

The height of each bread sample was measured, and the results are shown in Figure 4.5. There were significant differences in the height of the bread samples ($p < 0.05$). The okara bread sample that was most similar in height to the control was the one with 100% hydration. In contrast, the 90% and 80% hydration breads had similar heights, while the bread made with wet okara (instead of okara flour) differed from all other samples. In this physical characteristic, the amount of water added to the formulation was the key factor affecting the bread height. While all samples, except the control, contained okara, the water content varied. Apart from the content of gluten, the difference in height among breads can be primarily attributed to the incomplete hydration of gluten proteins. One of the main components of okara flour is fibre which absorbs some of the water intended for gluten hydration. As a result, if gluten proteins are not completely hydrated during the formation of bread dough, the gluten network cannot form properly, leading to reduced elasticity and extensibility. This negatively impacts the final structure of the bread (Philip et al., 2022). However, when the hydration level was 100% in breads containing 20% okara flour and 80% wheat flour, the height of the bread was not significantly different from the control as shown in Figure 4.5, indicating that sufficient hydration allows for proper gluten protein hydration, maintaining the bread's structure.

Another component affected by the lack of water is starch. In gluten-free bread formulations, the amount of water added is typically increased to allow starch to fully gelatinize during baking. This helps improve dough's gas retention abilities, as increased water content raises the viscosity of the dough (Bender & Schönlechner, 2020). The 100% hydration bread appeared to have the best viscosity among all the okara bread samples, as its height wasn't significantly affected, indicating it retained the most gas.

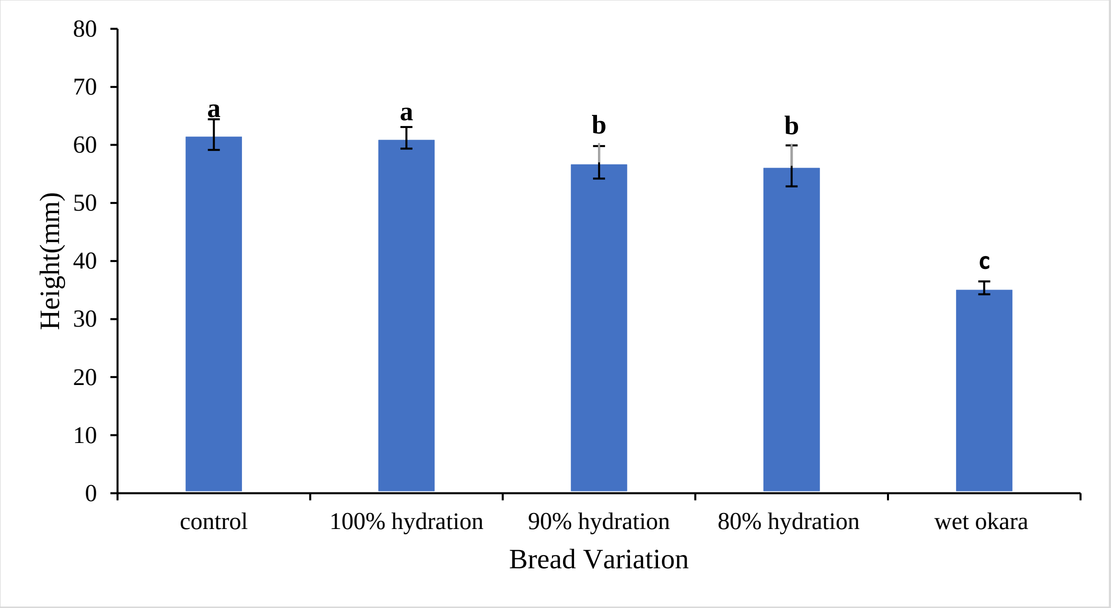


Figure 4.5 Height of the bread samples with varying hydration levels and okara content.

Values are presented as mean ± standard deviation. Significant differences between the samples are indicated by different lower-case letters ($p < 0.05$).

4.4.1.4 Textural properties

The texture properties of the bread samples were evaluated using Texture Profile analysis, assessing hardness, springiness, cohesiveness, gumminess, chewiness, and resilience. The results are shown in Table 4.7. There were no significant differences ($p > 0.05$) between the bread samples for springiness, cohesiveness and resilience, suggesting that the substitution of 20% wheat flour with okara flour did not negatively affect the texture. To identify the okara bread sample most similar in textural properties to the control bread (100% wheat flour), hardness and fracturability were considered. Also shown in Table 4.7, the bread with 90% hydration was the least similar to the control in these two factors.

Table 4.7 Textural properties of bread samples

Texture	Control	100 % hydration	90% hydration	80% hydration	wet okara
Hardness (g)	1314±601^{b}	1125±714^{b}	2423±1191^{a}	1801±601^{ab}	1509±816^{ab}
Fracturability(g)	1352±633^{b}	1164±756^{b}	2508±1257^{a}	1850±620^{ab}	1539±838^{ab}
Adhesiveness (g/s)	0.16±0.07^{a}	1.38±0.78^{ab}	2.48±2.03^{b}	0.44±0.40^{a}	0.30±0.25^{a}
Springiness	0.91±0.02^{a}	0.92±0.04^{a}	0.88±0.04^{a}	0.89±0.03^{a}	0.88±0.02^{a}
Cohesiveness	0.78±0.05^{a}	0.74±0.10^{a}	0.74±0.06^{a}	0.77±0.05^{a}	0.80±0.03^{a}
Gumminess	1004±388^{bc}	766±359^{c}	1746±737^{a}	1376±456^{ab}	1189±632^{abc}
Chewiness	918±352^{bc}	700±322^{c}	1542±647^{a}	1233±398^{ab}	1053±566^{abc}
Resilience	0.53±0.05^{a}	0.47±0.11^{a}	0.47±0.04^{a}	0.51±0.06^{a}	0.51±0.03^{a}

Values are presented as mean ± standard deviation. Different lower-case letters in the same row are significantly different ($p < 0.05$). n=9 for all samples.

It was initially expected that okara bread samples would have higher hardness values than the control (wheat bread) because okara flour has a higher protein content than wheat flour, and higher protein content typically results in a harder crumb texture (Gallagher et al., 2003). However, in this study, out of the okara bread samples, the 90% hydration sample was the only one that was significant different in hardness ($p < 0.05$) to control, meaning that protein content didn't affect hardness in this study. The hardness values of the okara bread samples were lower than expected, which can be attributed to two factors: of the increased water content and the presence of HMPC.

Firstly, an increase in water content leads to lower hardness values (Gallagher et al., 2003). This is confirmed with the 100% hydration bread having the lowest hardness value (1125) and highest water content. In this study, the moisture contents of the bread formulations were pretty similar, which could explain most of the okara breads having similar hardness values to the control bread. Based on the moisture content of freeze-dried okara (8.46%), wet okara (74%) and wheat flour (14%) and water added to the formulations; the 100% hydration formulation had the highest moisture content (54.02%)

followed by 90% hydration (51.70%), 80% hydration (49.15%), wet okara (48.29%) and control (45.48%). Secondly, HMPC has an impact on bread hardness due to its great water and gas retention abilities. The increase in gas retention improves the stability of gas cell walls in bread which prevents moisture loss during baking, obtaining a softer crumb (Crockett et al., 2011). Similarly, its great water-binding capacity helps avoid water loss in storage and delays starch retrogradation by hydrogen bonding with starch and replacing the gluten proteins (Sabanis & Tzia, 2011). The behaviour of HMPC and similar moisture content between formulation could explain the similarities in hardness between control sample and the okara bread samples with the exception of 90% hydration sample.

Cohesiveness provides insight on how the bread behaves during mastication, indicating whether it crumbles upon initial deformation or forms a bolus. For bread, a high cohesiveness value (>0.4) is desirable because it indicates that the bread doesn't crumble easily when compressed (Matos & Rosell, 2012). In this study, all the bread samples exhibited high cohesiveness values, which were greater than those (0.32 for 5%, 0.36 for 7.5% and 0.37 for 10% okara flour) reported by Ostermann-Porcel et al (2017c). The inclusion of okara flour at 20% appears to enhance this textural property, as Ostermann-Porcel et al. (2017c) observed lower values when using a maximum of 10 % okara flour.

Fracturability refers to a food product's tendency to crumble or fracture when a small force is applied. This property is commonly measured in baked goods and depends on the product's hardness and cohesiveness. Among all the bread samples, the 90% hydration sample had the highest hardness value and as result, the highest fracturability.

Springiness refers to the rate at which a food product returns to its original state after a force has been applied (Carocho et al., 2020). In the case of bread, springiness provides information about the freshness of the bread. Samples with low springiness values are associated with a fragile crumb structure and shorter shelf life (Tóth et al., 2022, Matos & Rosell, 2012). In this study, all bread samples showed no significant differences in springiness. When compared with other bread types studied by Carocho et al. (2020), the okara bread samples had similar springiness values to Bavaria bread samples.

The adhesiveness values of the bread samples were low when compared to stickier products with adhesiveness values above -25 g/s such as potato (-73.74) (Wong et al., 2023). This is typical, as Carocho et al. (2020) explained that bread is not considered a very adhesive food product. Adhesiveness refers to the tendency of a food product to adhere to the teeth when chewing, and bread typically doesn't adhere to the teeth.

Chewiness is the result of the all the other textural properties, as it is defined as the energy needed to chew a food. For example, the higher the hardness value, the higher the chewiness value, as a harder bread requires more energy to chew (Carocho et al., 2020). This is confirmed by the results obtained in this study; the hardest bread was the 90% hydration sample, which also had the highest chewiness value (1542).

Resilience, similar to springiness, measures how the food product recovers after a deforming force is removed, focusing on both force and speed (Carocho et al., 2020, Chandra & Shamasundar, 2015). In this study, all bread samples showed no significant differences in resilience. When compared with other bread types studied by Carocho et al. (2020), the bread samples had similar resilience values to oat and rye bread. On the other hand, when compared to other gluten-free bread samples studied by Matos & Rosell (2012), the okara bread samples were most similar to the bread made with corn starch as wheat flour substitute.

Gumminess is the product of hardness and cohesiveness (Chandra & Shamasundar, 2015). High gumminess values are obtained from high hardness values. This is confirmed by the results obtained in this study, all bread samples were significantly similar in terms of cohesiveness while the 90% hydration sample had the highest hardness value, as well as highest gumminess value (1542). Whereas 100% hydration sample had the lowest hardness and gumminess value (766).

In terms of texture properties, all the okara bread samples with the exception of the 90% hydration samples are good alternatives to wheat flour bread. These okara bread samples are significantly similar to the control bread in all textural properties.

The sensory evaluation of okara bread samples and cookies was not conducted due to the unexpected and swift closure of the food technology programme on the Albany campus and the delay in ethics approval . Unfortunately, this event created time constraints on the project, and it was decided that the primary aim when developing the bread samples and cookies was to quantify the specific physical attributed such as height, colour, spread factor and texture measurements instead of subjective sensory perceptions.

4.4.2 Physical attributes of cookies

Okara flour was also used in cookie preparation to investigate its functionality as an ingredient. The experimental trials conducted were to explore a full substitution of wheat flour with okara flour, chia seeds gel instead of egg, inclusion of corn flour and adjustments in shortening content to assess their impact on the cookie's physical and functional properties. Figure 4.6 shows the visual appearance of the okara cookies prepared by baking at 170 °C for 15 minutes.

In this study, three different okara cookie formulations were compared. When attempting to develop 100% okara flour cookies, the resulting structure was fragile, as shown in Figure 4.6A and Figure 4.6B. Comparing the pictures of each variation in Figure 4.6, it can be observed that the V1 cookies are filled with cracks and break easily. The V2 variation appears improved texture with fewer cracks, but the issues persists. By increasing the fat content and reducing the amount of okara flour, the V3 formulation was achieved, resulting in cookie with no visible cracks. The lack of gluten in the formulations seems to affect the binding of ingredients. However, fat is responsible for holding the ingredients together in the cookie dough and contributes to key properties such as mouthfeel, spread, flavour, lubricity, and tenderness (Devi & Khatkar, 2016, Culetu et al., 2021b). The issues observed in V1 and V2 likely stem from insufficient fat to effectively bind the cookie dough components, resulting in surface cracks. By increasing the fat content, as in V3, the structure of the cookie appears to be significantly improved.

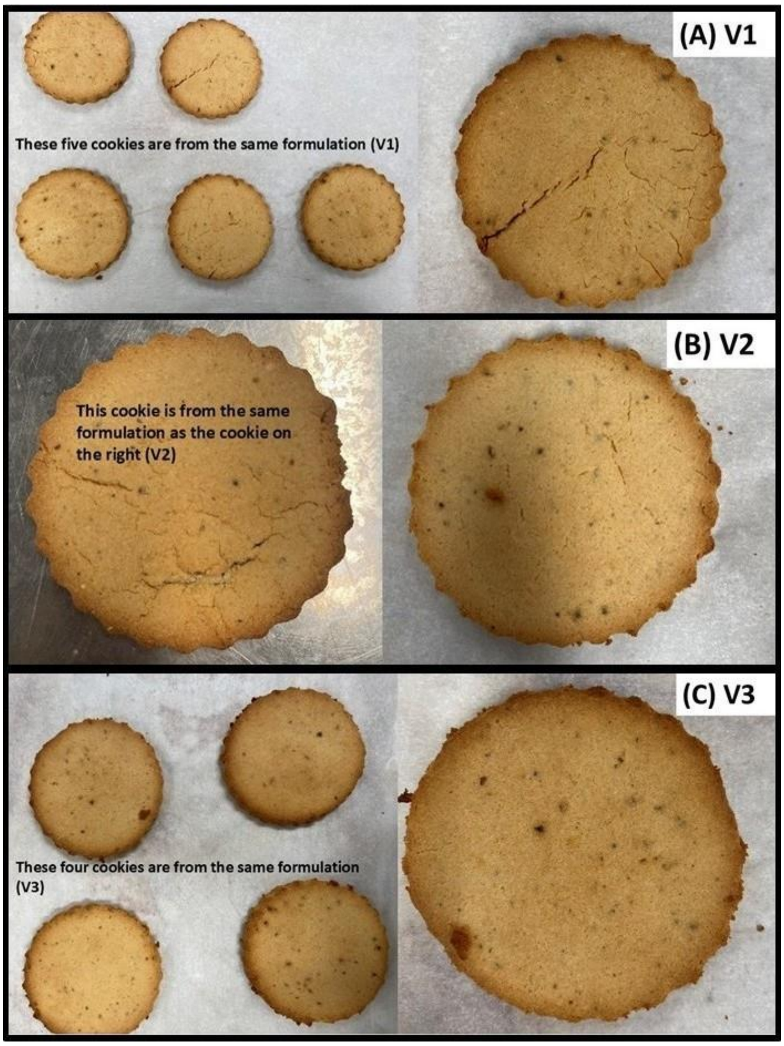


Figure 4.6 Visual appearance of okara cookies: (A) Formulation V1, (B) Formulation V2 and (C) Formulation V3.

4.4.2.1 Colour

The results of the colour measurements for okara flour cookies are shown in Table 4.8 and are expressed in CIELAB coordinates. As described earlier, L* refers to lightness, a* to redness/greenness, b* reflects yellowness/blueness, C* denotes the intensity or saturation of the colour and h° represents the hue or perception of the colour. Based on visual observation, the colour of the okara cookie samples can be described as fairly light with a noticeable red-yellowish bright tone. This visual impression is supported by the data in Table 4.8 where the L* values (69.44, 71.51, 67.89) suggest a light colour, as values closer to 100 correspond to a whiter appearance. The positive a* and b* values confirm the presence of red and yellow tones, respectively. Additionally, the C* value indicates colour intensity which is fairly saturated, while the hue angle (h°), which falls between 0 and 90 °C, confirms as the perception of a red-yellowish tone. Specifically, an h ° of 0 corresponds to a fully red tone, while 90° corresponds to yellow.

The three cookie variations (V1, V2, V3) show no significant differences in their L*, b* and C* values. However, V3 is different in terms of a* and h°. V3 has a higher a* value (8.46 ± 0.70), indicating a more reddish, which aligns with its smaller hue angle (71.27 ± 1.52). This difference may be due to V3 containing more shortening, as more fat content leads to more oil release during baking, the concentration of sugars increases and the Maillard reaction is more intense as a result (Mancebo et al., 2015). Osterman-Porcel et al. (2017b) reported similar L*, a* and b* values for cookies made with 50% okara flour as maximum substitution of wheat flour and Lee et al., (2020) had similar L and b* vales with 40% okara flour.

Table 4.8 Surface colour of okara-based cookies.

Variation	**L***	**a***	**b***	**C**	**h°**
V1	69.44±3.14^{ab}	7.44±0.77^{b}	24.66±0.96^{a}	25.77±1.03^{a}	73.29±1.48^{a}
V2	71.51±1.26^{a}	6.84±0.59^{b}	24.50±0.99^{a}	25.43±1.04^{a}	74.49±1.10^{a}
V3	67.89±1.74^{b}	8.46±0.70^{a}	24.81±0.93^{a}	26.22±0.93^{a}	71.27±1.52^{b}

Values are presented as mean ± standard deviation. Different lower-case letters in the same column indicate significant difference ($p < 0.05$). n=9 for all samples.

4.4.2.2 Spread factor

The spread factor is considered a reflection of a cookie's quality, as it depends on the diameter and height of the cookie. Table 4.9 shows the diameter, height and spread factor of the three variations of okara-based cookies. No significant differences ($p > 0.05$) were found in the spread factor, height, and diameter of the samples. The height of the cookies could vary slightly due to the thickness of the rolled dough, which may not always be uniform. To minimize this variation, an adjustable rolling pin with a 6 mm thickness was used. The diameter remained consistent because the same cookie mould was used for all three cookie variations.

Table 4.9 Spread ratio of the cookies.

Variation	Diameter (cm)	Height (cm)	Spread factor
V1	5.72 ± 0.04^a	0.57 ± 0.04^b	9.82 ± 0.65^a
V2	5.76 ± 0.18^a	0.64 ± 0.09^a	9.08 ± 1.00^a
V3	5.80 ± 0.18^a	0.63 ± 0.07^{ab}	9.34 ± 0.73^a

Values are presented as mean ± standard deviation. Different lower-case letters in the same column are significantly different ($p < 0.05$). n=8 for all samples.

The spread factor of cookies is influenced by the expansion of the dough. It has been noticed that when cookies are made with flours high in protein and fibre, the spread factor is reduced. These types of flours bind strongly to the free water in the dough, increasing its viscosity and limiting dough expansion and elasticity. As a result, the spread factor is restricted, and the cookies have a tighter structure (Hooda & Jood, 2005, Agrahar-Murugkar et al., 2015, Lee et al., 2020, Park et al., 2015). This is consistent with the findings of Agrahar-Murugkar et al. (2015) and Park et al. (2015), where wheat flour was fully replaced with composite flour and fresh okara, respectively. In contrast, the cookies made by Lee et al. (2020) had higher spread ratios because they only substituted 20-40% of the wheat flour with okara flour.

Another component that affects spread ratio is fat. Fat contributes to the volume of the cookie via aeration (Ostermann-Porcel et al., 2017b). The type of fat used in the cookie formulation also has an influence on the spread ratio. Jacob & Leelavathi (2007) noticed that cookies made with oil, as opposed to shortening, spread more during baking. This is because shortening contains hydrogenated fat, which forms beta crystals and impairs dough aeration, resulting in a smaller spread ratio. These authors observed that cookies made with sunflower oil spread during the first 7 minutes of baking, while cookies with shortening stopped spreading within the first 5 minutes. Additionally, Devi & Khatkar (2016) found all-purpose shortening resulted in the lowest spread factor compared butter and margarine. This is because butter and margarine have lower melting points, causing them to melt faster and release their lubricant effect sooner, allowing the dough to spread more easily (Devi & Khatkar, 2016). This is why, in the study reported by Lee et al (2020), the biscuits had a higher spread ratio, as their formulation used oil as the fat source.

4.4.2.3 Texture Analysis of Cookies

The textural profile of the cookies was obtained through Texture Profile Analysis, which provided results for hardness, springiness, cohesiveness, gumminess, chewiness, and resilience for each sample. These results are shown in Table 4.10. The only textural property where all three samples were statistically the same ($p > 0.05$) was springiness.

Table 4.10 Textural properties of okara cookies.

Variation	**V1**	**V2**	**V3**
Hardness (g)	1259.5 ± 338.7^{a}	1849.2 ± 606^{b}	787.2 ± 313.6^{c}
Springiness	0.47 ± 0.09^{a}	0.55 ± 0.38^{a}	0.62 ± 0.19^{a}
Cohesiveness	0.08 ± 0.07^{b}	0.05 ± 0.05^{b}	0.25 ± 0.26^{a}
Gumminess	75.9 ± 112.5^{a}	84.1 ± 100.4^{b}	263.4 ± 272^{b}
Chewiness	43 ± 69.8^{b}	48.5 ± 61.6^{b}	200.8 ± 225.1^{a}
Resilience	0.04 ± 0.05^{b}	0.02 ± 0.02^{b}	0.16 ± 0.14^{a}

Values are presented as mean ± standard deviation. Different lower-case letters in the same row are significantly different ($p < 0.05$). n=21

For each variation, three batches of cookies were prepared. Texture measurements were conducted on seven cookies per batch, totalling 21 measurements per variation. Despite maintaining consistent preparation protocols, high variability in textural properties was observed within the same sample. This variability can be attributed to several factors, including the broad particle size distribution of the okara powder, which may lead to uneven hydration, and variations in mixing efficiency, resulting in inconsistent ingredient distribution. Additionally, differences in dough handling, shaping, and baking conditions may have further contributed to the observed textural differences.

All samples were significantly different ($p < 0.05$) in terms of hardness, with V1 having the highest value and V3 the lowest. Sugar is one of the components responsible for the hardness in cookies because, after baking, sugar recrystallises as the cookies cool (Xu et al., 2020). However, in this project, all cookie samples contained the same amount and type of sugar, meaning it isn't responsible for the observed differences in hardness. The other two ingredients that influence textural properties are flour and fat.

Firstly, fat can reduce the hardness of cookies by interfering with air incorporation, thus reducing the size of air cells. The values of hardness depend on the amount and composition of the fat used. If fat content in the formulation is reduced, the hardness decreases (Devi & Khatkar, 2016, Culetu et al., 2021b). This effect is evident in Table 4.10, where V3, which has a higher fat content than the other two samples, exhibits a lower hardness value. The hardness could have been even lower if a different type of fat, such as oil or butter, had been used, as these fats have a lower content of hydrogenated fats, which results in a more viscous and cohesive dough, leasing to softer dough and cookies (Jacob & Leelavathi, 2007, Culetu et al., 2021b). Secondly, the composition and particle size of the flour affect hardness. Ostermann-Porcel et al. (2016) noted that when okara content increased in the formulation, the hardness values also increased as a result of okara's high protein content, which leads to a more viscous and less extensible dough, thus yielding a harder cookie. Mancebo et al. (2015) observed that using coarser flour instead of finer flour led to an increase in hardness. In the case of cookie samples in this study, the same flour was used, so the effect can be attributed to the amount of okara flour used in V1 and V2. More okara flour results in a less elastic dough, less spread in the cookies, and a greater force required to fracture the cookies.

Cohesiveness provides information about how well the cookies behaved after a second deformation. This textural property depends mostly on gluten development in the dough, which can be limited if the sugar content is too high because it can reduce protein-water interactions. Since all the cookie samples are gluten-free, cohesiveness depends on the strength of the structure achieved without gluten. A high cohesiveness value means the cookie breaks more easily when stress is applied (Taylor et al., 2008, Bakare et al., 2020). This is confirmed by V3, which has the highest cohesiveness value and the lowest hardness value, while V1 and V2 have lower cohesiveness values and their structures are crumblier.

The gumminess and chewiness of the cookies depend on their hardness. Nasiri et al. (2023) reported that when inulin content is increased in gluten-free biscuits, hardness values are higher, leading to higher gumminess and chewiness values. The results of V3 contradict this, as it has lower hardness values but the highest values for chewiness and gumminess.

Finally, resilience is how well the cookie goes back to its original shape after compression. V3 appears to be more resilient than the other 2 samples.

4.4.3 Physical appearance of coating agents

Okara flour was also attempted as a fry coating agent in this study. The zucchini slices after frying are shown in Figure 4.7. Figure 4.7A shows the zucchini slices coated with a wheat flour and bread crumb mixture, while Figure 4.7B shows the zucchini slices coated with a combination of okara powder and okara bread crumb. It can be observed that using okara instead of wheat flour resulted in better coating outcomes. Figure 4.7B shows superior coating performance with no signs of detachment from the zucchini slices. In contrast, the samples coated with wheat flour and bread crumbs showed signs of detachment. When tasted, the zucchini slices coated with okara powder and okara bread crumb had a better flavour and a crunchier texture compared to those coated with wheat flour and bread crumb. This suggests that okara-derived ingredients (flour or bread crumbs) may be a more effective coating agent. According to Voong et al. (2018), a good coating agent should improve the texture of the product by preventing dehydration, reducing oil content and promoting colour development during frying. In this study, both coating agents facilitated browning, but okara was more successful in achieving a crispy texture and preserving the water content of the zucchini slices, as observed through individual personal sensory evaluation.

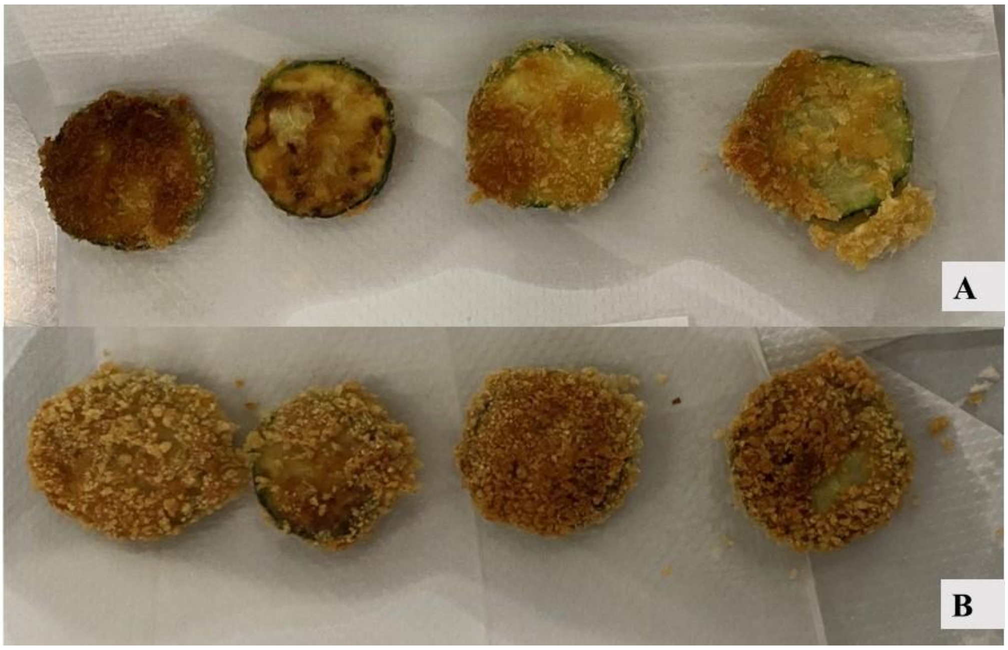


Figure 4.7 Zucchini slices after frying: (A) coated with wheat flour and bread crumbs, and (B) coated with okara powder and okara bread crumbs.

4.5 Conclusions

Among the bakery products tested, the only one in which okara powder could be used as a 100% substitute for wheat flour was cookies. For bread, full substitution was not possible due to the absence of gluten, the maximum level of flour substitution was 20%. When okara powder was included in the bread formulation, the hydration level or amount of water added needed to be increased to achieve similar results to regular bread. The hydration levels compared were 80, 90 and 100% based on total flour content. The best hydration level for bread with a 20:80 okara-wheat flour ratio was 100% because it yielded the most similar results to bread made with 100% wheat flour in terms of crumb structure, height, and texture properties. The 100% hydration okara bread allowed for complete hydration of the proteins, resulting in a similar height, a less compact structure, and ideal texture properties, particularly in terms of hardness, fracturability, adhesiveness, gumminess, and chewiness.

The use of fresh okara instead of dried okara for the bread formulation produced better results than expected, although the height suffered, which is an important characteristic of bread. On the other hand, cookies were successfully made using 100% okara powder as a substitute for wheat flour, and they could be made without dairy products or egg by using chia seed gel and coconut shortening. However, one of the main challenges in developing the cookie formulation with okara powder was the appearance of cracks, due to poor binding of the ingredients, mainly because okara powder has a low water-holding capacity. The best formulation out of the three samples was V3 because it had a higher fat content, which helped reduce cracking. This formulation also exhibited better textural properties, particularly lower hardness values, which are desirable for cookies.

One characteristic of the cookies was that their spread factor was lower compared to other studies, which could be attributed to the type of fat used. Okara powder also demonstrated to be an effective coating agent for frying purposes. For further studies, it would be recommended to use oil instead of shortening to increase the spread factor in cookies. Additionally, exploring other types of bread, such as sourdough or French bread, would be beneficial to investigate whether more okara powder could be incorporated. Sensory testing should also be conducted for all the products to obtain consumer preference regarding the formulations, rather than relying solely on experimental results. Furthermore, evaluating the oil-holding capacity of okara and testing it as a coating agent for other food products, such as chicken, meat and vegetables beyond zucchini, would be worthwhile.

Chapter 5. Overall Conclusions & Recommendations

5.1 Research Outcomes

This research project was conducted to explore the potential of revalorising okara into an ingredient for bakery products and other food applications by characterizing dry okara powders and evaluating their impact on physical and textural properties of the products.

Different drying conditions were applied to fresh okara to determine the optimal drying treatment to obtain functional and nutrient-rich okara powder. This study provided fundamental information on the best drying conditions, based on process time and their effect on the physicochemical characteristics of the powder. The drying treatments tested included freeze-drying and convection oven drying at various temperatures (50, 60, 70, 80, 90 and 100°C). Their effects on proximate composition, particle size distribution, microstructure, water activity, water holding capacity, solubility, bulk density, flowability and colour were compared. Results indicated that drying conditions significantly affected drying time, moisture content, particle size distribution, water activity, water holding capacity, solubility, bulk density, and colour.

The okara powders were found to have better nutritional compositional compared to wheat flour, with higher protein and fibre content, as well as all essential amino acids and some essential fatty acids. Freeze-drying treatment was shown to be a viable method for obtaining dry okara, but it was not the most efficient, as it took the longest time, resulting in a lower overall efficiency. However, this was due to the use of a small-scale lab freeze dryer. While freeze-dried okara powder had the best water-holding capacity, essential for food applications; it also had the highest moisture content and poor solubility. On the other hand, overall, convection oven drying at 90 °C and 70 °C was found as the most efficient methods, as they required less time to produce to dry okara, with low moisture content, small particle sizes, acceptable water-holding capacity, low water activity, and poor flowability.

To explore the application of okara powder as an ingredient in food products, it was tested as a substitute for wheat flour in bread, cookies and frying coatings. In bread making, it was determined that a complete substitution of wheat flour with okara powder is not feasible. The maximum substitution was 20 %. Three bread formulations containing 20% okara powder and 80 % wheat flour were tested at different hydration levels (80, 90 and 100%) and compared with bread made with 100% wheat flour (control bread). The impact of hydration level was noticed in the bread's height, crumb structure, hardness, and gumminess, with the addition of 20% of okara powder at 100% hydration yielding the best results in terms of crumb structure and height due to higher protein hydration. In contrast, the 80% and 90% hydration formulations exhibited more compact crumb structures and lower height as incomplete protein hydration resulted in an underdeveloped protein network. The use of wet okara instead of dried okara in bread formulation was possible, but the resulting bread had less height and stability.

For cookies, a complete 100% substitution of wheat flour with okara powder was achievable with modifications to the formulation. Three different formulations (V1, V2, and V3) were tested to determine the best formulation based on textural properties, spread factor, colour and physical appearance. A main challenge in cookie formulation was poor bonding, which resulted in cracks in the cookies. This issue was resolved in formulation V3 by adding more fat and reducing the amount of okara powder. The higher fat content improved textural properties, particularly hardness, making V3 the optimal formulation.

In conclusion, the most effective drying conditions for revalorising okara into powder were achieved by convection oven drying at 90°C or 70°C. For breads, a 100 % hydration level is optimal when using a 20% okara powder substitution to achieve the desired height, crumb structure, and texture. For cookies, a 100% okara powder substitution requires 15% fat content to produce cookies with better structure and prevent cracking. The okara powder obtained in this study is a nutritious and functional alternative to wheat flour, offering significant potential for use in bakery products. The revalorisation of okara can contribute to food security and provide a new gluten-free alternative for the market.

5.2 Recommendations

The aim of this project was to identify the best method for drying fresh okara and obtaining a powder that could be used as a wheat flour alternative in food production. The experiments focused on the effect drying conditions had on the physicochemical characteristics of okara powder and its impact on bread and cookie formulation. The results showed that different drying conditions affect the stability, water removing efficiency, colour, particle size, solubility and water-holding capacity of the powder. Additionally, okara powder can be used as a complete flour substitute for cookies, which hasn’t been reported in the literature. Cookies present fewer challenges because gluten is not as critical for structure as it is in bread; instead, the balance between fat content and okara is crucial for achieving a successful structure. For bread, a maximum substitution of 20% okara powder is recommended to obtain an acceptable product, with a hydration level of 100% for complete protein hydration.

The particle size of okara powders plays an important role in the functional properties of the flour. It affects bulk density and flowability. The powders obtained in this study were larger than those reported in the literature and those known for wheat flour, possibly because a coffee grinder was used instead of a laboratory mill. Therefore, it is recommended to grind the dried okara using various laboratory mills and compare the particle sizes obtained, along with an analysis of any changes in the other physical characteristics of the powders.

Additionally, the poor water-holding capacity and solubility of okara powders make it challenging to use them in products where water binding is essential, such as bread. It would be beneficial to explore composite flours by combining okara other less popular gluten-free flours and study their nutritional composition and functional properties in food products.

Hydrocolloids are commonly used in gluten-free formulations to obtain better results. In this study, only HMPC was used to improve the structure on bread and cookies. Therefore, it is recommended to investigate the use of other hydrocolloids at the same concentration to identify which ones perform best for each product, based on textural and structural properties. Proximal analysis of the okara powders confirmed that they are not nutritionally inferior to wheat flour. However, a proximate analysis of the food products would provide additional evidence on the health benefits of okara powder as a wheat flour substitute. It would also be helpful to conduct shelf-life studies on bread and cookies containing okara powder to determine ideal packaging and storage conditions for future commercialization.

While this study applied okara powder as an ingredient in food products, a formal sensory analysis was not conducted. The best formulations were chosen based on experimental results, but it is recommended to complement these experimental results with consumer feedback.

The project has demonstrated that okara powder can be successfully used as a wheat flour alternative in bakery products. However, there are still gaps in the application of this by-product. Therefore, further experimentation is needed to explore the use of okara powder in other food applications, such as salad dressings, by evaluating its oil retaining capacity.

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Appendices

Appendix 1 Okara powder comparisons

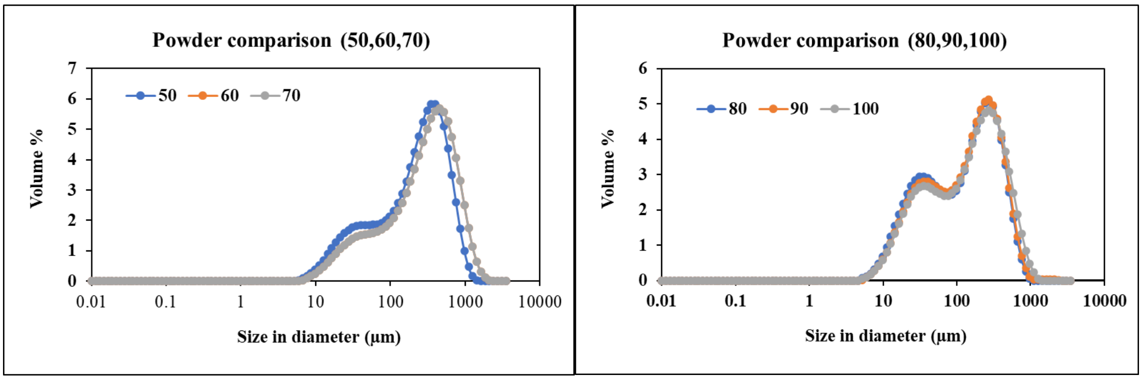

Figure A1.1 Particle size distribution comparison of okara powders obtained by convection oven drying at 50 °C, 60 °C, 70 °C, 80 °C, 90 °C and 100 °C.

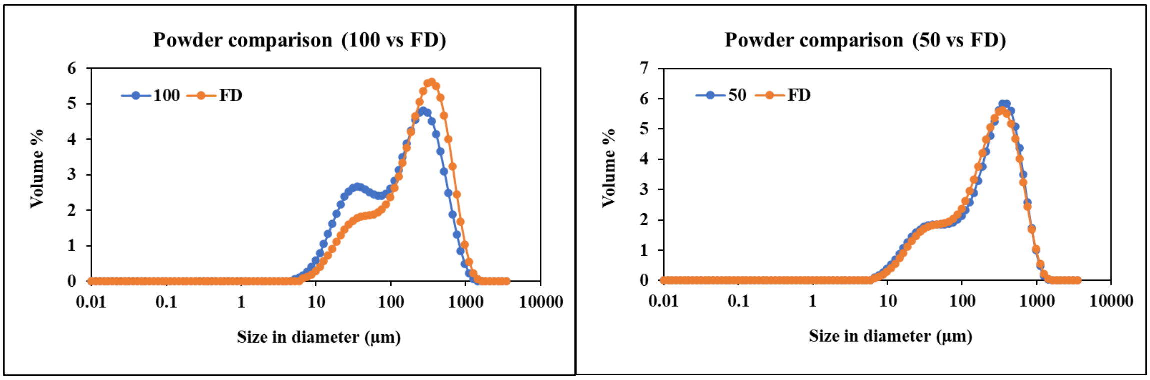

Figure A1.2 Particle size distribution comparison of okara powders obtained by convection oven drying at 50 °C, 100 °C and freeze-drying,

Appendix 2 Okara suspension particle size comparisons

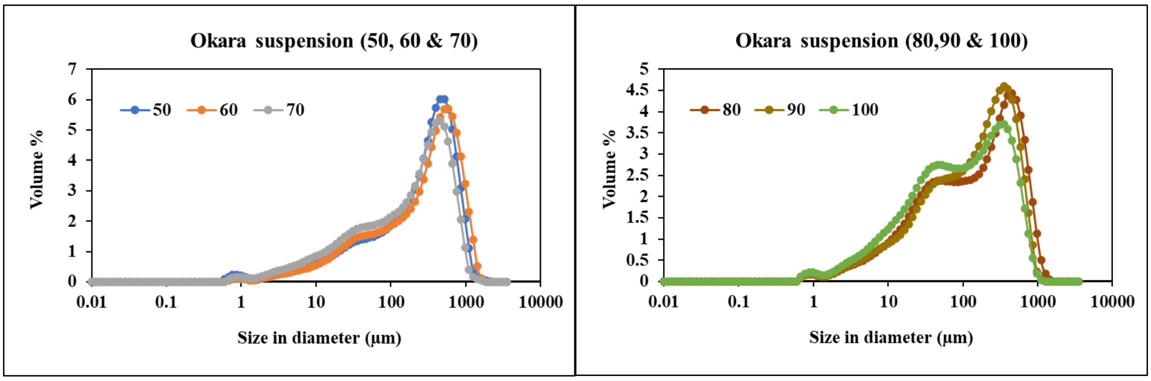

Figure A2.1 Particle size distribution comparison of okara suspensions where the okara powders used are O50, O60 and O70, O80, O90 and O100.

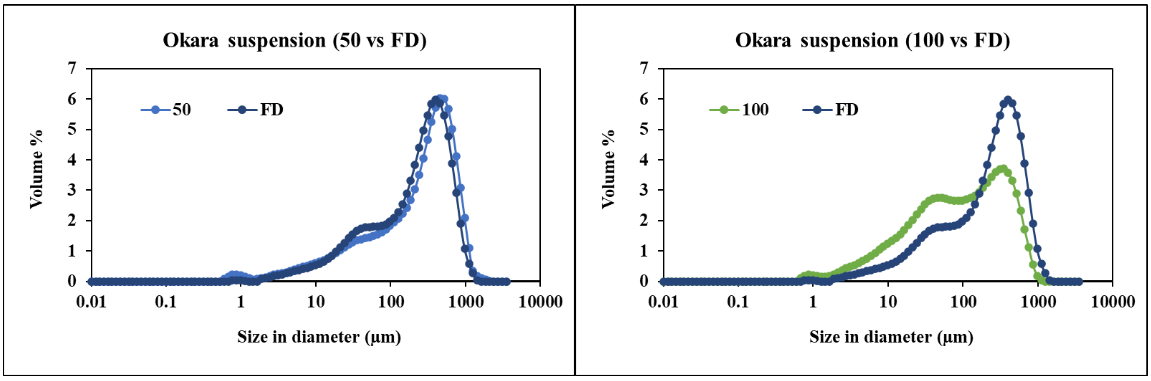

Figure A2.2 Particle size distribution comparison of okara suspensions where the okara powders used are O50, O10 and FD.

Appendix 3 Particle size comparison of powder and suspension

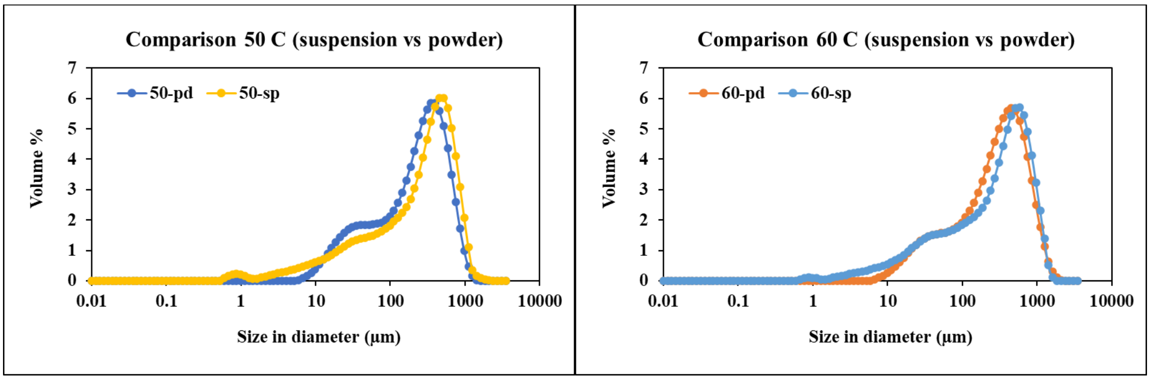

Figure A3.1 Particle size distribution comparison of okara powder and suspension when the drying condition is convection oven at 50 °C and 60 °C.

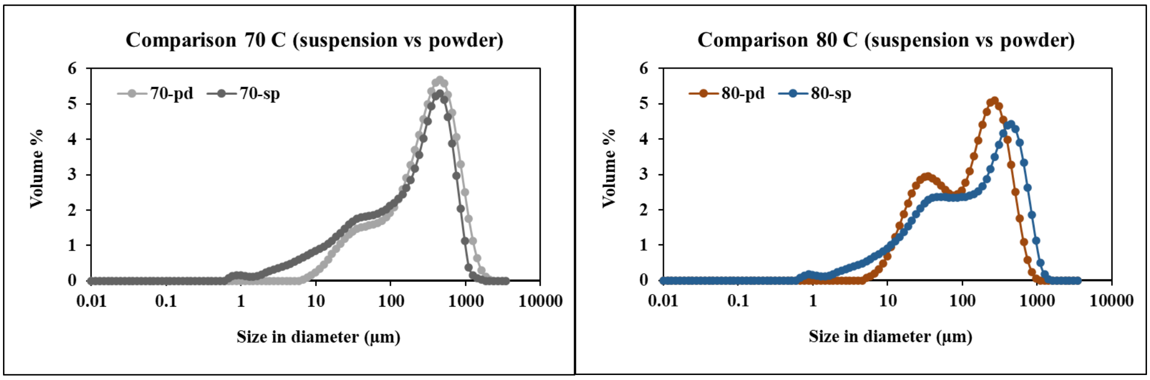

Figure A3.2 Particle size distribution comparison of okara powder and suspension when the drying condition is convection oven at 70 °C and 80 °C.

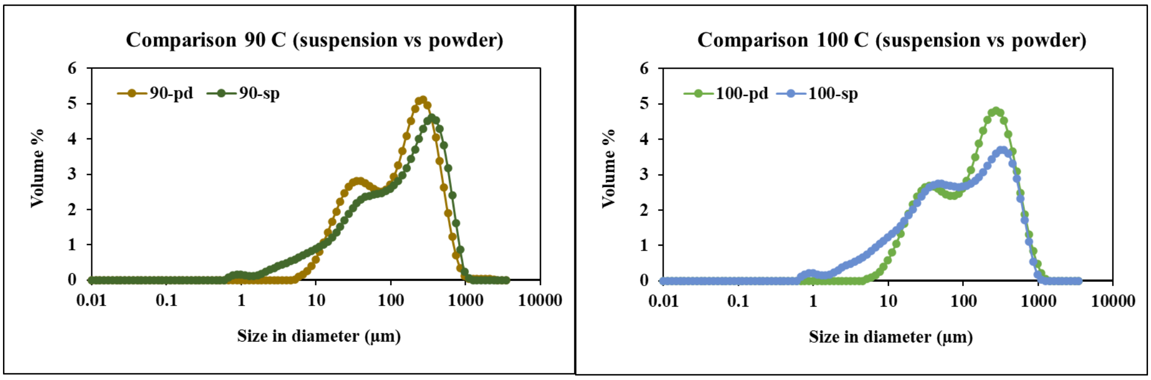

Figure A3.3 Particle size distribution comparison of okara powder and suspension when the drying condition is convection oven at 90 °C and 100 °C.

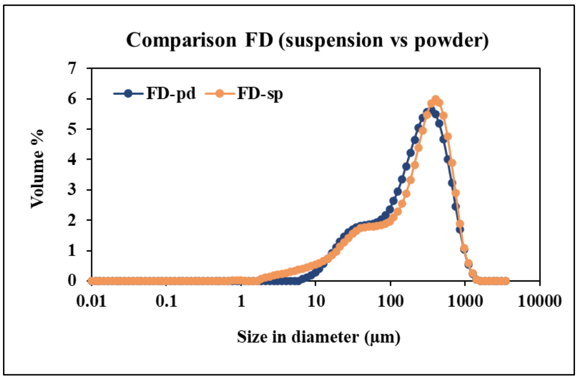

Figure A3.4 Particle size distribution comparison of okara powder and suspension when the drying condition is freeze-drying.

Appendix 4 Carr Index and Flowability Relationship. Adapted from Singh & Kumar (2012).

Carr Index	Hausner Ratio	Flow Character
≤10	1.00-1.11	Excellent
11-15	1.12-1.18	Good
16-20	1.19-1.25	Fair
21-25	1.26-1.34	Passable
26-31	1.35-1.45	Poor
32-37	1.46-1.59	Very poor
>38	>1.60	Very, very poor

Appendix 5 Testing okara powder amount for bread formulation

Ingredients (%)	**Bread Test**			
	1	**2**	**3**	**4**
Wheat Flour	90	50	75	75
Okara flour	10	50	25	25
Water	64	128	85	100
Yeast	1	1	1	1
Oil	3	3	3	3
Sugar	2	2	2	2
Salt	1.5	1.5	1.5	1.5
HMPC	-	1.5	1.5	1.5

Figure A5 Preliminary trials breads with different wheat: okara flour ratios and hydration levels (1) 90:10, 64% hydration level (2) 50:50, 128% hydration level (3) 75:25, 85% hydration level and (4) 75:25,100% hydration level