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Strategies for Shelf-Life Extension and Novel Food Product Development

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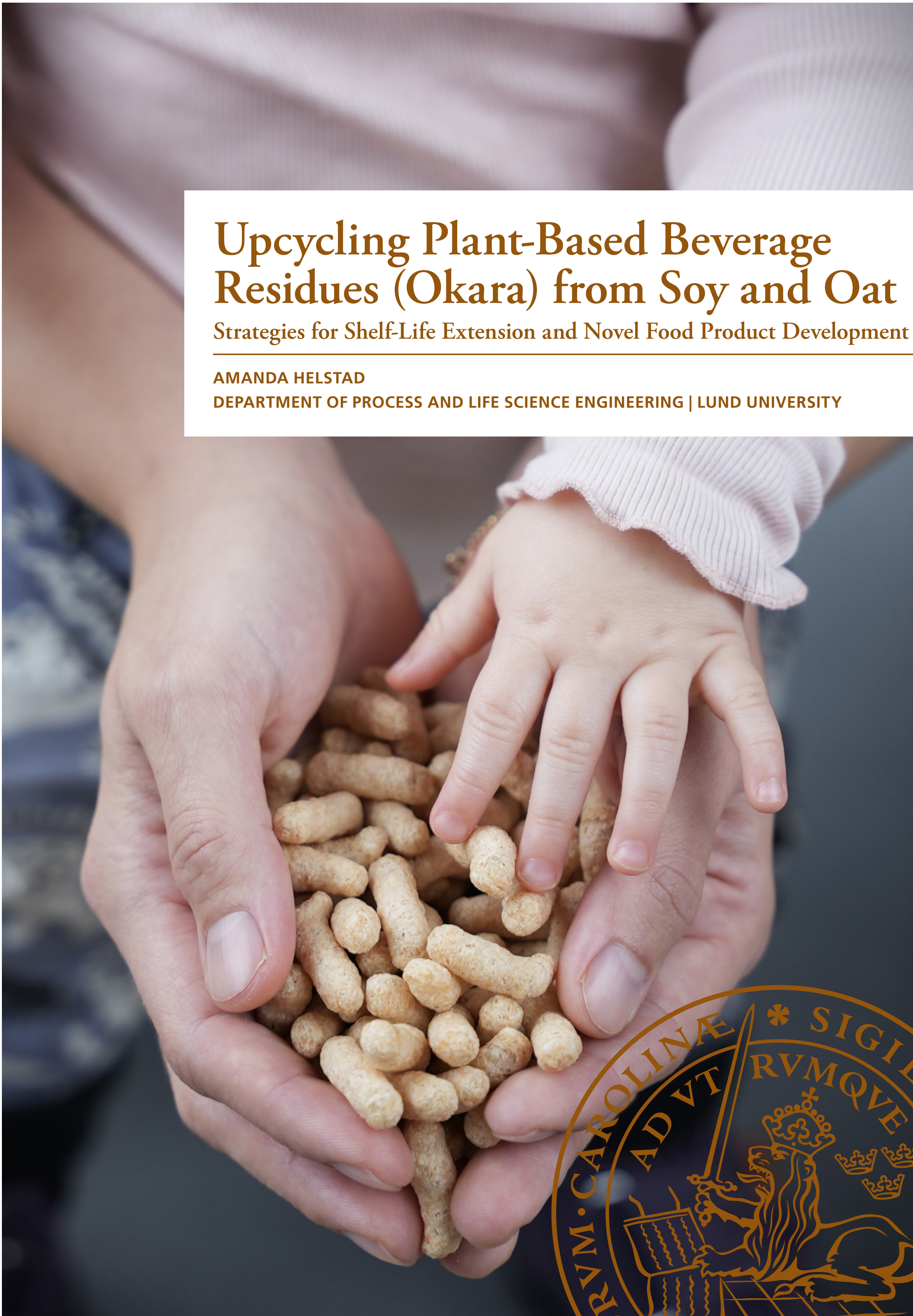

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Strategies for Shelf-Life Extension and Novel Food Product Development

Amanda Helstad

LUND UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Engineering at Lund University to be publicly defended on 13th of June at 9.00 in lecture hall A at The Chemical Centre, Department of Process and Life Science Engineering, Naturvetarvägen 14, Lund.

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Title and subtitle: Upcycling Plant-Based Beverage Residues (Okara) from Soy and Oat: Strategies for Shelf-Life Extension and Novel Food Product Development

Abstract:

In the plant-based beverage industry, a residue called okara, is generated in large quantities in the liquid-solid separation step. Despite the high nutritional value of okara from soy and oat beverages with respect to fiber and protein content, these residues are generally discarded or used as animal feed or compost. It would be more sustainable to utilize okara for human consumption, however, the main issue is the short shelf-life due to its high moisture content and water activity. The aim of this doctoral thesis was therefore to investigate strategies to extend the shelf-life of soy and oat okara and to find appropriate food applications to reduce food losses and thus improve the circular economy of plant-based beverages.

High-pressure processing was evaluated to improve the shelf-life of soy and oat okara at 200 MPa, 400 MPa, and 600 MPa. It was found that a treatment at 600 MPa could successfully extend the shelf-life for up to 2 weeks for soy okara, and almost 4 weeks for oat okara at storage in 4°C. High-pressure processing can therefore be a potential pasteurization technique for soy and oat okara to achieve a microbiologically safe ingredient. However, more research is required to optimize the process and to further investigate the microbiological species present in soy and oat okara to exclude any potential food safety risks. A beneficial nutritional outcome was also observed for the 600 MPa treatment, increasing the soluble fiber fraction of the total dietary fiber content in both soy and oat okara to 4.4% and 9.2%, respectively.

Dried oat okara was co-extruded together with corn grits to produce an expanded snack prototype. Five formulations were extruded (corn grits and dried oat okara ratios: 100:0, 90:10, 80:20, 70:30, 60:40) at three different feed moistures (14%, 16%, and 18%). Dried oat okara was successfully incorporated up to 30%. A threshold was reached at 40% dried oat okara, resulting in low specific mechanical energy input, melt temperature and pressure, leading to hard and dense extrudates with low expansion and porosity.

Dried oat okara and hempseed protein concentrate were co-extruded to produce a high-moisture meat analog. It was performed with a novel wet-feeding method of the hempseed protein concentrate, where the hemp slurry was fed through the water inlet. Three different feed moistures (49%, 52%, and 54%) and screw speeds (500 rpm, 700 rpm, and 900 rpm) were tested at a temperature profile of 40-70-110-130°C. The lowest feed moistures (49% and 52%) and the highest screw speeds (700 rpm and 900 rpm) were also tested for a higher temperature profile of 40-70-120-150°C. It was possible to achieve a high-moisture meat analog with thin-layered and fibrous structures, where dried oat okara could contribute up to 64% of the total protein. A higher feed moisture resulted in decreased hardness and chewiness, while a higher temperature profile had a reverse effect as it increased the degree of texturization. Change of screw speed did not have any consistent influence on the textural attributes.

Okara from soy and oat have great potential to become nutritious ingredients in a large variety of food products. In this doctoral thesis, applications for snacks and high-moisture meat analogs were explored, but okara could also be suitable for various bakery goods. There are, however, still issues regarding the microbial safety of okara that need to be further investigated. Upcycling of okara reduces food losses in the food industry by using all of the processed crops for food, leading to a better utilization of resources.

Keywords: oat, soy, okara, high-pressure processing, extrusion, expanded snack, high-moisture meat analog

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“The more you know, the more you realize you don’t know.”

– Aristotle

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Abstract

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Popular science summary

Plant-based beverages can seem to be modern food products which have popped up in our grocery shops lately, however, plant-based beverages are an old invention. For example, the soy beverage was invented in China for over 2,000 years ago, not known exactly when. Much later, at Lund University, Sweden, during the 1990s, the recipe and the process for oat beverage were developed, where the main goal was to produce a beverage similar to milk for people with lactose intolerance or milk protein allergy. It has a lower content of saturated fats in comparison to cow's milk, which reduces the risk for cardiovascular diseases, and it also turned out to have between 16-41% lower greenhouse gas emissions compared to milk. There are therefore many benefits to plant-based beverages. However, when plant-based beverages are produced, a residue called *okara* is generated. The name okara comes originally from the soy beverage residue and has, for example, been fermented to traditional food products, such as the Japanese *koji* or the Chinese *meitauza*.

Today, there are enormous amounts of plant-based beverages that are produced each year, generating about 18 million tons of soy okara and 228,000 tons of oat okara. To compare, it is about the same as 1,800 Eifel towers in weight. The most common handling of these types of residues is unfortunately to discard or use as animal feed. It is a shame, as okara from both soy and oat have a high protein and fiber content and could be nutritional ingredients in various food products. The problem is its high moisture content which makes it susceptible to bacterial growth, making it deteriorate quickly. This residue has therefore become a massive food waste problem in the plant-based beverage industry.

In this doctoral thesis, the objective has been to investigate different strategies to improve the microbial stability of okara from soy and oat and therefore reduce food waste. Novel food products have also been investigated where oat okara could be a suitable ingredient, and if incorporated in a popular food product it would increase the availability of oat okara.

High-pressure processing was tested on both soy and oat okara to investigate if the microbial stability could be improved. Pasteurization is usually performed with heat during a certain time to kill bacteria and is performed on products such as milk to extend the shelf-life. However, pasteurization with heat can be difficult to apply on materials such as okara due to its texture, whereas it contains a lot of water but resembles a dough that can be formed with the hands. High-pressure processing is however not sensitive to these types of attributes and was therefore a promising method. Bacteria are sensitive to heat, but also to pressure, which is why high pressure can be used as a pasteurization process. It was possible to extend the shelf-life up to 2 weeks for soy okara and 4 weeks for oat okara stored in a fridge (4°C) if pasteurizing with a pressure of 600 MPa, which is a substantial improvement as okara usually deteriorates within 24 hours at room temperature.

Novel snacks and meat analogs were developed with oat okara as an ingredient. Soy okara was not investigated as the production of soy beverage was discontinued at the factory of our collaboration partner, The Green Dairy, during the project. A meat analog is a ‘fake meat’, a vegan product based on plant protein that is supposed to resemble real meat with similar texture and flavor. Meat analogs are usually based on soy protein and can be found on the market, for example, as Oumph. When producing snacks or meat analogs, an extruder is usually used. In an extruder there are two screws that knead a dough at high temperatures and pressures. With different settings, ingredients, and moisture contents, it is possible to produce several types of food products.

To produce the expanded snacks, oat okara was mixed with corn grits. Corn is suitable as a helping ingredient as it contains a high amount of starch, which is important for the expansion of snacks. It was possible to add up to 30% oat okara in the snack formulation which increased the nutritional profile of the snacks with a higher protein and fiber content. The prototype resembles lentil snacks that can be found in the shops today, where corn has been extruded together with a protein rich ingredient (lentils) to improve the nutritional content, but also to increase the crispiness.

The meat analog was extruded with oat okara mixed with hemp protein. Hemp is a plant that was commonly cultivated in the past and was used for the production of, for example, ropes and textiles. However, due to the fact that hemp also could be used as a drug, it became forbidden to cultivate. Through plant breeding, *industrial hemp* is now available, which does not contain the drug substance and has therefore started to be cultivated in Sweden again on a small scale. From the hempseeds, it is possible to extract hemp oil, and the residue after the oil has been liberated from the seeds is called hempseed press cake. Our research group has developed a method for extracting protein from this hempseed press cake, and it was this hempseed protein that was extruded together with oat okara. Hence, two residues were mixed into a novel and exciting food product. A meat analog was successfully produced with thin-layered fibers that resemble meat, where up to 64% of the total protein was contributed by oat okara.

By limiting the waste of residues from the food industry and instead utilizing them to develop novel plant-based food products, sustainable development can be promoted. It reduces food losses and increases the availability for plant-based alternatives to meat. Reduced animal product consumption can in turn decrease emissions and land use.

Populärvetenskaplig sammanfattning

Växtbaserade drycker kan verka som en modernitet som har tillkommit i våra matbutiker på senare år här i Sverige. Men exempelvis sojadryck är något som uppfanns i Kina för nästan 2 000 år sedan, men man vet inte exakt när. Långt senare, vid Lunds universitet på 1990-talet, utvecklade man ett recept och en process för havredryck där målet var att skapa en mjölkliknande dryck för människor med laktosintolerans eller mjölkproteinallergi. Havredrycken visade sig ha mellan 16-41% lägre utsläpp av växthusgaser jämfört med komjölk, samt lägre innehåll av mättade fetter än mjölk, vilket minskar risken för hjärt-och kärlsjukdomar. Det finns därför många fördelar med växtbaserade drycker, men när dessa produceras bildas också en restprodukt som kallas för *okara*. Namnet okara kommer ursprungligen från sojadryckens restprodukt och har traditionellt fermenterats till matprodukter så som japansk *koji*, eller kinesisk *meitauza*.

Idag produceras enorma mängder växtbaserad mjölk som genererar ungefär 18 miljoner ton soja-okara och 228 000 ton havre-okara varje år. Som jämförelse kan nämnas att denna mängd viktmässigt motsvarar ungefär 1 800 Eiffeltorn. Tyvärr slängs ofta de här restprodukterna eller används som djurfoder. Det är synd, då okara från både soja och havre har ett högt protein- och fiberinnehåll, och skulle därmed kunna vara en näringsrik ingrediens i olika matprodukter. Problemet är dess höga vattenhalt som gör den känslig för bakterietillväxt och snabbt gör den dålig. Denna restprodukt har därmed blivit ett stort matsvinn i den växtbaserade dryckesindustrin.

I den här avhandlingen har målet varit att undersöka olika sätt att kunna förlänga den mikrobiella hållbarheten för okara från soja och havre och därmed minska matsvinnet. På grund av att många inte känner till okara och för att det dessutom är en ovanlig råvara, har även två olika matprodukter undersökts som skulle kunna passa med havre-okara som ingrediens. Om havre-okara skulle kunna bli ett tillskott i en populär produkt, skulle det också öka intresset och tillgängligheten.

Högtryckspastörisering testades för att undersöka om den mikrobiella hållbarheten på okara från både soja och havre skulle kunna förlängas. Vanligtvis brukar man pastörisera med värme under en viss tid för att döda bakterier, vilket görs på exempelvis mjölk för att förlänga hållbarheten. Men pastörisering med värme kan vara svårt att applicera på material som okara på grund av dess konsistens. Okara innehåller en hel del vatten men är samtidigt så fast i konsistensen att det liknar en deg som man kan forma med händerna. Högtryckspastörisering påverkas inte av hur fast eller flytande ett material är vilket gjorde denna process till en lovande kandidat. Bakterier är känsliga för värme, men också för tryck, vilket utnyttjades i denna pastöriseringsmetod. Det var möjligt att förlänga hållbarheten med upp till 2 veckor för soja-okara och 4 veckor för havre-okara förvarad i kyl (4°C) om man pastöriserade med ett tryck på 600 MPa, vilket är en klar förbättring då okara brukar bli dåligt inom ett dygn i rumstemperatur.

Nya varianter av snacks och köttanaloger utvecklades med havre-okara som ingrediens. Soja-okara undersöktes inte då vår samarbetspartner, Det gröna mejeriet, slutade producera soja-dryck under projektets gång. En köttanalog är ett ”fejk-kött”, en vegansk produkt baserad på växtprotein som ska efterlikna riktigt kött med liknande textur och smak. Köttanaloger är vanligtvis baserade på sojaprotein, och finns på marknaden som exempelvis Oumph. När man producerar snacks och köttanaloger använder man en så kallad extruder. I en extruder använder man två skruvar som knådar en deg under höga temperaturer och tryck. Med olika inställningar, ingredienser och vattenhalter kan man producera många olika typer av matprodukter.

För att tillverka snacks blandades havre-okara med majs. Majs passade som en hjälpande ingrediens då det innehåller mycket stärkelse som är viktigt för att få matprodukten att expandera till exempelvis fluffiga snacks. Det gick att tillsätta hela 30% havre-okara till snacks, som på köpet också blev mer näringsrika med ett högre fiber- och proteininnehåll. Prototypen skulle kunna liknas vid de linsbågar som man kan hitta i affärerna idag där man har extruderat majs med en proteinrik ingrediens (linser) för att öka näringsinnehåll, men också för att få en ökad krispighet.

Till köttanalogen extruderade vi havre-okara med hampaprotein. Hampa är en växt som man odlade mycket förr i tiden för att tillverka bland annat rep och textilier. På grund av att hampa också kunde användas som drog så blev det till slut olagligt att odla växten. Genom växtförädling finns idag *industrihampa* som saknar ämnet som kan användas som drog, och har därmed börjat odlas på landsbygden i Sverige igen i liten skala. Från hampfrön kan man pressa ut hampolja, och efter det att man har pressat fröna återstår en annan restprodukt som kallas för hampfrökaka. Från hampfrökakan har vårt forskningsteam lyckats utveckla en metod för att utvinna hampaprotein, och det var detta protein som extruderades tillsammans med havre-okara. Två restprodukter sattes därmed ihop till en ny och spännande matprodukt. Det gick att producera en köttanalog med tunna fibrer som liknade kött, där upp till 64% av det totala proteinet i produkten kom från havre-okara.

Genom att försöka utnyttja restprodukter från industrin, som istället skulle slängts eller blivit djurfoder, till att utveckla nya spännande växtbaserade produkter, främjas den hållbara utvecklingen. Det minskar matsvinnet när man får en större användningsgrad av hela grödan och ökar tillgängligheten för växtbaserade alternativ till kött. Minskad köttkonsumtion kan i sin tur leda till minskad mängd växthusgasutsläpp och utnyttjande av land.

List of papers

Paper I **Helstad, A.**, Marefati, A., Ahlström, C., Rayner, M., Purhagen, J., & Östbring, K. (2023). *High-pressure pasteurization of soy okara*. Foods, 12(20), 3736.

Paper II **Helstad, A.**, Marefati, A., Ahlström, C., Rayner, M., Purhagen, J., & Östbring, K. (2023). *High-pressure pasteurization of oat okara*. Foods, 12(22), 4070.

Paper III **Helstad, A.**, Landers, M., Larsson, E., Burleigh, S., Majumdar, A., Rayner, M., Östbring, K., & Purhagen, J. *Co-extrusion of oat okara and corn grits into expanded snacks*. Manuscript.

Paper IV Zahari, I., Purhagen, J. K., Rayner, M., Ahlström, C., **Helstad, A.**, Landers, M., Müller, J, Eriksson, J & Östbring, K. (2023). *Extrusion of high-moisture meat analogues from hempseed protein concentrate and oat fibre residue.* Journal of Food Engineering, 354, 111567.

Author's contribution to the papers

Paper I Amanda Helstad designed and planned the study with Karolina Östbring, Jeanette Purhagen, and Marilyn Rayner. Amanda Helstad performed the experiments, analyzed the data, and wrote the manuscript. The manuscript was revised together with the co-authors.

Paper II Amanda Helstad designed and planned the study with Karolina Östbring, Jeanette Purhagen, and Marilyn Rayner. Amanda Helstad performed the experiments, analyzed the data, and wrote the manuscript. The manuscript was revised together with the co-authors.

Paper III Amanda Helstad designed and planned the study with Karolina Östbring and Jeanette Purhagen. Amanda Helstad performed the experiments, analyzed the data, and wrote the manuscript. The manuscript was revised together with the co-authors.

Paper IV Amanda Helstad contributed to the design and planning of the study. Amanda Helstad performed the protein extraction of hempseed press cake together with co-authors and analyzed the oat okara raw material. The manuscript was revised together with the co-authors.

Other related publications

Helstad, A., Forsén, E., Ahlström, C., Mayer Labba, I. C., Sandberg, A. S., Rayner, M., & Purhagen, J. K. (2022). *Protein extraction from cold-pressed hempseed press cake: From laboratory to pilot scale.* Journal of Food Science, 87(1), 312-325.

Zahari, I., Ferawati, F., Purhagen, J. K., Rayner, M., Ahlström, C., **Helstad, A.**, & Östbring, K. (2021). *Development and characterization of extrudates based on rapeseed and pea protein blends using high-moisture extrusion cooking.* Foods, 10(10), 2397.

Zahari, I., Ferawati, F., **Helstad, A.**, Ahlström, C., Östbring, K., Rayner, M., & Purhagen, J. K. (2020). *Development of high-moisture meat analogues with hemp and soy protein using extrusion cooking.* Foods, 9(6), 772.

Abbreviations

HPP	High-pressure processing
MA	Malt extract agar
TSA	Tryptic soy agar
MRS	De Man Rogosa and Sharpe agar
VRBD	Violet Red Bile Dextrose agar
DSC	Differential scanning calorimetry
RVA	Rapid Visco Analyzer
WHC	Water holding capacity [ml/g]
OHC	Oil holding capacity [ml/g]
n.d.	Not detectable
SEM	Scanning electron microscopy
SME	Specific mechanical energy [kJ/kg]
WAI	Water absorption index [-]
WSI	Water solubility index [-]
SEI	Sectional expansion index [-]
N_{sr}	Spatial frequency of ruptures [peaks/mm]
F_{cr}	Average crushing force [N]
W_c	Crispness work [N mm/peak]
HME	High-moisture extrusion
LME	Low-moisture extrusion
HMMA	High-moisture meat analog
TVP	Texturized vegetable protein
HPC	Hempseed protein concentrate
TPA	Texture profile analysis
db	Dry basis
wb	Wet basis

Introduction

The demand for plant-based beverages and dairy alternatives has increased remarkably over recent years, which is indicated by their extensive utilization in recipes (Sethi et al., 2016). The dairy alternatives market has been valued at USD 27.0 billion in 2023 and is expected to reach USD 43.6 billion by 2028 (a CAGR of 10.1%) (MarketsAndMarkets, 2024). The trend is driven by consumers' concerns about animal welfare and greenhouse gas emissions but is also motivated by the positive health effects of a plant-based diet, which reduces the intake of saturated fats and consequently lowers the risk for cardiovascular diseases and stroke (Westhoek et al., 2014). The variety of plant-based beverages is large and is based on plant sources such as soybean, rice, hazelnut, cashew, almond, peanut, coconut, etc. (Aydar et al., 2020). In Sweden, locally cultivated crops are being used for plant-based dairy beverages, for example, oat and faba bean.

In the production process of plant-based beverages, a residue rich in fiber and protein, also known as *okara*, is formed in the liquid-solid separation step. With the increasing production of plant-based beverages, the total mass of produced okara is also increasing. Even though okara could be utilized in food applications as a source of fiber and protein, it is generally discarded or used as animal feed. One of the main obstacles being the short shelf-life of approximately 12 hours at room temperature (Azanza and Gascon, 2015). Microorganisms present in these residues can rapidly proliferate due to favorable growth conditions such as readily available nutrients, high moisture content (Li et al., 2012), and high water activity (Voss et al., 2018).

The soy beverage has a long history and has been a traditional drink in Eastern Asia for almost 2,000 years, but it is not known exactly when it was invented. It became popular in China in the eighteenth or nineteenth century (Du Bois, 2018). There are numerous studies and patents published on soy okara, which is indicative of the severity of this waste problem (Figure 1), but so far, there is still no universal solution for the management and utilization of soy okara or similar residues. Studies have investigated, for instance, bioactive compounds (Stanojevic et al., 2013; Kamble and Rani, 2020), biovalorization (Vong and Liu, 2016), biotransformation (Vong et al., 2017; Santos et al., 2018), fermentation (Zhu et al., 2008; Vong et al., 2016), drying (Taruna and Jindal, 2002; Wachiraphansakul and Devahastin, 2005; Ostermann-Porcel et al., 2017), and extrusion (Rinaldi et al., 2000; Shi et al., 2011) of soy okara.

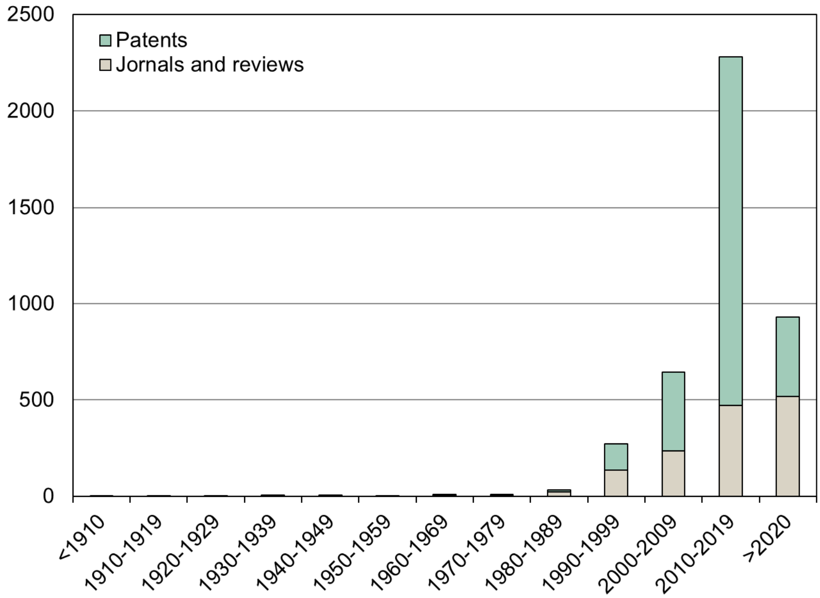


Figure 1. Patents, journals, and reviews found in Sci-finder using the search term “okara”. In total, 4,188 items were found.

The oat beverage is a more novel variant of plant-based drink, developed in the 1990s at Lund University, Sweden (Lindahl et al., 1994). The aim was to produce a milk analog for people with lactose intolerance or milk protein allergy, and the analog also turned out to have between 16-41% lower greenhouse gas emissions compared to cow’s milk (Röös et al., 2016). Due to the novelty, there are only few studies made on the oat residue, which has various names in the literature, such as “oat mill waste” (Patsioura et al., 2011), “oat slurry”(Röös et al., 2016), “oat press cake” (Aiello et al., 2021), or “oat pulp” (Le et al., 2025). However, for the sake of consistency, it will be called *oat okara* in this doctoral thesis. The earlier studies on oat okara have focused on the recovery of β-glucans with ultrafiltration (Patsioura et al., 2011) and enzymatic treatment combined with protein extraction (Aiello et al., 2021). Thus, focusing on the extraction of valuable biomolecules in oat okara, however, still leaves another residue behind.

The food system contributes between 19-29% of the global greenhouse gas emissions (Vermeulen et al., 2012) and processing losses of crops are estimated to be around 8% (Bajželj et al., 2014). The aim of this doctoral thesis was therefore to investigate strategies to improve the shelf-life of soy and oat okara and to find appropriate food applications to reduce the processing losses and thus improve the circular economy of plant-based beverages.

Background

At the beginning of this PhD project, data regarding the characteristics and functionalities of soy and oat okara were investigated to attain a better understanding of the material. However, it was not included in any scientific paper, but will instead be presented in this synopsis.

The industrial collaboration partner has been The Green Dairy (Karlshamn, Sweden), who provided both soy and oat okara to all studies. Starting the project, both soy and oat beverages were produced at The Green Dairy, but as the project proceeded, the production of soy beverage was discontinued. Therefore, most studies focused on oat okara (papers II, III, and IV). A recirculating fluidized bed dryer for oat okara was also developed at The Green Dairy during the project, which provided dried oat okara for the studies in papers III and IV.

Production processes of plant-based beverages

The production of plant-based beverages of soybeans differs from oats, since beans are protein-rich while oats are starch-rich. However, the production process for the two different starting materials begins similarly with dehulling and soaking of the beans or grain followed by a grinding step (McClements and Grossmann, 2022). Thereafter, the soybeans are cooked, generally around 100°C (Ikya et al., 2013), while the oats undergo an enzymatic treatment (glucosidases) to hydrolyze the starch granules, which reduces the viscosity and increases the yield of the beverage (Deswal et al., 2014). After 1-2 hrs, the enzymes are inactivated at around 95°C. A liquid-solid separation follows the cooking or enzymatic steps, performed in a decanter centrifuge where the soluble extract, the plant-based beverage, is separated from the solid residue, okara (McClements and Grossmann, 2022). The general production processes for soy and oat beverages are summarized in Figure 2.

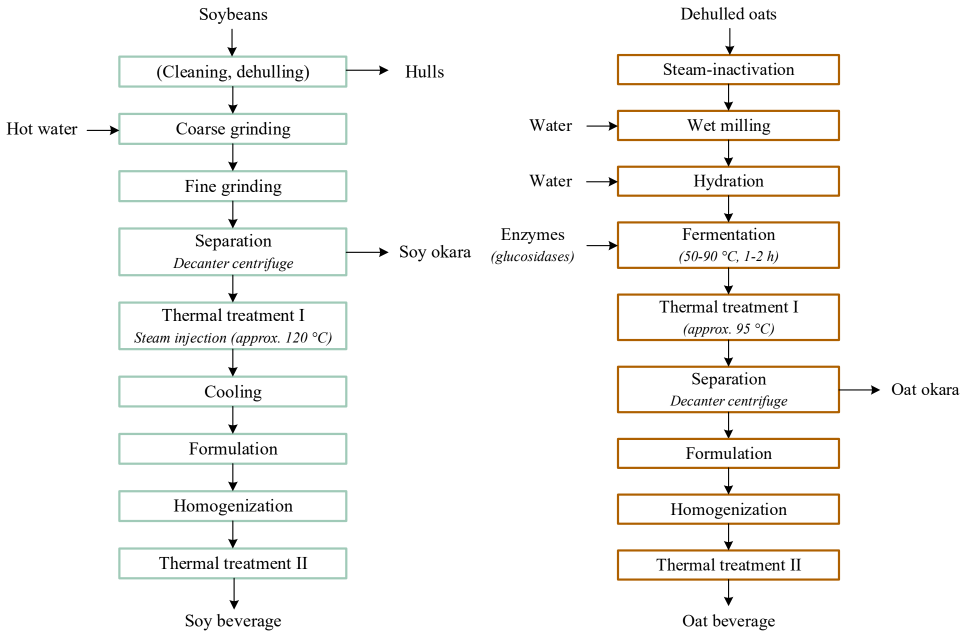


Figure 2. General production processes for soy and oat beverages (McClements and Grossmann, 2022).

For every processed kilogram of soybean, approximately 1.1 kg of soy okara is generated (Khare et al., 1995). This results in a production of approximately 18 million tons of soy okara each year on a global scale (calculated based on an annual world production of 350 million tons of soybeans, where 4.7% go to soy beverage and tofu processing) (FAO, 2019; Ritchie and Roser, 2021). For every kilogram of oats processed, around 0.45 kg of oat okara is co-produced (Röös et al., 2015), generating up to 228,000 tons of oat okara globally each year (Le et al., 2025).

Appearance and composition of soy and oat okara

The soy and oat okara are the solid fractions from the decanter centrifuge in the production process and could be seen as the fiber fraction that is separated from the plant-based beverage. Depending on the raw material, production process, and decanter settings, the composition of okara can vary. The soy and oat okara had a higher protein content and lower fiber content compared to other studies (Patsioura et al., 2011; Li et al., 2012). The soy and oat okara also contained a high amount of water, 76% and 54%, respectively (Table 1). This composition leads to a fibrous and wet appearance and texture (Figure 3).

Figure 3. Pictures of soy okara (left) and oat okara (right).

The main macronutrient for soy and oat okara was the protein content (41.8%, db and 52.1%, db, respectively). The soy okara had approximately the same protein content as its beans, while the oat okara had a higher protein content compared to its grain. The same trend was observed for fat and ash content. The carbohydrate content was reduced, and the fiber content increased for both soy and oat okara compared to their raw materials. The fiber content was mainly composed of insoluble fiber (Table 1).

Table 1. Proximate composition of whole soybean and oat grain, as well as their respective okara. Values are expressed on dry basis.

	Soybean		Soy okara		Oat grain		Oat okara	
Protein [%]	39.9	38.1	15.2-33.4	41.8	14.8	14.7	25.7	52.1
Fat [%]	21.8	20.7	8.3-10.9	22.9	7.3	7.7	14.3	14.1
Total dietary fiber [%]	10.2	14.1	42.4-58.1	26.9	11.3	11.0	37.1	15.8
Insoluble fiber [%]	-	-	-	26.9	-	-	-	15.8
Soluble fiber [%]	-	-	-	n.d.	-	-	-	n.d.
Carbohydrate [%]	22.9	11.4	3.8-5.3	4.2	64.6	64.1	17.1	12.3
Ash [%]	5.3	4.5	3.0-4.5	4.2	2.0	2.4	5.7	5.8
Water [%]	8.5	11.2	81.7-84.5	76.2	10.8	9.5	65.0	54.3
Reference	USDA, (2018)	*	Li et al., (2012)	*	USDA, (2020)	*	Patsioura et al., (2011)	*

*Unpublished data, Eurofins Food & Feed Testing Sweden, Lidköping
n.d. = not detectable

Dietary fiber

In the latest survey of adults' eating habits in Sweden, it was concluded that seven out of ten people do not eat sufficient amounts of dietary fiber in their diet (SwedishFoodAgency, 2010). Fiber was the second most abundant component in both soy and oat okara, after protein (26.9% and 15.8%, respectively, Table 1), and could therefore be an ingredient to enrich various food products with fiber. Fiber is defined as carbohydrates that cannot be digested by the human small intestine. They have positive physiological effects, such as lowering blood cholesterol, improving gastrointestinal mobility, regulating glucose and lipid metabolism, and stimulating bacterial metabolic activity. Fiber is often divided into insoluble and soluble fibers. Insoluble fiber is non-fermentable with no or limited fermentation by the bacteria in the large intestine. It generally has a high water-holding capacity and can increase transport in the intestines, which is helpful against constipation. Soluble fiber is instead fermentable and contribute with substrate for the bacteria in the large intestine. They are usually viscous and can slow down the transport in the intestines, which can reduce the glycemic response and plasma cholesterol (Samaan, 2017).

Oats contain a soluble fiber, β-glucan, which is known for its health-promoting effects, such as cholesterol control and modulation of glucose and insulin responses. However, the β-glucans are mechanically sensitive and can be degraded by high shear rates (Duss and Nyberg, 2004). Even though no soluble fiber content was detected in the oat okara (Table 1), the β-glucan content was investigated, as it is not always detected in a soluble fiber analysis. The oat okara still contained β-glucans after the production process (4%, db), where no significant difference in the β-glucan content between the oat kernels and the oat okara was found (Figure 4). Oat okara might therefore have some health-promoting effects, although it was not further studied in this doctoral thesis and falls outside the scope.

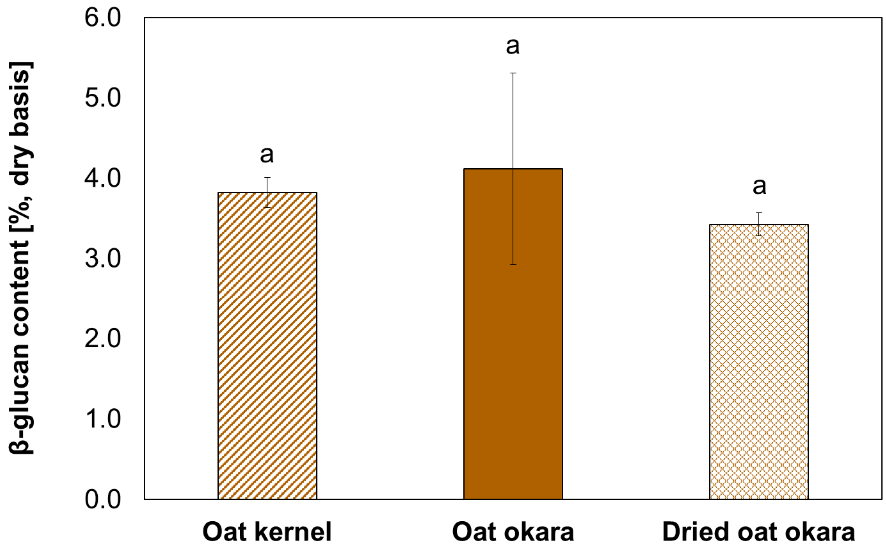


Figure 4. The β-glucan content in oat kernel, oat okara, and dried oat okara. No significant differences were found between the samples ($p > 0.05$), indicated with lowercase letters.

Antinutrients

Many plants contain antinutrients, which are substances that can restrict the digestion and absorption of nutrients. This is therefore an important factor in the new era of plant-based foods, where the removal or inactivation of these substances is important not to interfere with the normal digestion process (McClements and Grossmann, 2022). With adequate knowledge, food processing, and modern biotechnology techniques, it is possible to regulate these substances. However, antinutrients can also exhibit possible beneficial effects, although concentration-dependent effects must be carefully considered (Popova and Mihaylova, 2019).

A literature review on antinutrients was therefore performed for the raw materials used in this doctoral thesis. A summary of the reported antinutrients is available in Table 2. The phytic acid content in the hempseed protein concentrate (HPC) was analyzed and presented in another related publication (Helstad et al., 2022), but that study was not included in this doctoral thesis.

Table 2. Reported antinutrients in the raw materials used in this doctoral thesis.

Raw material	Antinutrients reported	Reference
Soybean	Phytic acid (1387 mg/100 g)	Ambawat and Khetarpaul, (2018)
	Oxalate	Chai and Liebman, (2005)
	Trypsin inhibitors	Bau et al., (1997) and Vagadia et al., (2017)
	Lectins	
	Saponins	
	Isoflavones	
Soy okara	Phytic acid (843 mg/100 g)	Ambawat and Khetarpaul, (2018)
	Trypsin inhibitors	Li et al., (2012)
	Lectins	
	Saponins	
Oat grain	Phytic acid	Bhawna Mehta and Sudesh Jood, (2018)
	Polyphenols	
Oat okara	No data	
Hempseed press cake	Phytic acid	Pojić et al., (2014)
	Trypsin inhibitors	
	Glucosinolates	
	Condensed tannins	
Hempseed protein concentrate (HPC)	Phytic acid (595 mg/100 g)	Helstad et al., (2022)
Corn	Phytate	Sokrab et al., (2011)

Phytic acid and *phytate* are abundant in cereals, legumes, and oil seeds and act as storage molecules for phosphorus and cations (Reddy et al., 1982). The molecular structure of phytic acid is a myo-inositol molecule with six phosphate groups carrying twelve negative charges. These negative charges can bind di- and trivalent cations such as Ca, Mg, Fe, Zn, Cu, and Mn into a stable complex, also known as phytate (salt of phytic acid) (Pallauf and Rimbach, 1997) (Figure 5). The phytate mineral complexes are insoluble and indigestible, leading to poor bioavailability of minerals (Zhou and Erdman Jr, 1995). Phytate can be degraded by the enzyme *phytase*, which has been a supplement in diets for monogastric animals to improve phosphate utilization. Phytase may also be used in the processing and manufacturing of foods to enhance mineral bioavailability (Greiner and Konietzny, 2006). Phytic acid and phytates can also be reduced with other processes such as soaking, fermentation, cooking, and germination (Popova and Mihaylova, 2019).

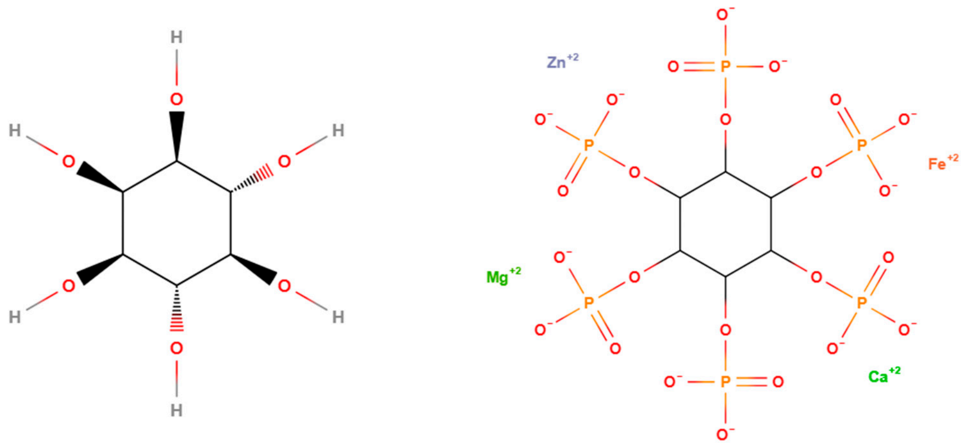


Figure 5. Myo-inositol (left) and phytate (salt of phytic acid) binding cations (right). The chemical compounds were illustrated in molview.org.

Oxalic acid can form insoluble salts or esters with iron, calcium, and magnesium, called *oxalates*. By binding these minerals, they become nutritionally unavailable (this is similar to phytic acid and phytate). Calcium oxalate can also be responsible for the formation of kidney stones. Oxalate content can be reduced by cooking and fermentation (Popova and Mihaylova, 2019).

Trypsin inhibitor is a type of protease inhibitor, having the ability to inhibit the proteolytic activity of digestive enzymes, including trypsin, chymotrypsin, and elastase (Makkar et al., 2007). The inhibitory effect leads to an overproduction of digestive enzymes, whereas the pancreatic functions are altered and can lead to pancreatic hypertrophy or hyperplasia (Vagadia et al., 2017). Trypsin inhibitors can be inactivated by heat treatment (moist heating being the most effective), fermentation and germination (Makkar et al., 2007).

Lectins are carbohydrate-binding (glycol) proteins that can survive digestion and disrupt lipid, carbohydrate, and protein metabolism. Oral acute toxicity of lectins is generally nausea, bloating, vomiting, and diarrhea (Vasconcelos and Oliveira, 2004). Lectin activity can be reduced by soaking and boiling (Pusztai and Grant, 1998).

Saponins have a bitter taste and can be toxic in high concentrations (Popova and Mihaylova, 2019). They can inhibit digestive enzymes such as amylase, glucosidase, trypsin, chymotrypsin, and lipase. Saponins can be reduced by cooking and fermentation (Samtiya et al., 2020).

Isoflavones belong to the class of phytoestrogens, which have structural and functional similarities to human estrogen (Munro et al., 2003). They are considered to be endocrine disruptors, having the potential to cause adverse effects on human or animal health. On the other hand, they have also shown numerous health benefits linked to their estrogenic activity, for example, when it comes to breast cancer, prostate cancer, cardiovascular diseases, and menopausal symptoms (Křížová et al., 2019). Isoflavone content can be reduced by soaking and cooking (Fernandez-Lopez et al., 2016).

Polyphenols are phenolic compounds that can form complexes with protein, lowering the bioavailability of these compounds (Khattak et al., 2007). *Tannins* are large and complex phenolic compounds (Crozier et al., 2006). Polyphenol content can be reduced by cooking and fermentation (Popova and Mihaylova, 2019).

Glucosinolates are non-toxic compounds, but when degraded by the enzyme myrosinase (present in the human intestinal tract), they can form compounds such as thiocyanates and goitrin. These compounds can inhibit iodine utilization by the thyroid gland, which in turn can lead to a decreased synthesis of thyroid hormone and cause goitre (hypothyroidism) (Felker et al., 2016). Glucosinolate levels can be reduced by thermal processing (Oerlemans et al., 2006).

Characteristics and functionalities of soy and oat okara

Water activity and moisture sorption isotherms

Water activity is an important parameter for storage stability of foods, where high water activities (0.7-1.0) promote microbial growth (Singh and Heldman, 2014). Both soy and oat okara had water activities of approximately 1.0, which highly contributes to their short shelf-lives.

The relationship between water activity and moisture content for soy and oat okara can be visualized in moisture sorption isotherm graphs (Figure 6), measured with a sorption balance instrument (Aquadyne DVS-2HT, Quantachrome Instruments, Boynton Beach, FL, USA). To reduce microbial growth, the water activity should be below 0.7 (Singh and Heldman, 2014). According to the results, soy and oat okara need to be dehydrated to a moisture content of 7.6% wb (8.2% db) and 7.5% wb (8.1% db), respectively, to reach a water activity of 0.7. Practically, it means that 674 g water/kg soy okara and 495 g water/kg oat okara would need to be removed to ensure a microbiologically stable product.

Sorption hysteresis is a common phenomenon for dried food materials where the moisture content for a certain water activity in the desorption is generally higher than in the adsorption. The okara residues were no exceptions (Figure 6). Incomplete wetting of the porous okara residues could be explained by changed contact angles between okara and water during adsorption (Al-Muhtaseb et al., 2002).

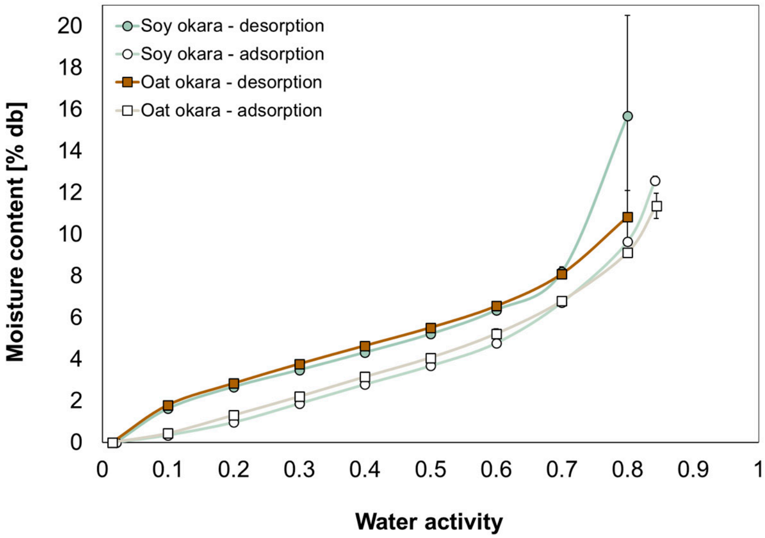


Figure 6. Moisture sorption isotherms (at 22°C) for soy okara (circle) and oat okara (square). Filled markers represent the desorption curves, and non-filled markers represent the adsorption curves.

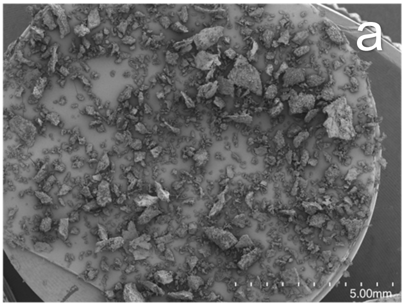
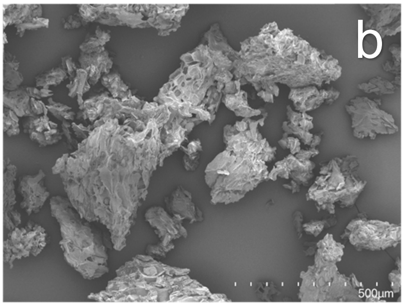
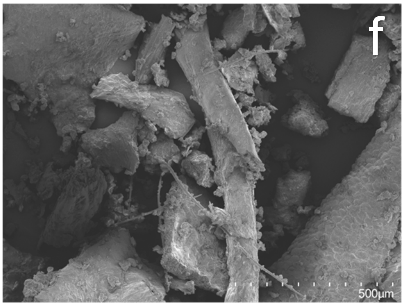
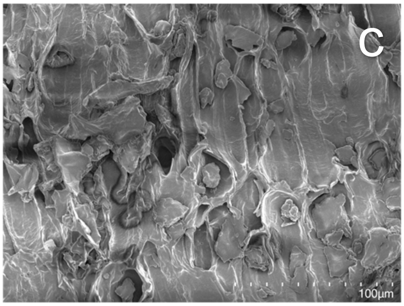
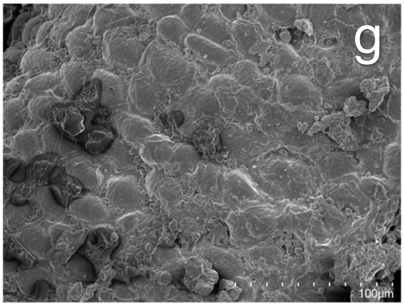
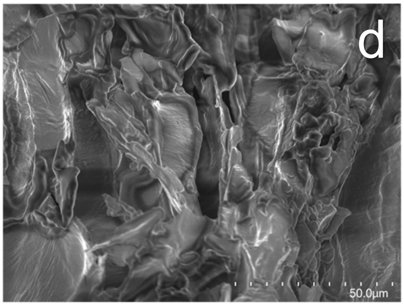
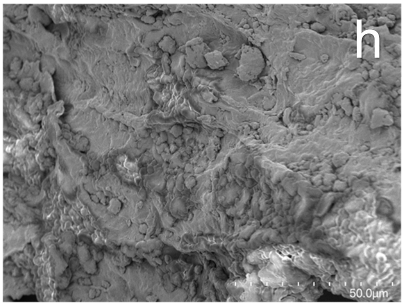
pH value

The pH is also an important factor for microbial growth and was measured to 6.68 ± 0.02 for soy okara and 6.28 ± 0.02 for oat okara (pH spear, Eutech Instruments). This is a favorable pH for several harmful microorganisms, e.g., *Bacillus Cereus* and *Salmonella spp*., which can cause foodborne illnesses (Gourama, 2020).

Microstructure

Scanning electron microscopy (SEM) micrographs were taken on freeze-dried soy and oat okara. A large particle size distribution was visualized where bigger flakes and smaller particles could be observed (Table 3a, e). The soy okara (Table 3b, c, d) had a porous surface, which most likely is a buildup of cell wall remnants, mainly composed of insoluble fiber (Hughes and Swanson, 1989). In oat okara, starch granules appear to have grouped and gelatinized in the hydrothermal treatment in the production process, which promoted swelling of granules (Table 3g, h) (Miranda et al., 2019; Duque et al., 2020). Rod-shaped fibers were also observed in the oat okara (Table 3f), which could derive from the pericarp, the outer layer of the oat grain, which is not removed in the dehulling process. The porous exterior of the okara residues is favorable for microorganisms to immobilize and grow (Xiudong et al., 2016), which can cause problems for the shelf-life, but can also be useful for fermentation of okara.

Table 3. SEM micrographs of freeze-dried soy and oat okara (a, e: 10X, b, f: 100X, c, g: 500X, d, h: 1000X).

	Soy okara	Oat okara
10X		
100X		
500X		
1000X		

Thermal and pasting properties

In a differential scanning calorimetry (DSC) analysis, several smaller endothermic peaks (< 1 mJ/mg) were identified in soy okara between 50-100°C. Some of these peaks could correspond to the identified peaks in a previous study of soy okara protein isolates where peaks around 60°C were partially denatured protein or non-protein transitions, peaks between 73.4-74.1°C were globulin conglycinin (7S), and peaks between 87.0-90.2°C were globulin glycinin (11S) (Ma et al., 1996). However, the small enthalpies in the DSC thermograms imply that most of the protein in the soy okara had been denatured in the plant-based beverage production. Larger peaks (1.48-5.18 mJ/mg) between 130-160°C were also observed, most likely attributed to the evaporation of bound water in dietary fiber (Liu et al., 2021).

Among cereals, prolamins are generally the main protein. However, in oats, globulins stand for the main fraction (50-80%), where the salt-soluble 12S globulin, avenalin, is the most abundant (Boukid, 2021). In the DSC analysis of oat okara, a distinct peak at 110°C (1.78-8.02 mJ/mg) was present, which corresponds to the protein avenalin with a denaturation temperature between 114–116°C (Ma and Harwalkar, 1988; Cheng et al., 2023). A high proportion of this protein must therefore have endured the production process temperatures of approximately 95°C and can still be native, which creates opportunities for protein extraction or extrusion of high-moisture meat analogs (HMMA). Oat starches usually have peak temperatures between 58-67°C with enthalpies around 9 mJ/mg (Autio and Eliasson, 2009). However, only small (< 1 mJ/mg) and distributed peaks over a broad range of temperatures (48-85°C) were observed, concluding that most starch had already been gelatinized or enzymatically degraded in the production process. The small peaks could be a visualization of retrograded starch forming a variety of crystallites of different stability and size, consequently broadening the endothermal peak (Hoover and Senanayake, 1996).

The soy and oat okara were also analyzed for their pasting properties with a Rapid Visco Analyzer (RVA). It determines the viscosity of a sample during heating (50-140°C) and cooling (140-50°C), simulating a cooking or extrusion process. The analysis can reveal the required temperature to achieve a viscosity change.

Soy and oat okara were run at 85% moisture content in the RVA (Figure 7), where soy okara had an initial high viscosity that decreased with increasing temperature. Two viscosity plateaus were identified around 110 and 140°C (approximately 2,250 mPa s and 1,600 mPa s, respectively). The oat okara showed a large viscosity peak around 120°C (approximately 3,560 mPa s), which corresponds well to the denaturation temperature of the protein avenalin (110°C) detected in the DSC analysis. Moreover, as the starch in oat okara has already been gelatinized or enzymatically degraded in the production process, as concluded from the DSC and visualized in the SEM micrographs, it did not generate any larger viscosity peak.

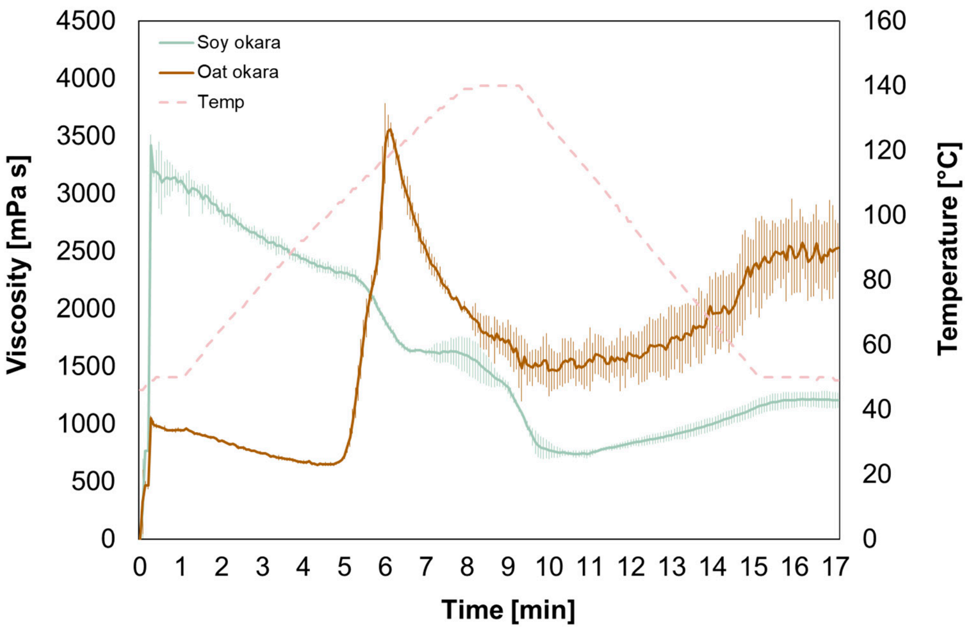


Figure 7. Average viscosity for soy okara (green) and oat okara (yellow) at 85% moisture content. The error bars represent the standard deviation, n = 3.

Current uses of okara

Most of the soy okara produced today has no current use other than disposal in landfills or first-generation recycling, using it for animal feed or composting (Choi et al., 2015). However, it has also been reported that soy okara is being used as a food ingredient in a wide variety of products such as bread, pasta, cookies, soups, and snacks, and as a substitute for marzipan (Schved and Hassidov, 2010). Some studies have evaluated the effect of including soy okara in extruded foods to fortify food products with dietary fiber and protein (Rinaldi et al., 2000; Shi et al., 2011). Soy okara can furthermore be found in traditional fermented food products such as the Japanese koji (Matsuo, 1999) and the Chinese meitauza (Zhu et al., 2008).

The oat okara is mainly used for animal feed but has also been used for soil improvement, composting or converted into energy as biomethane or by incineration (Oatly, 2023).

Challenges and limitations for the utilization of okara

Even though okara has a good nutritional profile, and several studies have reported various applications of soy okara as a food ingredient, the waste of okara is still a problem. The potential of okara as a food ingredient is immense, but the issue is not the ideas of what to use it for but rather the industrial handling of it. The biggest obstacles for the industry to manage okara narrow down to five main issues:

- The short shelf-life – The okara contains water and nutrients, has a high water activity, neutral pH, and porous structure. These characteristics create a beneficial environment for microorganisms, quickly deteriorating the okara.
- Microbial spores – The temperature in the production process of plant-based beverages reaches up to approximately 100°C before the okara is separated from the liquid. Microbial spores can survive these temperatures, and as the temperature slowly cools in the okara after the production process, with available water and nutrients, the spores can sporulate and grow almost unlimitedly and spoil the okara.
- Difficult to pump and transport – The okara is a semi-solid waste, thus, it is neither a liquid nor a solid. These types of materials are difficult for conventional processing lines and pasteurization methods to process.
- Decentralized and small-scale production – Many factories producing soy beverages operate on a small scale. This makes it difficult to establish any larger or more profitable business with soy okara, and the upcycling of the material therefore becomes too expensive. Producers are also often dispersed over wide areas, making it difficult to collect enough soy okara for centralized processing (Li et al., 2012).
- Economy – In many cases, the processing of okara is costly and rarely a profitable food ingredient or product. This does not motivate companies to make further investments and establishments for okara.

Possible solutions

To make okara a more valuable and available food ingredient, efficient processing strategies are required. There are numerous studies describing efforts and strategies on how to preserve okara, a few examples are:

- Dehydration – reduces the water content and water activity, which in turn inhibits microbial growth (Taruna and Jindal, 2002; Wachiraphansakul and Devahastin, 2005; Lee et al., 2016; Guimarães et al., 2020).

- Fermentation – controls the microbial growth for a certain strain of bacteria, fungi, or yeast (Vong and Liu, 2016).
- Freezing – stops the microbial growth but does not eliminate the initial microbial counts (Voss et al., 2018).
- Thermal pasteurization – inactivates microorganisms (Ezaki et al., 2003).
- High-pressure processing – a non-thermal pasteurization method that reduces microbial growth in thick and otherwise difficult food products to handle, such as guacamole and hummus (Balasubramaniam et al., 2016).
- Extrusion – pasteurizes and dehydrates the okara, inhibiting microbial growth (Rinaldi et al., 2000; Shi et al., 2011; Aussanasuwannakul et al., 2022).

Thermal dehydration treatments have a positive impact on the shelf-life of soy okara (Voss, Rodríguez-Alcalá et al. 2018). However, the process is expensive and requires a considerable amount of energy as it involves the removal of large quantities of water to achieve a shelf-stable product. Fermentation is a good strategy to control microbial growth in okara and is a good medium for many microorganisms, although additional research is needed for scaled-up fermentation, particularly under non-sterile conditions, to make it more relevant to the industry (Vong and Liu 2016). It can also be noted that fermentation can alter the flavor profile. Freezing is a good storage strategy for okara when it comes to microbial growth, but with the large quantities of okara that need to be managed in a factory, freezing will require a considerable amount of energy and can be rather costly. It can also be difficult to handle frozen okara in a subsequent processing step, where it will need to be transported and thawed in a sterile and effective way. Thermal pasteurization of soy okara has not been found in any study, but there is one issued patent on it. It suggests feeding soy okara into a scraper-type heat exchanger where it is exposed to temperatures up to 120°C and thereafter cooled and packed aseptically (Ezaki et al., 2003). A promising preservation method, however, it might be difficult to sell soy okara as it is, due to the fact that it is an unusual ingredient to use. To increase value, the industry might need to develop their own products in-house to create a market for it.

High-pressure processing had not been considered previously for soy and oat okara with the aim of lengthening the shelf-life. Pressure is not dependent on product thickness as compared to thermal pasteurization, and due to the fact that okara is a semi-solid material, high pressure could therefore be an easier alternative. This was therefore investigated for both soy (paper I) and oat (paper II) okara. Extrusion has been a successful processing strategy for soy okara but has not been investigated for oat okara before. Two studies were therefore performed on this possible solution, using oat okara in an extruded snack prototype (paper III) and an HMMA prototype (paper IV).

High-pressure processing

High-pressure processing (HPP) is a non-thermal pasteurization technology and serves as an alternative to traditional thermal pasteurization. HPP can reduce the number of viable microorganisms, but in contrast to thermal pasteurization, it does not alter the sensory or nutritional qualities of food products and can therefore maintain a fresh-like quality. HPP is most commonly applied to food products such as fruit juices, smoothies, and guacamole, where vitamins, flavors, and colors are desirable attributes. At an industrial scale, HPP is a relatively young unit operation in food production and became popular around the year 2000 (Balasubramaniam et al., 2016). HPP Nordic (Landskrona, Sweden) kindly provided access to the HPP equipment used in this doctoral thesis (Figure 8).

Figure 8. The HPP equipment used in this doctoral thesis at HPP Nordic.

During HPP, vacuum-sealed products are subjected to high pressures within a pressure vessel. The pressure is applied with a pressure-transmitting fluid, typically water, which is compressed with a pump and intensifier. A typical process time generally lasts 5 minutes, with pressures ranging from 200-600 MPa (Huang et al., 2017) (Figure 9).

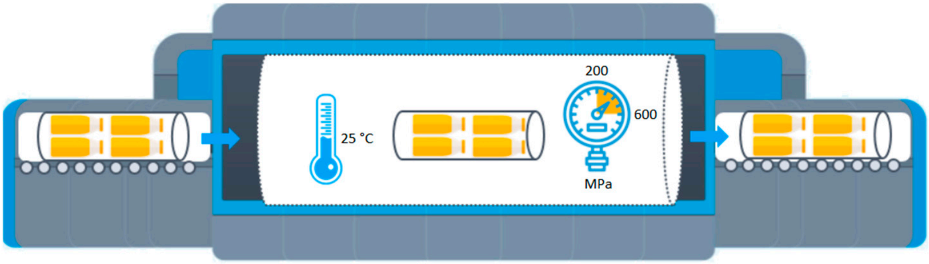


Figure 9. Overview of a general HPP. Modified illustration adopted from Thyssenkrupp, (2024).

Although HPP involves significant initial investment costs, it offers advantages in terms of reduced processing time and lower energy consumption compared to conventional thermal methods (Balasubramaniam et al., 2016). For instance, when comparing a thermal treatment with a 100 L vessel pressurized to 400 MPa, the energy requirement is 1920 kJ, whereas a retort using 1 kg of saturated steam at 120°C requires 2700 kJ (Deplace, 1995). However, HPP is conducted in a batch-wise manner, which makes it challenging to apply on high-speed production lines and can make it rather costly. Additionally, there remains a lack of fundamental knowledge regarding production conditions for HPP foods, such as pressure-resistant characteristics and indicator microorganisms for food safety (Huang et al., 2017).

Microbial inactivation mechanisms of HPP

The microbial inactivation mechanisms of HPP are not yet fully understood, but it is believed that the treatment can damage or alternate cell membranes, nucleoids (storage for genetic material in prokaryotic cells), ribosomes (protein factory) and proteins (Balasubramaniam et al., 2016). The denaturation of protein is caused by water being pushed into the proteins' core by pressure, which destabilizes the hydrophobic interactions and forces protein to unfold (Knorr et al., 2006). However, due to the fact that covalent bonds are resistant to pressure, the primary and secondary protein structures are negligibly affected (Heremans and Smeller, 1998).

Microbial inactivation by HPP is influenced by factors such as the type of microorganism (bacteria, yeasts, or molds), its form (vegetative cells, spores, gram-positive or gram-negative), as well as the strain, species, genus, and growth phase (Balasubramaniam et al., 2016). It is implied that different microorganisms exhibit varying levels of sensitivity to HPP, with gram-negative bacteria being the most sensitive, followed by yeasts, gram-positive bacteria, and bacterial spores being the most resistant (Shigehisa et al., 1991).

Structural modification of molecules by HPP

High pressure has little to no effect on molecules associated with food qualities such as nutritional content, flavor, and color. However, HPP can alter the structure of larger molecules, including polysaccharides, nucleic acids, proteins, and enzymes (Balci and Wilbey, 1999). As a result, the physical and functional properties of the food can therefore change, affecting characteristics such as melting point, solubility, and viscosity (Balasubramaniam et al., 2016).

Protein in food plays an important role in several functional properties such as emulsification, gelation, foaming, solubility, viscosity, and wettability. All functions are related to the proteins' physicochemical and structural properties, for example, the amino acid sequence, size, shape, and net charge (Damodaran, 1994). Applying high pressure to proteins and enzymes leads to different degrees of protein structure modifications. As high pressure has a disruptive effect on intramolecular hydrophobic and electrostatic interactions, the quaternary and tertiary structures start to unfold, and subunits dissociate (Balasubramaniam et al., 2016) (Figure 10). High pressure can, therefore, alter several functional properties of protein and consequently also alter the food product.

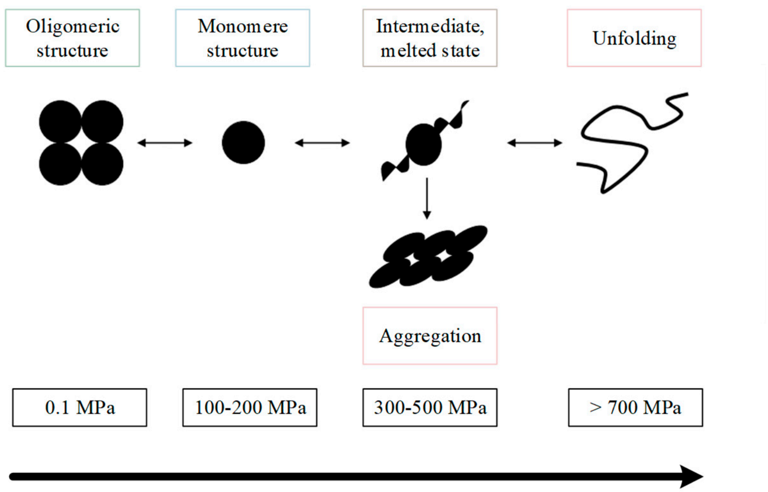


Figure 10. Protein structure modification by HPP. Modified illustration adopted from Balasubramaniam et al., (2016).

Fiber can also be affected by HPP, where the soluble fiber fraction can increase, and properties such as water-holding and oil-holding capacities can be altered (Xie et al., 2017). In one study, soy okara was subjected to high pressure (200 MPa and 400 MPa) at 30°C and 60°C, which increased the soluble fiber fraction and improved the swelling capacity, water retention capacity and oil retention capacity (Mateos-Aparicio et al., 2010).

Extrusion technology

Extrusion cooking has been used extensively in the food industry to produce products such as pasta, breakfast cereals, baby foods, pet foods, snack foods, and meat analogs (Akdogan, 1999; Alam et al., 2016). Extrusion can be regarded as a process that involves a combination of several unit operations that simultaneously transport, mix, heat, and shape polymeric and non-polymeric materials (Bouvier and Campanella, 2014). Extruders include one or two screws which are encased in a barrel that is either grooved or smooth. The screw(s) rotate(s), driven by a motor, where varying screw speeds can be applied. The dry ingredients are fed into a hopper at the beginning of the screw(s) and is mixed with water, added via the water inlet after the hopper. Along the barrel, there are different heating zones that add heat to the process. At the end of the screws and barrel, there is typically a die where the material is forced out of the extruder (Figure 11). The dies can have various shapes and temperature profiles depending on the desired end product (Ganjyal, 2020).

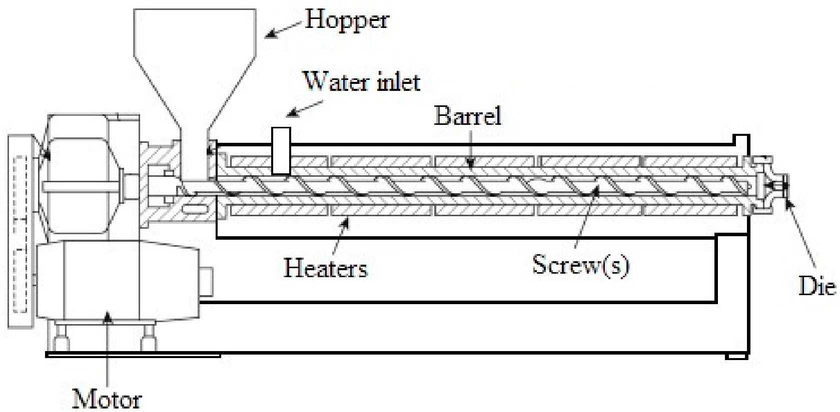


Figure 11. Overview of an extruder. Modified illustration adopted from Ebnesajjad and Khaladkar, (2005).

In single-screw extruders, the shear on the material is generated as it is rubbed in the interface between the barrel and screw, where the barrel can be grooved or spiral-walled (Bouvier and Campanella, 2014; Ganjyal, 2020). However, in twin-screw extruders, the barrel is usually smooth, and the shear is generated by the particles in the material being rubbed against each other as they are conveyed by the screws. The twin-screw extruder is more efficient in generating shear; thus it can provide a higher degree of cooking in a food material compared to a single-screw extruder. A single-screw extruder is therefore more commonly used for simple food products such as pasta, breakfast cereals and pet food, while twin-screw extruders can handle more complex food products such as expanded snacks and texturized proteins (Ganjyal, 2020). Twin-screw extruders can also be divided into different classifications depending on the direction of rotation of the screws; co-rotating (same direction) or counter-rotating (opposite direction). Additionally, the screws can also be intermeshing or non-intermeshing, which indicates the position of the screws in relation to each other. When screws are intermeshing, the flights of the two screws overlap each other to a lesser or greater degree, while non-intermeshing screws are tangent to each other and not overlapping (Figure 12). Intermeshing co-rotating twin-screw extruders are recognized as self-wiping extruders, which can prevent the build-up of ingredients and can handle sticky and difficult-to-convey food ingredients. They are therefore popular in food processing (Ganjyal, 2020). Co-rotating screws can also be operated at higher speeds in comparison to counter-rotating screws, which can provide higher shear rate, greater throughputs, and better mixing (Adekola, 2016). Counter-rotating twin-screw extruders are usually used for food products where uniform distribution of particles is desired in the dough mix, for example, in the confectionery industry (Ganjyal, 2020). Non-intermeshing twin-screw extruders have a similar conveying process to that of a single-screw extruder (Bouvier and Campanella, 2014).

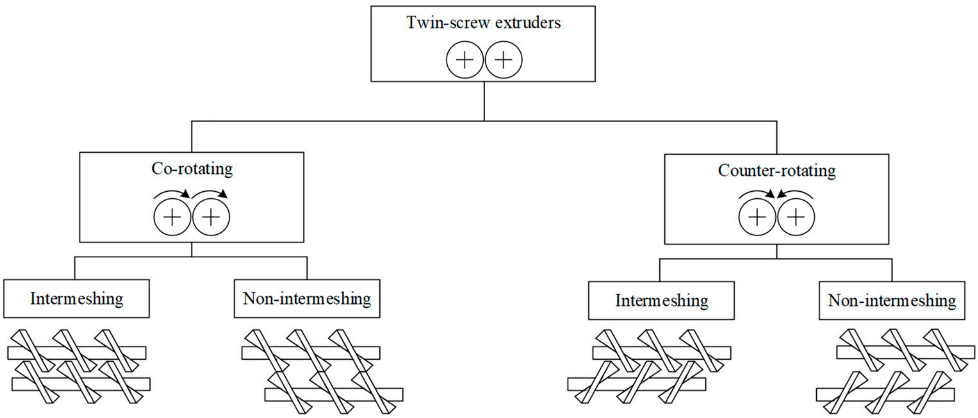
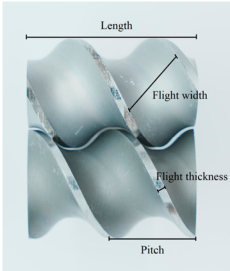
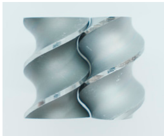
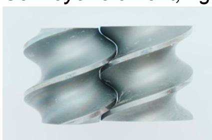
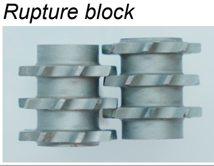
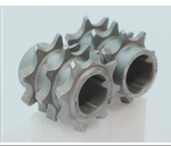


Figure 12. The different classifications of a twin-screw extruder.

The screw(s) used in an extruder can have various configurations. A single-screw usually has a fixed design made by the manufacturer. However, twin-screws are generally modular where various screw elements can be mounted on the screw shafts to form unlimited screw profile designs which ensures a high level of process flexibility (Ganjyal, 2020). The screw elements have various designs for different functions, a few examples for an intermeshing co-rotating twin-screw extruder are presented in Table 4 (Bouvier and Campanella, 2014).

Table 4. Process functions for common screw elements and kneading blocks for intermeshing co-rotating twin-screw extruders (Bouvier and Campanella, 2014).

Process functions	Type of screw element or kneading block
General screw element terminology.	
Screw elements for feed and transport sections – intermediate mixing, low shearing and high free volume. Usually positioned in the beginning of the screw. Can be found in various lengths and pitches. ➢ Larger pitches = higher transport efficiency ➢ Shorter pitches = generates pressure in shorter distance (Ganjyal, 2020)	*Conveyor element, right-handed pitch, 30 mm*  *Conveyor element, right-handed pitch, 20 mm*  
Screw elements for melting and shearing sections – intense shearing and high material retention.	*Inverse element, left-handed pitch, 2x10 mm* 
Kneading blocks for melting, mixing, and shearing sections – medium shearing and mixing, intermediate material retention. Can be found in various disc thicknesses and staggering angles. ➢ Larger disc thickness = lower distributive mixing performance. ➢ Larger staggering angle = longer residence time. (Zhang et al., 2009)	*Bilobe kneading block, 45° staggering, 30 mm*  *Bilobe kneading block, 45° staggering, 20 mm*  
Mixing element – intense mixing.	*Rupture block*  

In this doctoral thesis, a laboratory twin-screw extruder with co-rotating intermeshing screws was used (TwinLab-F 20/40, Brabender GmbH & Co.KG, Duisburg, Germany), presented in Figure 13.

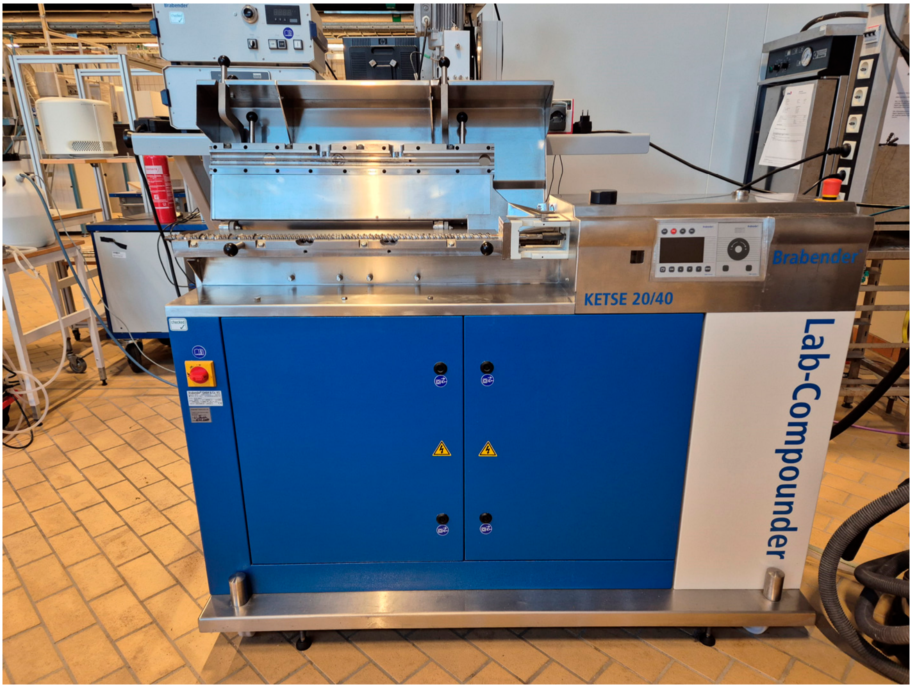


Figure 13. The laboratory twin-screw extruder with co-rotating intermeshing screws used in this doctoral thesis (TwinLab-F 20/40, Brabender GmbH & Co.KG, Duisburg, Germany).

Extruders often operate in conditions of extremely high temperatures and shear. The processed material is therefore subjected to thermomechanical forces, which influence the molecular structure and properties of the food biopolymers. For example, starch gelatinization and melting, protein denaturation, and complexations between ingredients (e.g., amylose-lipid complexation). The intense processing conditions also cause physicochemical reactions to occur, such as starch degradation (or starch dextrinization) and protein aggregation (Bouvier and Campanella, 2014).

Extrusion is a complex process that depends on several parameters, which can be divided into three different groups:

- Process parameters
- System parameters
- Product parameters

The *process parameters* are related to the extruder equipment (screw configuration, barrel temperature and type of extruder), parameters associated with the product formulation (moisture content, raw material characteristics, particle size distribution) and operational variables (screw speed, feed rate, and die-head configuration). The process parameters influence the *system parameters,* which include material residence time and mechanical and thermal energy input. The mechanical input is usually expressed and measured as specific mechanical energy (SME). The system parameters, in turn, affect the *product parameters* such as texture, taste, color, expansion, and solubility (Figure 14) (Bouvier and Campanella, 2014).

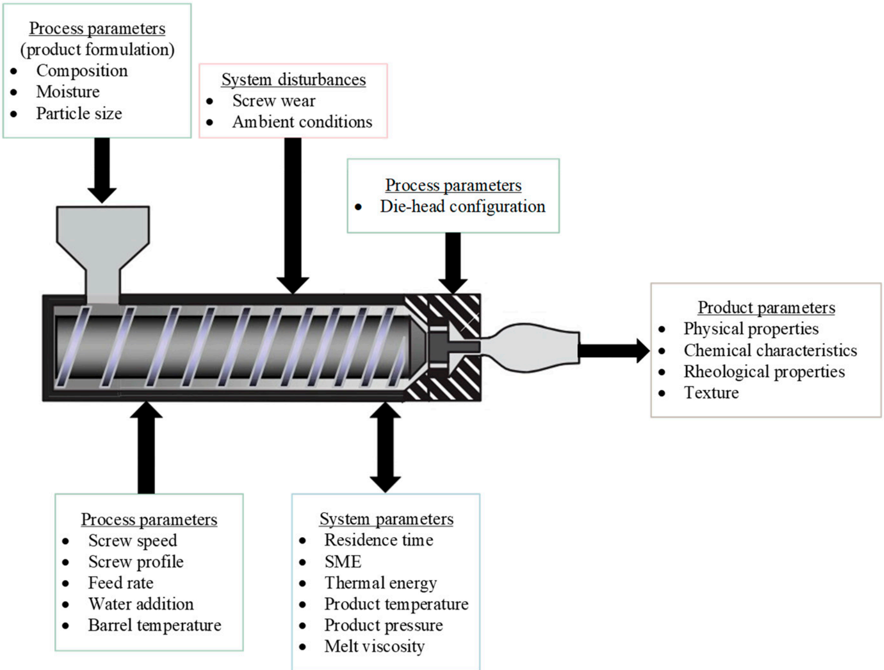


Figure 14. Overview of the extrusion process, system, and product parameters. Modified illustration adopted from Bouvier and Campanella, (2014).

The SME is a key process parameter describing the efficiency of an extrusion process with energy input per unit mass [kJ/kg]. It is influenced by several parameters, including process temperature, screw profile and die-head configuration. SME is a helpful unit when comparing the effect of different treatment conditions on a raw material or when designing an extrusion process scaling up from pilot to large-scale production (McClements and Grossmann, 2022). The SME has been calculated according to Equation 1 in this doctoral thesis (Fang et al., 2014).

$$SME = \frac{2\pi \cdot n \cdot T_{max}\left(\frac{\%T_{tot} - \%T_{idle}}{100}\right)}{MFR} \left[\frac{kJ}{kg}\right] \quad (1)$$

Where n is the screw speed [min^{-1}], T_{max} is the maximum torque of the extruder, T_{tot} is the total torque measured during a run [%], T_{idle} is the idle torque at the set screw speed [%], and MFR is the mass flow rate [g/min].

The idle torque was measured for several screw speeds, running the extruder empty with no screws and has been plotted in Figure 15. The idle torque is subtracted to exclude the friction torque of the drive train (Godavarti and Karwe, 1997).

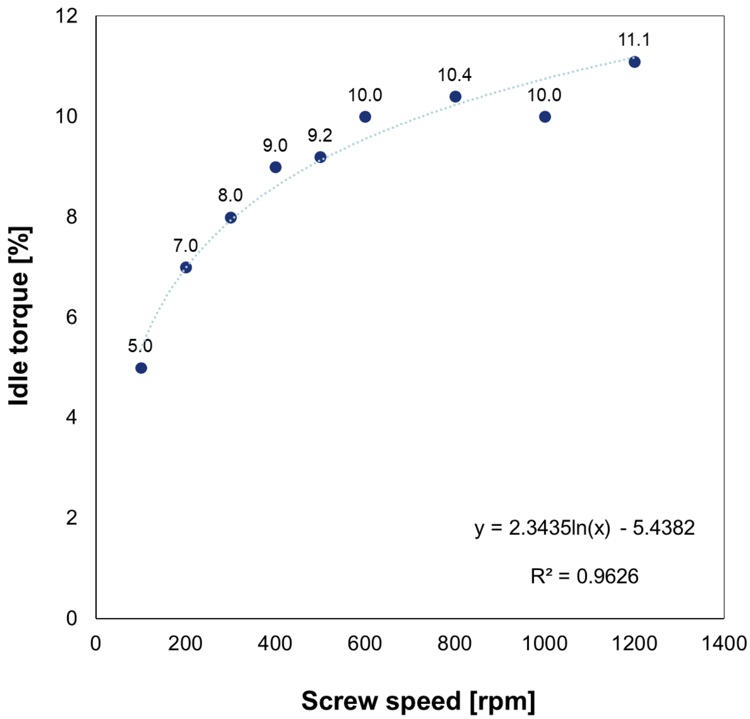


Figure 15. The idle torque for the extruder used in this doctoral thesis at several screw speeds. Used for the calculation of SME

Low- and high-moisture extrusion in food applications

Extrusion processing has been utilized extensively in the food industry, producing products such as pasta, breakfast cereals, baby foods, pet foods, snack foods, confectionary items, chewing gum, modified starch, and texturized vegetable protein (TVP) (Akdogan, 1999; Alam et al., 2016). Almost all of these applications are extruded at low to intermediate moisture contents (< 40%) (Akdogan, 1999), which is also known as low-moisture extrusion (LME). TVP products are meat analogs extruded at lower moisture and are slightly expanded, yielding a porous structure. This product requires further processing but can in the end resemble meat products such as burgers, sausages, or nuggets (Wagner and Ganjyal, 2024). At the beginning of the 1980s, high-moisture extrusion (HME), also known as wet extrusion, was developed. It is extrusion at higher moisture levels (> 40%) which has been used to produce meat-, seafood-, and cheese-analogs (Akdogan, 1999; Bouvier and Campanella, 2014). The texture of high-moisture meat analogs (HMMA) can resemble whole cuts of meat and require minimal processing after extrusion. This product is often distributed frozen in its fully hydrated state.

In this doctoral thesis, both LME and HME applications have been investigated for oat okara. The focus has been on the development of an expanded snack including oat okara and corn grits (LME, paper III), and an HMMA including oat okara and HPC (HME, paper IV).

Expanded snacks

In the production of expanded snacks, a starch-rich material is used, operating at low moisture contents (below 30-32%, wb) (Bouvier and Campanella, 2014), commonly using a die head with a small circular opening of 1-7 mm in diameter, also known as the nozzle size (Figure 16).

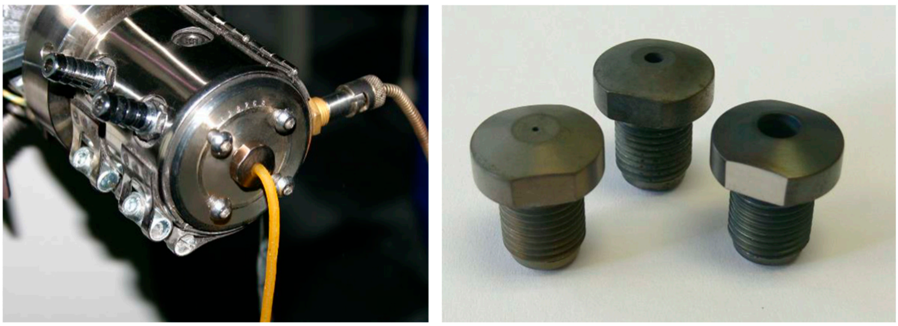


Figure 16. Circular die heads (courtesy of Brabender/Anton Paar TorqueTec).

Extrusion-cooking of starchy materials leads to a series of structural changes. Starch is composed of two different semi-crystalline macromolecules, amylose and amylopectin, which are stored in granules. In the extrusion process, the granules lose their crystallinity and granular integrity, followed by depolymerization of their starch polymers (or dextrinization), leading to an amorphous, cooked starch melt. The formation of complexes can also take form between amylose and lipids (Bouvier and Campanella, 2014). As the extrusion-cooking continues, the starch melt is exposed to high pressures (30-160 bar) and temperatures (130-160°C). In these conditions, water is still mixed with the melt in a liquid state. When the melt finally emerges from the small opening in the die, it is exposed to a sudden pressure drop, and the water flashes off and expands the melt with its water vapor pressure. Depending on the matrix elasticity and viscosity, the melt can expand quickly and form an open foam structure (Figure 17). This cellular structure defines the texture and is an important quality attribute of an expanded snack (Bouvier and Campanella, 2014).

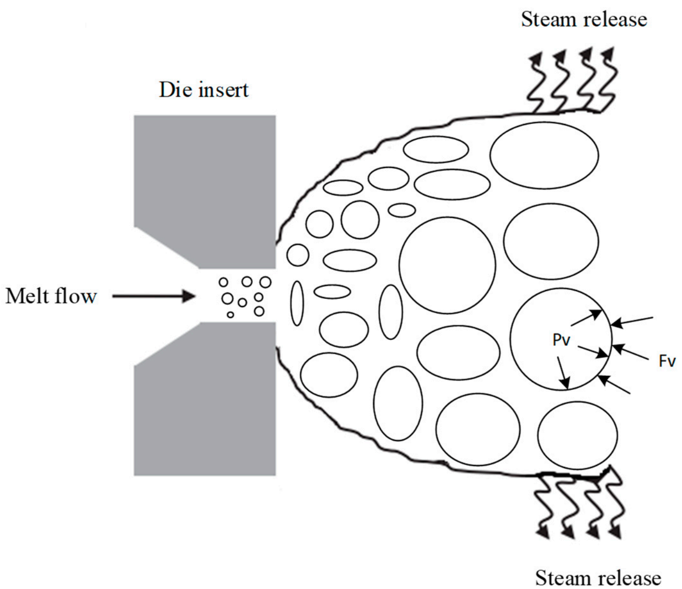


Figure 17. A schematic representation of steam-induced expansion at the die exit. Pv is the vapor pressure in a bubble, and Fv is the resistance force due to melt viscosity. Modified illustration adopted from Della Valle et al., (1997) and Bouvier and Campanella, (2014).

For the extrusion of snacks, a cutter is mounted at the end of the die. As the extrudate emerges from the die, it is quickly cut off flush to the die exit, producing pieces of the expanded extrudate (Figure 18).

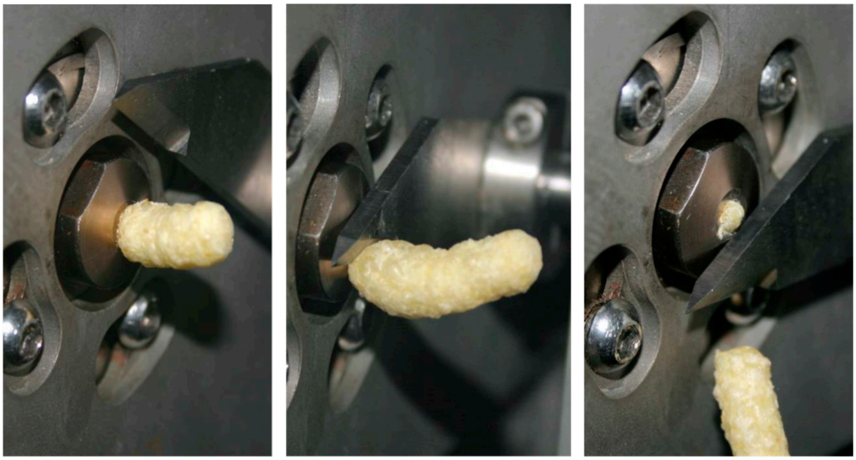


Figure 18. Expanded extrudate emerging from the die exit, cut off by a cutter (courtesy of Brabender/Anton Paar TorqueTec).

Dietary fiber, especially insoluble fiber, cannot undergo the same transformation as starches do in an extrusion process. They have a more rigid molecular structure as they have additional hydrogen bonding in their molecular chains, leading to higher melting temperatures. It has therefore been hypothesized that insoluble fiber act as inert components in an extrusion process (Ganjyal, 2020). However, studies show that fiber can be fragmented and converted from insoluble to soluble fiber under some conditions of shear and heat in an extrusion process (Redgwell et al., 2011; Yan et al., 2015; Sayanjali et al., 2017). If adding more than 25% fiber in a formulation, it will have negative effects on the expansion of extrudates (Bouvier and Campanella, 2014). It is hypothesized that fiber is not compatible with the starch phase, which could lead to a phase separation, increasing the extensional viscosity of the melt. The viscosity is closely related to the formation of cellular structure and expansion of an extrudate, whereas a too high melt viscosity can reduce bubble growth and expansion (Pai et al., 2009).

Protein are more complex biomolecules in comparison to starches as they have a wider range of functions, making them more reactive and unpredictable in an extrusion process. When added in a starch-based formulation, they can alter the melt expansion criteria, but whether the expansion is increased or reduced depends on the properties of the raw materials (Bouvier and Campanella, 2014).

High-moisture meat analogs

In the extrusion process for HMMA, a protein-rich material is used, operating at high moisture contents (> 40%) (Wagner and Ganjyal, 2024). A cooling die is crucial for the formation of the fibrous structure of the final product (Wittek et al., 2021), visualized in Figure 19.

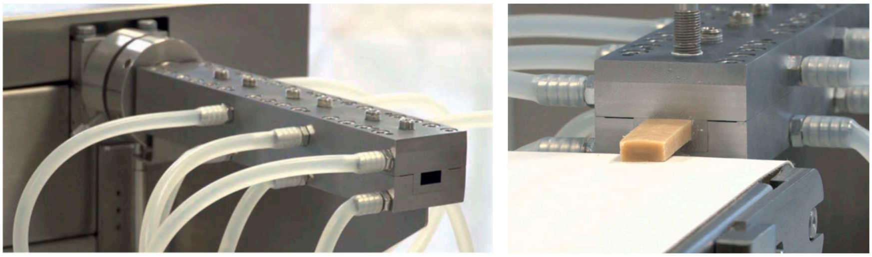


Figure 19. A cooling die for extrusion of HMMA (courtesy of Brabender/Anton Paar TorqueTec).

The extrusion cooking for HMMA begins with the feeding of a protein-rich material and water, followed by a mixing zone where the protein starts to slightly unfold. Thereafter, in the cooking zone, the protein is exposed to high temperatures (> 130°C) and shear and unfold completely to a molten state where hydrophobic and sulfhydryl groups are exposed. The unfolded protein can therefore form new chemical bonds (mainly disulfide bonds, hydrogen bonds, and hydrophobic interactions), leading to cross-linking of proteins, and a new 3D-network structure takes form. The cross-linked molten material is thereafter transported to the cooling zone in the cooling die, where it turns to a more rigid state, and a new fiber structure can take form (Figure 20) (Zhang et al., 2024).

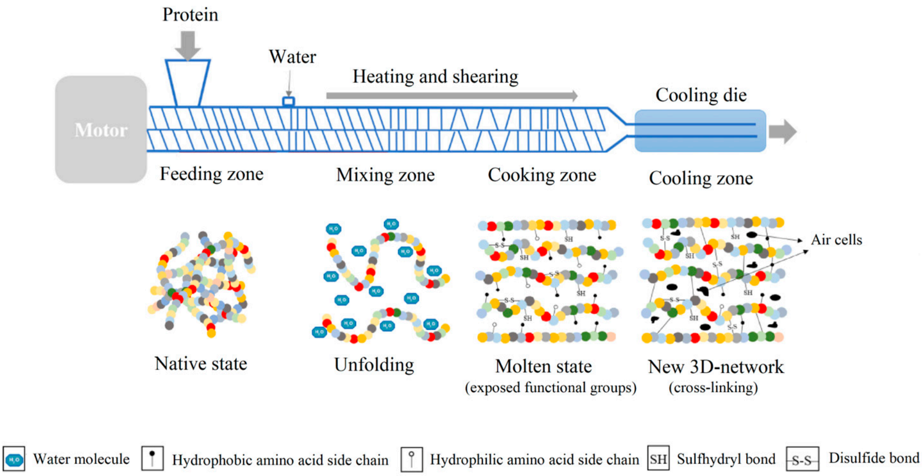


Figure 20. Schematic overview of an HMMA extrusion cooking process with protein conformation changes. Modified illustration adopted from Ferawati et al., (2021) and Sengar et al., (2023).

When the temperature of the melt decreases in the cooling die, the viscosity increases and the flow velocity decreases. The cooling occurs faster close to the wall of the die compared to the center of the melt, and a sheared velocity profile is therefore attained, thus creating a velocity gradient, leading to the typical profile of a HMMA fiber structure (Figure 21). The exact mechanisms of the formation of the fibrous structure are however currently not well understood (Murillo et al., 2019). There is an ongoing discussion that phase separations seem to play an important role in the formation of these structures, interrupting the formation of protein-protein interactions and forming anisotropic structures. Further research is still required to fully uncover the molecular and physicochemical origins of meat analogs' structure formation (McClements and Grossmann, 2022).

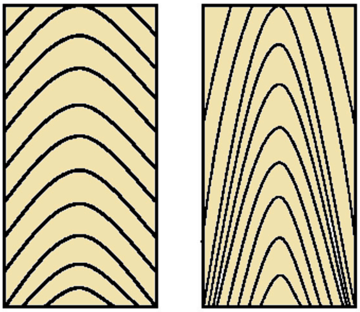


Figure 21. A flat velocity profile with a rough surface leading to short and big fibers (left) and a well-developed velocity profile with a smooth surface leading to long and numerous thin fibers (right). Modified illustration adopted from Bouvier and Campanella, (2014).

Aim and objectives

The overall aim of this doctoral thesis was to upcycle soy and oat okara by extending the shelf-life and to investigate possible food applications, developing novel foods. Successful upcycling would reduce okara waste and improve the circular economy of plant-based beverages. The aim was divided into the following specific objectives:

- Screening of the composition and functional properties of soy and oat okara to obtain a better understanding of the residues' challenges and opportunities to be formulated into novel food applications (synopsis).
- Investigate and evaluate if high-pressure processing could extend the shelf-life of soy and oat okara (papers I and II).
- Evaluate the extrudability of dried oat okara in co-extrusion together with corn grits into an expanded snack prototype (paper III).
- Evaluate the extrudability of dried oat okara in co-extrusion together with hempseed protein concentrate into a high-moisture meat analog prototype (paper IV).

Delimitation of doctoral thesis

An overview of what has been included and not included in this thesis is presented in a mind map (Figure 22). The focus of this doctoral thesis was the process engineering (HPP, LME, and HME) and functionality aspects of soy and oat okara. Nutritional aspects, such as antinutrients and β-glucans, were not included in the scope, but have been discussed in the synopsis. The β-glucan content was analyzed together with Abhinav Majumdar and Jose Zambrano (Division of Pure and Applied Chemistry, Lund University) with results presented in the synopsis. The moisture sorption isotherms, presented in the synopsis, generated information about the relationship between moisture content and water activity in soy and oat okara and was important for the development of the recirculating fluidized bed dryer at The Green Dairy in cooperation with Elajo. The dried oat okara that was used in papers III and IV was produced in that dryer. The X-ray microtomography of expanded extrudates (paper III) was performed by Emanuel Larsson and Stephen Burleigh (Department of Experimental Medical Science, Faculty of Medicine, Lund University), both scanning and analysis. The extraction of hempseed protein, which was used for the extrusion of HMMA (paper IV), is described in an earlier publication (Helstad et al., 2022), but it was decided not to be included in the scope of this doctoral thesis.

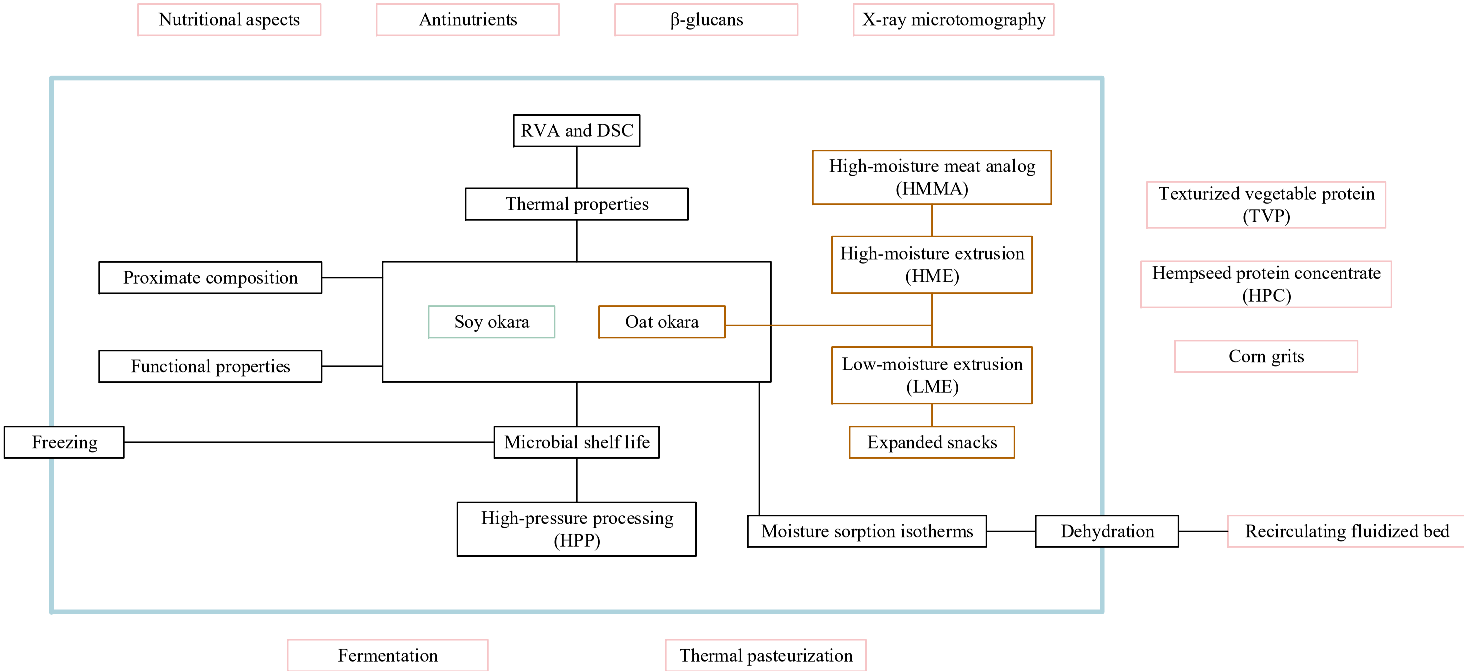


Figure 22. Delimitation mind map presenting the topics included in the scope (inside the blue box) and topics not included (pink boxes, outside the blue box) in this doctoral thesis.

Methodology

Raw materials

- Wet soy okara provided by The Green Dairy (paper I).
- Wet oat okara (paper II) and dried oat okara (papers III and IV) provided by The Green Dairy.
- Corn grits (C1) purchased from Cornexo GmbH (Freimersheim, Germany) (paper III).
- Hempseed protein concentrate (HPC) extracted from hempseed press cake, where the press cake was a kind gift from Gunnarshögs Gård AB (Hammenhög, Sweden) (paper IV).

Microbiological control of soy and oat okara (papers I and II)

It was investigated if high-pressure processing could improve the shelf-life of soy and oat okara.

Soy and oat okara were collected from The Green Dairy and were frozen and stored (-18°C) at the University for practical reasons. Soy beverage and oat beverage were not produced at the same dates in the factory, and it was not possible to synchronize the production dates for the two okara types with the HPP facility, therefore, freezing of the okara was necessary. For the HPP, the okara was thawed and separated into four groups of treatment: no treatment (reference), 200 MPa, 400 MPa, and 600 MPa. After the treatments, the samples were stored for 2 and 4 weeks at 4°C for a shelf-life study. To evaluate the microbial starting point and the effect of freezing, crude okara was plated directly from the factory (kept cooled at 4°C until it arrived in the lab). Figure 23 summarizes the experimental design.

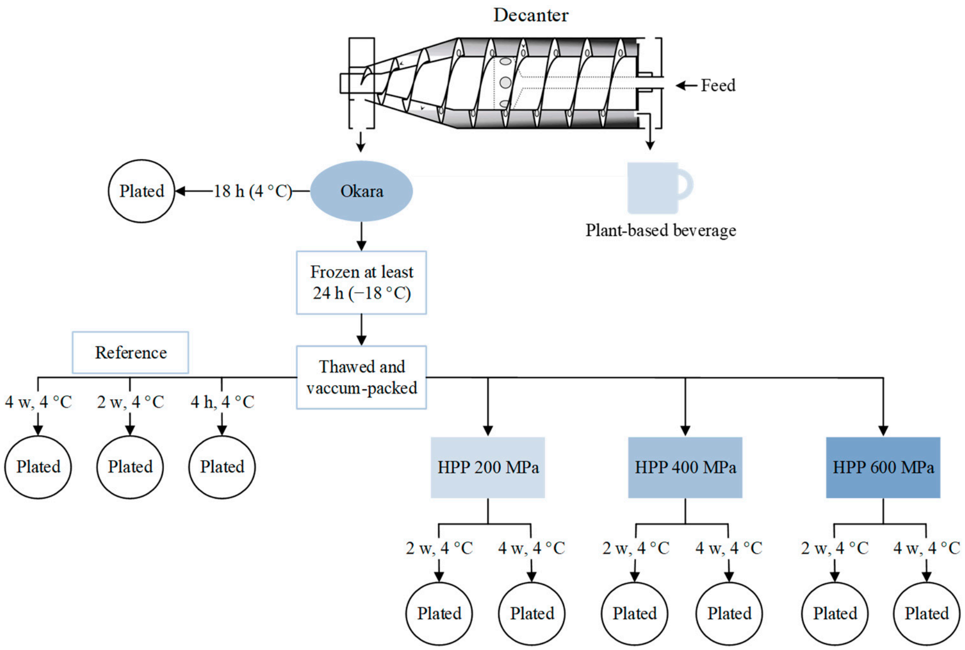


Figure 23. The experimental design for the shelf-life studies in papers I and II.

The microbial analysis included four different agar types:

- Tryptic soy agar (TSA) – Total aerobic count
- Malt agar (MA) – Yeast and mold
- De Man Rogosa Sharpe agar (MRS) – Lactic acid bacteria
- Violet red bile dextrose agar (VRBD) – Enterobacteriaceae

Several other properties were also investigated before and after HPP to evaluate any changes in the pasting properties, thermal properties, water activity, and water- and oil-holding capacities. Microscope imaging with scanning electron microscopy (SEM) was used to see any structural changes after HPP. The soluble and insoluble fiber content were also investigated, evaluating if HPP could increase the soluble fiber content.

Co-extrusion of dried oat okara and corn grits (paper III)

It was investigated if dried oat okara could be co-extruded with corn grits to produce an expanded snack.

Five formulations of corn grits and dried oat okara were co-extruded: 100:0, 90:10, 80:20, 70:30, and 60:40 (corn grits:oat okara) mixed on a weight basis (wb). The formulation of 100:0 was used as a reference. A laboratory co-rotating intermeshing twin-screw extruder (KETSE 20/40D, Brabender GmbH & Co.KG, Duisburg, Germany) was used. For each formulation, three different feed moistures (14%, 16%, and 18%) were tested with a temperature profile of 40-70-110-140-160-160°C (zone 1-2-3-4-5-6). A mass flow of 8 kg/h and a screw speed of 500 rpm were set, and the nozzle size was 3 mm. A summary of all settings is presented in Table 5.

Table 5. Summary of the different formulations and extruder settings in paper III.

Formulation (Corn grits: Oat okara)	Screw speed (rpm)	Mass flow (kg/h)	Feed moisture (%)	Temperature (°C) (Zone 1-2-3-4-5-6)
100:0	500	8	14 16 18	40-70-110-140-160-160
90:10			14 16 18	
80:20			14 16 18	
70:30			14 16 18	
60:40			14 16 18	

The raw material was analyzed for proximate composition, particle size, water activity, water absorption index (WAI), water solubility index (WSI), thermal properties, and pasting properties. This generated a good understanding of the extruded raw material, which could explain some of the extrudate results.

The extrudates were analyzed for water activity, WAI, WSI, thermal properties, pasting properties, sectional expansion index (SEI), density, texture, porosity, pore sizes, and cell wall thicknesses. The data generated a good overview of the characteristics and quality of the snacks, making it possible to compare them to similar extrudates in other studies.

Co-extrusion of dried oat okara and hempseed protein concentrate (paper IV)

It was investigated if dried oat okara could be co-extruded with HPC to produce a HMMA. It was performed with a novel feeding procedure which was not described in detail in the published paper due to a patent application at the time for submission of the manuscript. The HPC that was produced on site, based on the study by Helstad et al., (2022), had a high moisture content (82%). Instead of drying the HPC slurry to a powder, just to rehydrate it again in the extruder, the water feed was exchanged to the HPC slurry, contributing with both water and protein. The dried oat okara was fed normally as a powder in the hopper.

Dried oat okara and HPC were co-extruded at three different targeted feed moistures (60%, 63%, and 66%) and screw speeds (500 rpm, 700 rpm, and 900 rpm), at a temperature profile of 40-70-110-130-60°C. A higher temperature profile of 40-70-120-150-60°C was also tested for the lower targeted feed moistures (60% and 63%) with the two highest screw speeds (700 rpm and 900 rpm) to investigate possible changes in the texture of the extrudate. All trials were performed with a total mass flow of 4 kg/h and the details are summarized in Table 6.

The novel wet-feeding of HPC resulted in a water feed that contributed with both solids and water, and not only water which is the normal case. The actual feed moistures were therefore calculated after the trials which resulted in slight uneven numbers (Table 6).

Table 6. Summary of the different formulations and extruder settings in paper IV.

Total mass flow [kg/h]	Targeted feed moisture [%]	Wet feed [kg/h]	Dry feed [kg/h]	Actual feed moisture [%]	Protein contribution (HPC: Oat okara)	Screw speed [rpm]	Temperature (°C) (Zone 1–2–3–4-cooling die)
4	60	2.3	1.7	49	36:64	500	40-70-110-130-60
						700	
						900	
	63	2.4	1.6	52	39:61	500	
						700	
						900	
	66	2.5	1.5	54	42:58	500	
						700	
						900	
	60	2.3	1.7	49	36:64	700	40-70-120-150-60
						900	
	63	2.4	1.6	52	39:61	700	
						900	

The raw materials were analyzed for proximate composition, thermal properties, and pasting properties. The data generated was helpful for deciding the extruder settings, especially the temperature profile.

The HMMAs were analyzed for color and texture properties. Microscopic imaging with SEM was also performed as a complement to understand the texture properties better.

Analytical methods

Several analytical methods have been used during this PhD project. A summary of all methods is presented in Table 7, with a short description of each method. A more detailed description of the methods for differential scanning calorimetry, Rapid Visco Analyzer (RVA) and texture analysis can be found in the next three sections.

Table 7. Summary of all analytical methods used in this doctoral thesis.

Analysis	Material	Description	Reference	Paper
Proximate composition				
Ash (g/100 g db)	All raw materials.	Samples were weighed before and after incineration in a furnace at 550°C for 16 h.	AOAC 923.03	I-IV
Carbohydrate (g/100 g db)	All raw materials.	Calculated by difference. Carbohydrate = 100 – moisture – ash – fat – protein.		I-III
Fat (crude) (g/100 g db)	All raw materials.	Solvent extraction in a semi-automatic Soxtec apparatus (Tecator AB, Höganäs, Sweden), using petroleum ether as solvent.	AOAC 920.39	I-IV
Moisture content (g/100 g db)	All raw materials.	Samples were weighed before and after drying in an oven at 103 ℃ for at least 16 h.	AACC 44-15A	I-IV
Protein (g/100 g db)	All raw materials.	Dumas combustion method (N conversion factor 6.25).	AOAC 990.0	I-IV
Starch (including free glucose) (g/100 g db)	Corn grits and oat okara.	Eurofins Food & Feed Testing Sweden, Lidköping. Internal method, spectrophotometry.		III
Total dietary fiber (g/100 g db)	Soy and oat okara, corn grits.	Eurofins Food & Feed Testing Sweden, Lidköping.	AOAC 991.43 mod.	I-III
Insoluble fiber (g/100 g db)	Soy and oat okara.	Eurofins Food & Feed Testing Sweden, Lidköping.	AOAC 991.43 mod.	I, II
Soluble fiber (g/100 g db)	Soy and oat okara.	Eurofins Food & Feed Testing Sweden, Lidköping.	AOAC 991.43 mod.	I, II
Functional properties				
Oil holding capacity	Soy and oat okara.	The sample was mixed with rapeseed oil, vortexed, incubated and centrifuged. The supernatant was decanted. Samples were weighed before and after.	(Aziah et al., 2012)	I, II
Water holding capacity	Soy and oat okara.	The sample was mixed with deionized water, vortexed, incubated and centrifuged. The supernatant was decanted. Samples were weighed before and after.	(Aziah et al., 2012)	I, II

Water absorption index (WAI)	Expanded snacks.	A milled and sieved sample was mixed with deionized water and thereafter centrifuged. The sediment was weighed. $WAI = \frac{w_{sediment}}{w_{dry\ solids}}$	(de Mesa et al., 2009)	III
Water solubility index (WSI)	Expanded snacks.	A milled and sieved sample was mixed with deionized water and thereafter centrifuged. The supernatant was dried and weighed. $WSI = \frac{w_{dissolved\ solids\ in\ supernatant}}{w_{dry\ solids}} \times 100$	(de Mesa et al., 2009)	III
Microbiological plating				
Tryptic soy agar (TSA)	Soy and oat okara.	Cultivation of total aerobic count. Incubated aerobically at 30°C for 72 ± 6 h.		I, II
Malt agar (MA)	Soy and oat okara.	Cultivation of yeast and mold. Incubated aerobically at 25°C for 5-7 days.		I, II
De Man Rogosa Sharpe agar (MRS)	Soy and oat okara.	Cultivation of lactic acid bacteria. Incubated anaerobically at 37°C for 72 ± 6 h.		I, II
Violet red bile dextrose agar (VRBD)	Soy and oat okara.	Cultivation of Enterobacteriaceae. Incubated aerobically at 37°C for 24 ± 1 h.		I, II
Imaging				
X-ray microtomography	Expanded snacks.	X-ray tomography imaging was performed at the 4D Imaging Lab, Lund University, using an RX Solutions EasyTom150 microtomograph (Chavanod, France).		III
Scanning electron microscopy (SEM)	Freeze-dried soy and oat okara.	Scanning electron microscope (SEM; Hitachi SU3500, Tokyo, Japan) at 5 kV.		I, II
Thermal properties				
Differential scanning calorimetry (DSC)	Soy and oat okara.	See paragraph *Differential scanning calorimetry*		I-IV
Pasting properties	Soy and oat okara.	See paragraph *Rapid Visco Analyzer*		I-IV
Other analyses				
Color properties	HMMA	Values of L* (lightness), a* (greenness to redness), and b* (blueness to yellowness) were measured with a colorimeter (Konica Minolta CR-400, Osaka, Japan).		IV

Density	Expanded snacks.	Measuring the volume and weight of a sample. $V_{extrudate} = \pi r^2 l - (\pi r^2 d - \frac{4}{3}\pi r^3)$ r: radius of the extrudate [cm] l: length of the extrudate [cm] d: diameter of the extrudate [cm]		III
Particle size	Corn grits and oat okara.	Sieving using mesh sizes of 1250 µm, 1000 µm, 800 µm, 600 µm, 400 µm, 300 µm, 250 µm, 200 µm, 150 µm, 100 µm, and 50 µm.		III
Sectional expansion index (SEI)	Expanded snacks.	$SEI = \frac{{d_e}^2}{{d_d}^2}$ d_e: diameter of the extrudate d_d: diameter of the die exit	(Alvarez-Martinez et al., 1988)	III
Texture properties	Expanded snacks and HMMA.	See paragraph *Texture analyzer*		III, IV
Water activity	Soy and oat okara, corn grits, and expanded snacks.	Measured with a water activity meter (AquaLab Ver 3TE, Decagon Devises, Pullman, WA, USA) at 20°C. Calibration with standard salt solutions: 13.41 M LiCl (0.250 aw), 8.57 M LiCl (0.500 aw), and 6 M NaCl (0.760 aw).		I-III

Differential scanning calorimetry

The thermal properties of the raw materials in this thesis were analyzed with a DSC (Seiko 6200 DSC, Seiko Instruments Inc. Shizuoka, Japan). The DSC can measure the phase transitions of biopolymers, such as protein denaturation or starch gelatinization, and melting. It is a technique where the difference in energy required to increase the temperature of a sample as compared to an inert reference is measured. A phase transition in a sample will require a larger amount of heat compared to a reference, and this difference can be translated into enthalpy (Bouvier and Campanella, 2014). This has been important to measure, especially for the extrusion studies (papers III and IV), to know at which temperatures protein denatures and starch gelatinizes to set the right temperature settings in the extrusion cooking. It was also an instrument to follow the extent of denaturation of protein in okara samples after HPP (papers I and II), which in turn can be an important factor for further processing.

In the HPP studies (papers I and II), wet oat okara was analyzed in amounts of 4-6 mg together with Milli-Q water (1:1 w/w), and wet soy okara samples in amounts of 9-12 mg with no additional water. This resulted in a water content of approximately 75%; a sample and water ratio of 1:3. In the extrusion studies (papers III and IV), all samples were analyzed in amounts of approximately 2 mg together with Milli-Q water (1:3, w/w). The pans were sealed and analyzed in the DSC according to the settings presented in Table 8. An empty pan was used as a reference. A holding time of 1 minute was set for each run to achieve a stable baseline for all samples. After the measurement, the dry matter content was measured by puncturing the pans and letting them dry in an oven at 105 °C for at least 16 hours.

Table 8. The DSC settings used in this doctoral thesis.

	Start [°C]	Limit [°C]	Rate [°C/min]	Hold [min]	Sampling [s^{-1}]
	25	25	10	1	0.2
End step	25	160 or 200	10	0	0.2

Rapid Visco Analyzer

The pasting characteristics were measured with a high-temperature Rapid Visco Analyzer (RVA 4800, Perkin Elmer, Waltham, MA, USA). An RVA continuously measures the viscosity of a sample during continuous stirring at a controlled temperature profile (heating and cooling). It can be used to investigate several properties, such as the suitability of cereals for further processing, characterization of protein-based materials, or the degree of cook for extruded products (Bouvier and Campanella, 2014). The test profile used in this thesis is presented in Table 9.

Table 9. Test profile for the RVA in this doctoral thesis.

Time	Settings	
00:00	Temp	50°C
00:00	Speed	960 rpm
00:10	Speed	160 rpm
01:00	Temp	50°C
06:50	Temp	140°C
09:20	Temp	140°C
15:10	Temp	50°C
17:10	End	

Texture analyzer

The texture properties of the expanded snacks and HMMA extrudates were evaluated with a texture analyzer (TVT-300XP, Perten Instruments AB, Hägersten, Sweden).

Two variants of measuring the texture properties of expanded extrudates have been found. Either to measure one extrudate at a time by puncturing with various cylindrical probes (2-5 mm diameter, 38 mm diameter), compression speeds (40 mm/min, 0.4 mm/s, 10 mm/s), and distances (6 mm, 70% of extrudates' original height) (Van Hecke et al., 1998; Ding et al., 2006; de Mesa et al., 2009). Or, using a 5-blade Kramer shear cell to measure extrudates in bulk with a compression speed of 2 mm/s (Peressini et al., 2015; Aussanasuwannakul et al., 2022). Depending on the method, different variables are evaluated. If extrudates are measured one by one, the extrudates must be somewhat homogeneous, otherwise there will be an incorrect representation of the whole production sample. Another parameter of interest can be how the product is usually eaten, several at a time or one at a time, to understand the mouthfeel.

As the expanded snacks in paper III were homogenous and will probably be eaten one by one, they were also measured one by one. Three different probes were used to achieve a broad dataset. A cylinder probe (3 mm diameter) puncturing 5 mm at a speed of 0.5 mm/s, a craft blade knife probe (height 76 mm, width 80 mm) cutting 5 mm at a speed of 0.5 mm/s, and a triangular blade probe (height 117 mm, angle 60°) cutting 12 mm at a speed of 0.5 mm/s. Five extrudates of each sample were analyzed for all probes.

The data collected from the analysis of the expanded snacks was the area under the curve (S, N mm), the number of peaks (n, threshold strength ≥ 100 g), and the probe travel distance (d, mm) to calculate the spatial frequency of ruptures (N_{sr}, Equation 2), the average crushing force (F_{cr}, Equation 3), and the crispness work (W_c, Equation 4) (Van Hecke et al., 1998; Bouvier and Campanella, 2014; Azzollini et al., 2018).

$$N_{sr} = \frac{n}{d} \; [\frac{peaks}{mm}] \qquad (2)$$

$$F_{cr} = \frac{S}{d} \; [N] \qquad (3)$$

$$W_c = \frac{F_{cr}}{N_{sr}} \; [\frac{N\,mm}{peak}] \qquad (4)$$

The texture properties of the HMMA samples were determined with Texture Profile Analysis (TPA), which is a double compression test that can provide insight into how samples behave when chewed. (Johnson, 2023). Texture properties such as hardness, springiness, cohesiveness, resilience, and chewiness can be measured. Hardness defines how hard the product is and can be determined by the maximum force of the first compression (Equation 5). Springiness reveals how well the product physically springs back after the first compression (Equation 6). Cohesiveness describes how well the product withstands a second compression relative to its resistance from the first compression (Equation 7). Resilience defines how well the product regains its original height after the first compression (Equation 8). Chewiness is a dependent parameter and calculated as hardness times cohesiveness times springiness (Equation 9) (Kantanen et al., 2022; Johnson, 2023).

$$Hardness\ [N] = maximum\ force\ of\ first\ bite \qquad (5)$$

$$Springiness\ [-] = \frac{distance\ of\ the\ detected\ height\ during\ the\ second\ bite}{distance\ of\ the\ detected\ height\ during\ the\ first\ bite} \qquad (6)$$

$$Cohesiveness\ [-] = \frac{area\ under\ the\ deformation\ curve\ of\ the\ second\ bite}{area\ under\ the\ deformation\ curve\ of\ the\ first\ bite} \qquad (7)$$

$$Resilience\ [-] = \frac{upstroke\ energy\ of\ first\ compression}{downstroke\ energy\ of\ first\ compression} \qquad (8)$$

$$Chewiness\ [N] = hardness \cdot cohesiveness \cdot springiness \qquad (9)$$

The HMMA samples were prepared for the TPA in sizes of 2 × 2 cm with 7 mm thickness and were double compressed 2 mm using a cylindrical probe (18 mm diameter) at a speed of 2 mm/s. From the TPA, the hardness, springiness, resilience, and chewiness were evaluated. At least three measurements were performed on each sample.

A cutting test was also performed, both transversal and longitudinal, on HMMA sample sizes of 2 × 2 cm (transversal) and 5 × 2 cm (longitudinal) with 7 mm thickness. They were cut 5 mm deep using a knife blade (height 117 mm, width 67.5 mm) at a speed of 2 mm/s. Transversal cutting was done in the direction of the width of the sample, whereas longitudinal cutting was done in the direction of the length of the sample (Figure 24).

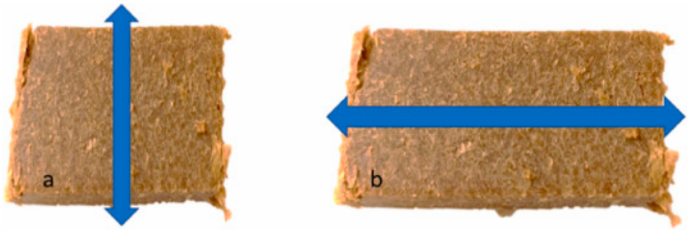


Figure 24. Transversal (a) and longitudinal (b) cutting of HMMA samples.

Statistical methods

All data has been presented as mean ± standard deviation based on duplicate measurements or more. The statistical analyses were performed using the software Minitab (Minitab Inc.; State College, PA, USA). The data were always checked for normality before choosing tests for detecting significant differences. For parametric data sets, the Tukey's test was used, and for non-parametric data sets, the Kruskal-Wallis test was used. Results were considered statistically different if $p < 0.05$.

Main results and discussion

This doctoral thesis is based on four different studies. papers I and II focused on extending the shelf-life of soy and oat okara with HPP. papers III and IV focused on the development of novel food products, incorporating dried oat okara in an expanded snack (paper III) and an HMMA (paper IV).

Microbiological control of soy and oat okara (papers I and II)

Shelf-life study

The microbial content was analyzed for the reference and pressure-treated soy and oat okara after 2 and 4 weeks to evaluate if HPP was able to increase the shelf-life of soy and oat okara. *Enterobacteriaceae* was below the detection limit for the crude samples and references after 2 weeks in both soy and oat okara and was therefore not further investigated in the shelf-life study.

For practical reasons, the soy and oat okara needed to be frozen before the HPP trials. The references that had been frozen had a significantly lower ($p < 0.05$) microbial growth compared to their crude samples on all agars (except for the content of lactic acid bacteria in soy okara, which was low in both samples with no significant difference, $p > 0.05$) (Figures 25, 26, 27). The microbial reduction by freezing cannot be distinguished from the HPP effect in these studies.

For soy okara, the HPP treatment of 600 MPa had a significantly lower microbial growth in all agars ($p < 0.05$) compared to the reference and treatments of 200 MPa and 400 MPa after 2 and 4 weeks (Figures 25a, 26a, 27a). The reference and the treatments at 200 MPa and 400 MPa almost reached a stationary phase after 4 weeks (log 7-8 CFU/g), while the treatment at 600 MPa remained in its exponential phase (log 6-7 CFU/g). The treatment at 400 MPa had significantly lower microbial growth of yeast and mold, and lactic acid bacteria after 2 weeks ($p < 0.05$) compared to the reference and 200 MPa but reached the same microbial load after 4 weeks.

For oat okara, the growth of total aerobic count was significantly reduced ($p < 0.05$) by the treatment of 600 MPa after 2 (log 4.0 CFU/g) and 4 weeks (log 5.2 CFU/g,

Figure 25b) compared to the reference. The treatment of 200 MPa and 400 MPa significantly increased ($p < 0.05$) the growth of total aerobic count after 2 weeks compared to the reference. After 4 weeks, the growth seemed to plateau for the 200 MPa treatment (log 7.2 CFU/g), closely followed by the 400 MPa treatment (log 6.6 CFU/g) and reference (log 6.6 CFU/g). After 4 weeks, soy okara treated at 600 MPa had the lowest microbial growth. For yeast and mold, the microbial growth was below the detection limit after 2 weeks for all treatments (log 2.3-2.8 CFU/g, Figure 26b). After 4 weeks, yeast and mold increased in numbers in the reference, 200 MPa, and 400 MPa treatments (log 3.7-4.6 CFU/g), but the microbial growth for the 600 MPa treatment was still below the detection limit (log 2.3 CFU/g). The growth of lactic acid bacteria followed approximately the same trend as for yeast and mold. However, the treatment of 400 MPa had the same effect as the 600 MPa treatment, with microbial growth below the detection limit (Figure 27b).

The microbial growth after the 200 MPa treatment was not significantly different ($p < 0.05$) from the reference on any agar in the soy okara. This could be explained by the fact that only the quaternary structure of protein is disrupted at 200 MPa, and microbes can therefore quickly recover (Balasubramaniam et al., 2016) In the oat okara, limited growth of lactic acid bacteria at 400 MPa and 600 MPa treatments was observed, which was in line with a previous study with similar results (Sohn and Lee, 1998). Even though lactic acid bacteria are gram-positive (with thicker cell walls compared to gram-negative bacteria), they did not have a stronger resistance to HPP in comparison to yeast and mold in this study. However, the sensitivity also depends on the strain of bacteria and the growth cycle (Balasubramaniam et al., 2016).

Different food products have different requirements for the limitation of microbial growth and should be considered carefully. Based on maximum limitations for various ready-to-eat foods, the total aerobic count should be limited to log 5-6 CFU/g, yeast and mold to log 4-5 CFU/g, and lactic acid bacteria to log 9 CFU/g (Stannard, 1997; Centre for Food Safety, 2014; FSANZ, 2016). Both soy and oat okara, treated at 600 MPa, could therefore be considered microbiologically safe after 2 weeks of storage, being below or at the limit for microbial growth on all agar types (Figures 25, 26, 27). However, after 4 weeks of storage, consumption of soy okara would not be recommended (above limits for both total aerobic count and yeast and mold), but it could still be possible for oat okara. The oat okara reference, with no treatment, could also be considered safe to eat after 2 weeks of storage (log 4.9 CFU/g) but not safe to eat after 4 weeks (log 6.6 CFU/g), looking at the total aerobic count. A shelf-life of 1-2 weeks would therefore only require freezing and vacuum-packing for oat okara, but if a longer shelf-life would be required, an HPP treatment of a minimum of 600 MPa could be considered. HPP treatments of 200 MPa and 400 MPa would not be appropriate as they did not have any effect (soy okara) or increased microbial growth (oat okara).

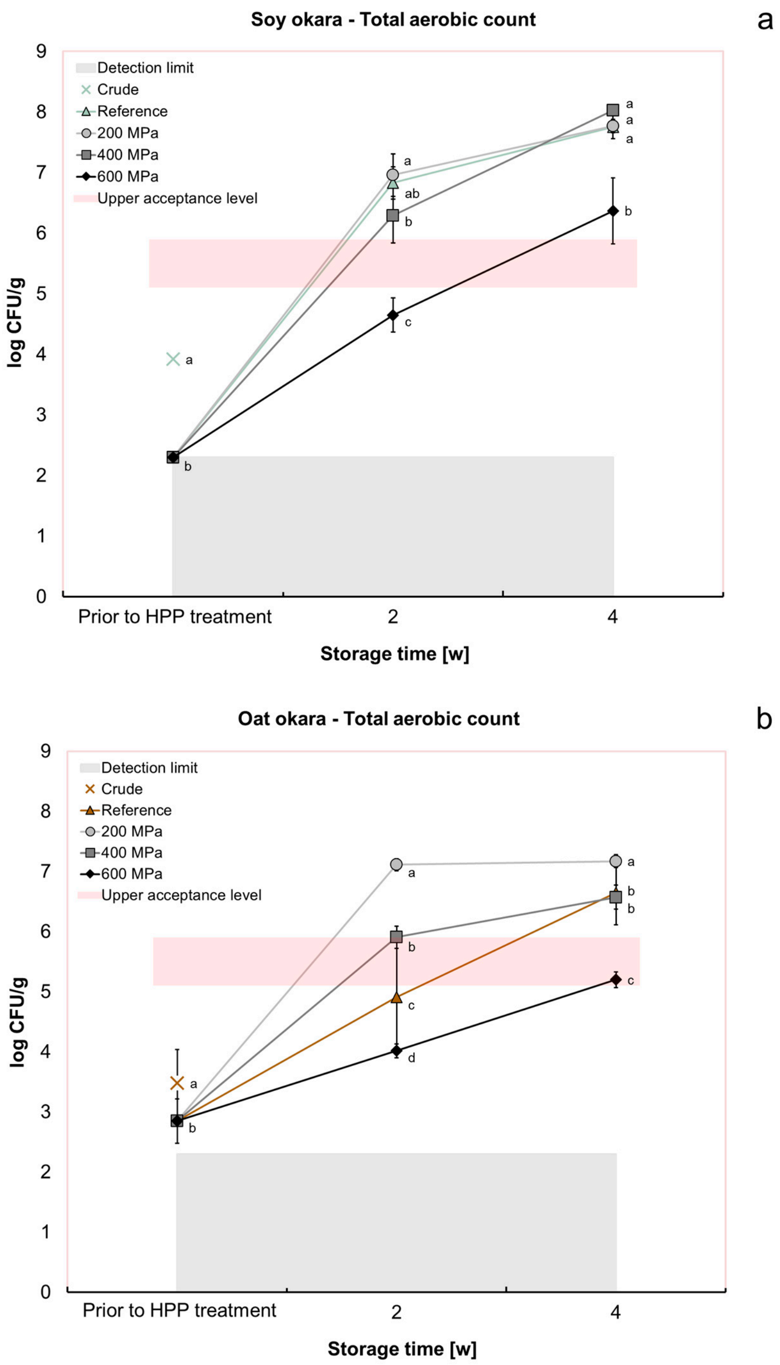


Figure 25. The total aerobic count in (a) soy okara and (b) oat okara. Data with different letters are significantly different, $p < 0.05$, $n = 6$.

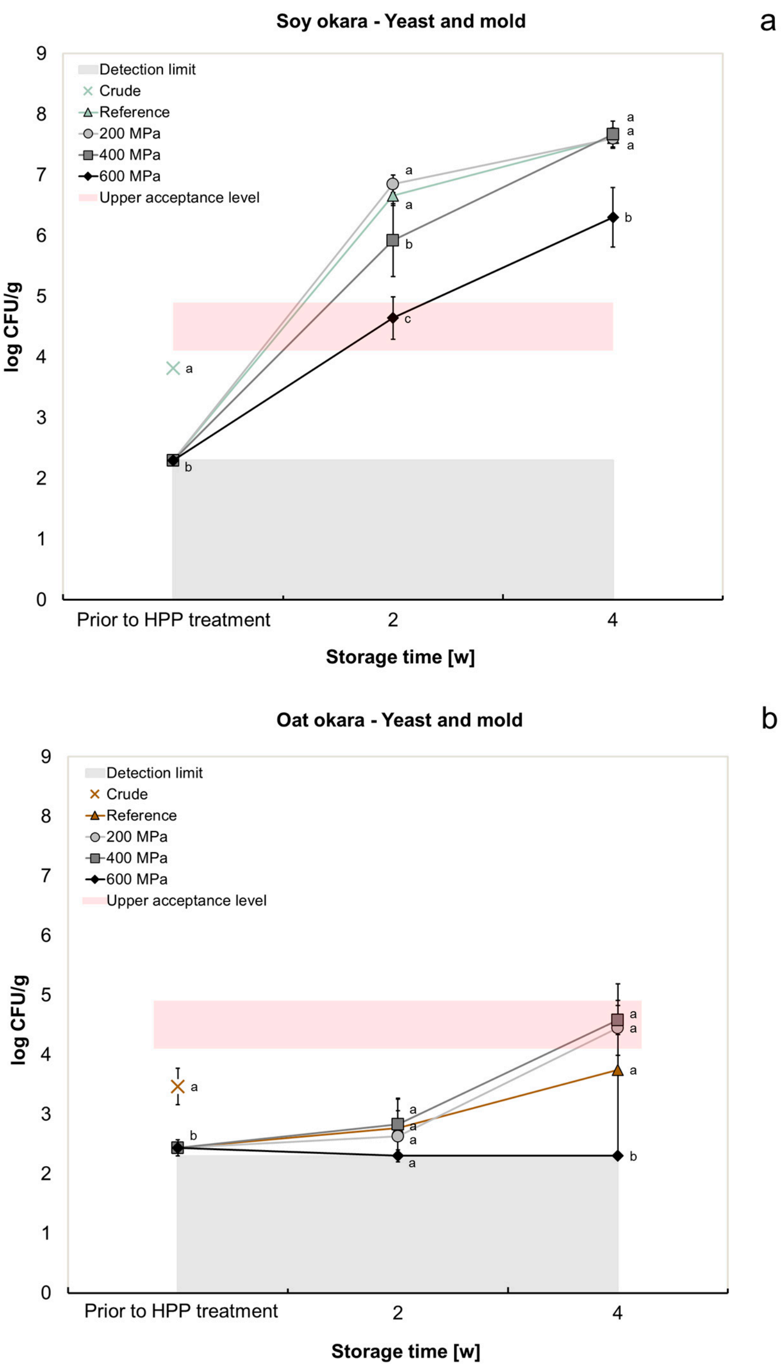


Figure 26. Yeast and mold content in (a) soy okara and (b) oat okara. Data with different letters are significantly different, $p < 0.05$, $n = 6$.

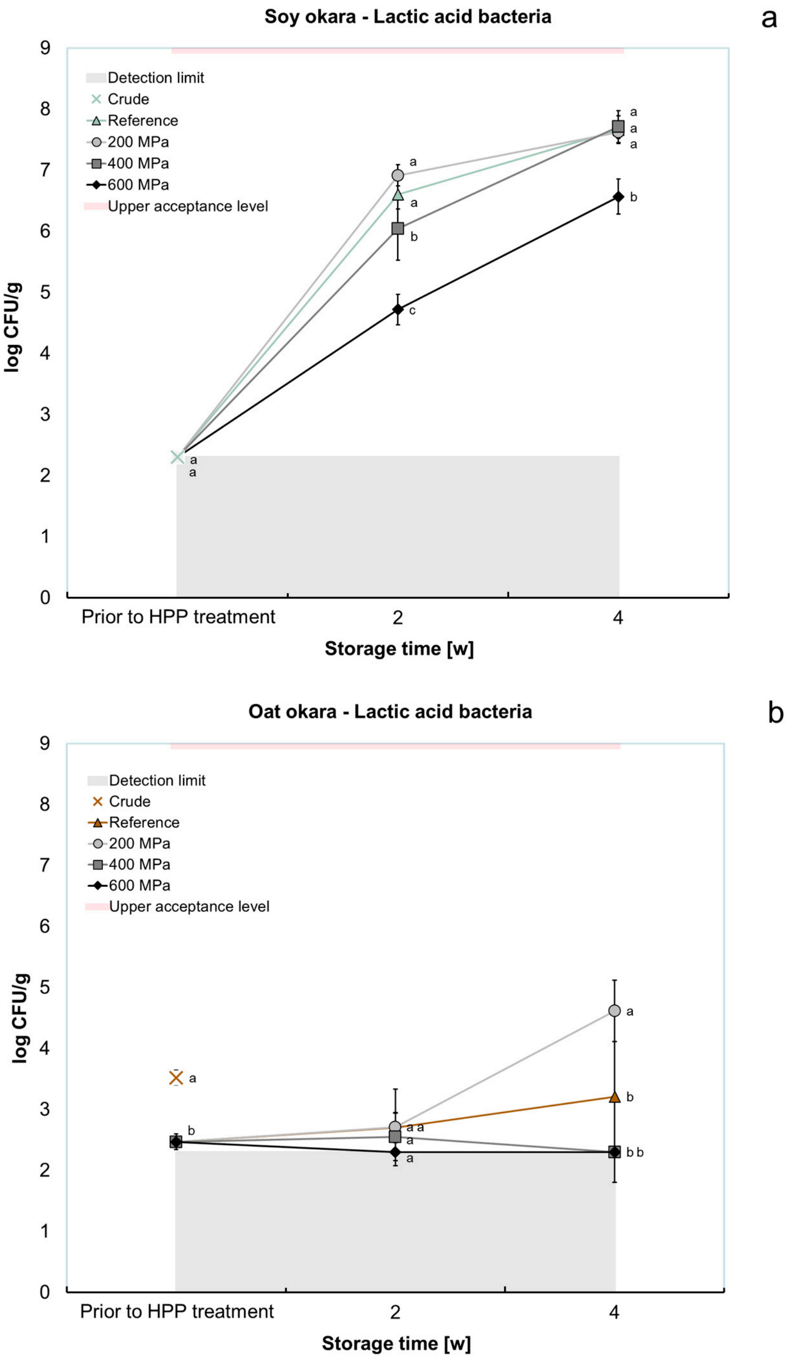


Figure 27. Lactic acid bacteria content in (a) soy okara and (b) oat okara. Data with different letters are significantly different, $p < 0.05$, $n = 6$.

Dietary fiber content

According to the dietary fiber analysis conducted by Eurofins, the total dietary fiber content consisted only of insoluble fiber in the reference prior to HPP treatment for both soy and oat okara (Table 10). These are conflicting results compared to other studies where soy okara has had a total dietary fiber of 54–55% consisting of 4.2–4.7% soluble fiber (Redondo-Cuenca et al., 2008; Mateos-Aparicio et al., 2010), and where oat okara has had a soluble fiber fraction of 12% of the total dietary fiber (Aiello et al., 2021).

There was no increase in soluble fiber content in soy okara for the 200 MPa and 400 MPa treatments; however, for the 600 MPa treatment, the soluble fiber content went from 0% to 1.1%, resulting in a soluble fiber fraction of 4.4% (Table 10). A redistribution from insoluble to soluble fiber has previously been observed in soy okara after HPP treatments at 200 MPa and 400 MPa (at 30°C and 60°C), which in turn affected the water and oil-holding capacities (Mateos-Aparicio et al., 2010). For oat okara, the soluble fiber content increased for all HPP treatments to 0.7% (200 MPa), 1.1% (400 MPa), and 1.7% (600 MPa), which is a soluble fiber fraction of 3.8%, 6.3%, and 9.2% of the total dietary fiber, respectively (Table 10).

Table 10. Total dietary fiber content, including insoluble and soluble fiber content in reference and all HPP-treated soy and oat okara samples on a dry basis.

		Reference	**200 MPa**	**400 MPa**	**600 MPa**
Soy okara	Total dietary fiber [%]	26.9 ± 4.0	24.5 ± 3.7	24.4 ± 3.7	24.9 ± 3.7
	Insoluble fiber [%]	29.4 ± 4.4	25.1 ± 3.8	24.5 ± 3.7	23.8 ± 3.6
	Soluble fiber [%]	n.d.	n.d.	n.d.	1.1 ± 0.2
	Fraction soluble fiber [%]	0	0	0	4.4
Oat okara	Total dietary fiber [%]	15.8 ± 2.36	18.1 ± 2.72	17.4 ± 2.62	18.5 ± 2.78
	Insoluble fiber [%]	16.0 ± 2.40	17.4 ± 2.61	16.3 ± 2.45	16.8 ± 2.52
	Soluble fiber [%]	n.d.	0.7 ± 0.11	1.1 ± 0.17	1.7 ± 0.26
	Fraction soluble fiber [%]	0	3.8	6.3	9.2

n.d. = not detectable

Thermal properties

The thermal properties were investigated to evaluate the effect of HPP on protein and fiber in soy and oat okara.

The soy okara had generally small peaks (<1.0 mJ/mg), which were difficult to interpret (Figure 28a). Denaturation temperatures of 68°C and 88°C have previously been linked to the main globular protein in soy, β-conglycinin and glycinin (Renkema and van Vliet, 2002). The peaks at these temperatures were not distinct, neither in the reference nor the HPP-treated soy okara (Figure 28a), which indicates that most of these proteins have already been denatured in the production process of soy beverages, where temperatures up to 100°C can be reached (Ikya et al., 2013).

The thermal decomposition temperature for soluble fiber in soy okara is around 86.6°C (Chen et al., 2014), and endothermic peaks around 130–150°C are attributed to the evaporation of bound water in dietary fiber (Liu et al., 2021). A peak at 132.4°C was observed for the reference but was not present for the HPP-treated soy okara (Figure 28a). Changes in the amount of bound water, fiber structure, and redistribution from insoluble to soluble fiber may have altered the melting temperatures, explaining the disappearance of the peak.

In the reference for oat okara, a distinct peak at 111.7°C (7.52 mJ/mg) was detected, which most probably represents the 12S globulin in oat, avenalin, with a denaturation temperature between 114–116°C (Ma and Harwalkar, 1988; Cheng et al., 2023) (Figure 28b). Avenalin is an oligomeric protein (Ma and Harwalkar, 1988) and at 200 MPa, its subunits may have dissociated but been able to reassociate after treatment. However, hysteresis of conformational changes can occur when this happens (Boonyaratanakornkit et al., 2002). This could explain the broader peak and lower enthalpy (4.58 mJ/mg) that was observed for 200 MPa (Figure 28b). At 400 MPa and 600 MPa, the enthalpy decreased further to 1.23 mJ/mg and 0.13 mJ/mg, respectively, concluding that an increase in irreversible denaturation of protein occurred with increasing pressure. At 600 MPa, another endothermic peak appeared at a higher temperature (133.1°C), which could possibly represent protein aggregates formed by protein-protein interaction of unfolded protein (He et al., 2014). The protein denaturation was proportional to the pressure, where the highest pressure (600 MPa) induced the largest protein denaturation, corresponding to the largest reduction in microbial content (Figures 25b, 26b, 27b).

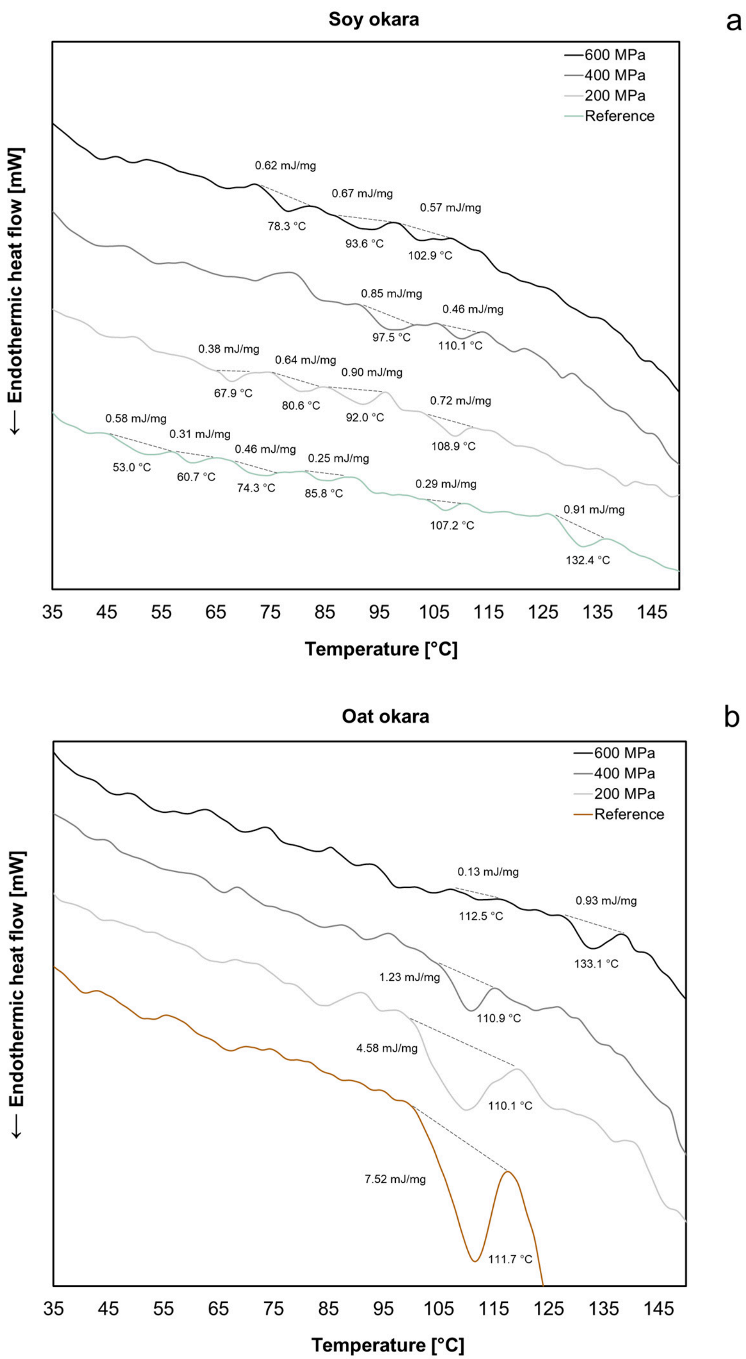


Figure 28. DSC thermograms for (a) soy okara and (b) oat okara. Reference (soy okara: green, oat okara: yellow) and HPP-treated okara at 200 MPa (light gray), 400 MPa (gray), and 600 MPa (black).

Pasting properties

Viscosity was examined during heating up to 140°C, followed by cooling, in order to evaluate any effects on the pasting properties of soy and oat okara after HPP. Pasting properties can bring useful information in product development.

The initial viscosity of soy okara was high (3000–3500 cP) due to the physical resistance, whereafter continuous heating reduced the viscosity (Figure 29a). The reference had two small peaks in viscosity around 100°C (2500 cP) and 140°C (1700 cP). The soy okara treated at 200 MPa had a small viscosity peak around 130°C (1900 cP), while the soy okara treated at 400 MPa and 600 MPa had larger viscosity peaks around 140°C (2100 cP and 1900 cP, respectively) (Figure 29a, peaks marked with arrows). The soluble fiber content increased slightly for the soy okara treated at 600 MPa (Table 10), which could explain the larger viscosity peak that appeared for that sample, as soluble fiber generally has a higher viscosity and a better ability to form gels (Mudgil, 2017) compared to insoluble fiber. However, the 400 MPa treatment had an even larger viscosity peak but without any increase in soluble fiber. This means that HPP might have another unknown effect on the fiber in the 400 MPa and 600 MPa treated samples (which also was indicated in the DSC thermograms, Figure 28a) inducing this viscosity change.

For the reference and HPP-treated oat okara at 200 MPa, a viscosity peak around 120°C was observed (Figure 29b). The peak corresponds well to the protein avenalin, which was identified in the DSC thermograms (Figure 28b). For HPP treatments of 400 MPa and 600 MPa, the viscosity peak disappeared, which confirms altered protein conformation and protein denaturation. The initial noise of 600 MPa was most likely due to the physical resistance of highly compressed oat okara containing larger chunks of material. In earlier RVA measurements (up to 95°C) performed on oat flour, a gelatinization peak at 95°C (approximately 3000 cP) has been observed (Cheng et al., 2023). That gelatinization peak was not detected in this study, indicating that all starch in the oat okara had already been gelatinized or enzymatically degraded in the oat beverage production process.

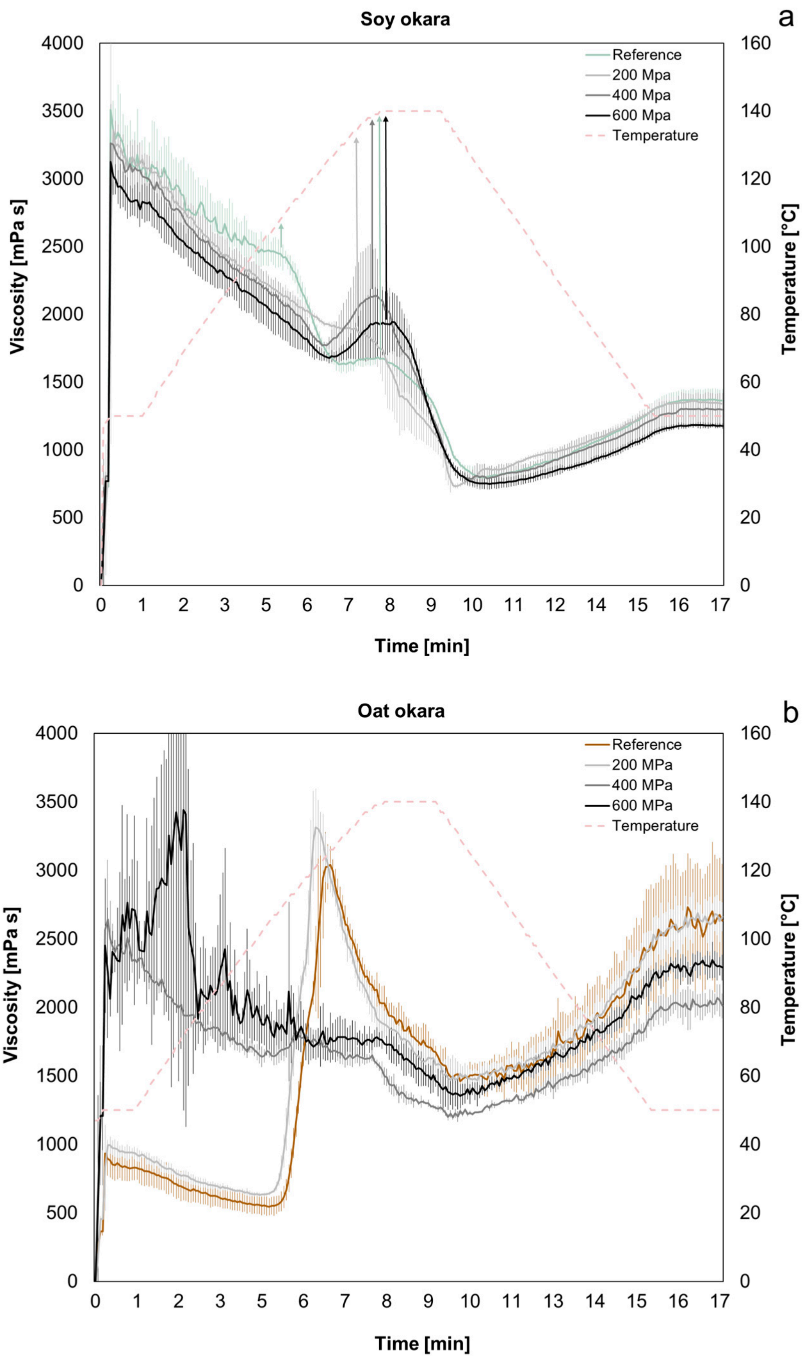


Figure 29. The average viscosity is presented for (a) soy okara and (b) oat okara. Reference (soy okara: green, oat okara: yellow) and HPP-treated okara at 200 MPa (light gray), 400 MPa (gray), and 600 MPa (black). The error bars represent the standard deviation, n = 3.

Water and oil holding capacities

The total dietary fiber of soy okara was mainly insoluble (with a slight increase in soluble fiber after the 600 MPa treatment, Table 10). Insoluble fiber has a porous matrix that can absorb, swell, and entrap water and has therefore a generally high WHC (Mudgil, 2017). The WHC of the soy okara reference was 6.03 ± 0.14 mL/g and was not affected by HPP (Figure 30). The WHC was comparable to wheat bran (6.6 mL/g) and oat bran (5.5 mL/g) and was considered high. A material with high WHC can be utilized as a functional ingredient to reduce the energy content and to adjust the texture and viscosity of formulated foods (Grigelmo-Miguel and Martín-Belloso, 1998).

The soy okara reference had an OHC of 0.75 ± 0.05 mL/g (Figure 30), and after treatment at 200 MPa, the OHC was significantly reduced (0.66 mL/g) ($p < 0.05$). However, the OHC for soy okara treated at 400 MPa (0.71 ± 0.03 mL/g) and 600 MPa (0.68 ± 0.01 mL/g) did not significantly differ ($p > 0.05$) from either the reference or the soy okara treated at 200 MPa. Therefore, HPP had a limited impact on the OHC, and the difference would probably not affect a final product.

The oat okara also mainly consisted of insoluble fiber (with a small increase of soluble fiber with increasing pressure, Table 10), but had an overall lower WHC compared to soy okara. The 200 MPa treatment gave a significantly lower WHC ($p < 0.05$) compared to the oat okara reference and 400 MPa and 600 MPa treatments (Figure 30). This outlier is somewhat difficult to comprehend. There might have been a loss in WHC as the proteins underwent conformational changes after HPP (DSC results, Figure 28b), affecting the 3D network, which is an important factor for WHC in solid foods (McClements and Grossmann, 2022). Even though an increase in protein denaturation followed the treatments of 400 MPa and 600 MPa, the WHC recovered and was as high as the reference. The increase in soluble fiber might have compensated for the possible loss of protein 3D network. The same phenomenon was observed in an earlier study where soy okara was subjected to high pressure (200 MPa and 400 MPa), and an increase in soluble fiber as well as water retention capacity was observed (Mateos-Aparicio et al., 2010).

The OHC of oat okara was not affected by HPP ($p > 0.05$) and had an overall higher OHC compared to soy okara between 1.2–1.3 mL oil/g (Figure 30).

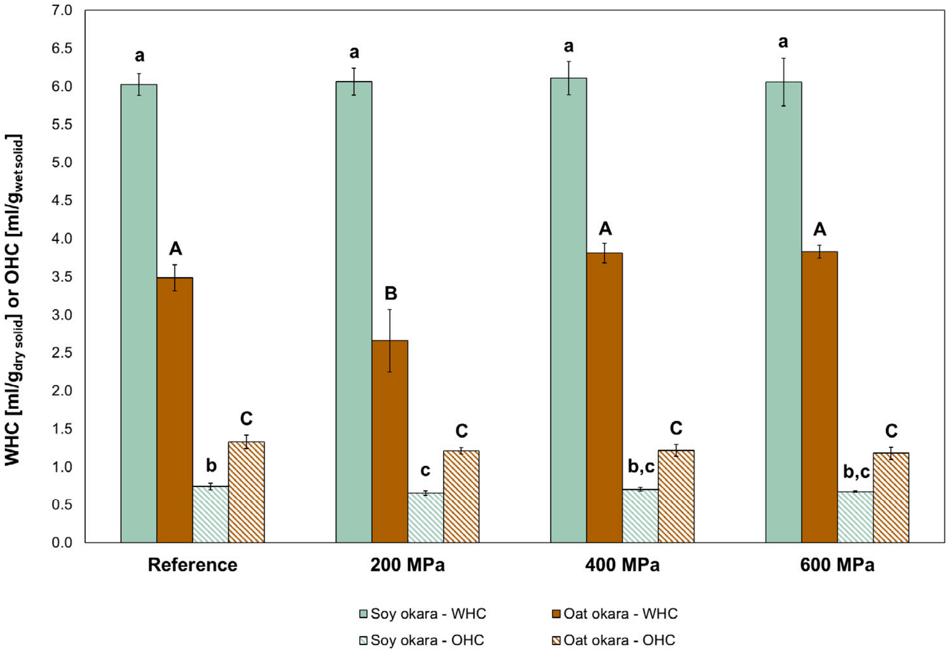


Figure 30. WHC (solid) and OHC (striped) results for reference and HPP-treated soy okara (green) and oat okara (brown). Data with different letters are significantly different, $p < 0.05$, $n = 3$ (lower-case letters for soy okara and upper-case letters for oat okara).

Co-extrusion of dried oat okara and corn grits (paper III)

Proximate composition and process conditions

Both input material and process conditions can affect the properties of extrudates. With increasing proportions of oat okara, the starch content decreased, and the protein and fiber content increased in the formulations (Table 11). For good expansion, the starch content should be at least 60% (Bouvier and Campanella, 2014), and a fiber content above 25% may have drastic and negative effects on the expansion of extrudates (Pai et al., 2009). High fiber content can also result in characteristics such as increased density, firmer textures, reduced crispiness, and consequently, less desirable products (Robin et al., 2012). Protein, being more complex and multifunctional compared to polysaccharides, makes them more reactive and less predictable in an extrusion cooking process. They can therefore alter the melt expansion criteria in a starch-based formulation (Bouvier and Campanella, 2014).

Table 11. The proximate composition of corn grits, oat okara, and all the formulations on a dry basis (n = 3).

	Corn grits	90:10*	80:20*	70:30*	60:40*	Dried oat okara
Protein [%]	7.17 ± 0.55	10.3	13.5	16.6	19.8	38.7 ± 1.16
Fat [%]	0.82 ± 0.21	1.32	1.83	2.34	2.85	5.91 ± 0.05
Fiber [%]	4.25 ± 0.17	6.31	8.37	10.4	12.5	24.9 ± 0.63
Starch (including free glucose) [%]	80.4 ± 0.17	74.6	68.8	63.1	57.3	22.6 ± 0.92
Other carbohydrates [%]**	6.45	6.17	5.89	5.61	5.33	3.64
Ash [%]	0.91 ± 0.03	1.25	1.59	1.93	2.27	4.31 ± 0.06
Water [%]	12.9 ± 0.14	12.1	11.4	10.6	9.84	5.24 ± 0.03

*Calculated from the proximate composition of corn grits and oat okara
**Calculated by difference

With an increase of oat okara in the formulations, changing the proximate composition, the process conditions also changed (Table 12). The melt temperature, pressure, and SME had a decreasing trend for all feed moistures when the proportion of oat okara increased. There are correlations between SME and starch structural changes, which in turn can affect the expansion of starch-based extrudates (Bouvier and Campanella, 2014). Lower feed moisture generally resulted in higher SME, melt temperature, pressure, and lower moisture content in the extrudates (Table 12).

Table 12. The melt temperature, pressure, and specific mechanical energy (SME) for each extrusion trial and moisture content of extrudates.

Extrudate formulation (Corn grits: Oat okara)	Feed moisture [%]	Melt temperature [°C]	Pressure [bar]	SME [kJ/kg]	Moisture content [%]
100:0	14	194 ± 0.1	158 ± 2.2	415 ± 13	4.40 ± 0.05^{h}
	16	186 ± 0.5	141 ± 1.5	348 ± 12	6.03 ± 0.07^{f}
	18	182 ±0.4	86 ± 2.3	525 ± 13	7.61 ± 0.04^{d}
90:10	14	190 ± 0.3	155 ± 2.1	362 ± 11	4.81 ± 0.10^{g}
	16	185 ± 0.2	137 ± 1.9	317 ± 10	6.38 ± 0.07^{e}
	18	179 ± 0.4	112 ± 10.4	286 ± 33	8.68 ± 0.03^{b}
80:20	14	190 ± 0.2	155 ± 4.6	332 ± 14	4.82 ± 0.07^{g}
	16	182 ± 0.4	131 ± 1.9	305 ± 10	6.39 ± 0.02^{e}
	18	178 ± 0.3	112 ± 1.2	262 ± 10	8.37 ± 0.06^{c}
70:30	14	187 ± 0.1	166 ± 2.8	302 ± 13	4.94 ± 0.05^{g}
	16	182 ± 0.3	137 ± 2.7	266 ± 16	6.35 ± 0.09^{e}
	18	176 ± 0.1	101 ± 2.3	236 ± 10	8.58 ± 0.04^{bc}
60:40	14	164 ± 0.5	106 ± 1.2	258 ± 8	5.89 ± 0.13^{f}
	16	159 ± 0.3	84 ± 1.6	227 ± 10	7.55 ± 0.18^{d}
	18	156 ± 1.0	71 ± 3.1	212 ± 12	9.08 ± 0.20^{a}

Expansion and microstructural properties

Photos of all extrudates in Table 13 presents the extrudates shape and geometry.

Table 13. Pictures of all expanded extrudates.

Feed moisture	18%	16%	14%
100:0			
90:10			
80:20			
70:30			
60:40			

A higher proportion of oat okara in the formulations reduced the SEI and porosity of the extrudates (Figure 31, Table 14) which also can be visualized in Table 13. It can be an effect of the fiber and protein content oat okara contributes with, as it can have an inhibiting effect on the expansion of starch-based extrudates (Aussanasuwannakul et al., 2022).

With higher proportions of oat okara, the density increased, especially for the formulation of 60:40 and suggests a threshold for the addition of oat okara (Figure 31). This is in agreement with the proximate composition of the 60:40 formula, where the starch content was below the threshold of 60% and also had the highest fiber content (Table 11). Fiber tends to be more rigid in comparison to starch-based polymers and can inhibit expansion (Kowalski et al., 2016). The fiber in oat okara is also mainly composed of insoluble fiber (Helstad et al., 2023), which can retain water in the fiber matrix in an extrusion process and reduce the steam flash-off at the end of the die (Kowalski et al., 2016). This effect can be confirmed as the moisture content in the 60:40 extrudates was significantly higher compared to the other extrudates (Table 12). The feed moisture had a limited effect on both SEI and density, suggesting that the proportion of oat okara played a more significant role in influencing these properties.

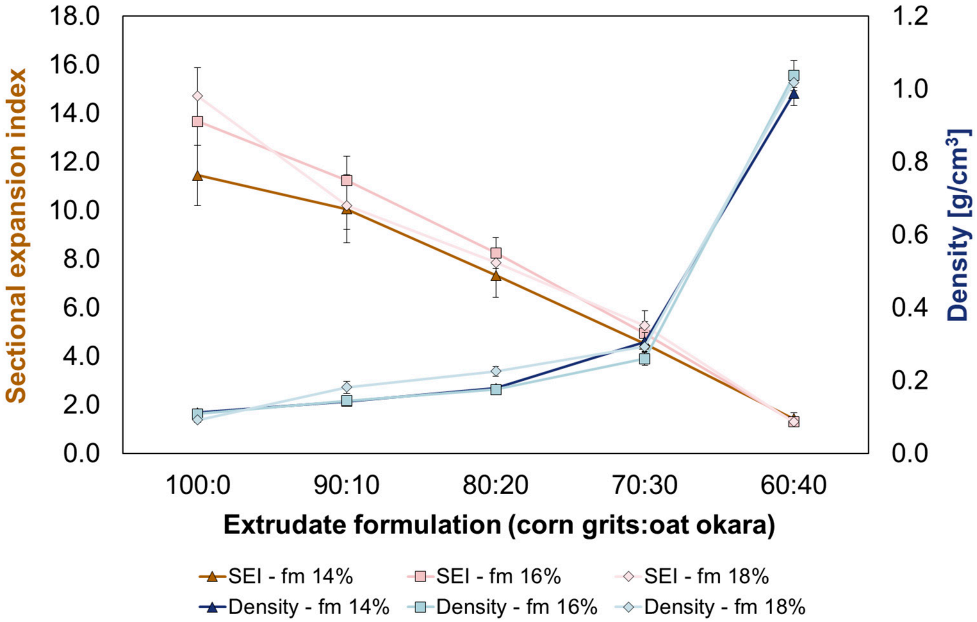


Figure 31. Sectional expansion index (SEI, brown) and density (blue) plotted against extrudate formulations (corn grits:oat okara). Feed moistures (fm) of 14% (triangle), 16% (square), and 18% (diamond). The error bars represent the standard deviation, n = 3.

Table 14. The porosity and number of pores for extrudates run at 16% feed moisture.

	100:0	90:10	80:20	70:30	60:40
Porosity [%]	90.1	88.3	85.3	78.7	24.3
Number of pores	1,587	1,535	1,774	1,767	24,974

In the X-ray microtomography results, it was also concluded that an increase of oat okara reduced pore sizes and increased the local cell wall thickness (Figure 32), and is visualized in the cross sections (Figure 33) and 3D volume renderings Table 15). This corresponds well to the trends of decreasing SEI and increasing density.

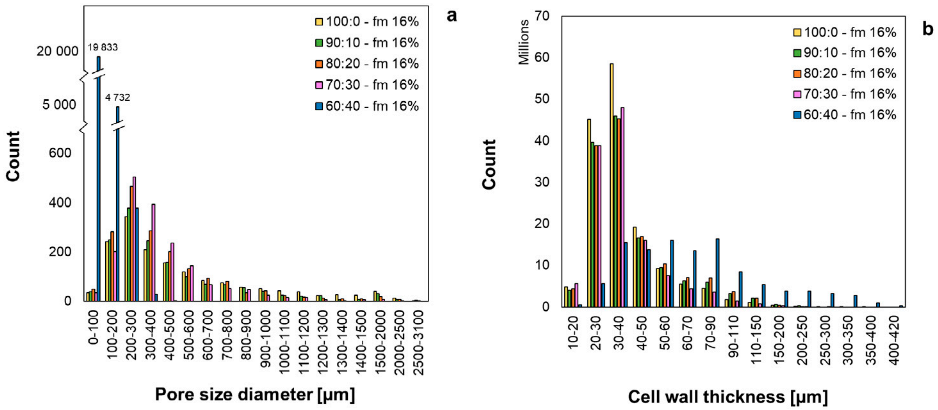


Figure 32. X-ray microtomography results for extrudates produced with 16% feed moisture, presenting (a) the pore size distribution as the equivalent diameter of each pore and (b) the local thickness of the cell walls.

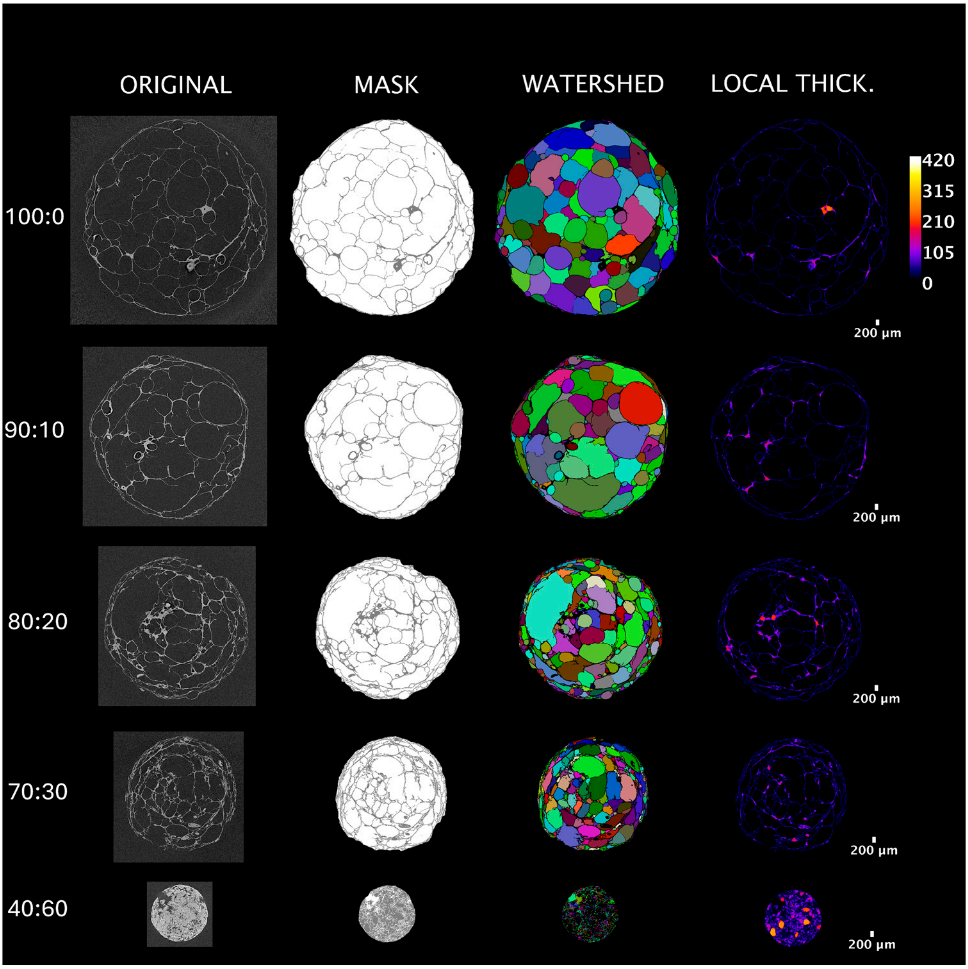
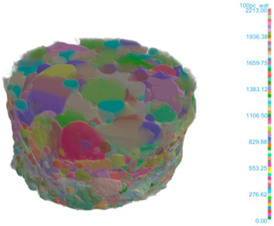
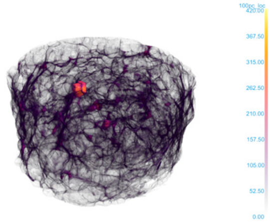
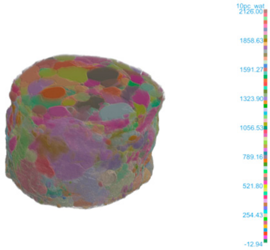
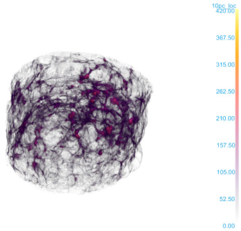
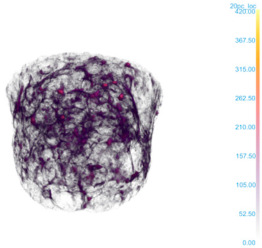
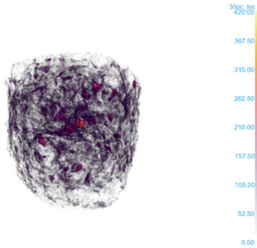
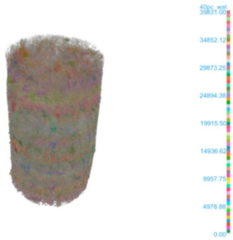
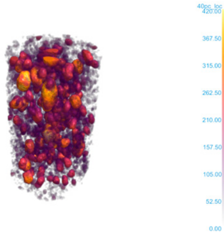


Figure 33. Cross-sectional images obtained via X-ray microtomography slices, illustrating the general pore structure of extrudates produced with 16% feed moisture. The first column shows the original cross-section image from the scanning. The second column displays the segmented mask image, closing of the material structure and pores within the sample from the external air. In the third column, watershed segmentation has been applied to distinguish individual pores, each assigned a unique identifier for the calculation of pore sizes. The fourth column presents the local thickness of the cell wall structures within the samples. The color scale is the same for all the samples.

Table 15. 3D-volume renderings with some transparency of the individually separated macro pores and the local cell wall thickness, where light yellow is thicker and dark purple is thinner cell walls.

Formulation	Individually separated macro pores	Local cell wall thickness
100:0		
90:10		
80:20		
70:30		
60:40		

Thermomechanical properties

The raw materials and milled extrudates were analyzed with RVA to evaluate the degree of cook for the expanded extrudates. Extrusion is a degradative process that affects the polymer size, interaction, and network formation, and this process history can be reflected in their viscosity profiles (Crosbie and Ross, 2007).

The raw materials of 100:0, 90:10, and 80:20 had dominant viscosity peaks around 130°C and high final viscosities, while their corresponding extrudates had cold swelling peaks around 50-80°C and low final viscosities (Figure 34). The cold swelling peaks are associated with the swelling capacity and shear resistance of particles, as well as the dissolution rate of starch polymers (Bouvier and Campanella, 2014). The shift in viscosity profiles implies that the starch has undergone significant mechanical shear during the extrusion process, leading to extensive degradation and structural and molecular alterations of the starch granules (Ilo et al., 1999).

The dried oat okara did not have a high viscosity peak as it had already been processed, which reduced the viscosities substantially in the raw material formulations of 70:30 and 60:40. No viscosity shift was therefore observed between the raw materials and extrudates in those formulations (Figure 34). The cold swelling peaks for these formulations were also low, indicating that a high degree of their carbohydrate polymers were still insoluble after extrusion. It is not surprising, as oat okara contributes with a high insoluble fiber content (24.9%, db). The extrudates with a higher proportion of oat okara were also subjected to the lowest SME in the extrusion cooking process (Table 12), which may have resulted in less depolymerization in the extrusion process (Bouvier and Campanella, 2014). This was confirmed by the water solubility index (WSI) results (manuscript III, Figure 5) which decreased with an increasing proportion of dried oat okara.

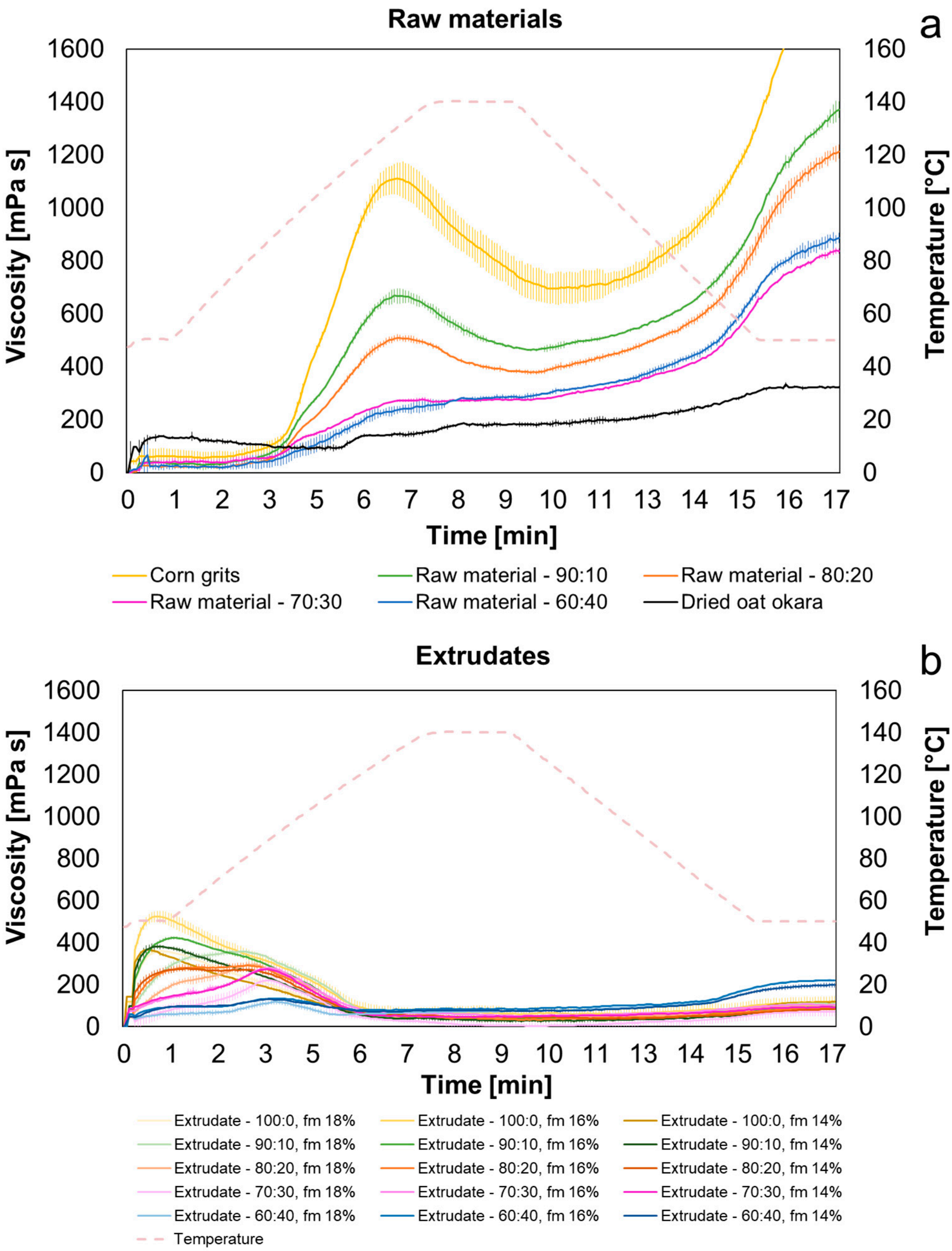


Figure 34. Graphs presenting the average viscosity for (a) raw materials and (b) milled extrudates (100:0: yellow, 90:10: green, 80:20: orange, 70:30: pink, 60:40: blue, and dried oat okara: black). The error bars represent the standard deviation, n = 2 (fm = feed moisture).

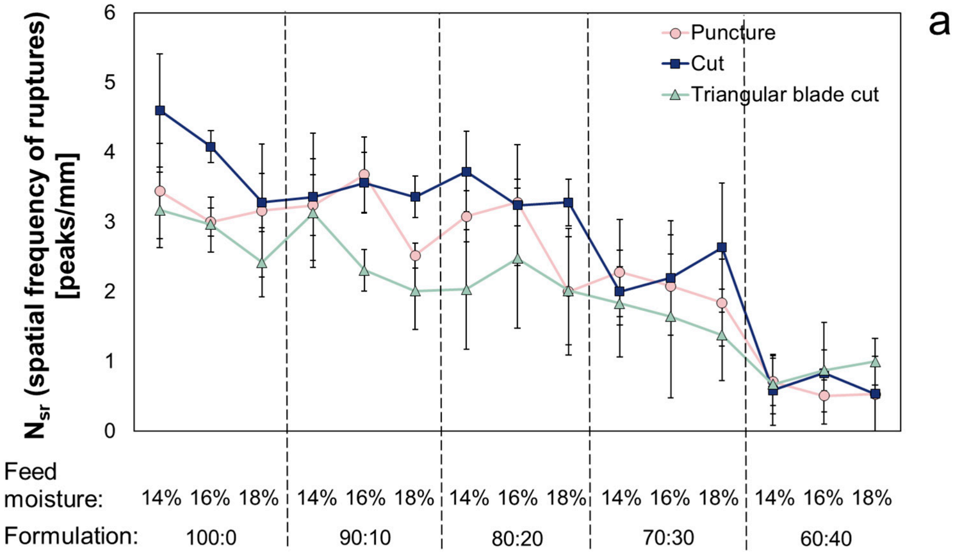
Mechanical properties

The spatial frequency of ruptures (N_{sr}), average crushing force (F_{cr}), and crispness work (W_c) are presented for all methods that were used in Figure 35 (puncture, cut, and triangular blade cut). The frequency of ruptures instead had a decreasing trend as the proportion of oat okara increased, independent of the method used. A lower feed moisture had an increasing trend for the frequency of ruptures in all extrudates and methods, except the 60:40 formulation. This implies that an increase of oat okara and moisture reduced the number of cells in the extrudates (Figure 35a). However, as was visualized from the X-ray microtomography, the number of pores actually increased (Table 14, Figure 32b), concluding that the texture analyzer was not sensitive enough to recognize the smallest pores. Although, when consuming snacks, the smallest pores will not be recognized. Therefore, the texture analyzer provides a better overview of the actual crispiness of the sample, which is not always equal to the number of pores.

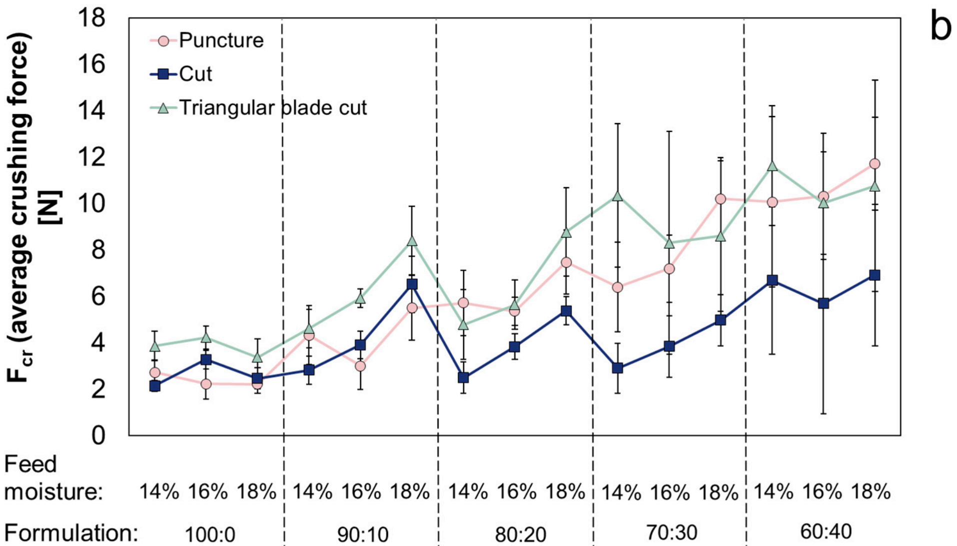
The average crushing force had instead an increasing trend with a higher proportion of oat okara in the extrudate, leading to an increased hardness (Figure 35b). It can be correlated to the increased cell wall thickness (Figure 32a) and density, together with decreasing expansion (Figure 31). It was especially clear in the methods of triangular blade cut and puncture. Increase in crushing force and hardness is a typical outcome for expanded snacks when fiber (Lotfi Shirazi et al., 2020) or protein (de Mesa et al., 2009) is incorporated into a starch-based formulation. When feed moisture increased, the average crushing force also increased, which was mainly visualized in the cut method (Figure 35b).

The crispness work also showed a slight increasing trend when the proportion of oat okara was increased, resulting in a higher crispiness. However, the 60:40 formulation had a much higher crispness work compared to the other formulations since it required much higher force to simply break the extrudate in two pieces. This was therefore not representative of the actual crispiness (Figure 35c). There were only small differences in crispness work between the feed moistures of the extrudates. This property is dependent on both average crushing force and frequency of ruptures (Equation 4), which had a decreasing and increasing trend, respectively, which appeared to have evened out the differences.

The three different methods visualized the large general trends similarly for all texture properties that were calculated (Figure 35). The cut method, however, uses a sharp probe with less contact area compared to the triangular blade cut probe and might have been more sensitive, recognizing more cells (higher frequency of ruptures), but also required less force to cut through the extrudates (lower crushing force). The standard deviations are, however, rather large in all methods, which makes it difficult to compare and visualize the advantages and disadvantages.



a
N_{sr} (spatial frequency of ruptures) [peaks/mm]
Puncture
Cut
Triangular blade cut
0
1
2
3
4
5
6
Feed moisture:
14% 16% 18% 14% 16% 18% 14% 16% 18% 14% 16% 18% 14% 16% 18%
Formulation:
100:0
90:10
80:20
70:30
60:40



b
F_{cr} (average crushing force) [N]
Puncture
Cut
Triangular blade cut
0
2
4
6
8
10
12
14
16
18
Feed moisture:
14% 16% 18% 14% 16% 18% 14% 16% 18% 14% 16% 18% 14% 16% 18%
Formulation:
100:0
90:10
80:20
70:30
60:40

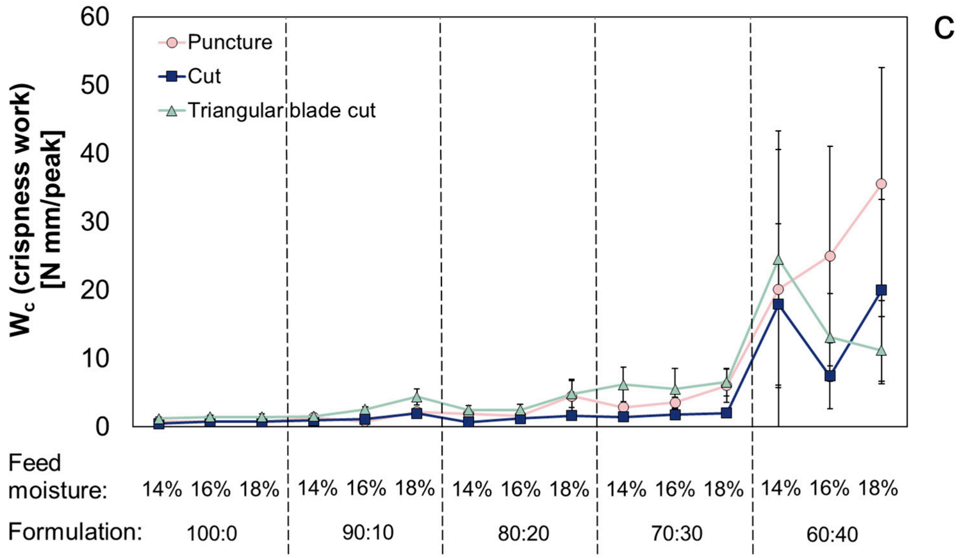


Figure 35. Texture properties for all extrudates and methods (puncture: pink, cut: blue, triangular blade cut: green), presented as (a) N_{sr} (spatial frequency of ruptures, (b) F_{cr} (average crushing force), and (c) W_c (crispness work). The error bars represent the standard deviation, n = 5.

The particle size distribution of corn grits was between 300 and 600 µm (d_{50} = 474 ± 2 µm), and for dried oat okara between 300 and 1250 µm (d_{50} = 829 ± 6 µm). A previous study found that smaller particle sizes had a significant effect on crispiness by increased expansion, pore size, porosity, and reduced hardness (Alam, Järvinen et al. 2014). The rather large particle size distribution of oat okara can therefore have contributed to the reduced expansion, pore size, porosity, and increased hardness (Figures 31, 32, 33, Tables 14, 15).

Co-extrusion of dried oat okara and hempseed protein concentrate (paper IV)

Proximate composition and process conditions

The proximate composition of hempseed protein concentrate (HPC) and dried oat okara is listed in Table 16. In the calculations based on the protein concentrations in this study, the standard conversion factor of 6.25 was used. There is, however, an ongoing discussion of using more accurate crop-specific conversion factors than the standard to retrieve more accurate protein contents (Mariotti et al., 2008). The amount of protein (86%) and fat (9.0%) was higher in HPC than in the dried oat okara (41% and 6.9%, respectively), and the ash content was higher in dried oat okara (5.2%) compared to HPC (2.3%). HPC had almost no carbohydrate content (2.9%), while the dried oat okara had a much higher content (47%).

Table 16. Proximate composition of hempseed protein concentrate (HPC) and dried oat okara on a dry basis. The protein conversion factor was 6.25.

	Hempseed protein concentrate	Dried oat okara
Protein [%]	86 ± 0.3[a]	41 ± 0.5[b]
Fat [%]	9.0 ± 0.6[a]	6.9 ± 0.4[b]
Carbohydrate [%]*	2.9	47
Ash [%]	2.3 ± 0.1[b]	5.2 ± 0.2[a]

*Calculated by difference

The SME values ranged from 63-185 kJ/kg depending on the operating conditions, such as temperature profile, feed moisture and screw speed (Table 17). However, the SME was significantly lower compared to previous work from our research group, for example, rapeseed protein concentrate co-extruded with yellow pea isolate (412-1276 kJ/kg) (Zahari et al., 2021), commercial hempseed protein concentrate co-extruded with wheat gluten and chickpea protein concentrate (672-1015 kJ/kg) (Zahari et al., 2023), and yellow pea protein isolate co-extruded with faba bean protein concentrate (992-1259 kJ/kg) (Ferawati et al., 2021). It is hypothesized that the low SME could be a result of the novel feeding of the HPC, where the protein were still in a hydrated state and might have facilitated the texturization, leading to less energy required. The particle size distribution might have had an effect as well, as the dried oat okara has a rather large and inhomogeneous particle size ranging from 300-1250 µm (d_{50} = 829 ± 6 µm) (paper III). The effect of particle size has been reported to play a significant role for LME, but has not been extensively investigated for HME (Singh and Koksel, 2021). For expanded snacks, a smaller particle size generates higher SME (Sharifi et al., 2021), and the large particle size of dried oat okara might therefore have had a decreasing effect on SME in this study as well.

A higher screw speed resulted in higher SME at the same feed moisture level, and a higher feed moisture at the same screw speed resulted in lower SME for both temperature profiles (Table 17), which is in agreement with previous studies (Fang et al., 2014; Caporgno et al., 2020; Ferawati et al., 2021; Zahari et al., 2021; Kantanen et al., 2022). Higher screw speeds generate higher shear rates, thus increasing SME (Akdogan, 1999). A higher feed moisture has been related to the reduction in viscosity and serves as a lubricant, thus decreasing the friction between the extruded material and the screws, resulting in a lower SME (Lin et al., 2002; Chen et al., 2010; Mateen et al., 2023). The higher temperature profile was found to generate lower SME values, which could be expected as a higher temperature would lead to lower melt viscosity (Osen et al., 2014). However, it has also been discussed that higher temperatures can increase viscosity due to increased protein denaturation and texturization, thereof requiring higher SME (Lin et al., 2002). The temperature is therefore not only dependent on the melt viscosity, but also on the friction during texturization (Osen et al., 2014).

Table 17. Average process conditions, presenting the melt temperature and SME for the extrusion of HMMA.

Temperature profile (°C)	Actual feed moisture [%]	Screw speed [rpm]	Melt temperature [°C]	SME [kJ/kg]
40-70-110-130	49	500	123 ± 0.1	79 ± 12
		700	125 ± 0.1	147 ± 65
		900	126 ± 0.1	185 ± 33
	52	500	120 ± 0.0	72 ± 12
		700	122 ± 0.2	120 ± 52
		900	124 ± 0.0	137 ± 33
	54	500	119 ± 0.1	63 ± 12
		700	120 ± 0.1	95 ± 33
		900	121 ± 0.1	110 ± 22
40-70-120-150	49	700	137 ± 0.1	109 ± 27
		900	137 ± 0.1	132 ± 18
	52	700	136 ± 0.1	107 ± 18
		900	136 ± 0.1	109 ± 17

Thermal properties

The thermal properties were used to characterize the denaturation temperatures and enthalpies of HPC and dried oat okara. The HPC exhibited an endothermal peak around 85.2-85.9°C, which most likely corresponds to the hempseed storage protein edestin (Wang et al., 2008). The dried oat okara had a higher thermal denaturation temperature around 111.7-112.6°C, similar to wet oat okara (presented in synopsis and paper II), corresponding well to the 12S globulin, avenalin, in oat (Ma and Harwalkar, 1988; Cheng et al., 2023) (Figure 36). This indicated that the cooking zone with the highest temperature in the extruder should be set to at least 115°C if all protein in the materials is to be denatured in the extrusion process.

The primary peak in HPC had high enthalpies around 7.85-12.4 mJ/mg, and the dried oat okara had lower enthalpies around 2.68-4.13 mJ/mg (Figure 36). The enthalpies are a measure of how much of a sample's protein is still in its native state (Arntfield and Murray, 1981). This means that the HPC is contributing with more native protein compared to the dried oat okara. According to the present research, protein need to be native to be able to form the typical fiber structure of an HMMA by the cross-linking of unfolded protein (Zhang et al., 2024).

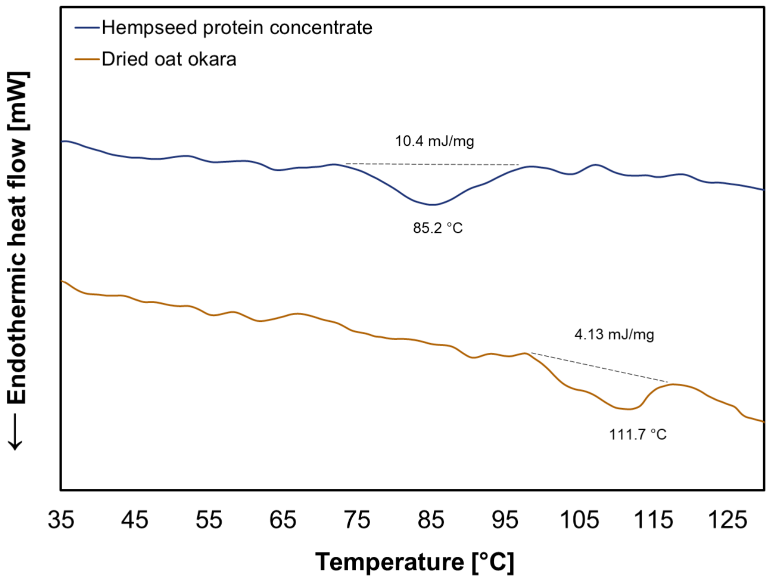


Figure 36. DSC thermograms for hempseed protein concentrate (HPC, blue) and dried oat okara (brown).

Pasting properties

The pasting properties of HPC and dried oat okara are presented in Figure 37. The viscosity of HPC increased dramatically when the temperature reached 100°C, indicating the commencement of cooking and a reduction in protein solubility due to protein denaturation. This agreed well with the thermal properties of HPC with a denaturation temperature between 85.2-85.9°C (Figure 36). When the protein denatured in the HPC, a phase separation also occurred, where the cooked protein built up a solid mass that adhered to the rotating paddle, resulting in almost non-existent viscosity at the end of the RVA run.

The HPC had a larger peak viscosity (6550 mPa s) than the dried oat okara (1000 mPa s) due to its high native protein content (Figure 37). The peak for the dried oat okara (around 120°C) was most likely corresponding to its protein avenalin, which was observed in the DSC thermograms (Figure 36). The peak is, however, much lower compared to the peak for wet oat okara (3560 mPa s, Figure 7, synopsis), which could be a result of partial protein denaturation in the drying process. Even though dried oat okara also had a high carbohydrate content (Table 16), no gelatinization peak was observed, indicating that most starch has already been gelatinized or enzymatically degraded in the oat beverage production (corresponding well to previous results on wet oat okara, Figure 7, Figure 29b). The viscosity increased gradually upon cooling for the dried oat okara, implying formation of new networks.

The heat that is required for a phase transition is measured by DSC, while the physical change in a material is measured by RVA. Energy is required before a physical change can take place, which explains why the pasting effects occurred at slightly higher temperatures than the denaturation temperatures for both HPC and dried oat okara. To achieve the same viscosity effects in the extrusion process, it is therefore suggested to set the temperature to at least 120°C, as was measured by the RVA, to achieve a viscosity change.

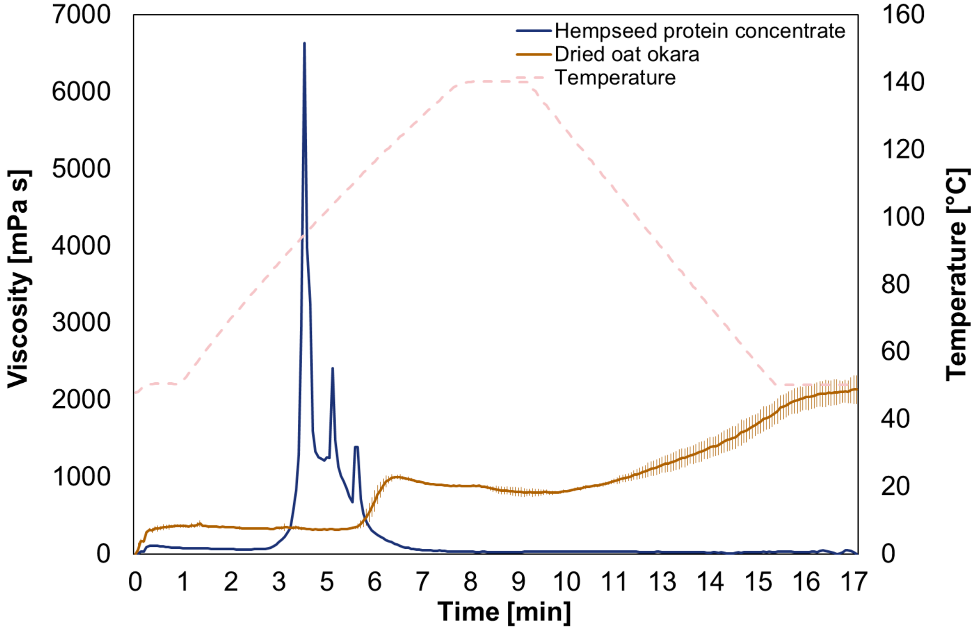


Figure 37. Pasting properties for hempseed protein concentrate (HPC, blue) and dried oat okara (brown).

Visual appearance

The HMMAs produced were texturized into thin-layered and fibrous structures similar to that of actual meat (Table 18). The cooling die was essential for avoiding expansion of the extrudate that would otherwise result from the evaporation of superheated water (Samard et al., 2019).

The HMMAs produced with the lower temperature profile (40-70-110-130°C) were folded in half, where white patches of oat okara were visible. The higher temperature profile (40-70-120-150°C) showed HMMAs with thinner and more distinct fibrous layers when folded in half, and the oat okara was not as prominent as in the lower temperature profile. The higher temperature profile appeared to cook and melt the dough more efficiently, reducing the brittleness that was observed at the lower temperature profile due to the oat okara.

Table 18. The visual appearance of all produced HMMAs, folded in half.

40-70-110-130°C			
	Actual feed moisture [%]		
	49%	52%	54%
500 rpm			
700 rpm			
900 rpm			
40-70-120-150°C			
	49%	52%	
700 rpm			
900 rpm			

Texture properties

An increase in feed moisture resulted in a reduction in the product's hardness, chewiness, and transversal cutting strength (Figure 38). This is in accordance with another study where a higher feed moisture leads to lower viscosity, elasticity, and temperature in the dough, which can lead to incomplete protein denaturation and texturization, resulting in softer extrudates (Lin et al., 2000). The same properties increased with the higher temperature profile (40-70-120-150°C), indicating a greater degree of protein denaturation and texturization. The change of screw speed did, however, not have any consistent influence on the textural attributes and cutting strength of the HMMAs produced. The springiness and resilience of the HMMAs did not differ significantly from each other when compared statistically (0.8-0.9 and 0.4-0.6, respectively, $p > 0.05$, paper IV, Table 4).

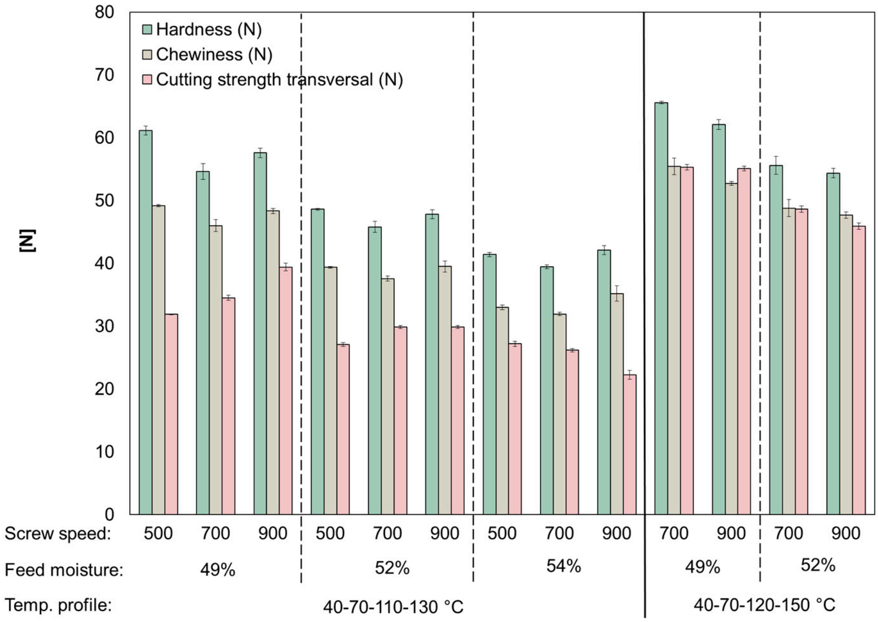


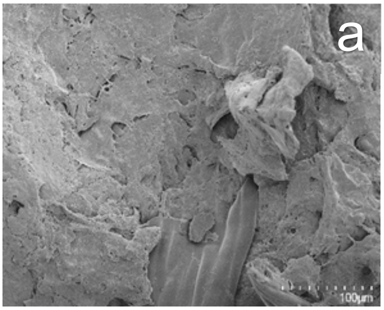
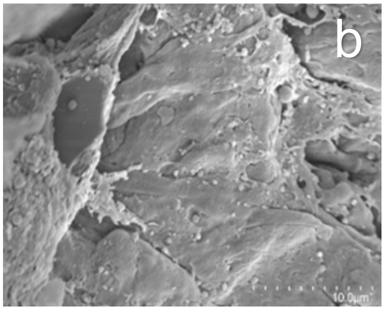
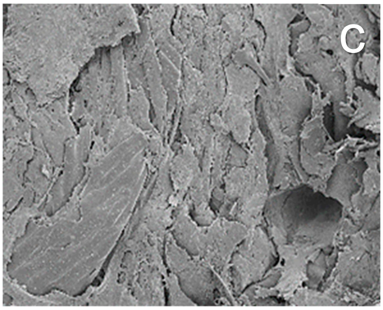
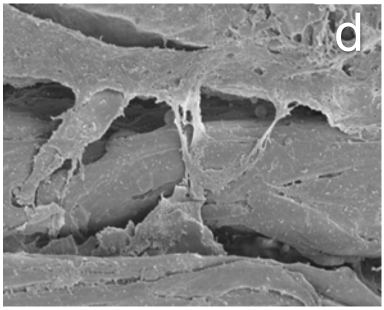
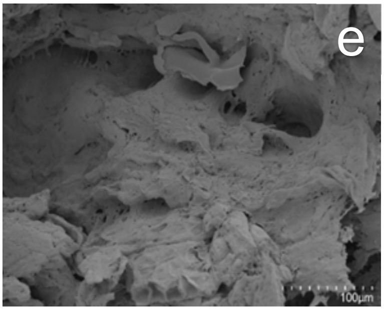
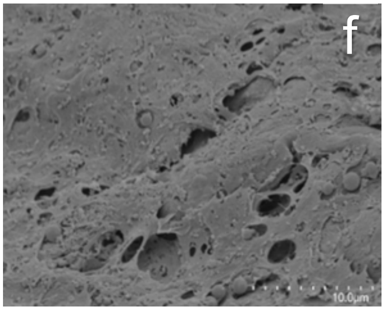
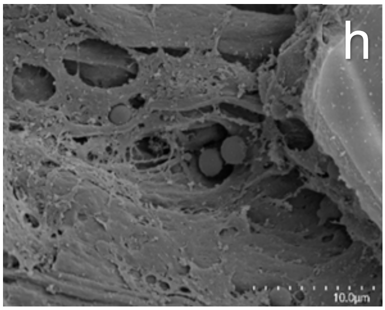
Figure 38. Texture properties of hardness (green), chewiness (yellow), and transversal cutting strength (pink).

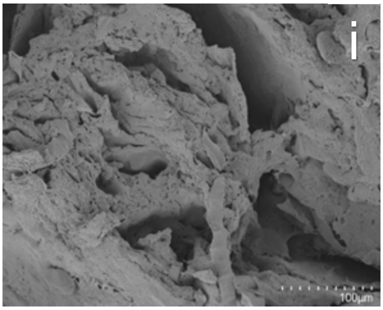
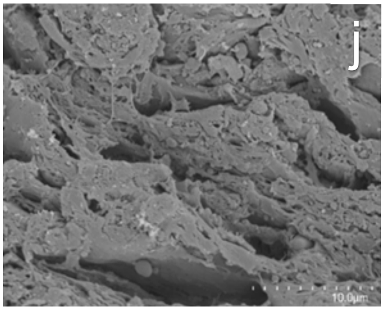
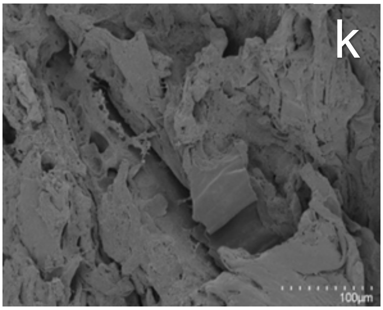
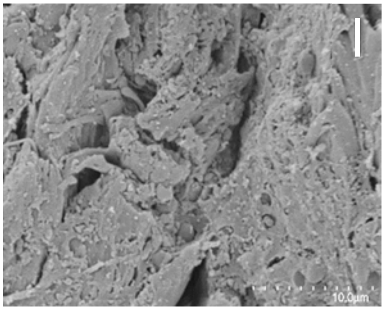
Microscopic imaging

Scanning electron microscopy (SEM) was used to attain a better understanding of the texture changes due to different extrusion parameters and to determine if the oat okara retained its integrity. All HMMAs produced had fibrous and thin-layered structures (Table 19). Lower feed moisture resulted in a compact structure, while a higher feed moisture increased the number of air cells formed, resulting in a more porous structure (Table 19f, h). When increasing the screw speed from 500 to 900 rpm, it resulted in layers that seemed to form larger fiber bundles (Table 19c, d, g, h).

Small white spots were seen in the samples with the lower temperature profile (Table 19b, d, f, h), which most likely is the oat okara. The higher temperature profile seems to have induced thinner layers, and the white spots were less prominent, which also was observed for the visual appearance of the HMMAs with the higher temperature profile (Table 18).

Table 19. SEM micrographs for all HMMAs produced with 300X and 4000X magnification (fm = feed moisture).

40-70-110-130°C		
	300X	**4000X**
49% fm **500 rpm**		
49% fm **900 rpm**		
54% fm **500 rpm**		
54% fm **900 rpm**		

40-70-120-150°C		
	300X	**4000X**
52% fm **700 rpm**		
52% fm **900 rpm**		

Conclusions

A 600 MPa treatment successfully extended the shelf-life up to 2 weeks for soy okara and almost 4 weeks for oat okara at 4°C. However, further studies are required to increase the knowledge regarding which the most resistant relevant pathogen is to design the optimal process criteria for both soy and oat okara. If pressure is included in a future pasteurization method, it should be higher than 400 MPa, as 200 MPa and 400 MPa did not have any effect (soy okara, paper I) or increased microbial growth (oat okara, paper II). A positive nutritional side-effect of HPP at 600 MPa was the increased soluble fiber fraction of the total dietary fiber content in both soy and oat okara to 4.4% and 9.2%, respectively.

Dried oat okara was successfully co-extruded with corn grits into an expanded snack. It was possible to co-extrude up to 30% dried oat okara with corn grits before the expanded structure collapsed. With the formulation of 40% oat okara, the starch content was below 60%, which led to a drop in SME, melt temperature, and pressure, resulting in hard and dense extrudates with low porosity. With the addition of oat okara in a snack product, it is possible to increase the nutritional profile with fiber and protein (paper III).

It was possible to co-extrude two underutilized side-streams from the food industry, dried oat okara and HPC, into an HMMA with thin-layered and fibrous structures with a temperature profile of 40-70-120-150°C. The novel wet-feeding method of HPC was successful, where the protein was still in a hydrated state and might have facilitated the texturization. The dried oat okara could contribute up to 64% of the total protein, but also with fiber, which otherwise is not present in meat. Investigating these unique raw materials into HMMA prototypes provides diversity in the food industry for HME (paper IV).

What started as a project to reduce food losses in the plant-based beverage industry also resulted in the development of novel plant-based foods. Both these aspects are important for the future of sustainable foods. As the food system contributes between 19-29% of global greenhouse gas emissions (Vermeulen et al., 2012), it is important that food losses are minimal, utilizing as much as possible of the harvested crops. Moreover, a reduction in animal product consumption results in reduced emissions and land use (Röös et al., 2017), which is why it is important that the food industry enhances the development of plant-based foods that are more available, nutritious and tasty.

Future perspectives

To ensure microbial safety in okara, it is necessary to identify the microorganisms more specifically in order to optimize a pasteurization method, not only assessing the total aerobic count, yeast and mold, and lactic acid bacteria. The spore-forming bacteria *Bacillus Cereus* have previously been detected in soy okara (Voss et al., 2018) and in a batch of oat beverages (FSN, 2022). *Bacillus Cereus* is a common contaminator of agricultural products due to its abundance and ability to form spores and could therefore represent a substantial part of the total aerobic count (Figure 25). *Bacillus Cereus* can cause foodborne illnesses and create heat-stable toxins if microbial growth is not controlled (Deswal et al., 2014), where a satisfactory limitation of growth is log 2-3 CFU/g (Centre for Food Safety, 2014; FSANZ, 2016). In brewers spent grain, a similar residue to okara from beer production, the spore-forming bacteria *Clostridium Botulinum* have also been present (van Deventer et al., 2020), which can produce neurotoxins with the ability to interfere with the transmission of nerve impulses. Botulism can cause symptoms including disturbances in vision, speech, and swallowing, and without treatment, the mortality rate ranges from 10 to 65% (Ting and Freiman, 2004). Spores of *Clostridium Botulinum* are the most heat-resistant pathogenic microorganisms and are, therefore, a common target organism in pasteurization (TetraPak, 2022), and should probably also be so for okara.

Bacterial spores are difficult to inactivate with HPP, and the treatment can trigger spore germination (Black et al., 2007). To combat spore-forming bacteria, one strategy could be to combine pressure and heat treatment, also known as pressure-assisted thermal sterilization (Balasubramaniam et al., 2016). When HPP triggers spore germination and the bacteria return to their vegetative state, it also makes them vulnerable to heat treatment (Black et al., 2007). In an earlier study, it was observed that the inactivation rate of *B. Stearothermophilus* spores in mashed broccoli increased when combining high pressure (600 MPa) with thermal treatment (> 80°C) (Ananta et al., 2001). The germination effect by HPP could also explain the significantly higher growth of total aerobic count for 200 MPa and 400 MPa treatment compared to the reference in oat okara (Figure 25b).

Extrusion might be able to act as a heat treatment to enable a safe okara product, where the residence time in the extruder could act as the pasteurization time. The process criteria for foods with *Clostridium Botulinum* as the most resistant relevant pathogen is 137°C during 4 sec (TetraPak, 2022). The snacks produced reached melt temperatures above 156°C, and the HMMAs produced with the higher temperature profile of 40-70-120-150°C (49% feed moisture) reached a melt temperature of 137°C (Table 17). The snack product must therefore have exceeded these process criteria, but the HMMA might just be on the limit. Increasing the temperature even more, to 160°C in the highest temperature zone for the HMMA, might be enough to reach the process criteria, but this needs to be further investigated. Due to the fact that it was successful to extrude both an expanded snack and an HMMA including dried oat okara, it should also be possible to extrude wet oat okara and use the extrusion cooking as a pasteurization method. The feeding of the wet oat okara will, however, need to be considered as it can be problematic to achieve an even feeding flow as it is sticky and chunky in comparison to dry powders. A possible solution could be to mix the wet okara with an additional dry ingredient to increase the dry matter, which might simplify the feeding. Wet oat okara could also be co-extruded in various combinations of plant proteins and starches to optimize the nutritional profile, taste, and texture into products such as HMMA, TVP, snacks, or breakfast cereals. TVP is an interesting product to investigate further, as it has the advantage of having a lower moisture content in comparison to HMMAs, which is in favor of the shelf-life.

When incorporating dried oat okara in the extrusion of snacks, it mainly had a disturbing effect on the expansion and porosity. Dried oat okara is a rather inert material, as the starch has already been gelatinized and has a high fiber content (25%, db, Table 11), which mainly absorbs water. It therefore had a dramatically negative effect on the expansion of the extruded snacks, which limited the addition of dried oat okara. There are, however, potential improvements that can be made, such as reducing the particle size of the raw materials, as this has been found to improve expansion.

The addition of fiber in an HMMA formulation can increase the melt viscosity as fiber has a good water-holding capacity, which can alter the flow properties of the HMMA melt as it enters the cooling die, affecting textural properties (Wagner and Ganjyal, 2024). There can therefore be a limitation of how much dried oat okara that can be added in a formulation, but as the dried oat okara also contributed with native protein (up to 64% of the total protein content), it might be possible to include even more than what was investigated in paper IV, which can be an interesting future study.

When working with plant-based foods, it is important to remember the antinutrients present in the raw materials. In the literature study, it was found that phytic acid/phytate is present in oat grain, hempseed press cake, and corn grits, and is most likely present in our final extrudate products. The phytate mineral complexes lead to poor bioavailability of minerals, such as iron (Zhou and Erdman Jr, 1995). The prevalence of iron deficiency among fertile women in Europe has been estimated to be 10-30% (Hercberg et al., 2001), and can be caused by insufficient iron intake from the diet or by poor absorption (Zimmermann et al., 2005). In Western countries, women are twice as likely as men to be vegan or vegetarian (Modlinska et al., 2020), which makes it important for the plant-based food industry not to neglect these antinutritional factors. Especially for food products, such as HMMAs, that are supposed to replace meat, which otherwise is a good source of iron. Although meat is a concentrated source of nutrients, it has major negative consequences for land and water use and environmental change and has also been linked to cardiovascular diseases (Godfray et al., 2018). It is therefore time for plant-based foods to reduce the consumption of meat, but not at the cost of human health. To increase the nutritional content in plant-based foods, it could be possible to include an extra processing step to reduce the phytate content, such as an enzymatic treatment with phytase (Greiner and Konietzny, 2006), fermentation or cooking (Popova and Mihaylova, 2019), and should be the focus in future studies.

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About the author

AMANDA HELSTAD has a Master of Science degree with a major in Engineering Biology from Linköping University. She included environmental engineering in her master studies through exchange studies in Hamburg, as environment and sustainability are subjects close to her heart. Her doctoral studies and research investigated the upcycling possibilities of the residues from soy and oat beverage production (okara). The aim was to extend the shelf life and to explore appropriate food applications to reduce food losses and thus improve the circular economy of plant-based beverages. The work was conducted in collaboration with The Green Dairy and IKEA, and in cooperation with HPP Nordic and Brabender/Anton Paar TorqueTec. It was found that high-pressure processing was able to extend the shelf life of okara, and that it was possible to incorporate oat okara into extruded expanded snacks and high-moisture meat analogs.

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