Functional properties of an oat fiber fraction

- and its effect in vegan burgers

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Functional properties of an oat fiber fraction- And its effect in vegan burgers

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Abstract

Cereal industries give rise to extensive quantities of fiber side streams, of which the main part goes to animal feed. However, valorisation of these side streams could lead to more efficient raw material utilisation, and at the same time, have potential to be used as ingredients, for new healthy plant-based foods. The purpose of this study was to characterize functional properties of an oat fiber fraction (OFF) side stream, and investigate its effect in vegan burgers, in terms of burger integrity, texture and sensory properties. Regarding the functional properties of the OFF, its dry matter before and after centrifugation was 23.6% and 18.8%, respectively. It had a water holding capacity of 3.9 g water per g solid OFF and an oil holding capacity of 4.2 g oil per g OFF. No swelling or emulsifying capacity of the OFF was observed, and its viscosity varied between 67 000-80 000 cP. OFF burgers were formulated, in which texturized vegetable protein was substituted with OFF in the concentrations 0%, 25%, 40% and 75%. Burgers with wheat and potato fiber were also formulated in the same concentrations, and commercially available burgers were included for comparison. Overall, an increase in OFF concentration resulted in increased burger cook loss, diameter, and thickness reduction. From texture profile analysis, an increase in OFF concentration in general led to a decrease in texture parameter values. However, OFF25 burgers had similar hardness to OFF0 burgers. Finally, OFF burgers were assessed for hardness and chewiness in a sensory evaluation, which included a triangle test together with an affective test. The number of right and wrong answers from the triangle tests, as well as preferred samples, varied between the two evaluation rounds. Significant differences, regarding correct answers from triangle test, between OFF25 compared with OFF75 were observed. Furthermore, these two formulations when assessed for hardness, were assigned scale numbers significantly different from each other and the other OFF samples. In conclusion, due to the complex nature of the OFF, there is a call for more research, including method and product development. This is of importance, in order to further understand its functional properties and effect in vegan burgers.

Keywords: oats, dietary fiber, plant-based meat analogue, vegan burger, functional properties, cooking measurements, texture profile analysis, sensory evaluation

Sammanfattning

Spannmålsindustrin ger upphov till stora mängder fibersidoströmmar, varav merparten går till djurfoder. Valorisering av dessa sidoströmmar kan dock leda till effektivare råvaruanvändning och samtidigt ha potential att användas som ingredienser för nya hälsosamma växtbaserade livsmedel. Syftet med denna studie var att karakterisera funktionella egenskaper hos en sidoström i form av en havrefiberfraktion (OFF) och undersöka dess effekt i veganska burgare avseende burgarintegritet, textur och sensoriska egenskaper. Gällande funktionella egenskaper hos OFF var dess torrsubstans före och efter centrifugering 23,6 % respektive 18,8 %. Dess vattenhållandeförmåga var 3,9 g vatten per g fast OFF och dess oljehållandeförmåga 4,2 g olja per g OFF. Den uppvisade ingen svällnings- eller emulgerande förmåga och dess viskositet varierade mellan 67 000–80 000 cP. OFF-burgare formulerades, där texturerat vegetabiliskt protein substituerades med OFF i koncentrationerna 0%, 25%, 40% och 75%. Även burgare med vete- och potatisfiber formulerades i samma koncentrationer och kommersiellt tillgängliga burgare inkluderades för jämförelse. Sammantaget resulterade en ökning av OFF-koncentration i burgare i ökade tillagningsförluster samt minskning i diameter och tjocklek. Från texturprofilanalys, innebar en ökning av OFF-koncentrationen i allmänhet till en minskning av texturparametervärden. Dock hade OFF25-burgare liknande hårdhet som OFF0-burgare. Slutligen analyserades OFF-burgare för hårdhet och tuggmotstånd i en sensorisk utvärdering, som innefattade ett triangeltest tillsammans med ett affektivt test. Antalet rätta och felaktiga svar från triangeltesterna, liksom föredragna prover varierade mellan de två utvärderings omgångarna. Signifikanta skillnader, avseende korrekta svar från triangeltestet mellan OFF25 och OFF75 observerades. Vidare tilldelades dessa två burgarformuleringar skalnummer som skiljde sig signifikant från varandra och de övriga OFF-burgarna vad gäller hårdhet. På grund av dess komplexa natur fordras sammanfattningsvis vidare forskning om OFF, inklusive metod- och produktutveckling. Detta är av betydelse för att ytterligare få kunskap om dess funktionella egenskaper och effekt i veganska burgare.

Nyckelord: havre, kostfiber, växtbaserad köttanalog, veganburgare, funktionella egenskaper, tillagningsegenskaper, texturprofilanalys, sensorisk utvärdering

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Abbreviations

AACC	American Association of Cereal Chemistry
AX	Arabinoxylan
cP	Centipoise
EC	Emulsifying capacity
DF	Dietary fiber
DM	Dry matter
MW	Molecular weight
NSP	Non-starch polysaccharides
OFF	Oat fiber fraction
OHC	Oil holding capacity
SC	Swelling capacity
TPA	Texture profile analysis
TVP	Texturized vegetable protein
WHC	Water holding capacity

1. Introduction

1.1 Background about plant-based meat analogues, oats and Lantmännen

Today's meat production has a vast environmental footprint in terms of land and water exploitation, as well as contributing to greenhouse gas emissions (Gerber et al. 2010 see Sun et al. 2021; Bruinsma 2009 see Sun et al. 2021; Evans & Yarwood 2007 see Sun et al. 2021). Moreover, a diet characterized by a high intake of meat which contains saturated fatty acids, cholesterol and salt, is associated with an increased risk of obesity, cardiovascular diseases and colorectal cancer (Springmann et al. 2018 see Boukid & Castellari 2021; Mohamed et al. 2017 see Boukid & Castellari 2021; Desmond 2006 see Boukid & Castellari 2021). To achieve more sustainable meat alternatives, both from an environmental and nutritional point of view, novel plant-based meat analogues are growing on the market. One such analogue product is plant-based burgers, which is estimated to grow globally with a compound annual growth rate of 22% between 2020 and 2030 (Boukid & Castellari 2021). Furthermore, developing meat analogues that are similar to conventional meat products in terms of nutrition and physical sensations such as texture, can improve consumers' meat substitution willingness in the transition to plant-based products (Kyriakopoulou et al. 2021).

Regarding the nutrition of these analogues, they are usually a good source of proteins, vitamins, and minerals but commonly lack dietary fiber (Sun et al. 2021). Generally speaking, consumers recognize dietary fiber as an important part of a healthy diet (Inglett et al. 2008). The recommended total intake of fibers per day among European countries ranges between 25–32 g for women and 30–35 g for men. Nevertheless, European national dietary survey data for intake of fiber, shows that the total fiber intake for men and women does not meet these recommendations (Stephen et al. 2017). Thus, to reach the recommendations and enhance the nutritional quality of meat analogue products, fortification with fibers would be advantageous. Cereal grains such as wheat, maize, rice, barley and oats are important sources of fibers (40-60%), proteins (25-50%) as well as essential vitamins and minerals (McKevith 2004 see Poutanen et al. 2022; Valsta et al. 2014 see Poutanen et al. 2022). However, since cereal grains for human consumption generally are incorporated into food products in a refined state, as a consequence of extensive milling, dietary fiber and minerals are lost. Also, extensive quantities of fiber side streams are generated, of which mainly goes to animal feed (Poutanen et al. 2022). However, these side streams including husks, hulls and brans (Alan 2017 see Valoppi et al. 2021) are rich in polysaccharides, proteins and antioxidants (Helkar et al. 2016 see Valoppi et al. 2021; Luithui et al. 2019 see Valoppi et al. 2021). Thus, valorisation of these side streams could lead to more efficient raw material utilisation, and at the same time, have potential to be used as ingredients, for new healthy plant-based food products (Sozer et al. 2017).

During the last ten years the release of new products with oats have seen a three times increase (Global New Products Database, Mintel 2020 see Lantmännen n.d.a). Also, the growing interest among consumers about functional foods, which are foods consumed as ordinary food, but which have documented health effects (Lantmännen n.d.b), has opened new possibilities in product development in which oats are incorporated (Strychar 2009). It is a familiar cereal among consumers and considered palatable with a well acceptable flavour (Lehtinen & Kaukovirta-Norja 2008). As a food ingredient, oats are used either as a whole grain for example in porridge and breakfast cereals or added as a specific ingredient such as fiber in bakery products (Salmenkallio-Marttila et al. 2011). Regarding oat fiber, there are studies in which it has been incorporated in meat products such as beef and chicken burgers to improve nutritional and cooking properties (Szpicer et al. 2020; Huber et al. 2016). However, to the authors knowledge, the use of oat fibers as a main ingredient in plant-based meat analogues and more specifically in vegan burgers, seems to be an unexplored area.

Furthermore, according to the market research Food Digest performed by Lantmännen, a Swedish agricultural cooperative, whole grain and vegetarian foods are factors consumers in the Nordic countries value highly (Janson 2021). Thus, the use of fibers from cereals such as oats, and incorporating it in vegetarian food products is a way to meet both consumer demands. Moreover, oats, in addition to rye, has a strong cultural tradition in the Nordic countries with superior cultivation conditions, which therefore makes it a contributor for sustainable consumption (Poutanen 2022).

Lantmännen Oats, which is part of Lantmännen group, produces oat ingredients for the nutrition, health and cosmetic industries (Lantmännen, n.d.c). Their oat milling process, which takes place at a production site in the town of Kimstad, includes several steps (Figure 1). Once the harvested oat grains arrive in the factory a dry milling process takes place, in which the grains are being dehulled to remove the oat husks. This gives the oat kernel, which is milled and sieved into oat bran or more finely milled into flour. The oat bran is further processed in a wet process, together with enzymes and water. Before decanting for dextrin, β-glucan and oat protein, an insoluble oat fiber fraction (OFF) is obtained, which currently is considered a side stream and used for biofuel production. However, its potential for food applications is being investigated. For example, the OFF has been incorporated in bread baking with successful results, giving more moisture, softness and cereal taste without off-flavours, as well as improving the bread shelf-life (Lantmännen 2021).

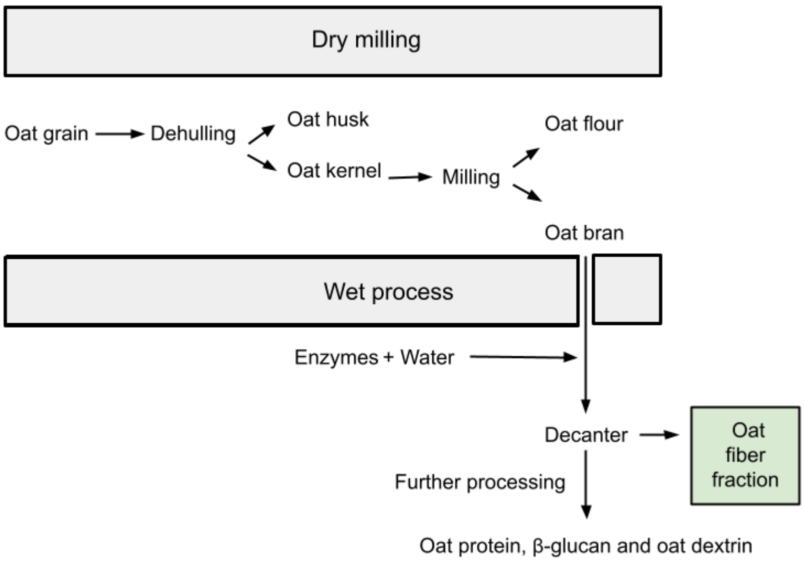

Figure 1.Processing steps for obtaining the OFF (Lantmännen 2022).

1.2 Objectives and scope

The main objectives of this study were twofold. Firstly, to characterize functional properties of the OFF and secondly to investigate its effect in vegan burgers regarding burger integrity, texture and sensory properties, by incorporating the OFF in different concentrations. The study can be seen as a first in depth investigation of the relatively unexplored OFF, in order to gain knowledge of its future applications in plant-based meat analogues.

2. Theoretical background

2.1 Cultivation, anatomy and nutrient composition of oats

Oat (*Avena Sativa*) is a cereal grain that belongs to the grass family *Gramineae.* It is primarily cultivated for use as animal feed, followed by food for human consumption and to a smaller extent for industrial, cosmetic, and pharmaceutical applications (Zwer 2004). Oats are grown in cold and moist climates, mainly in the northern hemisphere in countries such as Russia, Canada, United States, Finland and Poland. Among European countries, Finland and Sweden are the two main oat exporters (Strychar 2009). In Sweden, oats are the third largest grown cereal crop after wheat and barley, and in 2021 oats constituted 17% of the cereal land area (Swedish Board of Agriculture 2021). Approximately 0.7 million tons of oats are harvested yearly in Sweden, which represents around 13% of the total cereal harvest (Lantmännen, n.d.d).

The oat kernel or so-called groat of the oat grain (Figure 2), is morphologically more slender compared to the kernel of wheat and barley and is also covered with hairs, trichomes. The groat is protected by a hull, mainly consisting of the insoluble fibers hemicellulose and cellulose, as well as minor quantities of lignin and phenolic compounds (Miller & Fulcher 2011). The groat itself comprises the bran, the starchy endosperm and the germ. The bran layers include the aleurone, the sub-aleurone, the pericarp, seed coat and nucellus. The three latter layers consist to large extent of insoluble polysaccharides such as cellulose and hemicellulose, while the aleurone is rich in vitamins, minerals, phytate and antioxidants (Peterson et al. 1975 see Arendt & Zannini 2013; Marlett 1993 see Arendt & Zannini 2013; Kent and Evers 1994 see Arendt & Zannini 2013). The endosperm, apart from starch, also contains proteins and lipids while the endosperm cell walls, which are thin compared to cell walls surrounding the aleurone cells, are rich in β-glucan as well as some arabinoxylan (AX), cellulose, and glucomannan. The germ is a reservoir of mainly lipids and proteins, as well phytin (Miller & Fulcher 2011), which serve as the primary storage form of phosphorus.

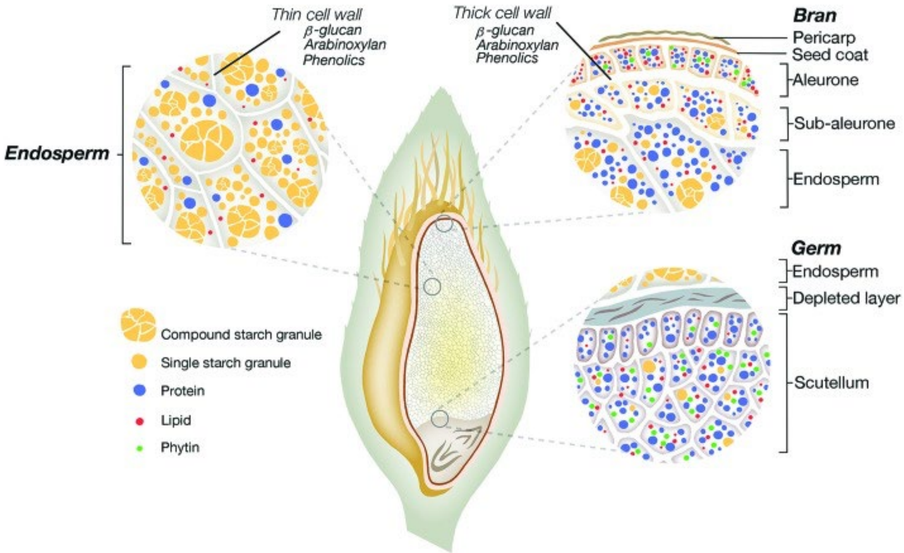

Figure 2.[Structural representation of the oat grain presenting different oat tissues (i.e., the bran, germ and endosperm) and the nutrient distribution/organisation within these tissues. Grundy et al. 2018 CC BY 3.0]

Oat starch, which constitutes approximately 40-60% of the oat groat (Paton 1977 see White 2009; Åman 1987 see White 2009; Hartunian- Sowa & White 1992 see White 2009; Doehlert et al. 2001 see White 2009), has some distinctive properties which differs from other cereal starches. For example, the oat starch is composed of small sized polyhedral granules that occur in both compounds (15-80 µm) or as single, simple granules (2-11 µm). The oat starch is also rich in lipids (Hartunian-Sowa & White 1992 see White 2011). These lipids can, however, impair the water-binding capacity, swelling and starch solubilization as well as forming amylose-lipid complexes which negatively impact the thickening ability of gelatinized oat starch (Swinkels 1985 see White 2011). Furthermore, oat starch has a low gelatinization temperature of 55°C (MacArthur & D'Appolonia 1979 see White 2009), slower rate of retrogradation and greater swelling power in comparison with other starch cereals (Doublier et al.1987 see White 2009; Gudmundsson & Eliasson 1989 see White 2009).

Among cereal grain-crops, oats are the one with the greatest protein content (Webster & Wood 2011). Proteins represent approximately 15-20 % of the oat groat weight, depending on growth environment and genotype (Peterson 1992 see Peterson 2011). Of the total protein content, globulins constitute around 80% and avenins, a type of prolamin in oats, represent circa 10-15% (Rasane et al. 2015). These storage proteins have a well-balanced amino acid composition, which provide both nutritional and functionality benefits of oats (Peterson 1992 see Peterson 2011). There is especially a high content of glutamic acid, which is characteristic for seed storage proteins. Though, in general oat proteins are nutritionally limited in the essential amino acids lysine, methionine and threonine

(Peterson 2011). These amino acids can however be found in legume proteins. Thus, to obtain all essential amino acids in a plant-based diet, cereal proteins such as oat protein, should preferably be complemented with legume protein (Poutanen et al. 2022). Moreover, since the avenins in oats lack certain immunogenic epitopes that otherwise are the cause of celiac disease in cereals such as wheat and rye, oats can be part of a gluten-free diet (Gilissen et al. 2016).

Regarding the oil content in oat groats, it varies between 2-13% depending on variety. Oat lipids contain many health promoting fatty acids and sterols. Approximately 80% of the fatty acids are unsaturated oleic, linoleic, linolenic and eicosenoic acids, while the most common sterol is β-sitosterol (Lehtinen & Kaukovirta-Norja 2011). The high lipid content can however be problematic during storage and processing since rancid off-flavours can be produced. Thus, a thermal treatment, the so-called killning together with steaming, takes place in order to inactivate enzymes such as lipases and lipoxygenases (Kahlon 1989 see Lehtinen & Kaukovirta-Norja 2011; Peterson 2001 see Lehtinen & Kaukovirta-Norja 2011).

2.2 Dietary fiber

Dietary fiber (DF) includes non-starch polysaccharides (NSP) that are found in plant materials such as cereals, fruits, vegetables and legumes. According to the definition by the American Association of Cereal Chemistry (AACC) from 2000:

"*Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation".*

DF can be divided into two categories, soluble and insoluble fiber, depending on their solubility in water. Soluble DF include for example pectin and gums, while the insoluble DF are for example cellulose and hemicellulose, lignin and resistant starch. Regarding DF composition and content in cereal grains, it varies depending on the specific anatomical compartment, processing and fractionation treatment (Maina et al. 2021) as well as genotype (Manthey et al. 1999).

2.2.1 DF in oats and their physiological effect

The DF in oats are approximately two-thirds insoluble and one-third soluble (Behall & Hallfrisch 2011). The health benefits of DF in oats are mainly attributed to the partially soluble DF β-glucan, which apart from oats also is abundant in barley. The (1→3) (1→4)-D-β-glucan is a linear polysaccharide composed of the glucopyranosyl units cellotriosyl and cellotetraosyl. The ratio of these two glucose units will affect the solubility, viscosity as well as gel formation of β-glucan. Oat β-glucan, which has a lower cellotriosyl to cellotetraosyl ratio, compared to the ratio in wheat and rye, is more soluble. Apart from solubility, β-glucan concentration and molecular weight (MW) are related to its physiological effect (Andersson 2021).

Among native cereal grains, oat β-glucan has the highest MW, of approximately $2x10^6$ g/mol (Wood 2011 see Maina et al. 2021). However, the weight is susceptible to processing and extraction methods (Beer et al 1996, 1997a, b see Salmenkallio-Marttila et al. 2011; Suortti et al. 2000 see Salmenkallio-Marttila et al. 2011; Ajithkumar et al. 2005 see Salmenkallio-Marttila et al. 2011). High molecular β-glucan weight is linked to increased viscosity, which in turn is related to health benefits (Kim & White 2013 see Du et al. 2019). These benefits include reducing postprandial glucose responses, slowing down gastric emptying and having a positive effect on satiety (Malkki & Virtanen 2001 see Welch 2011). Furthermore, high and medium MW β-glucan have been demonstrated to reduce serum LDL cholesterol in humans (Wolever et al. 2010). This cholesterol-lowering ability has allowed a health claim for oats and its soluble fibers (Behall & Hallfrisch 2011). However, in order to use the claim, food needs to deliver a minimum of 3 g of oat β-glucan per day (EFSA 2010).

Regarding the insoluble NSP cellulose and lignin, these, as previously mentioned, are mainly found in the oat hull. Cellulose, which is built up of β(1→4) linked D-glucopyranosyl units, is the principal structural component in plant cell walls (Maina et al. 2021). Lignin on the other hand, is composed of substituted phenylpropane units, and unlike the polysaccharides, it is hydrophobic (Theander et al. 1993). This makes lignin able to restrain water loss from vascular systems of plants (Holtzapple 2003 see Maina et al. 2021). Lignin can be both acid soluble and acid insoluble, the latter is also referred to as Klason lignin. Generally speaking, lignin is associated with fiber digestion restrictions (Sewalt et al. 1997 see Vivekanand et al. 2014). Unlike soluble DF, there is no authorized health claim for insoluble DF. However, a high consumption of the latter fibers, has been shown in observational studies to reduce the risk of heart disease and diabetes (Salmerón et al. 1997 see Stevenson & Inglett 2011; Wolk et al. 1999 see Stevenson & Inglett 2011).

2.3 Composition and microstructure of the OFF

As earlier described, the OFF from Lantmännen Oats' process in Kimstad, is a side stream from the wet process (Figure 1). It is a heterogeneous material and its texture is similar to a semi-solid paste in which irregular particles can be distinguished (Figure 3). It is composed of approximately 37- 41% DF, 25-30 % protein, 13-17% starch and 14-15% fat (Table 1). The DF in the OFF is to large extent insoluble, since more soluble DF such as β-glucan, has been separated out for further processing.

*Table 1. Macronutrient composition of three Kimstad OFF. Values are in weight percentage as their dry weight. [1]Via total N*5.7, [2] via acid hydrolysis and amino acid composition (Nofima 2020).*

Sample ID:	0901	2801	2802
Protein[1]	28.5	29.9	27.4
Protein[2]	26.9	28.8	25.9
Starch	16.7	13.1	15.8
Maltodextrin	0.5	1.2	0.4
Fat	14.5	14.3	14.5
Ash	5.8	5.9	5.9
AX	10.6	11.6	11.4
β-glucan	10.5	11.1	10.1
Cellulose	1.4	2.1	1.9
Rest NSP	2.1	2.1	2.2
Acid insoluble lignin	12	14.3	12.5
Total	101	104.5	100.6

The microstructure of the OFF has been characterized by light microscopy (Figure 4).

Figure 3.Camera photography of the OFF.

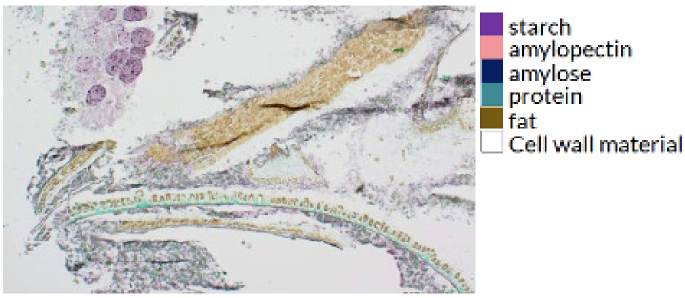

Figure 4.Microstructure of the OFF (RISE 2021).

The amino acid composition of the proteins in the OFF, is similar to the composition of native oat groat proteins (Figure 5).

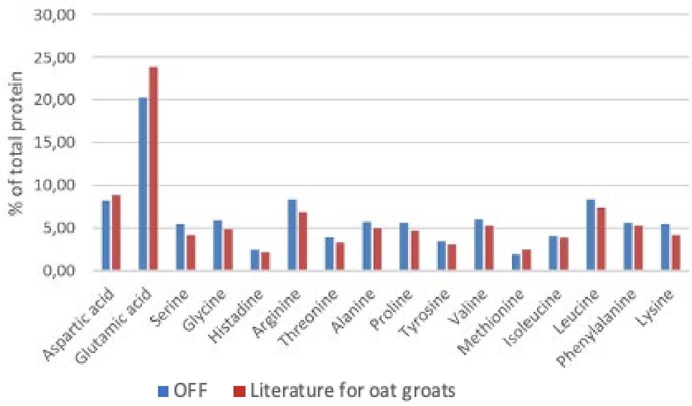

Figure 5.Amino acid composition as weight percentage of the proteins in OFF, compared with the composition in native oat groat proteins, compiled from literature (from Lantmännen 2021).

2.4 Functional properties of cereal components in food

Functional properties of foods are a result of their physicochemical characteristics, which reflect the interplay between food component structures, in the environment and condition in which they are being measured (Suresh & Samsher 2013 see Awuchi et al.2019; Kaur & Singh 2006 see Awuchi et al. 2019; Siddiq et al. 2009 see Awuchi et al. 2019). These components include for example starch, fiber and proteins. Moreover, functional properties depict the behavior of ingredients during cooking and how they influence the final food product, in terms of visual appearance, texture and taste. Examples of functional properties are for example swelling capacity (SC), emulsifying capacity (EC), gelatinization and viscosity (Awuchi et al. 2019).

Cereal grain components, apart from enhancing the nutritional profile of food products, can be used as functional ingredients in food systems. For instance, oat polysaccharides, such as starch and fiber, have the ability to retain water and fat in food matrices. This can be advantageous in order to reduce cook loss and for maintaining texture in food products. For example, the oat fiber β-glucan is used as a hydrocolloid and thickening agent to modify food texture (Wood 1986 see Salmenkallio-Marttila et al. 2011). It has been used as a fat substitute in beef-patties which improved fat and moisture retention (Piñero et al. 2008). In addition, when oat fiber together with carrageenan was added to frankfurters, the water holding capacity and emulsion stability was enhanced. Also, the total fat content was reduced from 30% to 5% (Hughes et al. 1997).

Regarding oil holding capacity (OHC) it refers to the amount of oil that fibers maintain after centrifugation with oil. The capacity is associated with surface properties, charge density and hydrophobicity of the fiber particles (Fleury & Lahaye 1991 see Elleuch et al. 2010). A large particle surface area is related to enhanced oil adsorbing ability (Mora et al. 2013). Moreover, hydrophobic fiber particles such as lignin show enhanced OHC (Kinsella 1976 see Dhingra et al. 2012). Another characteristic property of DF is their water holding capacity (WHC), which is the ability of biopolymers to retain water or water-based fluids, within the food structure (Cornet et al. 2021a see McClements & Grossmann 2021; Grasso et al. 2020 see McClements & Grossmann 2021). This ability is due to formation of a porous 3D-network that can hold water through capillary and hydration forces (Blackwood et al. 2000 see McClements & Grossmann 2021; Stevenson et al. 2013 see McClements & Grossmann 2021). When water is absorbed through the cell walls, the size of fiber particles will increase (Autio et al. 2001 see Kurek et al. 2015). The resulting fiber swelling is, among other things, determined by fiber matrix structure, which includes porosity and crystallinity (Auffret et al. 1994 see Dhingra et al. 2012). The WHC of fibers also has physiological effects, since it can increase fecal bulk, which in turn decreases intestinal transit time and counteracts constipation (Huber & BeMiller 2017 see Damodaran & Parkin 2017).

Furthermore, oat starch can contribute to gel and thickening properties of food products (Lillford 1997 see Webster & Wood 2011). The process of gelatinization occurs as a result of starch granule swelling, during heating in excess water. The process is influenced by the presence of proteins, lipids and organic acids (White 2011). One way to study gelatinization is by performing viscosity measurements. Viscosity is the internal friction of a fluid or its tendency to resist flow (Bourne 2002). The flow behavior is often an important parameter to describe product consistency and quality (Brookfield Engineering n.d.). With a rotary viscometer, a rotating sensing element, called spindle, measures the torque necessary to overcome the viscous resistance to the induced movement (Brookfield Engineering Labs n.d.a). The viscosity of polysaccharides such as β-glucan, are determined by structure and MW (Wood 2011). The viscosity, moreover, is related to polysaccharide molar mass, concentration and the capability to form aggregates (Gomez et al. 1997 see Du et al. 2019).

Functional properties of proteins are for example their WHC, ability to stabilize emulsions and provide texture (Poutanen et al. 2022). Several of these functional properties are enhanced and affected by protein solubility, which to large extent rely on water-protein interactions (Damodaran 2017). Similar to other plant proteins, oat protein solubility is poor at neutral and acidic pH (Mäkinen et al. 2017). The abundant globulin proteins in oats more specifically, are poorly soluble in water but rather salt soluble (Loponen et al. 2007). The capacity of proteins to entrap water gives appropriate texture in foods and is also related with juiciness and tenderness in fragmented meat products. Moreover, due to the amphiphilic nature of proteins they are able to act as surfactants in interfaces by reducing the surface tension. This protein surface activity often provides mechanical strength to emulsions and foams (Damodaran 2017). Furthermore, globulin proteins in oats have a denaturation temperature around 110°C thanks to hydrophobic interactions, which makes the proteins steady to thermal treatments in food systems. Oat globulin gelatinization takes place at 90-100°C (Ma and Harwalkar 1987 see Peterson 2011).

2.5 Texture measurements

Food texture, apart from flavor and appearance, is an important sensory characteristic when it comes to the food choice of consumers. However, texture is a complex concept. Apart from physicochemical properties, which is a result of the chemical and molecular composition of a food, it also includes the sensation from chewing food. Texture can be measured through two types of methods: objective instrument measurements and subjective sensory analysis (Liu et al. 2019).

2.5.1 Texture profile analysis

Plant-based meat analogues aim to resemble traditional meat in terms of bite, chewiness, juiciness and firmness (Kyriakopoulou et al. 2021). However, achieving a texture and bite for these analogue products is a major quandary (Sun et al. 2021). Texture profile analysis (TPA) is one instrumental method to assess meat texture (Novaković & Tomašević 2017). By performing TPA, an understanding of texture and how it should be formulated in food products to meet consumer acceptance, can be obtained (Bourne 2002).

During TPA, a texture analyzer instrument containing a probe, compresses a bite-size sample of food. Usually, the compression is performed in two cycles, mimicking the movement of the human jaw when for example chewing or biting (Liu et al. 2019). The instrument produces a force-time graph (Figure 6) where from several mechanical parameters can be estimated, depending on the probe used (Friedman et al. 1963 see Bourne 2002; Szczesniak et al. 1963 see Bourne 2002). Two common probes for assessing meat products are cylinder and shear probes. From a cylinder probe some common textural parameters derived are hardness, cohesiveness, springiness, gumminess and chewiness. Hardness is defined as the height of the force peak from the first compression cycle. Cohesiveness is the ratio of the positive force areas under the first and second compression cycles (A2/A1). Springiness is the distance that the food recovered its height during the time that elapsed between the end of the first bite and the beginning of the second bite (Bourne 2002). From these three parameters, gumminess and chewiness can be calculated. Gumminess is defined as the product when multiplying hardness with cohesiveness, while chewiness is the product when multiplying gumminess with springiness.

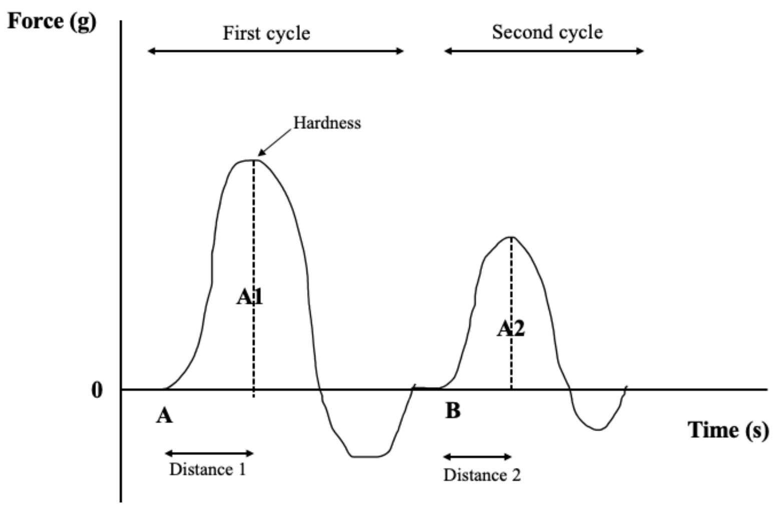

Figure 6. Force-Time graph derived from a TPA.

A shear probe such as a knife/guillotine blade is used in shearing tests, mainly for testing meat quality. In these tests a food sample is cut or so-called sheared (Liu et al. 2019). Two common parameters obtained from a shearing test are maximum shear force and work of shear which is the work to split a product into two fragments. The shearing action of these two parameters mimic the cutting of the incisors when food is being bitten in the mouth. This gives a good indication of meat hardness (Novaković & Tomašević 2017). Even though texture instrument measurements are quick, economical and provide reproducible as well as quantitative data, it is essential to legitimize the validity of the instrumental properties with sensory properties (Liu et al. 2019).

2.5.2 Sensory analysis

Sensory evaluation includes a collection of techniques to measure human response to food. In contrast to texture measurements with a texture instrument, human perception of texture includes the senses of sight (visual texture), touch (tactile texture) and sound (auditory texture). Oral-tactile texture involves the action of the lips, teeth, oral mucosa, saliva, tongue and the throat. Sensory methods can thus be argued to be more precise than non-sensory techniques (Bourne 2002). However, sensory evaluations require much time and effort. Furthermore, the data obtained from evaluations when using humans as measuring instruments, are usually highly variable since the variation between humans and for the same human can vary from day to day. Also, factors such as motivation and mood of the panelists are only partially controllable (Lawless & Heymann 2010).

Several sensory evaluation tests exist, with different objectives. One common group of tests are the so-called discrimination tests, which are used in order to understand if a difference between two products can be sensed (Lawless & Heymann 1999). Included in the discrimination tests are for example the triangle test. This test includes two samples from the same batch and a third from another batch. The panelist is served three samples and has the task to differentiate the odd sample within the trio. Ideally 25-40 panelists should participate in a discrimination test. Another type of sensory test is the affective, also called hedonic test, which seeks to find out about liking or disliking of a product. In this test, panelists make a choice based on liking among different products, which therefore provides information if there is a pronounced product preference among the participants. However, affective tests are not that informative regarding the degree of liking or disliking. A panel of 75-150 persons, which are common users of the product, is recommended to take part in the test (Lawless & Heymann 2010).

3. Materials and methods

3.1 Materials

OFF (see composition Table 1), a wheat fiber flour containing 35% DF (of which the main part was insoluble), a texturized vegetable protein (TVP) made of yellow pea with a protein content of 70% (Roquette Nutralys), and a functional blend of hydrocolloids with unknown composition (Solina AB) were provided by Lantmännen. Other burger ingredients were bought in a local supermarket and included rape seed oil (Eldorado Rapsolja), potato fiber (Semper Pofiber n.d.) containing 65 % fiber (of which two thirds were insoluble), and ice cubes (ICA). Also, three commercially available burgers from the supermarket, two plant-based burgers (P1 and P2) and one meat burger (M) were bought. The major ingredients in P1 were fungal and textured wheat protein, while P2 contained mainly soy and wheat protein. The M burger had a beef content of 81% and contained small amounts of potato fiber. Distilled water from Lantmännen Oats in Kimstad and tap water from Lantmännen's Head Office in Stockholm was used for measurements and formulation of burgers. Ingredients were used at room temperature.

3.2 Methods

The method was divided into five parts: characterisation of functional properties of the OFF, manufacturing of burgers with OFF and evaluating the burgers regarding cooking properties, texture and sensory characteristics.

3.2.1 Characterisation of functional properties of the OFF

Several characterisation methods were applied, which were performed in duplicates with two separate OFF batches.

<u>DM</u> of the OFF was determined according to an in-house method from Lantmännen Oats, by taring dishes containing sand and glass rods with a Sartorius LA230S analytic scale. Then 2 g of the OFF sample in duplicates was added to dishes and the weight of the dishes was noticed. Glass rods were used to homogenize the samples and ethanol was added to dissolve the samples further. The dishes were

afterwards placed in a 60°C hotbox oven with a fan overnight. Subsequently, samples were cooled to room temperature in a desiccator for 60 minutes. Then, the weight of the dishes containing the dried OFF samples were noticed and the average DM of two samples was expressed as a percentage by using (equation 1):

(1) $DM (\%) = ((m_3-m_1)/(m_2-m_1)) \times 100$

m_3 = weight after drying

$m_2 = m_1$+sample

m_1 =weight of dried dish with sand and glass rod

<u>SC</u> was analysed according to a method by Robertson et al. 2000 see Huber et. al 2016, with some modifications. 100 g OFF was added to 100 ml of distilled water in a graduated cylinder with an inner diameter of 3.5 cm. The cylinder was shaken by hand for the water and OFF to mix, and the occupied volume by the OFF was noticed. The mixture was left standing for 18 h at room temperature. Afterwards the volume occupied by the OFF was noticed once more.

<u>WHC</u> was determined by using the AACC (1999) method 56-30, but with larger quantities of material. 50 g OFF was added to 300 ml of distilled water in centrifugation tubes and the tubes were weighted. The fiber and water were mixed with a vortex mixer followed by centrifugation at 2000xg for 10 minutes. The supernatant was discarded, and the tubes were reweighted. WHC was expressed as grams of water per gram of solid material (Huber et al. 2016). As a separate WHC experiment, the pure OFF was centrifuged**,** without addition of water, at 2000xg for 20 minutes to investigate whether water was being expelled.

<u>OHC</u> was estimated according to the method by Wong & Cheung see Huber et al. 2016, with minor modifications. 20 g OFF was blended with 200 ml of rape seed oil in tubes which where weighted. Then the tubes were mixed with a vortex mixer. Afterwards the tubes were centrifuged at 2000xg for 30 minutes followed by removal of the supernatant. The tubes were reweighted and the OHC was calculated as grams of oil retained per gram of fiber.

<u>Viscosity</u> was measured according to an in-house method by Lantmännen Oats, at room temperature with a Brookfield Viscometer model DV 2 (Brookfield Engineering Labs. Inc Stoughton, Ma 02072 USA). A 07-disc spindle was set rotating with a constant speed of 20 revolutions per minute, inside a beaker containing 400 ml of OFF, and the centipoise (cP) was registered.

<u>EC</u> was evaluated by the method by Wong & Cheung 2005 see Huber et al. 2016, with some changes. 10 g OFF was added in centrifuge tubes with 125 ml of distilled water. The tubes were subjected to mixing with a vortex mixer for 1 minute. Then 125 ml rape seed oil was added to the mixture and the tubes were remixed. The

height of the oil and water layers as well as the total volume of the tubes was noticed. Afterwards, the tubes were centrifuged at 500xg for 10 minutes and the height of the layers were noticed once more. Also, after the supernatant was decanted, the pH of the remaining OFF was measured. EC was calculated as a ratio between the height of the two layers and the height of the tube volume.

3.2.2 Manufacturing of burgers

The specific proportion of ingredients for different burger formulations can be seen in Table 2. First, a reference burger (OFF0), without OFF, was formulated according to an in-house recipe and was composed of an emulsion mixture and TVP. The mixture was prepared by adding ice and water at a ratio of 1:1, in a thermomixer (TM6-Vorwerk) and mixing at high speed, with a temperature around 0°C for 30 seconds, until a slurry mixture was obtained. Then a functional blend of hydrocolloids was added under mixing for another 30 seconds. At the end, rape seed oil was slowly added while mixing at high speed until a gel like and white coloured emulsion was formed. The mixture was put in the refrigerator for 20 minutes before further use. Regarding the TVP, it was hydrated in water at a ratio of 1:3 (TVP: water) and was left at room temperature for about 30 minutes.

Table 2. Ingredients per 100 g for the different burger formulations.

	Ingredients					
Formulations	Emulsion mixture [a]	TVP	OFF	Potato fiber	Wheat fiber	Water
OFF0	37.0	63.0	-	-	-	-
OFF25	37.0	47.3	15.8	-	-	-
OFF40	37.0	37.8	25.2	-	-	-
OFF75	37.0	15.8	47.3	-	-	-
P25	37.0	47.3	-	3.8	-	12.0
P40	37.0	37.8	-	6.0	-	19.3
P75	37.0	15.8	-	11.2	-	36.1
W25	37.0	47.3	-	-	3.8	12.0
W40	37.0	37.8	-	-	6.0	19.3
W75	37.0	15.8	-	-	11.2	36.1

[a] Composed of ice, water, blend of hydrocolloids and rapeseed oil.
OFF25, OFF40, OFF75: total TVP of 63 g substituted with 25%, 40%,75% of OFF respectively.
P25, P40, P75: total TVP of 63 g substituted with 25%, 40% and 75% potato fiber and water respectively.
W25, W40, W75: total TVP of 63 g substituted with 25%, 40% and 75% wheat fiber and water respectively.

For formulation of other burgers than OFF0, the emulsion mixture concentration was kept on a fixed level of 37 g, while the TVP was substituted with increasing concentrations of OFF. The reason why the emulsion level was chosen to be kept constant was because it contains a functional blend of hydrocolloids that binds texturized proteins and enhances oil encapsulation. Furthermore, the emulsion mix also contains vegetable oil which provides juiciness, tenderness and flavour release (Savell & Cross 1988 see Kyriakopoulou 2021; Reig et al. 2008 see Kyriakopoulou 2021). By keeping the emulsion on a fixed level, the goal was to ensure a stable burger formulation, even when the level of TVP was substituted.

In order to decide which concentrations of OFF to be used for further testing, pilot burgers were formulated, in which the total amount of TVP (63 g) was substituted with OFF at five concentrations (15%, 25%, 40%, 75% and 100%). The three middle concentrations (25%, 40% and 75%) were then chosen to proceed with since the OFF25% and OFF40% burgers showed promising visual and tasting properties, while the OFF75% burger was included to have a burger with high OFF concentration. To compare the effect of OFF in burgers with other fibers, burgers with potato and wheat fiber were also formulated in the same three concentrations (25%, 40% and 75%). During potato and wheat fiber burger formulations, the fibers were added water, so that the DM corresponded to the same percentage as in the OFF. Each individual burger batter of 100 g was hand rolled to balls and pressed into burgers with a hamburger press (diameter of 10 cm).

3.2.3 Cooking measurements of burgers

To understand how cooking impacts burger integrity in terms of weight, diameter and thickness, burgers were examined before and after cooking. Before cooking, the raw burger weight (RBW) in grams, raw burger diameter (RBD) and raw burger thickness (RBT) in centimeters was measured. Then burgers were fried in 1 tablespoon of rape seed oil, 3 minutes per side. Afterwards the cooked burger weight (CBW), cooked burger diameter (CBD) and cooked burger thickness (CBT) was measured. Burger cook loss (equation 2) and diameter reduction (equation 3) were calculated according to Besbes et al. (2008):

(2) Cook loss (%) = ((RBW-CBW) x(100))/(RBW)
(3) Diameter reduction (%) =((RBD-CBD) x(100))/(RBD)

Burger thickness reduction (equation 4) was calculated according to El-Magoli 1996 see Dosh et al. 2016:

(4) Thickness reduction (%) = ((RBT-CBT)/(RBT)) x (100)

3.2.4 Texture profile analysis on burgers

TPA was conducted on fried self-formulated burgers and the three commercially available burgers P1, P2 and M using a TA.XT.plus C Texturometer (Stable Micro System). Six samples per burger were cut out with a cylindrical mold of 20 mm in diameter. The probes used were a cylinder probe with a diameter of 25 mm and a knife/guillotine shear blade from a standard blade set (HDP/BS, Stable Micro System). For the cylinder probe, a test-speed of 1 mm/s was used with a strain of 50% (Bis-Souza et al. 2018). For the shear blade probe a pre-set program with a cutting test specific for burgers was used, with a test speed of 5 mm/s (Stable Micro System). Before analysis the height was calibrated for each probe.

3.2.5 Sensory evaluation of burgers

To be able to compare the results from the TPA, regarding hardness and chewiness of OFF burgers, a sensory evaluation was performed, which included a triangle test combined with an affective test. The evaluation took place in a test kitchen at Lantmännen's head office in Stockholm. OFF burgers were formulated and fried on the same day of evaluation. OFF25 burger samples were included in each triangle test and compared against either OFF0, OFF40 or OFF75 samples. The motive for why OFF25 was chosen to be involved in each triangle test was because this formulation was considered promising in terms of texture.

From each burger eight sample pieces were cut out and held at room temperature before serving. Samples were blind labelled with random three-digit codes and served in a balanced sequence order (Appendix 2). The evaluation was performed by an untrained panel of ten individuals, of which eight were employees at Lantmännen within R&D, Marketing & Sales and in the Ingredient Application field. The two other panellists were university students studying nutrition. The panellists were familiar with vegan burgers to some degree since some of them were consuming these burgers occasionally. The panel was divided into two groups, with five persons in each group, which judged either hardness or chewiness. The panellists were placed in different parts in the kitchen to avoid influence from each other. Prior to the evaluation instructions were given orally and a written instruction paper (Appendix 3), in which each participant had to fill in their judgment was provided. Apart from burger samples, panellists were also given a glass of water to rinse in between evaluations and a glass to eliminate leftover sample pieces.

The evaluation was divided into two rounds, with a ten minutes pause in between. The reason for having two rounds was to get a repeated assessment from one and the same panellist. Moreover, in the second round the order of sample serving and the number of samples within each triangle trio could be altered, compared to the first round. Each panellist performed six triangle tests, with three tests per round, except for two panellists which only performed three tests since they had to leave after the first round. Forced choice was used, which means that panellists had to choose one odd sample even though no difference could be distinguished. When choosing the odd sample in the triangle tests, panellists were asked to comment on what made the odd sample different and assign each sample with a number on an attribute scale from 1 (low) to 9 (high). To facilitate the judgment on the scales, food references were given for each attribute. By letting the panellist number each sample, an understanding of the attribute intensities could be obtained and to what extent the attribute differences were perceived. Panellists also had to mark which sample they preferred for each triangle test, in order to get an idea of the overall burger liking.

3.3 Statistical analysis

A one-way analysis of variance (ANOVA) was conducted with IBM SPSS statistics (version 28.0.1.1) to study the effect of OFF, as well as potato and wheat fiber, on the cooking and texture properties of vegan burgers. Significant differences were considered at a p-value of <0.05. When a significant difference was noticed, a Post Hoc test (Tukey's test) was performed to determine which mean values were significantly different from each other. Due to a small n, independent groups and a lack of normal distribution, non-parametric methods were applied which included a Kruskal-Wallis and Mann-Whitney U test.

To analyse the results from the sensory evaluation, the number of right and wrong answers from the triangle tests, was calculated. For evaluation round one, the number of correct answers for each attribute, was compared to a table for critical values for number of correct answers, to establish significance (Roessler et al. 1978). For round two however, the number of triangle tests performed were below the minimum requirement to do the comparison. Furthermore, a two-way ANOVA with Minitab (version 19.2) was performed to study whether sample, panellist and evaluation round influenced the outcome, regarding the assigned scale number for samples evaluated for hardness and chewiness.

4. Results and discussion

4.1 Functional properties

The results from the measured functional properties of the OFF are summarized in Table 3. Regarding DM it was calculated to be approximately 24%. Since the OFF is the first side stream from the wet process (Figure 1), the OFF is wet by its nature, containing approximately 76% water. The presence of this water could be advantageous, since it can contribute to achieving mouthfeel and texture in food products (McClements & Grossmann 2021). Thus, incorporation of the OFF can be favourable for the sensory textural properties of the vegan burgers.

After centrifugation with water and removal of the supernatant, as part of determining the WHC, the DM of the OFF was approximately 19%. The water content in the OFF had thus increased with 5.0 percentage points. The supernatant removed after centrifugation, apart from the added water, might also contain water soluble material that was dissolved from the OFF during centrifugation. Methods which aim at determining water hydration properties by using centrifugal forces, and which lead to lost sample material after removal of the supernatant, can be seen as a method limitation (Frazzoli 2007).

Table 3.Functional properties of the OFF.

DM (%)	23.6
DM (%) after centrifugation and removal of supernatant	18.8
WHC (g water/g solid OFF)	3.9
SC (ml/g OFF)	n.d.
OHC (g oil/g OFF)	4.2
EC (%)	n.d.
Viscosity (cP)	67 000 - 80 000

Values are presented as averages from duplicate determinations.
n.d.=not detected.

When calculating the WHC, the already existing water content in the OFF had to be compensated for in the calculation (Appendix 1). The WHC was calculated to be 3.9 g water per g solid OFF. A similar WHC of 4.6 g water per g fiber, was observed for an oat fiber, containing 84% insoluble fiber, in a study by Huber et al. (2016). Because of the high water content in the OFF it can be argued to already exhibit a high WHC. Moreover, when the pure OFF was centrifuged, no water was expelled, which also demonstrated its good WHC.

No swelling of the OFF could be distinguished, since the volume occupied by the OFF sample before and after 18 hours in water, remained unchanged. The components in the OFF are most likely already saturated with water since they have swelled and absorbed water during the wet process (Figure 1). This might explain why the OFF does not swell further. To improve the SC of the OFF, chemical treatments in the future could be applied such as addition of acidic or basic solutions. The reason behind the increased SC of insoluble DF when adding these solutions, is the disruption of the cell wall order (Ozyurt & Ötles 2016).

To further investigate the hydration properties, WHC and SC of the OFF, one could reduce its particle size for example by homogenisation or grinding. Both positive and negative hydration effects have been demonstrating after grinding oat bran (Cadden 1987 see Frazzoli 2007; Mälkki 2001 see Frazzoli 2007). The positive effect of grinding is probable due to increased surface area for water uptake, while the negative effect is explained by the breakdown of fiber matrix, which among other things, decreases the space available for free water (Fleury & Lahaye 1991 see Frazzoli 2007). In a study by Valoppi et al. (2021), in which soluble and insoluble oat fractions were investigated, the WHC of the insoluble oat fraction was increased by thermal heat treatment and high-pressure homogenisation. However, high-speed homogenisation decreased the WHC of the insoluble oat fraction.

Regarding OHC, it was calculated to be 4.2 g oil per g OFF, when adjusted for DM (Appendix 1). The OHC of the oat fiber in the study by Huber et al. (2016), was 3.7 g oil per g fiber, which is comparable to the OHC in this study. Since the OFF is composed of approximately 14-15% fat, one possible scenario is that some of the fat might have dissolved into the oil of the supernatant during centrifugation. When afterwards discarding the supernatant, it might therefore have contained both the added oil and fat from the OFF. The calculated value of the OHC might thus deviate from the actual OHC of the OFF. Similar to increased WHC after high- pressure homogenisation, the OHC can be enhanced by homogenisation of fiber particles. This has for example been observed for high-pressure homogenised wheat bran (An et al. 2014 see Ozyurt & Ötles 2016).

Considering EC, no emulsion could be distinguished, since there was a distinct oil layer floating on top of a water layer, and the OFF was at the very bottom of the centrifugation tubes. The height of the water layer was also equal to the height of the oil layer before and after centrifugation. Thus, since the ratio did not change between these two layers and no emulsifying layer could be distinguished, the OFF did not show any EC. The formation of emulsions and maintaining their stability is to large extent attributed to proteins. Since the pH of the OFF on average was 7.0, after the supernatant was removed, the globulin protein solubility and emulsifying properties were likely poor, which might explain the lack of EC (Mäkinen et al. 2017). To improve the EC of the OFF, further investigation on how to adjust the pH in order to increase the solubility of OFF proteins, is needed.

When measuring the viscosity of the OFF the cP value did not reach an equilibrium, but rather fluctuated within an interval. The variation in viscosity is probably due to the inhomogeneity of the OFF, with its irregular particles which disrupted the measurement. To compare the viscosity of the OFF with other foods, water at 20°C has a viscosity of 1 cP, while a mayonnaise at 21 °C has a viscosity of 20 000 cP (Levine & Finley 2018). The viscosity of an 80% sucrose solution is approximately 40 000 cP (Bourne 2002). Thus, the viscosity of the OFF can exhibit close to twice as high viscosity as nearly a saturated solution of sucrose, and four times the viscosity of a mayonnaise. Since viscosity is highly temperature dependent (Brookfield n.d.b), one future investigation could be to measure the viscosity of the OFF at different temperatures. For example, heating to 55°C, the gelatinization temperature of oat starch, or heating to 90-100°C the gelatinization temperature of oat globulins, to observe a possible change in viscosity.

Overall, due to the complex composition of the OFF, with its high water content, the functional property results from this study are difficult to compare to other study results, on cereal fibers in burgers. The fibers investigated in these studies, are usually dry powders or flours with a low moisture content. For example, the methods used for determining WHC, SC, OHC and EC in this study by Huber et al. (2016), are applied for fibers with moisture contents in the range between 7-13%. In contrast, as mentioned above the OFF has a moisture content of around 76%.

4.2 Cooking measurements

Considering cook loss, an increase in fiber concentration within each fiber source, resulted in increased cook loss except for potato fiber, in which P40 showed higher loss than P75. Furthermore, W25 and W75 were significantly different from W40 and OFF0 in terms of cook loss (Table 4). The losses might be due to oil and water leaking out from the burgers upon heating. When a conventional piece of meat is being cooked, the cook loss in terms of weight, is due to leakage of meat fluids. These fluids, which contain water, protein, minerals and lipids mainly explain meat shrinkage (Hughes et al. 2017 see McClements & Grossmann 2021; Yu et al. 2017 see McClements & Grossmann 2021). In meat analogues on the other hand, TVP has the ability to bind water and thereby create emulsion stability. For example, TVP made of pea, has been demonstrated to bind water and fat (Kyriakopoulou et al. 2021). Consequently, the decreased level of TVP and substitution with increased level of fibers in this study, might explain the increased burger cook loss.

No significant difference regarding diameter reduction could be distinguished within each fiber source concentrations, when compared to OFF0. For OFF40, OFF75 and W75 the burger diameter instead increased. Also, for thickness reduction, no significant difference could be distinguished within each fiber source and its concentrations when compared to OFF0. Wheat fiber burgers showed the greatest thickness reduction, which was rather equal for all wheat fiber burger formulations. For the OFF burgers, an increased level of OFF resulted in increased thickness reduction. However, the reduction was less than in the OFF0 burgers. For potato fiber burgers, the thickness reduction decreased with increased fiber concentration.

Table 4. Cooking properties for burgers within each fiber source and its concentrations. The reference (OFF0) is included in the comparison with each fiber source and its concentrations.

	Cook loss (%)	Diameter reduction (%)	Thickness reduction (%)
OFF0	7.0±0.9^{a}	3.4±3.0^{a}	8.5±9.5^{a}
OFF25	7.7±0.4^{a}	1.0±1.8^{a}	2.2±3.9^{a}
OFF40	9.3±1.5^{a}	-2.5±5.6^{a}	7.0±7.2^{a}
OFF75	10.4±5.5^{a}	-0.7±0.6^{a}	7.8±8.4^{a}
P25	5.9±1.6^{a}	1.0±1.0^{a}	8.6±3.4^{a}
P40	6.6±2.0^{a}	1.7±2.1^{a}	6.0±11.4^{a}
P75	5.3±0.8^{a}	1.0±1.8^{a}	6.0±11.4^{a}
W25	11.3±1.8^{b}	3.4±1.2^{a}	13.3±11.9^{a}
W40	12.5±1.6^{a}	2.0±3.6^{a}	13.9±12.7^{a}
W75	18.1±3.2^{b}	-1.6±3.9^{a}	13.7±14.3^{a}

Values are expressed as average and standard deviation for samples measured in triplicates.
Different letters in the same column indicate significant differences among values ($p<0.05$) from a Tukey's Test.

The number of cooking measurements performed, was probably too small to detect significant differences at 0.05 level of significance, even though visual differences could be observed between burgers of different fibers and within the same fiber source (Figure 7). When visually studying the fried burgers, the OFF and potato fiber burgers in concentration 25% and 40% were most similar to the reference burger (OFF0), regarding burger thickness and diameter. On the other hand, wheat fiber burgers were floating out and became flat especially at a fiber concentration of 75%. A promising fiber combination for further investigation, to keep burger integrity similar to OFF0, might therefore be to combine the OFF with potato fiber, with a maximum concentration of 40%.

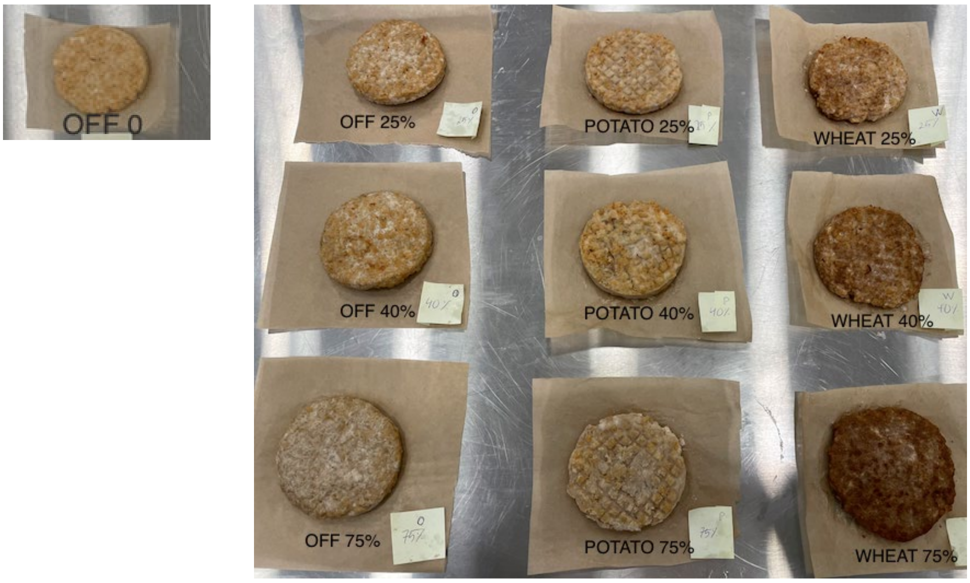

Figure 7. Fried burgers: upper left corner reference burger (OFF0), from left to right OFF, potato and wheat burgers with increasing fiber concentration (25%, 40%, 75%) up to down.

When comparing the cooking properties of the OFF0 with an average value of the concentrations 25%, 40% and 75% for each fiber source, burgers with potato fiber and OFF0 showed lowest cook loss (Table 5). Wheat fiber burgers however, showed the highest loss, which also was significant from the other fiber burgers and OFF0 (Table 5). The potato fiber as earlier mentioned contains 65% fibers, which are effective at absorbing large quantities of liquid (Semper n.d.). This might explain why the potato fiber burger exhibited lowest cook loss. Regarding diameter reduction, no significant difference was observed between the burgers of different fiber sources and OFF0. OFF burgers however, increased in diameter. Considering burger thickness reduction, wheat fiber burgers reduced the most in thickness, while potato fiber burgers showed lowest reduction followed by OFF burgers. No significant difference between the different fiber burger sources and the OFF0 was seen for this attribute.

Table 5. Cooking properties of burgers from different fiber sources and the reference (OFF0).

	Cook loss (%)	Diameter reduction (%)	Thickness reduction (%)
OFF0	7.0±0.9[a]	3.4±3.0[a]	8.5±9.5[a]
OFF	8.8±2.8[a]	-0.4±3.4[a]	7.6±8.4[a]
Potato	5.9±1.4[a]	1.2±1.5[a]	6.9±8.3[a]
Wheat	14.0±3.7[b]	1.3±3.5[a]	13.6±11.3[a]

Different letters in the same column indicate significant differences among values ($p<0.05$) from a Tukey's Test.
Values are expressed as averages with standard deviation of the concentrations 25%, 40% and 75% for samples measured in triplicates.

The procedure for determining burger cooking properties was subjected to sources of errors. For example, when formulating the burgers, the burger ingredients might have been unevenly distributed in the burger batter. This inhomogeneity might affect burger integrity during frying and thus the subsequent cooking properties. Further mixing of burger batter in a blender might improve the homogeneity. Furthermore, more precise measuring methods might be advantageous for future trials. Also, instead of frying each burger separately in a pan, frying in an oven might lead to more uniform burger cooking.

4.3 Texture profile analysis

The results from the TPA are summarized in Table 6 and 7. A general trend observed, was that when the OFF concentration increased, there was usually a decrease in the texture parameter values (Table 6). The reference burger (OFF0) was significantly harder compared to all other OFF burger formulations, except OFF25. Considering cohesion, springiness, gumminess and chewiness OFF0 also showed the highest values and was significantly different from all other OFF formulations. For maximum shear force and work of shear, no significant differences were detected between the OFF burger formulations.

Table 6. Texture parameter comparison between OFF burgers of different concentrations.

	Hardness (g)	Cohesion	Springiness (%)	Gumminess	Chewiness	Maximum Shear Force (kg)	Work of Shear (kg.sec)
OFF0	363.6±62.8[a]	0.3±0.0[a]	33.5±4.2[a]	105.2±21.3[a]	35.8±10.8[a]	0.9±0.4[a]	1.1±0.4[a]
OFF25	318.1±43.3[ac]	0.3±0.0[b]	27.9±2.8[c]	81.7±11.7[c]	22.7±3.7[c]	0.9±0.7[a]	1.3±0.9[a]
OFF40	282.4±54.4[bc]	0.2±0.0[b]	23.5±3.0[b]	65.9±16.6[bc]	15.5±4.4[bc]	0.6±0.3[a]	0.9±0.4[a]
OFF75	240.7±45.8[b]	0.2±0.0[c]	21.2±2.6[b]	48.4±12.9[b]	10.5±3.9[b]	0.5±0.2[a]	0.7±0.2[a]

Values are expressed as averages with standard deviation, from two separate burgers, with six samples per burger concentration.
Values with different letters in the same column indicate significant differences ($p<0.05$) from a Tukey's Test.

When comparing the parameters from the TPA between the different fiber sources and the reference (OFF0), the potato fiber burger formulation was significantly different from the OFF formulation and wheat in terms of hardness, cohesion, gumminess and chewiness (Table 7). The commercially available burgers P1, P2 and M were significantly different from the self-formulated burgers for all texture parameters, except cohesion and springiness. As earlier described, plant-based meat analogues aim to resemble, the bite or so-called hardness, and chewiness of traditional meat (Kyriakopoulou et al. 2021). In order to increase the hardness and chewiness of the self-formulated burgers, the ingredients used need to be optimized and complemented with other ingredients. Since the commercially available plant-based burgers, apart from their protein sources, also contain various stabilizers such as methylcellulose and carrageenan, which improve textural properties, they are not directly comparable to the self-formulated burgers. However, they can serve as a source of standards, when improving the texture of the OFF burgers in the future.

Table 7. Texture parameter comparison for burgers from different fiber sources, the reference (OFF0) and commercially available burgers (P1, P2 and M).

	Hardness (g)	Cohesion	Springiness (%)	Gumminess	Chewiness	Maximum Shear Force (kg)	Work of Shear (kg.sec)
OFF0 (n=12)	363.6±62.8^{a}	0.3±0.0^{a}	33.5±4.2^{a}	105.2±21.3^{a}	35.8±10.8^{a}	0.9±0.4^{a}	1.1±0.4^{a}
OFF (n=30)	273.4±53.8^{a}	0.2±0.0^{b}	24.1±3.6^{b}	64.1±18.0^{a}	15.9±5.9^{a}	0.8±0.5^{a}	1.1±0.6^{a}
Potato (n=12)	734.8±247.3^{b}	0.4±0.0^{cf}	38.0±4.7^{a}	267.4±98.1^{c}	103.7±42.5^{c}	0.7±0.3^{a}	1.0±0.2^{a}
Wheat (n=12)	282.3±36.1^{a}	0.2±0.0^{b}	22.6±3.0^{b}	58.0±10.9^{a}	13.3±3.9^{a}	0.7±0.2^{a}	0.9±0.1^{a}
P1 (n=6)	2149.6±684.5^{c}	0.4±0.0^{f}	46.8±2.7^{c}	839.4±224.1^{b}	391.0±96.9^{b}	2.6±1.5^{b}	4.4±2.1^{b}
P2 (n=6)	3010.1±647.7^{d}	0.3±0.0^{ac}	35.3±3.0^{a}	992.7±199.8^{d}	350.9±77.2^{b}	4.9±2.5^{c}	5.6±1.9^{b}
M (n=6)	2567.9±317.3^{e}	0.5±0.0^{g}	50.9±4.3^{c}	1157.7±95.4^{e}	590.0±78.8^{d}	3.0±2.1^{b}	4.7±2.8^{b}

Values with different letters in the same column indicate significant differences ($p<0.05$) from a Tukey's Test.
Average values and standard deviation of OFF concentrations 15%, 25%, 40%,75% and 100%. For potato and wheat fiber burgers values are averages of concentrations 25% and 40%. P1=fungal and textured wheat protein burger, P2=soy and wheat protein burger, M=beef burger.

Source of errors with the TPA are for example varying size and shape of the burger samples. The differences in sample geometry might have influenced the accuracy of the measurements. Also, the temperature of the burger samples might decreased during the time of testing, which could have affected the texture properties of the samples. Furthermore, for meat, the degree of cooking has an important effect on meat toughness. Therefore, it is necessary that all meat is cooked to the same degree when performing TPA. If the internal temperature is higher it will lead to a higher shear reading (Bourne 2002). If assuming that the same reasoning can be applied for plant-based meat analogues, the internal temperature of the burgers needs to be kept as uniform as possible during measurements.

4.4 Sensory evaluation

The number of right and wrong answers from the triangle tests, in other words, whether the odd sample could be distinguished or not, are summarized in Figure 8. The answers from the two evaluation rounds were rather inconsistent for both attributes. The reason for these results might be explained by the use of an untrained panel, without the sensitivity to detect differences for hardness and chewiness. Another explanation for the varying results, could be that the differences between the burger formulations, for the two attributes, were too small to be detected. The results from the TPA in terms of hardness, showed that OFF25 was only significantly different to OFF75. For chewiness, OFF25 was significantly different to OFF0 and OFF75 (Table 6). These few significant differences between the burger formulations detected by the Texturometer, might explain why panellists were not able to distinguish the odd samples easily. Consequently, panelists have likely guessed, with the probability of choosing the odd sample of 1/3 chance.

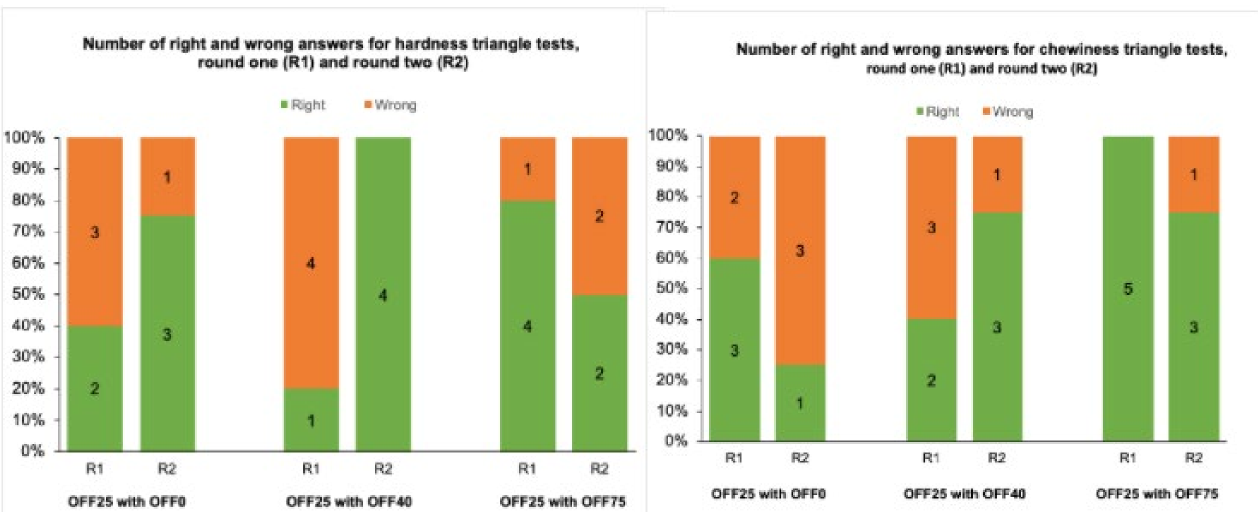

Figure 8. Number of right and wrong answers for hardness (bar charts to left) and chewiness (bar charts to right) triangle tests, round one and two.

When comparing the right answers from round one with a table for critical values for number of correct answers to establish significance (Roessler et al. 1978), there were no significant differences between samples OFF25 with OFF0 as well as between OFF25 with OFF40, for samples assessed for hardness. However, there was a significant difference between OFF25 with OFF75 ($p<0.05$). Similar to the hardness triangle tests, there were no significant differences when comparing the right answers to critical values for correct answers, between samples OFF25 with OFF0 as well as between OFF25 with OFF40 assessed for chewiness. There was on the other hand a significant difference between OFF25 with OFF75 ($p<0.04$).

Panellist comments regarding the odd sample from hardness and chewiness triangle tests are summarized in Appendix 4. For samples assessed for hardness, one panellist commented that OFF25 was perceived softer than OFF0. For the triangle test between OFF25 with OFF40, one comment was that the difference between these two formulations was hardly noticeable. OFF25 was commented to be more firm and not as "mousse-like" when compared to OFF75. These comments can be compared to the results for hardness from the TPA, which showed that OFF25 was the formulation most similar to the reference burger (OFF0) in terms of hardness, followed by OFF40 and then OFF75. Also, no significant difference was observed between OFF25 and OFF40, which is in line with the panellist comment, for these two formulations (Table 6).

For samples assessed for chewiness, OFF25 was commented to be slightly more "porridge-like" when compared to OFF0. For triangle tests between OFF25 with OFF40, one panellist wrote that these two formulations were "very similar". This is in agreement with the results from the TPA, since no significant difference between these two formulations was observed. OFF25 was commented to be firmer and less "porridge-like" when compared to OFF75. From the TPA results, OFF0 exhibited highest chewiness followed by OFF25, OFF40 and then OFF75 (Table 6). This is the same burger concentration pattern which also was shown for burgers assessed for hardness. One explanation for this correlation is that these two parameters are interdependent. As described in section 2.5.1, chewiness is the product of gumminess multiplied with springiness, while gumminess is the product of hardness multiplied with cohesiveness. Thus, a change in hardness will result in a change in chewiness. For example, with an increase in OFF from 25% to 75%, the hardness decreases which also results in lower chewiness. An explanation of this decrease from an ingredient point of view, is that TVP in plant-based products is responsible for the meaty and chewy texture (Kyriakopoulou 2021). When TVP is substituted with increased concentration of OFF, hardness, and consequently the chewiness decreases.

Regarding assigned numbers for samples from the hardness and chewiness scales, panelists have utilized the lower part of the scales. For samples assessed for hardness, the most frequently assigned numbers were two and three, while samples assessed for chewiness mainly were assigned number three and four. The difference between assigned numbers for a trio of samples, in a triangle test, for both attributes, were often one unit. This indicates that hardness and chewiness respectively, were considered rather equally between burger samples of different OFF concentrations. For some triangle tests, the same number has been assigned to all three samples. In these cases, the odd sample could not be distinguished (Appendix 5).

From a two-way ANOVA, no significant difference could be observed regarding assigned scale numbers for chewiness samples. However, there were significant differences in how samples were assigned scale numbers for hardness, adjusted for round and panellist ($p<0.001$). From a subsequent Tukey Pairwise comparison test, it was shown that OFF25 and OFF75 samples were assigned numbers significantly different from each other and to OFF0 as well as to OFF40 in terms of hardness (Table 8).

Table 8.Tukey Pairwise comparison test regarding assigned numbers for samples evaluated for hardness, adjusted for round and panelist.

Sample	N	Mean
OFF0	14	3.1^{a}
OFF40	13	2.9^{a}
OFF25	41	2.5^{b}
OFF75	13	1.9^{c}

Means that do not share a letter are significantly different.

The preferred samples assessed for hardness and chewiness, are shown in Figure 9. Similar to the number of right and wrong answers from the triangle tests, the preferred samples were varying between the two evaluation rounds. Regarding samples assessed for hardness, OFF0 were more frequently preferred than OFF25, while OFF40 seemed to have been preferred over OFF25 at least in round two. For preferred sample between OFF25 and OFF75, it appeared as OFF25 was more preferred in round one. For preferred chewiness samples, there seems to be no distinct preferred sample between OFF25 with OFF0 and OFF25 with OFF40. However, OFF25 appeared to be preferred over OFF75 at least in round one.

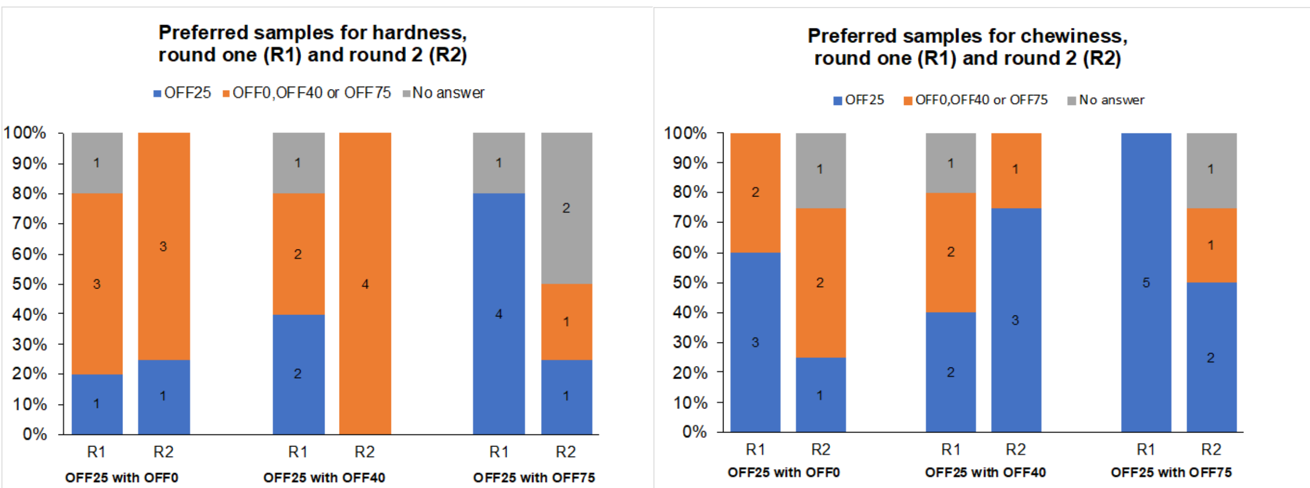

Figure 9.Preferred samples for hardness (bar charts to left) and chewiness (bar charts to right) from triangle tests, round one and two.

Source of errors during the sensory evaluation, apart from panel fatigue, is perhaps that panellists showed decreased responsiveness at the end of the evaluation. This decrease is due to adaptation which is caused by constant sample stimulation. Constant stimulation can be seen as a source of bias, since it is a process that causes a change in the response (Lawless & Heymann 2010). For future evaluations, fewer samples could be served and instead of panellists only judging one attribute, both attributes could be assessed, with one attribute per round. Also, the discrimination and affective tests could be performed separately at different testing days, to avoid panellists both judging sample differences and selecting preferred samples. When these two tests are combined, panellists can have a tendency to prefer the duplicate similar samples rather than the odd one. This tendency could be seen as a bias factor (Lundgren 1981).

To get more power in the study for the statistical reasonings, the number of panellists should preferably be bigger. For discrimination testes ideally 25-40 participants and for affective tests 75-150 participants. With a bigger panel, the chance to get a normal distribution will increase, which is necessary in order to perform, for example t-tests and different types of regression models. Another improvement for future sensory evaluations, could be to use the different OFF burger formulations as references on the attribute scales instead of other foods. Also, to perform a descriptive sensory evaluation test of the OFF burgers, with a trained panel would be valuable. This test is considered the most extensive and informative sensory evaluation tool, in which both quantitative and qualitative data can be obtained (Lawless & Heymann 2010). Several of the descriptive methods can also be used to interpret sensory instrumental relationships, which would be relevant for this study in order to compare with the TPA results.

5. Conclusions

The aim of this study was to characterize functional properties of the OFF and to investigate its effect in vegan burgers, in terms of burger integrity, texture and sensory properties by incorporating the OFF in different concentrations. Regarding the functional properties of the OFF, its DM was 23.6% before and 18.8% after centrifugation, respectively. It showed a WHC of 3.9 g water per g solid OFF and a OHC of 4.2 g oil per g OFF. However, no SC or EC of the OFF could be observed, and its viscosity varied between 67 000-80 000 cP. Considering burger integrity after cooking, an increase in OFF concentration in burgers, resulted in increased cook loss, diameter and thickness reduction. For burgers formulated with other fiber sources, potato fiber burgers showed lowest values for all cooking properties, while wheat fiber burgers showed highest cook loss and thickness reduction.

From TPA, it was observed that increased OFF concentration in burgers, overall led to a decrease in texture parameter values. The reference burger (OFF0) was significantly different from all other OFF burger formulations in terms of cohesion, springiness, gumminess and chewiness. The OFF25 burger had similar hardness to the OFF0 burger, which makes it a potential future burger. The commercially available burgers were significantly different to the self-formulated burgers for all texture parameters, except cohesion and springiness. The results from the sensory evaluation regarding right and wrong answers as well as preferred samples, were rather inconsistent between the two evaluation rounds. From triangle tests in round one between OFF25 and OFF75, there was a significant difference regarding number of correct answers for both hardness and chewiness. In terms of assigned sample numbers for the hardness attribute, OFF25 and OFF75 were assigned numbers significantly different from each other and the other OFF samples.

In conclusion, due to the complex nature of OFF with its high water content and inhomogeneity, the results from this study are difficult to compare to similar studies on cereal fibers in burgers. Thus, there is a call for more research, including method and product development. This is of importance, in order to further understand the functional characteristics of OFF and its effect on burger integrity, texture and sensory properties. Moreover, further research is required to be able to optimize the application of the OFF in future plant-based meat analogues, such as vegan burgers.

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Popular science summary

With a growing population, the production of sustainable food is a constant global challenge. As the population also becomes wealthier, a shift towards an animal-based diet is observed. However, meat production has a negative impact on the environment in terms of land and water use, as well as contributing to greenhouse gas emissions. In addition, excessive consumption of meat is associated with several health risks. As a response to these environmental and nutritional aspects of meat, novel so called plant-based meat analogues, have seen a rise in recent years. These analogues are usually made of vegetable proteins from pulses or cereals. Cereal grains such as wheat, maize, rice, barley and oats are important sources of fibers, proteins as well as essential vitamins and minerals. However, vast amounts of cereal side streams are generated from cereal industries. This can be seen as a loss of nutritious biomass. Utilisation of these side streams could instead lead to more efficient raw material applications, to develop new healthy plant-based food products.

Oat is a cereal grain that has received much attention especially due to its healthy and functional properties as a food ingredient in, among other things, plant-based food products. The aim of this Master Thesis was to characterize functional properties of an oat fiber fraction (OFF) and study its effect in vegan burgers. Initially, functional properties of the OFF were investigated, which included dry matter, oil and water holding capacity, viscosity as well as swelling and emulsifying capacity. Afterwards, burgers were formulated in which texturized vegetable protein was substituted with OFF in the concentrations 0%, 25%, 40% and 75%. OFF burgers were also compared to burgers made of potato and wheat fiber as well as to commercially available burgers. To understand how cooking affect burger integrity in terms of cook loss, diameter and thickness reduction, burgers were examined before and after cooking. Burgers were also analysed in terms of texture, which included texture profile analysis and a sensory evaluation. The latter consisted of a triangle and affective test, performed by a panel of ten individuals, in two evaluation rounds.

Regarding functional properties, the dry matter of the OFF before and after centrifugation was 23.6% and 18.8%, respectively. Its water holding capacity was 3.9 g water per g solid OFF and its oil holding capacity was 4.2 g oil per g OFF. However, no swelling or emulsifying capacity of the OFF could be observed and its viscosity varied between 67 000-80 000 cP. Due to the inhomogeneity of the OFF and its high water content, the functional property results are difficult to compare to similar study results.

The effect on burger integrity after cooking, showed that an increase in OFF concentration in burgers, overall resulted in increased cook loss, diameter and thickness reduction. In comparison, wheat fiber burgers exhibited higher cook loss and thickness reduction, while potato fiber burgers showed lower values for all cooking measurements. From the texture profile analysis, an increase in OFF concentrations in general led to a decrease in all texture parameter values. However, the OFF25 burger had a similar hardness to the OFF0 burger, which makes it a potential future burger. The commercially available burgers were significantly different from the self-formulated burgers for all texture parameters, except two parameters. Regarding the results from the sensory evaluation, the number of right and wrong answers as well as preferred samples, were rather inconsistent between the two evaluation rounds. Therefore, using a bigger and trained panel would be favourable in future evaluations.

Overall, there is a call for more research, including method and product development, in order to further understand the functional properties of the OFF and its effect in vegan burgers. From a broader perspective, the utilisation of industrial by-products such as the OFF side stream, could lead to improved circular material flows, which is one step along the way, to reach more sustainable food systems.

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Appendix 1-Calculations of WHC & OHC

Calculations are made from average values of duplicate determinations

WHC= g of water/g of solid material

50 g of OFF+ 300 ml water are mixed and centrifuged. Afterwards the supernatant is discarded.

Weight of tube + OFF+H2O= 435 g
Weight of tube + OFF (after supernatant being discarded) = 172 g
Supernatant (containing water and dissolved material from OFF): 435-172= 263 g

DM of OFF before centrifugation: 23.60%
DM of OFF after centrifugation (and removal of supernatant): 18.78%
Dissolved material from OFF during centrifugation: 23.6-18.78= 4.82%
Dissolved OFF material: 50 x 0,0482= 2.41 g
OFF left: 50-2.41= 47.59 g
Water left: (300-263-2.41)= 34.59 g

WHC = (34.59)/(47.59 x 0.1878)= <u>3.87</u> g water/g solid OFF

OHC= g of oil retained/g of fiber

20 g OFF + 200 ml oil are mixed and centrifuged. Afterwards the supernatant is discarded.

Weight of tube + OFF+ oil before centrifugation= 274 g
Weight of tube + OFF (after supernatant being discarded) =112 g
Oil discarded= 274-112= 162 g

Mass of oil added= 200 x 0.91=182 g
Oil retained: 182-162= 20 g

DM of OFF=23.6%
DM of 20 g OFF: 20 x 0.236= 4.72 g

OHC: 20g oil /4.72g OFF=<u>4.23</u> g oil/g OFF

Appendix 2- Severing order and sample coded numbers in triangle tests

HARDNESS			
Panellist 1			
Test 1	OFF25 (748)	OFF25 (624)	OFF0 (533)
Test 2	OFF25 (362)	OFF40 (133)	OFF25 (250)
Test 3	OFF75 (798)	OFF75 (427)	OFF25 (493)
Test 4	OFF40 (465)	OFF25 (776)	OFF40 (106)
Test 5	OFF25 (314)	OFF25 (701)	OFF75 (782)
Test 6	OFF0 (822)	OFF25 (655)	OFF0 (235)
Panellist 2			
Test 1	OFF75 (688)	OFF25 (223)	OFF25 (814)
Test 2	OFF25 (946)	OFF0 (114)	OFF0 (548)
Test 3	OFF40 (367)	OFF25 (815)	OFF40 (920)
Test 4	OFF25 (397)	OFF40 (780)	OFF25 (412)
Test 5	OFF25 (275)	OFF75 (680)	OFF75 (136)
Test 6	OFF0 (215)	OFF25 (234)	OFF25 (544)
Panellist 3			
Test 1	OFF40 (849)	OFF40 (970)	OFF25 (289)
Test 2	OFF75 (156)	OFF25 (245)	OFF75 (664)
Test 3	OFF25 (535)	OFF0 (244)	OFF25 (391)
Test 4	OFF0 (731)	OFF0 (641)	OFF25 (258)
Test 5	OFF25 (504)	OFF75 (442)	OFF25 (859)
Test 6	OFF25 (325)	OFF40 (403)	OFF25 (794)
Panellist 4			
Test 1	OFF0 (993)	OFF25 (548)	OFF0 (679)
Test 2	OFF25 (466)	OFF25 (350)	OFF40 (158)
Test 3	OFF75 (306)	OFF75 (938)	OFF25 (254)

Test 4	OFF40 (735)	OFF40 (326)	OFF25 (284)
Test 5	OFF25 (534)	OFF25 (910)	OFF75 (926)
Test 6	OFF25 (609)	OFF0 (733)	OFF25 (373)
Panellist 5			
Test 1	OFF75 (860)	OFF25 (664)	OFF25 (334)
Test 2	OFF25 (254)	OFF40 (181)	OFF25 (935)
Test 3	OFF0 (886)	OFF0 (198)	OFF25 (303)

CHEWINESS			
Panellist 1			
Test 1	OFF75 (508)	OFF75 (572)	OFF25 (541)
Test 2	OFF25 (846)	OFF0 (106)	OFF25 (382)
Test 3	OFF25 (406)	OFF40 (290)	OFF40 (712)
Panellist 2			
Test 1	OFF75 (797)	OFF25 (944)	OFF75 (879)
Test 2	OFF25 (485)	OFF0 (337)	OFF0 (455)
Test 3	OFF25 (560)	OFF25 (398)	OFF40 (888)
Test 4	OFF0 (346)	OFF25 (738)	OFF25 (523)
Test 5	OFF40 (338)	OFF40 (459)	OFF25 (115)
Test 6	OFF25 (144)	OFF25 (509)	OFF75 (202)
Panellist 3			
Test 1	OFF0 (986)	OFF0 (619)	OFF25 (528)
Test 2	OFF75 (601)	OFF25 (788)	OFF25 (613)
Test 3	OFF25 (207)	OFF40 (849)	OFF25 (306)
Test 4	OFF40 (218)	OFF40 (492)	OFF25 (175)
Test 5	OFF25 (958)	OFF0 (468)	OFF25 (618)
Test 6	OFF25 (484)	OFF75 (763)	OFF75 (856)
Panellist 4			
Test 1	OFF40 (831)	OFF40 (406)	OFF25 (197)

Test 2	OFF0 (181)	OFF25 (494)	OFF0 (672)
Test 3	OFF25 (254)	OFF25 (739)	OFF75 (568)
Test 4	OFF25 (680)	OFF25 (381)	OFF0 (470)
Test 5	OFF75 (593)	OFF25 (184)	OFF75 (499)
Test 6	OFF25 (211)	OFF25 (534)	OFF40 (356)
Panellist 5			
Test 1	OFF0 (562)	OFF25 (857)	OFF25 (958)
Test 2	OFF25 (872)	OFF25 (503)	OFF75 (928)
Test 3	OFF40 (601)	OFF25 (758)	OFF40 (903)
Test 4	OFF25 (955)	OFF25 (520)	OFF40 (980)
Test 5	OFF0 (515)	OFF0 (846)	OFF25 (679)
Test 6	OFF75 (673)	OFF75 (529)	OFF25 (105)

Appendix 3- Instructions sensory evaluation

Three samples are being served, of which two are identical. Evaluate all three samples in the order from left to right and mark which one is the odd one in terms of attribute* and write down in the comment section what makes the odd sample different compared to the other two. Also assign each sample with a number from 1 (low) - 9 (high) on the attribute scale (samples are allowed to have the same number). You must choose an answer, even though no difference can be distinguished please guess. Also mark the sample which is being preferred.

Prior to evaluation, please read the following definitions, references and evaluation techniques for your one assigned attribute.

Attribute*	Definition[a]	Reference scale[b]	Evaluation technique[c]
Hardness	Force required to compress a substance between molar teeth.	(1) Cream cheese (9) Hard candy	Place a sample between molar teeth, bite down and evaluate the force to compress the food.
Chewiness	Length of time required to masticate a sample at a constant rate of force to reduce it to a consistency which is suitable for swallowing.	(1) Rye bread (average nr of chews ca 10,3) (9) Chocolate toffee e.g. Riesen (average nr of chews ca 56,7)	Place a sample in mouth and masticate at the rate of one chew per second.

[a, c] (Bourne, 2007)

[b] (With minor modifications from Szczesniak et al. 1963 see Bourne 2007)

After evaluating a sample, discard it in the cup marked with the text “leftovers” and rinse mouth with water from the cup marked with “water” before evaluating the next sample.

The evaluation is divided into two rounds (with 3 tests/round). When you have finished the first three tests you are free to leave the room in a silent manner and wait outside until round two (for the last three tests).

On the next page please start by filling in your name. If any questions arise, please do not speak out, instead raise your hand.

ROUND 1-HARDNESS

HARDNESS TEST 1

Sample ID			
Which sample is the odd one in terms of **hardness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a hardness scale			
Which sample is preferred?			

HARDNESS TEST 2

Sample ID			
Which sample is the odd one in terms of **hardness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a hardness scale			
Which sample is preferred?			

HARDNESS TEST 3

Sample ID			
Which sample is the odd one in terms of **hardness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a hardness scale			
Which sample is preferred?			

ROUND 2- HARDNESS

HARDNESS TEST 4

Sample ID			
Which sample is the odd one in terms of **hardness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a hardness scale			
Which sample is preferred?			

HARDNESS TEST 5

Sample ID			
Which sample is the odd one in terms of **hardness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a hardness scale			
Which sample is preferred?			

HARDNESS TEST 6

Sample ID			
Which sample is the odd one in terms of **hardness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a hardness scale			
Which sample is preferred?			

ROUND 1- CHEWINESS

CHEWINESS TEST 1

Sample ID			
Which sample is the odd one in terms of **chewiness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a chewiness scale			
Which sample is preferred?			

CHEWINESS TEST 2

Sample ID			
Which sample is the odd one in terms of **chewiness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a chewiness scale			
Which sample is preferred?			

CHEWINESS TEST 3

Sample ID			
Which sample is the odd one in terms of **chewiness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a chewiness scale			
Which sample is preferred?			

ROUND 2- CHEWINESS

CHEWINESS TEST 4

Sample ID			
Which sample is the odd one in terms of **chewiness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a chewiness scale			
Which sample is preferred?			

CHEWINESS TEST 5

Sample ID			
Which sample is the odd one in terms of **chewiness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a chewiness scale			
Which sample is preferred?			

CHEWINESS TEST 6

Sample ID			
Which sample is the odd one in terms of **chewiness?**			
Comment: what makes the odd sample different compared to the other two?	-- -- --		
Assign each sample with a nr from 1 (low) - 9 (high) on a chewiness scale			
Which sample is preferred?			

Appendix 4- Panellist comments about the odd sample from triangle tests

(Translations are made from Swedish)

Comments in terms of hardness:

OFF25 as the odd sample when compared to OFF75:
“more distinct particles”
“more chews were needed”
“more firm and not as mousse-like”

OFF75 as the odd sample when compared to OFF25:
“much softer”
“more spongy and looser”
“felt somehow lighter, melted faster in mouth”

OFF25 as the odd sample when compared to OFF0:
“less spongy”
“couldn’t retain its consistency”
“loser, softer, perhaps a bit more creamier”

OFF0 as the odd sample when compared to OFF25:
“harder”
“no minor difference among the samples”
“felt a bit dryer but not harder compared to the other two samples”

OFF40 as the odd sample when compared to OFF25:
“slightly harder”
“better consistency which was well maintained longer”
“hardly no noticeable difference”

OFF25 as the odd sample when compared to OFF40:
“softer, fluffier”

Comments in terms of chewiness:

OFF0 as the odd sample when compared to OFF25:
"more to chew on"

OFF25 as the odd sample when compared to OFF0:
"slightly more porridge-like, though no major difference"
"more softer, slides away when chewing"

OFF25 as the odd sample when compared to OFF40:
"less porridge-like and easier to swallow"
"guessed, they were overall very similar"
"less chewable pieces inside"

OFF40 as the odd sample when compared to OFF25:
"slightly more porridge-like, though no major difference"
"had chewier, wooden parts"

OFF75 as the odd sample when compared to OFF25:
"porridge-like sample"
"more sticky"
"less chewable, was melting much easier in mouth"
"similar to slime, like a bean paste/mixture"

OFF25 as the odd sample when compared to OFF75:
"a bit more chunky"
"a bit firmer and less porridge-like"

Appendix 5- Answers from triangle tests, assigned scale numbers and preferred samples

HARDNESS

Paneliest	Round 1: OFF25 & OFF0						Round 2: OFF25 & OFF0					
	R/W*	O25	O25	O0	O0	Prefered sample	R/W*	O25	O25	O0	O0	Prefered sample
1	W	3	2	3		no pref.	W		3	3	3	O25
2	W		2	4	2	O0	R	2	2	3		O0
3	R	2	2	2		O25	R		2	3	3	O0
4	R		3	4	4	O0	R	3	3	3		O0
5	W		3	3	4	O0						

Paneliest	Round 1: OFF25 & OFF40						Round 2: OFF25 & OFF40					
	R/W*	O25	O25	O40	O40	Prefered sample	R/W*	O25	O25	O40	O40	Prefered sample
1	W	2	3	2		no pref.	R		3	3	3	O40
2	W		2	2	3	O40	R	2	2	3		O40
3	W		3	3	2	O25	R	2	2	3		O40
4	R	3	3	4		O40	R		3	4	4	O40
5	W	3	2	2		O25						

Paneliest	Round 1: OFF25 & OFF75						Round 2: OFF25 & OFF75					
	R/W*	O25	O25	O75	O75	Prefered sample	R/W*	O25	O25	O75	O75	Prefered sample
1	R		3	2	2	no pref.	R	3	3	2		no pref.
2	R	2	2	1		O25	W		1	3	1	O75
3	R		3	2	2	O25	R	2	2	2		no pref.
4	R		3	2	2	O25	W	3	2	2		O25
5	W	3	2	2		O25						

*answer for triangle test: R=right, W=wrong

CHEWINESS

Paneliest	Round 1: OFF25 & OFF0						Round 2: OFF25 & OFF0					
	R/W*	O25	O25	O0	O0	Prefered sample	R/W*	O25	O25	O0	O0	Prefered sample
1	W	3	3	3		O25						
2	R		4	5	5	O25	W	5	3	5		no pref.
3	R		3	4	4	O0	R	3	3	4		O0
4	R		3	2	2	O0	W	2	3	2		O0
5	W	2	1	1		O25	W		5	3	5	O25

Paneliest	Round 1: OFF25 & OFF40						Round 2: OFF25 & OFF40					
	R/W*	O25	O25	O40	O40	Prefered sample	R/W*	O25	O25	O40	O40	Prefered sample
1	W		3	3	3	no pref.						
2	R	3	3	4		O40	R		4	3	3	O25
3	W	3	4	3		O25	R		4	4	4	O25
4	R		1	3	3	O25	R	3	3	5		O25
5	W		1	2	1	O40	W	3	2	3		O40

Paneliest	Round 1: OFF25 & OFF75						Round 2: OFF25 & OFF75					
	R/W*	O25	O25	O75	O75	Prefered sample	R/W*	O25	O25	O75	O75	Prefered sample
1	R		3	2	2	O25						
2	R		5	3	3	O25	R	4	4	3		no pref.
3	R	4	4	5		O25	R		4	6	6	O25
4	R	2	2	3		O25	R		3	4	4	O25
5	R	2	2	1		O25	W		3	3	5	O75

*answer for triangle test: R=right, W=wrong

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