Kaunas University of Technology
Faculty of Chemical Technology

Impact of Extruded Soy and Oat Products on Chemical, Functional and Sensory Properties of Meat Analogues

Master's Final Degree Project

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Food Science and Safety (6211FX011)

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Summary

Press-cakes are by-products of vegetable drink production, usually discarded as bio-waste or used as animal feed. These press-cakes are rich in nutritious compounds like proteins, carbohydrates and minerals, which can be utilised to enhance the nutritional value and functionality of various plant-based food products giving rise to development of a circularity in the production processess. Along with nutrients, press-cakes also contain some anti-nutritional compounds like trypsin inhibitors, phytates and tannins. They usually descrease the biological value and assesibility of nutrients in the products, therefore need to be reduced. This thesis report aimed to carry out the extrusion process for soy and oat press-cakes and determine their impact on the chemical, functional and sensory properties when used in meat analogue matrix. The raw materials used in the project included soy and oat press-cakes received as a by-product from tofu (Soy t) and soy and oat (Soy d and Oat d) drink manufacturing. A low moisture extrusion process with four designed moistures and temperatures models were carried out to attain the aim.

The extrusion process made the Soy d and Oat d press-cakes darker, while extruded Soy t press-cakes became lighter in colour. The chemical analysis of untreated press-cakes and extruded press-cakes were performed and compared. The results showed a reduction in protein content due to degradation during the extrusion process. To study the effect of extrusion on the anti-nutrients, the biogenic amines, acrylamide, free fatty acids and trypsin inhibitor activity was analysed. The amount of biogenic amounts were within the limit safety limits. The amount of trypsin inhibited after extrusion showed a reasonable inhibition rate. The analysis of functional properties showed that the water-holding capacity and oil-binding capacity of extruded press-cake samples increased with elevated temperatures and humidity. These extruded press-cakes were further analysed by adding them in 3% and 6% in amounts to the meat analogue matrix containing texturised soy protein as the base. The chemical analysis of meat analogues containing extruded plant origin press-cakes showed a high presence of proteins and unsaturated fatty acids but a lower amount of saturated fatty acids and sugars when compared with control samples (without addition of extruded press-cakes). The anti-nutrient analysis showed that the addition of different amounts of press-cakes extruded at different humidity and temperature lowered the biogenic amines, free fatty acids and trypsin inhibitor activity but increased the acrylamide content. The analysis of functional properties revealed that the meat analogue containing 6% press-cakes extruded at a temperature of 120 °C and humidity of 45% or 60% showed the best functional technological properties when compared to that of control sample. The texture profile analysis depicted that the addition of extruded press-cakes to the matrix improved its hardness, springiness, and gumminess compared to the control meat analogue without extruded press-cakes. The colour measurements and sensory analysis scores had high acceptability of meat analogues with extruded press-cakes, making it an excellent product to be developed further for market launch.

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Santrauka

Išspaudos yra augalinių gėrimų gamybos šalutiniai produktai, paprastai išmetamos kaip biologinės atliekos arba naudojamos kaip gyvūnų pašarai. Jose gausu maistingų junginių, tokių kaip baltymai, angliavandeniai ir mineralai, kurie gali būti naudojami siekiant pagerinti įvairių augalinių maisto produktų funkcionalumą ir skatinti tvarų vystymąsi. Kartu su maistinėmis medžiagomis juose taip pat yra tam tikrų antimitybinių junginių, tokių kaip tripsinas, fitatai, oksalatai, lektinai ir taninai, kuriuos technologinio apdorojimo metu siekiama sumažinti. Šio darbo tikslas buvo atlikti sojos ir avižų išspaudų ekstruziją bei nustatyti ekstruduotų išspaudų įtaką cheminėms, funkcinėms ir juslinėms savybėms, kai jos naudojamos mėsos analogo matricoje. Analizei naudotos žaliavos buvo sojos$_t$, sojos$_d$ ir avižų$_d$ išspaudos, gautos kaip šalutinis produktas iš tofu (soja$_t$), sojos ir avižų (soja$_d$ ir avižos$_d$) gėrimų gamybos.

Siekiant tikslo buvo sukurti ir įgyvendinti keturi žemos drėgmės ekstruzijos modeliai su skirtingais drėgmės ir temperatūros parametrais. Ekstruzijos metu sojų$_d$ ir avižų$_d$ išspaudos tapo tamsesnės, o sojos$_t$ išspaudų spalva tapo šviesesnė. Atliktas neapdorotų ir ekstruduotų išspaudų cheminės analizės rezultatų palyginimas, kurie įrodė baltymų kiekio sumažėjimą, dėl jų skilimo ekstruzijos proceso metu. Siekiant įvertinti ekstruzijos poveikį antimitybinėms medžiagoms, buvo išanalizuotas biogeninių aminų, akrilamido, laisvųjų riebalų rūgščių ir tripsino inhibitorių kiekis. Rezultatai parodė, kad biogeninių aminų kiekis išspaudose svyravo saugiose ribose, o ekstruzijos inhibuoto tripsino kiekis parodė pageidaujamo intensyvumo sumažėjimą. Funkcinių savybių analizė parodė, kad ekstruduotų išspaudų vandens ir riebalų surišimo geba didėjo taikant aukštesnes temperatūras ir drėgmę. Ekstruduotų išspaudų poveikis buvo toliau analizuojamos, pridedant jų 3% ir 6% į mėsos analogų matricas, kurių bazinę receptūros dalį sudarė tekstūruoti sojos baltymai.

Cheminė mėsos analogų su ekstruduotomis išspaudomis matricų analizė parodė, kad jose gausu baltymų ir nesočiųjų riebalų rūgščių, tačiau mažiau sočiųjų riebalų rūgščių ir cukrų, lyginant su kontrole (be ekstruduotų išspaudų). Antimitybinių medžiagų analizė parodė, kad pridedant skirtingą kiekį išspaudų, ekstruduotų esant skirtingai drėgmei ir temperatūrai, sumažėjo biogeninių aminų, laisvųjų riebalų rūgščių ir tripsino inhibitorių kiekis, tačiau padidėjo akrilamido kiekis. Mėsos analogų matricų funkcinių savybių tyrimų rezultatai parodė, kad mėsos analogai, kuriuose buvo panaudotas 6% išspaudų, ekstruduotų 120 °C temperatūroje, 45% ir 60% drėgmėje kiekis, pasižymėjo geriausiomis funkcinėmis technologinėmis savybėmis. Tekstūros profilio tyrimų duomenys parodė, kad pridedant ekstruduotų išspaudų į matricą, pagerėja matricos tvirtumas, elastingumas ir tamprumas, lyginant su kontroliniu mėsos analogo bandiniu. Spalvos koordinačių tyrimų ir juslinės analizės rezultatai parodė, kad mėsos analogai su išspaudomis buvo gerai įvertinti, priimtini ir yra tinkamas produktas, kurį galima toliau vystyti ir pateikti vartotojų rinkai.

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LIST OF ABBREVIATIONS

AOAC – Association of Official Agricultural Chemists

BAPNA – Na-benzoyl-L-arginine 4-nitroanilide hydrochloride

DMSO – Dimethyl sulphur oxide

EFSA – European Food Safety Authority

EU – European Union

FAO – Food and Agriculture Organisation of the United Nations

FDA – Food and Drug Administration

FFA – Free fatty acids

GMO – Genetically modified organisms

HCl – Hydrochloric acid

HMEC – High moisture extrusion cooking

ISO – International Organisation for Standardisation

LDL – Low-density lipoprotein

MUFA – Monounsaturated fatty acids

NaOH – Sodium hydroxide

OBC – Oil binding capacity

PUFA – Polyunsaturated fatty acids

SEM – Scanning electron microscopy

SME – Specific mechanical energy

SPI – Soy protein isolate

TPA – Texture profile analysis

WHC – Water-holding capacity

INTRODUCTION

Consuming meat as a part of the diet has been considered healthy in recent times. However, producing meat in large numbers and consuming it worldwide has also given rise to environmental problems. Even though meat-eaters know the problems, the idea of consuming a lower amount of meat is met with dislike [1]. Despite the resistance towards non-meat consumption, many people around the world are moving towards accepting a meatless diet. In past five years, the population of vegans with in the United Kingdom climbed from 150,000 to 542,000. In the United States, around 19.6 million people consumed a vegan diet in 2017 [2]. There are a huge variety of vegetarian meat alternatives available in the market. Vegan burger and patty substitutes are often formed of soy or wheat protein, whereas vegetarian burger and patty substitutes are created from egg protein. [3]. The ingredients used in the meat alternative foods are healthy, having various benefits. They also make the choice of consumers higher, which can help the public's overall well-being [4]. One such product that was developed to counter high meat consumption was the meat analogues. They've been created to mimic the structure, appearance, and flavour of meat. It also has high fibre and low fat-content intake, sustainable results and diversity in diet. Replacing the diet with meat substitutes gives rise to intake of antioxidants or fibre, which are not usually present in meat [5]. They are usually produced from texturised vegetable proteins like soy, pea, wheat, faba bean and other plant-origin sources. Meat analogues can be made more sustainable and nutritious using plant origin press-cakes in the product matrix. Press-cakes are by-products of plant origin drink production used as animal feed or for producing biofuels. In open areas, an immense quantity of by-products is disposed of, contributing to environmental problems. Such press-cakes are rich in chemical composition, enabling them to manufacture valuable food items. It is possible to use them for formulating functional foods [6]. These rest raw materials can effectively boost the physio-chemical and functional properties of food product with suitable treatment methods [7]. Extrusion cooking has been used in industry to bring new products, and it has also been applied for vegetable and plant proteins.

Therefore, the aim of the master thesis was to understand the changes that occur in plant origin by-products when it undergoes the process of extrusion and the benefits of including the extruded press-cakes in the meat analogue matrix. The following tasks were carried out to achieve the main outcome:

1. Selection of the plant origin press-cakes for pre-testing of extrusion process based on their anti-nutritional factor and functional properties.
2. Pre-testing of extrusion process for soy (from German and Lithuanian supplier) and oat (German supplier) press-cakes.
3. Carrying out a low moisture extrusion process for the selected press-cakes with different extrusion temperature and humidity.
4. Evaluation of the chemical composition, including anti-nutritional factors and functional properties of the extruded soy and oat press-cakes.
5. Designing of the meat analogue matrices with industrial texturised soy base and inclusion of the extruded plant origin press-cakes.
6. Selection of the best meat analogue matrix based on the chemical, functional and sensory properties for further research.

1. LITERATURE REVIEW

Products that are developed to imitate the structure, appearance and flavour of meat are known as meat analogues. There have been many meat analogues, consumers due to beliefs, nutrition, and social reasons. The risk of *Escherichia Coli* or *Salmonella* infection is lowered. There is a cause for the reduction of animal slaughter for making meat-based food [5]. With various advantages, there are also a few disadvantages that follow. Some of them being the lack of Vitamin B12, which is only present in animal sources. At present, meat analogue products are more expensive as compared to products made from meat origin. The level of sodium content in these products is higher due to heavy processing. One concern that can arise while consuming these products is the risk of allergens caused by wheat, soy or nuts. Also, there is a risk of allergies that can occur from the additives used [8].

1.1. Protein sources from meat, plants and the rest raw materials

1.1.1. Meat

A new study released by the FAO found that meat consumption globally in 2016 accounted for 317 million tonnes. It is one of the essential elements of the human diet. Around 30% of the available calories in the EU are provided by animal products, a vital nutrient source [9]. Meat with 28 gram of protein per day is the primary protein source, followed by grain and dairy products. Proteins from aquatic animals, as well as plant-based sources like lentils, have been steady over time but less steady than meat. Previously, the two primary meat protein sources were cow meat and pig meat. From the 1960s to the 1990s, pig meat quantities increased steadily and remained constant, the demand for cow meat was seen only until 1990s and later dropped considerably [9].

On the other hand, the accessibility of protein from poultry meat has steadily increased over the years, surpassing that of cow meat and approaching that of pigmeat. The rising share of chicken meat and declining proportion of bovine meat is largely due to rising meat prices [9]. A range of factors influences consumption of some of them being fraudulent adulteration, substitution, and mislabeling activities which contribute to unfair competition in the meat sector [10]. Consumption of meat provides essential nutrients in the modern diet, such as proteins, minerals, and vitamins. Animal farming negatively affects greenhouse gas emissions, water footprint, contamination, and scarcity, leading to significant ecological impact [11]. Meat products that are processed have been created in response to the requirement to create meals with an extended shelf life. The processed meat products shelf life and physicochemical properties are based on the manufacturing process and their composition. These processed meat are usually sold in the form of slices. The handling and preservation of sliced products is critical for maintaining good food safety and quality in terms of biological and chemical dangers during manufacturing and storage [12]. Cooking tends to add a more rigid texture to meat by coagulation of myofibrillar and constriction collagen. Different physical, chemical, and mechanical methods for softening the texture of intact meat have therefore been identified, such as the use of thermal treatment, the treatment of exogenous enzymes such as papain, bromelain and actinidine, the use of high-power ultrasound, high-pressure treatment, electrical stimulation and blade tenderisation [13].

1.1.2. Protein sources from plants

1.1.2.1. Soybean

Glycine Max L.Merrill, also called soybean, is a part of the Leguminosae family and Papilionaceae subfamily. It is the most commonly grown grain in the world. Soybean uses nitrogen present in the atmosphere through nitrogen fixation, due to which its dependence on nitrogen fertilisers is low. Its primary use for commercial purpose was for its oil, but it is also used as a protein crop in recent years [14]. Soybeans contain 25-45% of proteins, and their seeds consist of 18-23% oil which makes it an important crop. Cultivation of soybean in four central countries like the United States, Argentina, Brazil and China yields 90% of the world's output [14].

On ripening of the soybean, the digestion of protein occurs, and the amino acids that are released are taken to the place of growth of the seedling. Soybeans have 2S albumin protein stored but only in small numbers [15, 16]. Soybean consists of about 35% of the carbohydrates, out of which most of them are non-starch polysaccharides. They also have oligosaccharides like stachyose and raffinose present [17]. They mainly contain insoluble dietary fibres. To utilise it as a dietary fibre supplement, polysaccharides that are soluble are used to change the physical properties of various products [18]. About 19% of oil is present in soybean, out of which the major component is triglycerides. They are distinguished by the high content of polyunsaturated fatty acid (PUFA), 55% of linoleic acid and 8% of α-linolenic acid, respectively. One of the essential fatty acids is the Linoleic acid which gives out essential nutrients and physiological uses [19]. Soybean is rich in Vitamin B in contrast to oats, even though it needs B12 and Vitamin C. Tocopherols, present in soybean oil, are useful for prevention of carcinogens [20, 21]. They contain α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol in small amounts. About ~5% of minerals are present in soybean. It also consists of potassium, phosphorus, calcium, magnesium, and iron. Soy ferritin can enhance good amounts of iron [21].

The soybean contains a about 3 milligram per gram dry weight of isoflavones which is a sub class of flavonoids [22]. Soybean contains three kinds of isoflavone namely daidzein, genistein and glycitein in three forms of glycosidic linkage together with their aglycone structure. They add to more than 90 per cent of total isoflavones. They apply numerous effects on health and are of great help for keeping up a healthy lifestyle [23]. Soybean oil consists of 0.3-0.4 grams of plant sterols per 100 grams. The significant parts of soy sterols include 53-56 percent β-sitosterol, 20-30 per cent campesterol, and 17-21 per cent stigmasterol [22]. Phospholipids make up 1-3 per cent of soybean oil, including 35 percent phosphatidylcholine, 25 per cent phosphatidylethanolamine, 15 percentage of phosphatidylinositol, and 5-10 per cent phosphatidic acid. During degumming, the phospholipids are removed from the oil and used as a food enhancer. They are polar triglycerides that contribute to the cell's structure [20, 21].

Fig. 1. Extruded soy protein isolate sample [24]

Soybeans, when defatted or dehulled, are subjected to protein isolation. Soy proteins are present in various food items, especially in conventional soy foods like soy milk, soy sauce or tofu. These foods utilise the soybeans as a whole as the source material, and food with soy protein in concentrates form as ingredients [25]. One of the significant product of soy includes the soy protein isolate which consists of 85–90 per cent dry protein. The composition and qualities of SPI vary substantially depending on the primary sources, processing conditions, and even the producer due to the difficulties of development. pH is the most influential factor that influences the configuration of soy proteins; pH-related structural changes occur at the quaternary and tertiary stages. Subsequently, commercialised SPI is typically not dissolvable in water [26]. Soy protein is a protein that is consumable by people after heat processing. When separated from soybeans through crushing or extrusion, as shown in **fig. 1**, it depicts the presence of various amino acids. Its primary form is called a soybean meal, and it consists of protein that is less than half by weight [26].

Soy flour or soy protein concentrates are transformed to texturised soy protein through the process of fibre spinning or extrusion to give it a texture that is similar to meat in order to produce meat analogues. All basic amino acids are found in texturised soy protein. The primary limiting amino acid is methionine, but significant amount of lysine can also be found. To make texturised soy protein products have the same PER value as casein, methionine can be added. The manufacturing processes for textured soy proteins allow for the incorporation of important nutrients into the food. [27]. Soybeans contain some non-nutritional components together with the nutritional and bioactive compounds, which could make the consumer's body metabolism alter and induce a negative impact on the protein's nutritional qualities [28]. A diet containing soybean will help lower blood pressure, and the According to the Food and Drug Administration, including 25 grams of soy protein a day in a low-fat, low-cholesterol diet can help reduce the risk of chronic disease. Soy can also help with menopause symptoms and osteoporosis risk. Breast, cervical, and prostate cancers can be prevented by consuming soy products [29].

1.1.2.2. Pea

Pisum sativum L., commonly called a pea, is cultivated principally in colder regions. Various cultivars of pea have been grown to produce full-grown, dry seeds, delicious and well-developed seeds [30]. Planting can be done from winter to late spring based on the regions grown as it is a yearly plant. The regular pea weighs between 0.1 and 0.36 gram [31]. The tender peas are utilised as a fresh vegetable. Field peas are used as the base for porridge and soup which are the staples of medieval cooking. Low-growing and vining varieties of peas are available. Vining cultivars produce slender shoots from leaves that wrap around any available support and grow to a height of 1-2 metres.

Based on the variety and weather conditions, field peas contain 20-30 per cent protein. Albumins and globulins make up 18-25 per cent and 55-80 per cent of the overall protein content respectively. These are the main protein forms in peas, with convicillin, prolamins and glutelins present in small quantities. Depending upon their sedimentation coefficients, globulins can be categorised as legumin (11S) and vicilin (7S). Pea is a 320-400 kDa protein that is made up of six unit pairs, each of which has a disulfide connection between an acidic and a basic constituent. Vicilin has three subunits since there are no disulfide linkages accessible [32]. Tryptophan, lysine, and threonine are more abundant in pea albumin, but arginine, phenylalanine, leucine, and isoleucine are more abundant in globulins. Peas are deficient in methionine and cysteine, but abundant in lysine, like many other grain legumes, therefore their essential amino acid distribution complements cereal grains [32]. Pea seeds are mostly made up of oligosaccharides, monosaccharides, polysaccharides, and disaccharides, and include 60-65 per cent simple sugars [33]. Peas also have a high dietary fibre content, which includes cellulose, pectin, hemicellulose, gelatin, mucilage, lignin, and starches. Depending on the variety, season, and locality, dry peas provide 17-27 per cent nutritional fibre [33]. Pea seeds contain 5-6 per cent sucrose and raffinose in terms of sugars [35]. Sucrose ranges from 2.2 to 2.6 per cent, while oligosaccharides, such as stachyose, range from 1.3 to 3.2 per cent, verbascose from 1.2 to 4.0 per cent, and raffinose from 0.2 to 1.0 per cent, depending on varietal and climate [33]. Pea seeds have a lipid content ranging from 1.2 per cent to 1.8 per cent, depending on the varietal, with oleic acid accounting for around 25 per cent of unsaturated fats and 50 per cent of linoleic acid. In addition to vitamins and minerals, peas are high in folic acid, pyridoxine, pyridoxamine , niacin, pyridoxal and riboflavin [33].

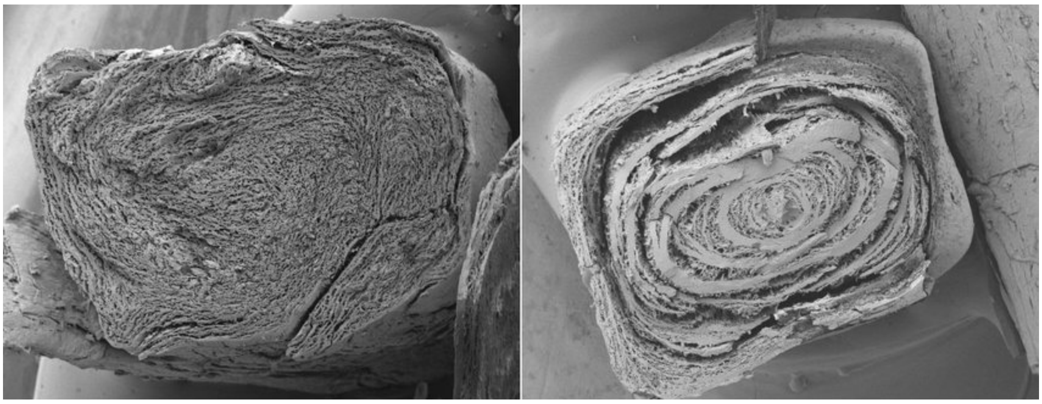

Fig. 2. Extruded pea flour [34]

Peas can be separated into carbohydrate, fiber, and protein-enriched items for use in the manufacturing of innovative foods, as shown in **fig. 2**, which uses pea flour to make texturised protein isolate using the extrusion technique. Pea protein isolates are a growing market for ingredients, partially because of consumer preferences and comparatively low costs compared to proteins extracted from animals. Pea protein is high in protein and carbohydrate, among other elements. Pea protein is also low in lipids and contains a variety of important vitamins and minerals [35]. They are not constant, despite the fact that they are high in protein. It is varied and is influenced by genetic variables as well as environmental influences such as soil and the climate in which peas are grown [36]. To suit human dietary demands, pea protein contains all necessary amino acids [37]. Peas typically have a protein content of 23.1–30.9 per cent, a fatty acid composition of 1.5–2.0 per cent, and minor elements such as nutrients, phytic acid, saponins, antioxidants, minerals, and oxalates [37]. They also comprise several protein types, including globulin, albumin, prolamin, and glutelins [37]. Albumins and globulins make up the majority of the peptides in the pea, accounting for 10-20 per cent and 70-80 per cent of the total protein [36]. The albumins are water-soluble and are thought to be digestive and enzymatic proteins, whereas the globulins are salt-soluble and are thought to be storage proteins. Legumin and vicilin are two types of globulins found in the 11S and 7S protein classes, respectively [38]. Vicilin proteins are trimers, while legumin is a hexameric polypeptide [38]. Pea proteins attract the food sector because of their low allergenicity, health benefits, and non-GMO nature. Peas include anti-nutritional elements that may impede digestion and have other possible negative consequences, yet they are nevertheless considered nutrient-dense foods that provide health advantages beyond basic nutrition [38].

1.1.2.3. Wheat

Triticum Spp. commonly called wheat began to be grown in the Mediterranean, and the Middle Eastern regions. Wheat is accustomed to cold, dry, and moderate environment. Conventional wheat is divided into five groups, each with different processing and end-use properties. Wheat has a dry weight of 31–35 gram per 1000 pieces and comprises roughly 80 per cent starch, 15 per cent protein, 2.5 per cent lipid, and 3.5 per cent fiber. [39] Amylose is found in 25–28 percent of wheat starch molecules. A-starch and B-starch particles are two types of molecular chains that are distinguished by their size. A-starch particles are large, measuring 20–45 micrometres and having a lenticular form, whereas B-starch granules are small, measuring 2–8 micrometers and having a globular structure. [39]. Wheat is a part of the genus *Triticum* of the family *Gramineae*. The source of wheat that are refined species began in ancient times. Development of wheat is found to have begun in Syria and the region once part of Palestine of the Middle East [40]. White and red wheat are the popular varieties of wheat. It comes in a variety of shapes and sizes. Dark, yellow, and blue wheat are further commercially insignificant yet promotes well-being. [40].

Fig. 3. Extruded wheat protein sample [41]

The wheat protein (**fig. 3**) belongs to one of the plant proteins most regularly used in food products. The protein level of completed wheat gluten items is regularly 75–82% (dry mass). Vital wheat gluten, texturised wheat gluten, and isolated wheat gluten can be utilised in meat imitation products. When hydrated, texturised wheat gluten has a structure that can be adjusted to imitate the visual and texture of meat. It has a good water-holding capacity [42]. Gluten protein usefulness relies upon the wheat source and the process cycle (segregation, drying and extrusion). Gluten protein is prominently appropriate for use as an additive without meat in meat items because of its unique properties. When blended with water it, result in viscoelastic mass. Specific significance to the meat manufacturers is the blending and film formation qualities of gluten, interacting with myosin [42]. Wheat proteins have been characterised traditionally based on solubility. Albumins are dissolvable in the water, while the globulins are dissolvable in the salt solution. On the other hand, gliadin dissolves in 70% ethanol, and glutenin dissolves in weak acid or base. Gliadin and glutenin account for about 80% of the proteins present in flour and are available in equivalent amounts. Gliadin and glutenin are the most significant determinants of the valuable properties of wheat flour. [43] Even while the genetic traits and features of plants such as wheat can be quickly modified, the gluten-containing prolamins must be processed within the small intestinal lumen after consumption; nonetheless, they are lengthy amino acid units high in proline and glutamine that are difficult for humans to digest. Glutenin and gliadin are both comprised of the same repeating polypeptide chain. Gliadins are further divided into subfractions by their electrophoretic motility. Individual gliadin peptides have a variety of natural characteristics that can all be implicated in the pathogenesis of gluten-related illnesses [43]. Ideal dough properties rely upon a balance between gliadin which adds to the thickness, and glutenin, which adds to the quality and flexibility. It is the interesting blend of these properties that makes up the valuable properties of the dough [43]. Gluten-containing grains like wheat make up a considerable part of the advanced Western eating regime. This is, to some degree, because of their satisfaction, ease to harvest and use into a wide assortment of products, the enormous scope for production, and high content of nutrition by weight [43].

1.1.2.4. Oat

Avena Sativa is also known as oat, is a part of a grain of cereals harvested for its seed. Oat milk and oatmeal have been usually used as feed for the livestock, while it also is suitable for consumption by humans. When eaten regularly, it can reduce blood cholesterol levels [44]. The morphology of the oat plant shows that it grows up to 1.2 metres in height. They have straight, smooth and cylindrical culms and a system of the fibrous root. The Leaves have a length and breadth of 150-400 mm and 60-80 mm, respectively [45]. Two overlapping husks make up a spikelet. Even though oat is a part of cereal grain, it belongs to only 1% of the production of cereals in the world. It is used for food and feed as it has good nutritional value and some additional health benefits. It can lower heart-related diseases and effectively enhance the response of blood glucose level. The biologically active compounds present in oats include β-glucan and phenolic compounds such as avenanthramides. The microstructure of oat gets affected due to processes like milling and food preparation, which also change the functional properties like fibre, protein, and starch components [45].

Fig. 4. Extruded oat protein [45]

The usage of oat and its products are highly seen in varied commercially available food products. The heat processing of oat protein (**fig. 4**) creates a unique flavour and aroma, paving the way for its use in baked food and cereal products. Flakes are made from the processing of whole grain oats and sectioned groats. The whole grain oats take a longer time to cook compared to the sectioned oats. Flour made of oat is used in many food products as they consist of antioxidant activity, leading to lipid oxidation during storage. Ground goats, when sieved coarsely, give oat bran which is used in cereals, biscuits, muffins and bread. Oat protein has large amounts of carbohydrates up to 66.3 g per 100 g portion, giving rise to 389 kcal. It also consists of 16.9 g and has minerals like magnesium, phosphorus, potassium, manganese, iron and calcium present [46].

The by-product rich in protein content produced from the Beta gluten is called oat protein concentrate. These concentrates are sustainable while also improving the economy [46]. Protein concentrate with 59 per cent to 75 per cent protein content was provided by wet milling of ground oats. The following fractions make up oat protein concentrate: 78 per cent alkali-soluble, 11 per cent alcohol-soluble, 2.9 per cent salt-soluble, and 4 per cent suspended and non-protein nitrogen. Compared to wheat, oat protein concentrate has a lesser alcohol-soluble protein content and a greater salt-soluble protein content [47]. Due to its high rheological properties, it is used as a thickener in sauces, soups, gravies and also as a meat extender. They are also utilised in making products which have less calorie and a high intake of fibre. Researches have depicted that oat proteins can be added to meals for celiac patients as the protein prolamine gets denatured during the thermal processing of groats. In Sweden, a milk drink made from oat protein is used as a supplement for lactose intolerant. Oat protein is also utilised as a substitute for fat in some low-calorie products [48].

1.1.2.5. Faba bean

Vicia Faba L, also called fava bean, is a part of the Fabaceae plant family. Though its origin is uncertain, it is harvested and used for consumption by humans. It is also utilised as a cover crop. Crops that are smaller in size and have harder seeds are used as a horse or other animal feed. These are known as tick bean or field bean [49]. It is harvested as a legume as it has an excellent yield and a high amount of protein level, about 25-40% [50]. Faba bean is a plant legume that can grow up to 1.5-2 meters in height. It consists of taproot and various fibrous lateral roots, which can cover 0.9 meters of soil. The stems of faba beans are rough, cylindrical and unbranched. They consist of tillers that are grown from their basal nodes. The leaves are 80 mm in length, but their leaflets have length and breadth of 60-80 mm and 20-40 mm, respectively. These legumes bear 30-40 mm long and are white with black or purple spots. Their fruit is cylindrical pods that are 100 mm long and has a diameter of 10-20 mm. These pods are green in colour when they are young and become darker in colour during maturity. These pods consist of oval beans, which have a unique hilum on their side [51]. Due to its ability to grow under low irrigation, it is grown in areas that have arid and semi-arid lands. Faba bean seeds are eaten dry, canned, froze or fresh. Countries that produce faba beans include China, Ethiopia, Australia, Egypt and some European countries. High consumption countries are reflected based on geographical distribution and breeding programs [51].

Fig. 5. Faba bean protein concentrate [51]

The seeds of faba bean are rich in carbohydrates, vitamins, fibre, minerals and proteins. However, its biological value is lowered due to the presence of tannins, phytic acid, lectins, activity of trypsin inhibitors and factors like vicine and convicine, which induce favism. In order to use these legumes effectively, it is essential to remove the antinutrients. These antinutrients can be eliminated by heat treatment like cooking, extrusion or boiling. They can also be eliminated via dehulling or soaking. However, the use of these treatments affects the physio-chemical changes in starch and amino acids. They also cause loss of vitamins and minerals, which fluctuates the nutritional properties [52].

The extraction of protein from fava bean flours provides fava bean protein isolates or concentrates. Concentrates can be made with either dry or wet processing (**fig. 5**), while isolates are only made with wet processes. Protein concentrates comprise 65 to 90 per cent (w/w) water or alcohol miscible proteins, as well as carbohydrates, fibres, and flavourings. Isolates, on the other hand, have a minimum of 90% proteins without fibres. With the increased proportion of protein extraction, V. faba components display a decline in aqueous solubility, which is followed by an improvement in fat and water binding capacities, as well as oil emulsification capability. Vegan/dairy-free foods containing V. faba proteins accounted for 0.45% of all foods containing plant proteins. V. faba components have a huge amount of potential in food products, particularly when it comes to partially or fully replacing conventional food ingredients in meatball analogues, sauces and sausages [53]. Fava bean also has some health benefits. It stores a high content of L-Dopa in various parts. L-Dopa is a dopamine generator that is frequently prescribed to treat Parkinson's disease and hormonal abnormalities [53].

1.1.3. Protein sources from the rest raw materials

The production of agri-foods, such as plant-origin drinks, has resulted in the creation of high amounts of biowaste called press-cakes. These press-cakes are used as feed for animals and the production of biofuels. An enormous amount of bio-products is disposed of in open territories, which prompts rise to environmental issues. These press-cakes are rich in chemical composition, due to which they can be utilised to make valuable food products [54].

1.1.3.1. Soy press-cakes

Soy press-cakes are the rest raw materials remaining from soybean after receiving the hydro-extractable fraction utilised to create tofu or soy drink. It is also known as okara, dreg or tofuzha. It consists of 10% fat, 50% fibre and 25% proteins [55]. Other components present include coumestans, phytates, lignans, phytosterols and isoflavones, which have health benefits like antioxidants, protection from heart-related diseases and chemopreventive properties for cancer. They also include 8% calcium, 7% iron, potassium, 6% magnesium, and some traceable sodium [56]. Soy press-cake has high nutritional value and can be recycled or reused for the recovery of essential components. However, instead, they are utilised as animal fodder or dumped as waste. The amount of chemical composition depends on the hydrophilic phase extracted and the addition of water for residual extraction. It also depends on the methods of production and cultivars [56]. There are some antinutrients present in soy press cakes as well. These include trypsin inhibitors, phytic acid, tannins and some oligosaccharides, which have to be lowered to improve the digestibility of the press-cakes for consumption [56].

1.1.3.2. Oat press-cakes

The by-product that arises due to the processing of oat drink results in the formation of oat press cakes. It is a good source of soluble fibres, namely beta-glucan, related to cholesterol reduction and cardiovascular disease prevention [57]. Oat press cakes contain about 12 to 24 per cent of total dietary fibre, based on variety, planting region, ecological conditions, and fertilisation. Soluble dietary fibre accounts for around half of total dietary fibre, whereas insoluble fibre accounts for the other half. Beta-glucan is the most essential part of soluble fiber. Insoluble fibres are made up of cellulose, lignin, and certain hemicelluloses. With a high water hydration potential, insoluble oat fibres are perfect bulking agents. They can be used in minced bovine and pork sausage products as carriers of minor fibre components, lipid replacers and absorbents of various flavor components [58]. Oat press cake consists of minerals like calcium, iron, magnesium, potassium and sodium, along with the composition of carbohydrates, proteins and fats, making it an ideal addition to meat analogues. [58] The phytate content in oat press-cake is relatively high compared to trypsin inhibitors. These phytates bind to minerals preventing their absorption by the body. Therefore, these antinutrients must be removed for successful mineral absorption [58].

1.1.3.3. Wheat press-cakes

The processing of wheat-based drink forms by-products called wheat press-cakes, and it has long been used as a part of animal feeds. Wheat press-cake has three physiological impacts: nutritional, physical, and antioxidant benefits in the digestive tract due to its phytochemical contents.The press cakes from wheat have higher antioxidant activity [59] They contain phytic acid, lignans, phenolic acids, vitamins, and minerals like calcium, iron, magnesium, potassium, and sodium other components, including carbohydrates, proteins and fats. Wheat press-cake constituents also provide beneficial effects on human health, such as preventing cancer and type 2 diabetes. They contain antioxidant capabilities, according to many research, including free radicals, metal ions chelates, and antioxidant enzymes [59]. Wheat press-cake is also rich in minerals like zinc and copper. It also contains more than half required intake of selenium per day and more than twice the daily value of manganese. It is nutrient-dense while still being low in calories. It is low in total conetn of fat, saturated fat, and cholesterol, and it is a decent source of plant-based protein, with 16 grams per 100 grams. Studies have shown that wheat press cake can minimise digestive symptoms like bloating and discomfort and is more efficient than other insoluble fibre like oats and certain fruits and vegetables at growing faecal bulk. It is also high in prebiotics, which are nondigestible fibres that serve as a food source for good gut bacteria, helping them multiply [59].

1.1.3.4. Coconut press-cakes

The coconut press-cake is formed as a by-product of the production of coconut drink. It is usually discarded as waste but, in reality, is a cheap alternative protein source in countries where the growth of coconut is high. The FAO has estimated that the total output of coconut in 2009 was up to 61.7 million, out of which 1/4th has been utilised to produce coconut milk. Even Though coconut does not consist of a good amount of protein, but its availability at no cost makes it an area of interest. It consists of 3-4% protein which can be received through the mechanical pressing of the endosperm of the coconut [60].

1.2. Traditional meat burgers and the use of plant proteins as analogues and extenders

1.2.1. Traditional meat burgers

Burgers are among the most common meat products sold globally, with 70 per cent meat muscle and 25–30 per cent fat as the main ingredients. Commercial burgers are typically made with beef (**fig. 6**) or chicken, but newer formulations contain various red meats such as deer [61]. Due to its simplicity, the meat burger is eaten by tons of people worldwide [80]. They are high in proteins, lipids, vitamins, minerals, and essential amino acids while being readily available, inexpensive, and ready to eat. However, a connection between comsuming meat and health issues like cardiovascular issues and hypertension exists [62]

Fig. 6. Traditional meat burger sample [63]

These health concerns worry a wide range of population, especially children around the world. Due to microbial activity and lipid degradation, they have a short shelf life. These factors affect the production of off-odours, rancid tastes, colour degradation, nutrient loss, and the presence of potentially toxic compounds, all of which make meat unfit for human consumption [64].

1.2.2. Meat extenders

Meat extenders are substances that do not contain meat and make meat products cheaper and protein-rich. There was insufficient demand for extended products in the high-end market as their sensory properties did not match meat products. However, it has been progressing in recent years due to spice mixtures or suitable plant-based additives, which can enhance the sensory properties of extended meat products, making them attractive in the low-end market and could lead to further advancement. To support healthy food production, new additives are incorporated into the meat industry from the dairy, bakery, and other food sectors. These additives aid in improving the fibre content of meat products [65].

Fibre additives have functional properties which increase water binding capacity and in the formation of a product having a creamy texture. Additives are also used to increase certain minerals like iron, magnesium and calcium in meat products. Production of blends containing beans, grains and manioc together with meat, internal organs and fat belong to the class of extended products. Burgers are essential blends of ground meat and animal fats, including the classic hamburger, which is only made of beef with low-fat content without extenders or binders. As there are no specific product cohesiveness or colour specifications, significant quantities of meat extenders are used in burgers [65].

The most widely used extender in the industrial meat production of burger patties is medium to coarse granular shaped soy concentrate as texturised vegetable protein, which has a meat-like texture when rehydrated. Breadcrumbs, rice, cassava, or potatoes are used as fillers in cheaper burger matrices and texturised vegetable protein as an extender. Hamburgers with 7.5% texturised vegetable protein, or 7.5% texturised vegetable protein, and 10% cassava starch was still considered equal to full-meat burgers in consumer acceptability tests in some regions. In burger-type products, cellulose fibre additives such as bamboo and potato fibres are also widely used with extenders such as texturised vegetable protein [65].

1.2.3. Meat analogues

Plant proteins like soy, wheat gluten and others are used to formulate meat analogues [66]. Meat analogues can be produced in circular disks, thin sheets, patties, long strips and other desired shapes. Their water-absorbing capacity increases at least three times when cooked in hot water for about 15 minutes. Their layer is similar to that of muscle meat. Analogues resembling products made from coarse ground meat usually consist of texturised proteins available in many colours and sizes. Characteristics found in products made of meat should be precisely added by the product developer to the analogue. Texture and flavour that are acceptable show significant advancement challenges [66]. Proteins from plant origin foods cost less than meat proteins. The high cost of meat has led the food industry to develop non-meat based proteins. Adding further, the availability of proteins from animals is low in many under-developed countries, due to which malnutrition of protein-energy is one of the major problems being faced. Due to diseases from animals like mastitis, low availability of animal protein and high demand for products with a low amount of saturated fat, demand for halal food due to religious belief and economic reasons, the pressure on the consumption of non-meat proteins is high. Some researchers believe there might be a rapid end to the meat business due to increasing vegetarianism and the influence caused by the animal rights movements [67].

Fig. 7. Meat analogues from plant protein [67]

The traditional method of producing meat analogues is carried out in two stages: preparation of emulsion and chunk formation. Blending, slicing, and emulsifying proteins, lipids, salts, and other components to generate a protein matrix that incorporates lipids and insoluble particulates is how an emulsion is made. The prepared emulsion is thermally treated under pressure as required. The pressure causes the protein chain to arrange and orientate, giving rise to a three-dimensional network. Denaturation of proteins occurs due to heating which makes the emulsion irreversible to maintain the shape of the final product. Meat analogues are also produced by the process of extrusion and fermentation. Extrusion has been used for many years. Meat analogues are produced at low moisture using a single screw extruder or high moisture using a double screw extruder [67]. **Fig. 7** depicts the texture of meat analogues produced from plant proteins. The fibrous structure is similar to that of traditional muscle meat. Many companies have started producing plant-based meat analogues, and one such company which offers soy-based burgers in retail stores is called Before the Butcher. The company beyond meat makes products from pea protein which are gluten-free and contain no GMO. Another very famous company, BurgerKing, introduced an impossible whopper, a burger made from meat analogue. It was quite successful and well accepted by the consumers. This paved the way for other companies like Dunkin doughnuts and KFC to enter the meat analogues market. In 2019, Nestle entered the plant-based meat analogues market by developing their "Awesome Burger" [66].

1.3. Treatments to optimise composition and functional technological properties

One source of proteins from plants will generally not consist of a total amino acid profile. Additionally, if these proteins are not cooked or treated, the digestibility lowers because of the antinutrients like tannins, trypsin inhibitors and lectins, which stop or hinder nutrient absorption. Mixing two or more vegetable proteins like grains and legumes can ensure that the total amino acid profile can be reached; however, the protein digestibility will not improve [68]. Even if the formulation of some products made of plant protein is carried out by the addition of enzymes to enhance protein digestibility, there is a possibility that these enzymes may be eliminated by stomach acid before they are functional in the intestines. The process of extrusion and fermentation helps in optimising digestion, nutrition and tolerability of plant protein sources, making it safe and healthy for consumption [68].

1.3.1. Extrusion cooking

With increasing demand for extruded products, there has been a rise in understanding and controlling the product's structure, its usefulness and health benefits that are brought by the extrusion process on food. An extruder could be considered a complex multivariate bioreactor where food material undergoes heating and applied stress created by the pivot of screws. These applied stressed give rise to changes in the material structure physically and chemically via melting, gelatinisation, denaturation, fragmentation, oxidation of lipids and flavour developments. **Fig. 8** displays the extrusion parameters, the processes that occur and the final product structure. The food extrusion process is beneficial, making it appropriate to manage the characterisation and control of the method and formed products. The extrusion process is of low-cost having various importance like the efficiency of the energy, absence of effluents needed and adaptability to a wide variety of ingredients and the structure and texture of products that can be created [69]. Extrusion cooking allows for the manufacture of meat analogues with the structure of muscular meat generated from plant or animal proteins [69]. Most commonly used raw materials for the meat analogue process include extrusion of soybean proteins, gluten from wheat, cottonseed proteins and proteins from other plants [70]. The process of extrusion is further classified into two types, namely: High moisture extrusion and Low moisture extrusion.

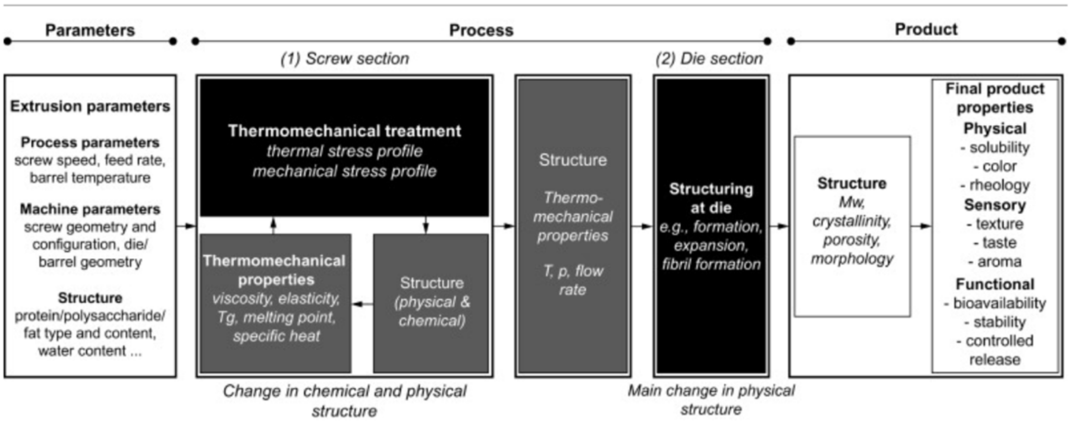

Fig. 8. Extrusion process stages [69]

1.3.1.1. High moisture extrusion cooking

The high Moisture Extrusion Cooking (HMEC) method is one promising breakthrough for extracting meat-like fibres using plant protein. Changes in moisture, temperature, pressure, and shear factor cause protein plasticization and texturization in a long cooling dye [71]. The combination of these process parameters causes molecular modification and synthetic reaction in the protein molecules, resulting in the three-dimensional configuration of the protein particulates being intact after extrusion [71]. The texture of high moisture meat mimics, which resembles muscle meat, is an important feature. The procedure of textural profiling was used to assess the completed protein products. The type of the fiber generated has been determined by assessing cutting efficiency in vertical and horizontal orientations, as well as flow direction in the cooling die [72]. Extruder reactions are used to evaluate the effects of protein components and extrusion factors on the resultant texture. When heat is applied to a protein, macromolecular changes occur, causing moisture in the extruder barrel and cooling die to soften, resulting in extrudate's textural qualities [72]. SME values are thus related to coherence within the extruder barrel which can be used to observe and compare changes in the proteinaceous matrices throughout extrusion texturisation. It is critical to understand the linkages between peptide source qualities, extruder reaction, and product quality in order to increase the durability of the fibre's process and creation during HMEC. Previous studies on reduced moisture extrusion of soy protein showed that the protein's qualities influence both the processing factors and the textural features. It's still unclear which qualities of the original source impact the extruder's reaction and result texture [72].

1.3.1.2. Low moisture extrusion cooking

Extrusion with low moisture content is widely used in the food industry [73]. Extruded foods are generally starch-based and low in calories [74]. Recently, there has been a surge in demand for extruded foods that are higher in protein and fiber [74]. During the process of low moisture extrusion, the components can undergo structural changes as a result of high temperatures and pressure, which can affect product attributes such as expansion and texture [75]. Adjusting the extrusion settings or feed content might also change their properties [75]. Whey proteins, wheat, and soy proteins are used to fortify the protein content of extruded mixtures. When a protein is added to the feed, it induces low expansion and have a more hard texture [75]. Proteins have been observed to affect the distribution of water and extensional consistency within the extruded melt, hence influencing extrudate behavior [76]. Throughout the extrudate, alterations in feed composition can affect the separation of continuous and dispersed phases [76]. Incorporating soy protein can result in denser cell walls, greater pores, and smaller pore diameters. A boost in breaking strength was seen with thickened cell walls. It has been postulated that at a certain protein concentration, the peptide acts as a filler in the starch matrix, reducing the flexibility of the mix and influencing the expansion [76]. Insoluble fibres, like protein, reduce extrudate expansion, and several fiber sources have also been investigated for extrusion of fortified fiber, such as cauliflower by-products, soy fibre, and sugar beet fibre [77]. SEM was used to investigate the smaller cell length and disintegration of air cells with fibre addition. Because it comprises thermomechanical unwinding and cross-links, the alterations in the secondary, tertiary, and quaternary forms of the polypeptide are thermally and mechanically reliant [77]. The expansion factors are affected by protein-protein interactions and the influence on water distribution in the melt. The relationship between protein configurations and process parameters is intricate and intriguing [74].

2. MATERIALS AND METHODS

2.1. Raw materials

Two samples of dried soy press-cakes, namely Soy d and Soy t (from Berief Food GmbH, Germany and JSC Sojalita, Lithuania, respectively) and a dried sample of oat press-cake, namely Oat d (from Berief Food GmbH, Germany), were obtained for analysis. All samples were received and stored at room temperature. The production process of the soy and oat press-cakes are presented in **appendix 1**.

2.2. Extrusion process

Pre-treatment: Soybean samples were hydrated approximately to 30% or 60% moisture and oat samples to 30% or 45% moisture before the processing. Hydrated samples were allowed to equilibrate overnight at 4 °C.

Extrusion: The coarse raw press-cake powders (Soy t, Soy d and Oat d) were extruded using a ZE25Rx40D-UTXmi co-rotating twin-screw extruder composed of eleven segmented barrels (KraussMaffei Berstorff GmbH, Germany). The screws had a diameter of 26.6 mm, a length to diameter ratio (L/D ratio) of 40:1, and 100 rpm was set as the screw speed. The barrel diameter was 26.9 mm, and a die head was with three circular holes of 3.5 mm diameter. The extrusion was carried out under four different conditions for each raw material by varying processing temperature and initial sample moisture. Two different temperature profiles throughout the eleven barrels were set as follows: 18, 65, 85, 95, 100, 105, 110, 110, 110 , 110 °C and product exit temperatures (recorded) or 18, 65, 85, 95, 105, 110, 120, 120, 120, 120 °C and product exit temperatures were recorded.

2.3. Preparation of meat analogues

Meat analogues were prepared by addition of 3% (w/w), and 6% (w/w) extruded press-cake samples to the matrix, containing industrially textured soy protein, water, emulsion, and oil. Additional spices were not added to the meat analogue matrix to recognise the base matrix's sensory properties. The emulsion was made by blending industrial emulsifier, water and oil. Two samples of each analogue were prepared to carry out an analysis of cooked and uncooked meat analogue samples. The prepared meat analogues were cooked in a fan-forced oven with cooking parameters set at 180 °C temperature for 20 minutes. It was adequate to reach an internal temperature of more than 75 °C. The cooked samples were wrapped using aluminium foil and placed in labelled trays. They were held at a temperature of 60 °C before using them for sensory analysis. The cooked samples were also utilised for texture and colour analysis.

2.4. Determination of the chemical composition of press-cakes and meat analogues

The chemical composition of the untreated press-cakes, extruded press-cakes and uncooked meat analogues were determined based on standard methodologies. The composition of the amino acid content of the extruded press-cakes and raw meat analogues were analysed using the A300 Amino Acid Analyser (MembraPure GmbH, Germany), which uses ion exchange chromatography including a reaction with ninhydrin in post-column and detection in the spectrum of the visible region. Analysis of fatty acid contents was carried out using gas chromatography methods (ISO 12966-1:2015 and ISO 12966-2:2017)[78]. Humidity was determined using the traditional oven method (ISO 712:2009) [79]. The saturated fatty acid contents were determined using the gravimetric method (AOAC 922:06

and AOAC 963.15:2003) [80]. Analysis of nitrogen and protein contents were carried out using the Kjeldahl method (ISO 20483:2006) [81]. The sugar contents were determined using chromatographic methods, and the salt content was determined using the titration method (ISO 3634:1979) [82]. Enzymatic gravimetric method was used for determination of fibre content (AOAC 985.29:1990) [83].

2.5. Determination of anti-nutritional factors for press-cakes and meat analogues

2.5.1. Determination of trypsin inhibitor activity

The anti-nutritional trypsin inhibitor activity was determined using the method described by Clifford et al. [84].

Sample preparation: 1 gram of each sample was weighed and transferred to a 50 mL tube. The samples were shaken with 50 mL of 10mM Sodium hydroxide. The pH of the formed slurry was adjusted using 1M NaOH and 1M HCl such that the pH lay between 9.4 - 9.6. The slurry was left overnight at 4C. Then, the samples were shaken and filtered out using the filter paper. The samples were now ready for further use.

Reagents: (a) Tris Buffer solution – 6.05 gram of Tris (hydroxy methyl) methylamine and 2.94 gram of Aqueous calcium chloride were dissolved in distilled water of 900 mL. The pH was adjusted such that it was 8.2 after the solution was diluted to 1000 mL. (b) BAPNA solution – 0.04 g of BAPNA was dissolved in 1 mL DMSO and diluted with Tris Buffer, which was previously heated at 37C, up to 100mL. This reagent was warmed at 37 °C when in use. (c) Standard trypsin solution – 0.04 g of bovine trypsin was dissolved in 1mM of HCL and diluted up to 2000mL.

Determination: Test tube of reagent blank-A was filled with 2 mL of distilled water, a test tube of standard -B was filled with 2 mL trypsin solution and 2 mL of distilled water, Sample blanks test tubes were filled with 0.33 mL of each sample with 0.66 mL of distilled water, and sample test tubes were filled with 0.33 mL of each sample with 0.66 mL of water and 2 mL trypsin solution. All test tubes were pre-heated for 10 minutes at 37 °C . Then, 5 mL of BAPNA solution was added and mixed. Once again, they were heated at 37 °C for 10 minutes. Then, 1 mL of Acetic acid was added to all test tubes to terminate the reaction. Trypsin solution of 2 mL was added to the reagent blank test tube and the sample blank test tubes. All the samples were centrifuged in Hettich Universal 320 R at 3000 x g for 10 minutes at 4 °C . After centrifugation, the absorbance of the samples was measured at 410 nm using the Hanover 3000 spectrophotometer. Three results of absorbance were noted and used for calculation of trypsin inhibitor activity:

$$TIA = \frac{2.63 * D * At}{S}\ mg/g \qquad (1)$$

Where,
D is the dilution factor
At is the change in absorbance
S is the weight of the sample

2.5.2. Determination of biogenic amines, acrylamide, peroxide values and free-fatty acids

The histamine, cadaverine, putrescine, tyramine and spermine content were determined by high-pressure liquid chromatography (ISO 19343:2017) [85]. The acrylamide was determined using gas chromatography methods, while the peroxide values were determined using iodometric determination with visual end-point detection (ISO 3960:2017) [86]. Determination of fatty acids and free fatty acids was via cold solvent method (ISO 660:2009) [87].

2.6. Colour analysis of extruded and non-extruded press-cakes

In a Minolta colourimeter (Minolta Colorimeter Model CR-400), colour measurements were made using an aperture of 8 mm in diameter. Relative to the illuminated area, the exposed area was sufficiently wide to prevent any light-trapping effect. A white tile was used to calibrate the instrument. After calibrating, the instrument was placed on different areas of the untreated and extruded press-cake samples. The results were recorded in terms of L, a and b values. Three measurements were taken for each sample and the average value was used.

2.7. Analysis of functional properties of non-extruded press-cakes, extruded press-cakes and meat analogues

2.7.1. Water holding capacity

Determination of water holding capacity was performed using the method described by Sadh et al. [88] with some modifications. 0.8 gram of sample was added to 15 mL pre-weighted centrifuge tubes. Then, the sample was diluted with 12 mL of distilled water and shaken manually for 1 minute. The tubes were centrifuged in Hettich Universal 320 R for 10 minutes at 3000xg. After centrifugation, the obtained supernatant was discarded, and the tubes were weighed again. The water holding capacity was expressed as a gram of sample bound per gram of sample. It was calculated using equation 2.

$$WHC = \frac{(Wst - Wt) - Ws}{Ws} \quad g/g \tag{2}$$

Where,
W_{st} is the weight of the tube with sample
W_t is the weight of the empty tube
W_s is the amount of sample used for analysis
WHC is the water holding capacity of the sample

2.7.2. Oil binding capacity

Determination of oil binding capacity was performed using the method described by Sadh et al. [88] with some modifications. 0.8 gram of sample was added to 15 mL pre-weighted centrifuge tubes. Then, 12 ml of rapeseed oil was added to the tube containing the sample and shaken manually for 1 minute. The tubes were centrifuged in Hettich Universal 320 R for 10 minutes at 3000xg. After centrifugation, the supernatant obtained was discarded, and the tubes were weighed again. The oil holding capacity was expressed as a gram of sample bound per gram of sample. It was calculated using equation 3.

$$OBC = \frac{(Ost - Ot) - Os}{Os} \quad g/g \qquad (3)$$

Where,
O_{st} is the weight of the tube with sample
O_t is the weight of the empty tube
O_s is the amount of sample used for analysis
OBC is the oil binding capacity of the sample

2.7.3. Bulk density

The bulk density of the untreated press cakes, extruded press-cakes and meat analogue samples was determined using the method described by Sadh et al. [88]. The bulk density was calculated as the ratio of the sample's mass per unit volume of sample (g/ml).

2.7.4. Cooking loss

The weight of raw meat analogue samples was noted. The meat analogues were cooked in a fan dried oven at 180 °C for 20 minutes. The samples were allowed to cool up to 60 °C. The weight of cooked meat analogue samples was noted. Calculation of cooking loss was done by subtracting the initial weight and the final weight, further dividing it by the original weight of the samples. The cooking loss was expressed in percentage.

2.8. Texture and colour analysis of meat analogues

2.8.1. Texture profile analysis

The texture profile analysis was carried out for samples of cooked meat analogues using Instron 3343 machine (Instron Engineering Group, High Wycombe, England), equipped with a load cell of 1kN. The samples were cut into 2.0×2.0×2.0 cm and were compressed perpendicularly using a cylindrical probe of 0.5 cm. The testing conditions were done via two cycles consecutively at a compression of 70% and cross-head movement at a speed of 1 mm/s. Calculation of hardness and chewiness was done following the method described by Bourne [89].

2.8.2. Colour measurements

In a Minolta colourimeter (Minolta Colorimeter Model CR-400), colour measurements were made using an aperture of 8 mm in diameter. Relative to the illuminated area, the exposed area was sufficiently wide to prevent any light-trapping effect. A white tile was used to calibrate the instrument. After calibrating, the instrument was placed on different areas of the cooked meat analogues samples. The results were recorded and compared with those of meat products. The results were recorded in terms of L, *a*, and *b* values. Three measurements on each sample were taken, and the average value was used.

2.9. Sensory analysis of meat analogues

A quantitative descriptive analysis of 15 point scale was performed by a consumer jury made up of 6 researchers. The jury analysed the produced meat analogues' perception characteristics and determined the optimal treatment conditions like suitable temperature and humidity for the extrusion process, a suitable amount of press-cake added in the matrix and optimal storage temperature of the raw meat analogues. The range of scores for the scale was between 1-15, where 1 = not intensive and 15 = very intensive. The overall acceptability was also reported using the 15 point scale based on appearance, odour, taste and texture, where 1 = not acceptable and 15 = highly acceptable. The jury's main goal was to produce sensory descriptors to describe the modelled meat analogues. The jury's sessions were conducted in the KTU Food Institute's sensory laboratory (Kaunas, Lithuania).

2.10. Statistical analysis

All the experiments were carried out in triplicates. The results were reported as mean ± standard deviation (SD) using Microsoft excel. The two-way analysis of variance (2-way ANOVA) was used for considering significant differences in extruded press-cake samples followed by a *post hoc* test using LSD (Least significant difference), and three-way analysis of variance (3-way ANOVA) was utilised to consider the significant differences in meat analogue samples to study the significance of independent factors as well as their interactions. At $p \leq 0.05$, the samples were considered to be statistically significant. The analysis was performed using IBM SPSS 27.0 software (IBM Corporation, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Process of extrusion and its effect on the colour measurements of plant origin press-cakes

The selection of soy and oat press-cakes were carried out based on their properties. These press-cakes consist of proteins, lipids, carbohydrates, and dietary fibres, which are major components required to enhance a product's nutritional value. However, they also contain anti-nutritional factors like trypsin inhibitor activity and biogenic amines, that need to be lowered before their usage. These plant origin press-cakes show good water holding and oil binding capacity due to hydrophilic chains and hydrophobic fatty acid chains. These properties need to be optimised before their use in the meat analogue matrix. Process of extrusion was carried out to optimise functional technological properties, such as water-holding, oil-binding, and sensory parameters of the press-cake samples and to check if the applied process can affect trypsin inhibitor activity which causes problems with digestion due to its anti-nutritional effect. The extrusion involved modelling the parameters: press-cake humidity and cooking temperature to get certain functional and sensory properties of the press-cakes. The experiment included the temperature-humidity models: T = 110 °C x H=30%; T= 120 °C x H=30%; T = 110 °C x H=60%; T= 120 °C x H=60%; T = 110 °C x H=45%; T= 120 °C x H=45%. The extrusion scheme and the process parameters presented in **appendix 2**.

3.2. The effect of extrusion on colour changes of the plan origin press-cakes

Colour can, in various ways, affect the experience of eating. When eating or drinking, most individuals first take visual details into account. In relation to the surrounding plate or jar, they consider how the food appears and rely on experience with similar foods before eating, making assumptions on the taste of the food and resulting satisfaction [90]. Colour measurements were performed in order to optimise the press-cakes so that when added to the meat analogue matrix, it does not influence the appearance of the final product but instead will create meat analogues having a close resemblence to meat burgers.

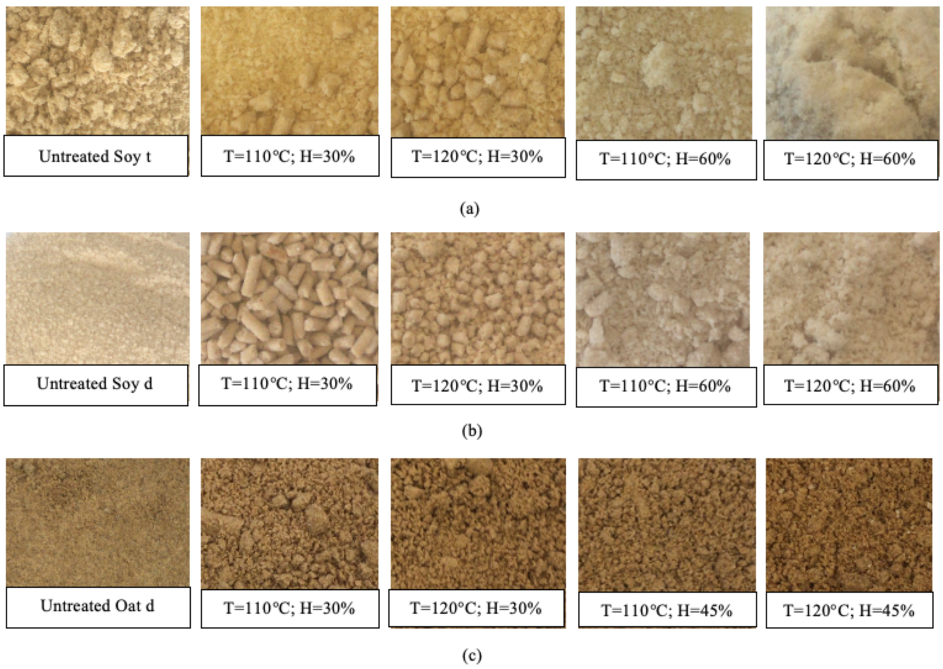

Fig. 9. Untreated and extruded press-cakes at different temperatures and humidities (a) Soy t (b) Soy d (c) Oat d

The colour of the samples was measured using the CIELab grading system, where *L* represents lightness, *a* represents the red or green coordinates and *b* represents blue or yellow coordinates. **Fig. 9** shows the colour of the press-cakes before and after extrusion. The results obtained were reported in **appendix 3**. The results depict that both untreated Soy d and Oat d press-cakes were brighter before undergoing the extrusion process. However, the extrusion process made the Soy t press-cakes brighter than their initial appearance. During the extrusion process, the colour changes were caused due to the Maillard reaction, caramelisation, hydrolysis, and pigment degradation [90]. The extruded Soy t, Soy d and Oat d press-cakes showed the maximum level of brightness at temperature and humidity of 110 °C and 30% with *L* values of 71.37, 59.90 and 57.66 respectively, whereas the press-cakes showed slightly darker appearance at the temperature of 120 °C and humidity of 45%. Lysine and other amino acids present in the raw material were likely to react with sugar reduction, preferred by processing conditions that resulted in the extruded products being darkened, as reported in a study conducted by Gutkoski and EL-Dash [91].

The values of brightness were all statistically significant as $p<0.05$. The *a* and *b* values resulted from the analysis showed that all values of extruded Soy d and Oat d press-cakes lied in the positive coordinates. This shows that the extruded press-cakes lie in the region of redness and yellowness, respectively. Soy t press-cakes at the temperature of 110 °C with 60% humidity showed negative a value, thereby lying in the green region. The redness value for Soy t and Soy d press-cakes decreased with increasing humidity but increased with an increase in the extrusion temperature lying between the range of 0.41 - 2.56 and 2.52 - 4.40, respectively. The values of redness for Oat d press-cakes increased with increasing temperature but showed low amounts of increase when compared to increase in humidity. The highest value of redness of 9.35 was noted at the temperature of 120 °C having a humidity of 45% whereas the lowest being 7.66 was noted at the temperature of 110 °C having a humidity of 30%. The yellowness of the Soy t press-cakes decreased with increasing temperature and humidity compared to Soy d, whose yellowness increased with increasing temperature but decreased with increasing humidity. In contrast, the yellowness of Oat d press-cakes decreased with increasing temperature but increased with increasing humidity. Both values of *a* and *b* showed statistical significance as $p<0.05$.

3.3. The effect of extrusion on nutritional value of plant origin press-cakes

The proximate analysis of the press-cakes is carried out to ensure the by-product has nutritional value that could be beneficial for the consumers. **Table 2** shows the nutritional content of plant origin press-cakes before and after extrusion. The humidity of Soy t press-cakes increased from 6.51% to 23.59% and further reached 55.18% after extrusion. Similarly, the humidity increased from 8.19 % to 22.23% in Soy d and 5.56% to 23.95% in Oat d press cake samples. The total fat content in both the soy press-cakes increased with increasing temperature but decreased with increasing humidity. However, in Oat d press-cakes, the fat content increased after extrusion but lowered as the temperature and humidity increased. The presences of unsaturated fatty acids were more than the saturated fatty acids, while the trans fatty acids were not detected. Observing the fatty acid analysis in **appendix 6**, the significant amount of saturated fatty acid present in plant press-cakes was palmitic acid. In contrast, the major amount of unsaturated fatty acid detected was ω-6 linoleic acid. The fatty acid analysis supports our findings in the proximate analysis. On comparing the press-cake samples, a high amount of fatty acid was observed in Soy d (13.1%) at the temperature of 110 °C and 30% humidity while the lowest was observed in soy t (4.2%) at the temperature of 110 °C at 60% humidity. This could have occurred due to the lowering of oleic acid and palmitic acid after the extrusion process. The lowering of saturated fatty acid lowers the risk of cardiovascular diseases. However, the high amount of ω-6linoleic acid in the plant press-cakes adds to health benefits [92]. In comparison with the plant press-cakes, the highest amount of ω-6 linoleic acid was observed in soy t having 57.49 % (T = 110 °C and H = 30%) while the lowest in oat d having 38.56% (T = 120 °C and H = 60%). The nitrogen content decreased after the process of extrusion. The impact of increasing extrusion temperature and humidity decreased the nitrogen content. This is on par with the results obtained for the amount of protein which also reduced with increasing temperature and humidity of extrusion. This occurred due to the degradation of some essential and non-essential amino acids. On observing **appendix 4** and **appendix 5** the degradation of amino acids is noted, supporting our proximate analysis. The major degradation was observed in oat d press-cakes. In soy t, essential amino acid histidine and valine increased at 30% humidity, while in Soy d, all essential amino acids increased at the temperature of 110 °C except phenylalanine which increased at the temperature of 120 °C. Similarly, while all non-essential and conditionally non-essential amino acids were lower in extruded soy t and oat d press-cakes, Soy d showed an increase in non-essential and conditionally non-essential amino acids at 110 °C temperature except serine which increased at 120 °C. While an increase was seen in Soy t and Soy d amino acid analysis, Oat d showed some amount of degradation of all amino acids after extrusion. This can be the reason for the lowest reduction of protein in oat d press-cakes. The sum of all the minerals in the substance is the complete ash. There are many health-promoting influences of natural plant minerals [93]. The process of extrusion increased ash content. They varied from 1.51% - 4.07%. The increasing temperature and humidity decreased the amount of ash content due to the loss of minerals. The total sugar content increased due to extrusion, but there was an impact with a change in process parameters. Increasing temperature decreased the total sugar content in oat d while the contrary was observed in the case of soy press-cakes. This occurred because, at high temperatures, the Maillard reaction and caramelisation take place, causing degradation of sugars. The Maillard reaction occurs when free amino acids and carbonyl groups present in reducing sugars react when the extrusion process takes place. It degrades the nutritional content of the product. This can be lowered by lowering the content of reducing sugars. The total sugar content also decreased with increasing humidity. Similar findings have been observed in the work of Woo Sik Koan et al. [94]. The salt content was negligible in all plant press-cakes at less than 0.03%. Dietary fibres are an essential aspect of nutritional value due to their effect on the betterment of health as their consumption leads to the lowering of several diseases. In the study conducted by Dhingra et al. [95], it has been stated that extrusion-cooking, fermenting, crushing, brewing, and frying change the physico-chemical characteristics of dietary fibre, thus increasing their functionality. This correlation has been noticed in our study in the case of oat d press-cakes. The dietary fibres in soy press-cakes decreased as the humidity increased but the dietary fibres increased with an increase in temperature. After extrusion, the lowest amount of dietary fibres was observed in oat d containing 10.8% (T = 120 °C and H = 45%), while the highest observation was noted in Soy d containing 30.1% (T = 110 °C and H = 30%). Slukova et al. [96] reported that after extrusion, the physico-chemical properties and the amount of fibres are dependent on the material, its composition and the process parameters. Increasing process parameters cause weak bonds within polysaccharide chains, making them split. Glycosidic linkages in the polysaccharides of dietary fibre can also be disrupted. The overall proximate analysis of press-cakes shows a positive effect after undergoing the extrusion process despite the degradation of some characteristics, making it an excellent source to improve the functionality of a food products.

Table 1. Data of the effect of extrusion on changes in nutritional value of the press-cakes

Indicator, %	**Untreated Soy t press-cake**	**Extruded Soy t press-cakes,**				**Untreated Soy d press-cake**	**Extruded Soy d press-cakes,**				**Untreated Oat d press-cake**	**Extruded Oat d press-cakes,**			
		30%		**60%**			**30%**		**60%**			**30%**		**45%**	
		110 °C	**120 °C**	**110 °C**	**120 °C**		**110 °C**	**120 °C**	**110 °C**	**120 °C**		**110 °C**	**120 °C**	**110 °C**	**120 °C**
Humidity	6.51±0.03	23.59±0.10	23.47±0.10	56.10±0.01	55.18±0.08	8.19±0.03	22.23±0.06	25.16±0.06	56.26±0.01	55.47±0.03	5.56+0.28	23.95±0.05	23.99±0.11	40.04±0.04	40.60±0.07
Fat	9.10±0.08	11.70±0.13	11.70±0.03	4.20±0.11	4.80±0.04	10.90±0.07	13.10±0.12	12.00±0.21	6.30±0.11	7.20±0.08	2.40±0.13	8.50±0.11	8.70±0.09	6.30±0.07	6.60±0.08
Saturated Fatty acid*	nd	1.71±0.03	1.78±0.01	0.81±0.02	0.88±0.04	nd	2.22±0.05	1.86±0.01	0.99±0.04	1.60±0.07	nd	1.55±0.01	1.49±0.09	1.10±0.07	1.10±0.08
MUFA	nd	2.21±0.06	2.27±0.02	0.93±0.04	1.35±0.04	nd	3.24±0.01	2.91±0.02	1.53±0.04	2.17±0.03	nd	3.49±0.02	3.55±0.02	2.63±0.04	2.86±0.03
PUFA	nd	7.78±0.03	7.65±0.04	2.46±0.01	2.57±0.01	nd	7.64±0.01	7.23±0.01	3.78±0.09	2.43±0.07	nd	3.44±0.03	3.65±0.05	2.57±0.05	2.64±0.06
Trans fatty acid	nd	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	nd	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	nd	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Nitrogen	5.28±0.05	4.11±0.01	4.10±0.04	2.35±0.06	2.42±0.06	4.37±0.03	4.17±0.08	4.05±0.04	2.37±0.06	2.45±0.06	11.24±0.04	4.44±0.05	4.89±0.06	3.91±0.06	4.11±0.04
Protein	33.90±0.40	25.70±0.28	25.60±0.28	14.70±0.14	15.10±0.70	27.30±0.4	26.10±0.56	25.30±0.84	14.80±0.14	15.30±0.14	70.30±0.63	27.80±0.43	30.60±0.54	24.40±0.31	25.70±0.67
Ash	3.38±0.80	2.67±0.10	2.68±0.04	1.51±0.04	1.52±0.09	3.30±0.56	3.21±0.14	3.11±0.03	1.83±0.07	1.81±0.03	1.35±0.03	4.32±0.07	4.29±0.01	3.39±0.04	3.37±0.04
Sucrose	2.56±0.08	2.47±0.08	2.54±0.07	1.10±0.10	0.26±0.11	0.13±0.08	1.17±0.08	1.07±0.07	0.28±0.09	0.40±0.11	0.02±0.03	0.98±0.09	0.99±0.09	0.86±0.11	0.95±0.07
Glucose *	0.03±0.04	0.26±0.07	0.25±0.05	0.26±0.11	0.20±0.05	0.02±0.03	0.20±0.04	0.21±0.07	0.20±0.08	0.20±0.01	9.69±0.07	9.93±0.05	9.78±0.13	6.77±0.05	7.30±0.08
Fructose *	0.02±0.02	0.32±0.05	0.38±0.06	0.31±0.10	0.49±0.05	0.02±0.05	0.28±0.07	0.26±0.05	0.24±0.03	0.21±0.06	0.14±0.06	1.11±0.05	1.22±0.03	0.93±0.03	1.02±0.07
Total Sugar	0.15±0.03	3.05±0.06	3.17±0.07	1.67±0.10	0.75±0.06	0.15±0.04	1.45±0.08	1.54±0.06	0.52±0.04	0.61±0.08	9.83±0.04	16.09±0.06	15.92±0.08	11.53±0.01	12.30±0.03
Salt	<0.03	<0.03	<0.03	<0.03	<0.03	nd	<0.03	<0.03	<0.03	<0.03	nd	<0.03	<0.03	<0.03	<0.03
Fibre	47.00±0.38	28.50±0.21	28.90±0.11	15.80±0.07	17.90±0.09	47.00±0.48	30.10±0.09	27.90±0.07	17.70±0.13	18.60±0.23	5.10±0.12	17.90±0.18	15.60±0.10	13.70±0.21	10.8±0.13

All values within the same row indicate significant differences for temperature and humidity independently, 2-way ANOVA, $p < 0.05$
* indicate that their interactions are not significant for all values within the same row, 2-way ANOVA, $p > 0.05$
nd – not detected

3.4. The effect of extrusion on anti-nutritional changes of plant origin press-cakes

3.4.1. The analysis of the effect of extrusion on trypsin inhibitor activity

Trypsin inhibitors in the digestive tract are responsible for impairing the function of trypsin enzymes. Inactivation of these inhibitors is possible at elevated processing temperatures [97]. The activity of trypsin inhibitor can be utilised as a potential measure of processing to get hypoallergenic properties of protein isolates. As the trypsin inhibitor is heat-labile, extrusion caused the trypsin inhibitor activity to degrade significantly.

Table 2. Trypsin inhibitor activity of the untreated plant press-cakes

Samples	TIA (mg/g dm)
Soy t	$0.83 \pm 0.00^{c,C}$
Soy d	$1.74 \pm 0.13^{e,E}$
Oat d	$1.26 \pm 0.01^{c,C}$

$^{a\text{-}e}$ within the same column indicate significant significance for temperature, 2-way ANOVA, $p < 0.05$
$^{A\text{-}E}$ within the same column indicate significant significance for humidity, 2-way ANOVA, $p < 0.05$
The interactions are significant for all values within the same column, 2-way ANOVA, $p < 0.05$

Table 3. Data of the effect of the extrusion on trypsin inhibitor activity changes in the press-cakes

Extrusion parameters		Soy t TIA (mg / g dm)	Soy d TIA (mg / g dm)	Extrusion parameters		Oat d TIA (mg / g dm)
H* (%)	T* (°C)			H* (%)	T* (°C)	
30	110	$0.83 \pm 0.01^{d,D}$	$0.87 \pm 0.00^{c,C}$	30	110	$2.31 \pm 0.01^{e,E}$
	120	$1.07 \pm 0.01^{e,E}$	$1.36 \pm 0.013^{d,D}$		120	$1.36 \pm 0.01^{d,D}$
60	110	$0.67 \pm 0.01^{b,B}$	$0.51 \pm 0.01^{a,A}$	45	110	$0.48 \pm 0.01^{a,A}$
	120	$0.64 \pm 0.01^{a,A}$	$0.74 \pm 0.01^{b,B}$		120	$0.61 \pm 0.01^{b,B}$

$^{a\text{-}e}$ within the same column indicate significant differences for temperature, 2-way ANOVA, $p < 0.05$
$^{A\text{-}E}$ within the same column indicate significant differences for humidity, 2-way ANOVA, $p < 0.05$
The interactions are significant for all values within the same column, 2-way ANOVA, $p < 0.05$
*H – humidity; T-temperature

From **table 2**, it can be observed that untreated Soy d and Oat d press-cakes showed higher content of trypsin inhibitor activity as compared to untreated Soy t press-cakes. After extrusion, some inhibition of trypsin in the plant press-cakes occurred, as presented in **table 3.** The trypsin inhibitor activity in Soy t showed the highest inhibitor activity of 1.07mg /g at the temperature of 120 °C and humidity of 30%, after which as the temperature and humidity increased, the TIA lowered. Similar results were noted in Soy d and Oat d press-cakes where maximum inhibitor activity of 1.364 mg/g and 2.31 mg/g were observed at 30% humidity and a temperature of 120 °C and 110 °C, respectively. The inhibitor activity lowered up to 0.64 mg/g dm in Soy t press-cakes, while the lowest activity was observed in Soy d (0.51 mg/g) and Oat d (0.48 mg/g) at the temperature of 110 °C and 60% humidity. The results show that as the extrusion temperature and humidity increased, there was an increase in the inactivation of trypsin inhibitor. This is in agreement with the study conducted by Liener et al. [98], where antinutrients such as trypsin lowered at elevated temperatures and humidities.

3.4.2. The analysis of the effect of extrusion on biogenic amines, acrylamide and free-fatty acid content

Nitrogenous compounds that occur naturally are synthesised by flora, fauna and microorganisms by the decarboxylation of amino acids. These compounds are called biogenic amines [99]. This analysis was performed to check the amount of biogenic amines in our press-cake samples as they cause health issues. The amount of biogenic amines present in plant press-cakes are presented in **appendix 7**. Effect of extrusion had a significant effect on histamine, putrescine and spermine content in Soy t and Soy d, while for oat d, the significant effect was noticed on putrescine content. In Soy t, the histamine content increased after extrusion. Spermine content as well increased after the process of extrusion but lowered with increasing temperature and humidity. The lowest amount of spermine was observed at 110 °C temperature and 60% humidity (27 mg/kg). The putrescine decreased, and the lowest amount was observed at 120 °C temperature and 60% humidity (7 mg/kg). Both cadaverine and tyramine content did not change after extrusion. In Soy d, similar effect as soy t was observed on histamine and spermine content whose lowest amount was <5 mg/kg (T = 120 °C and H = 60%) and 42 kg/mg (T = 110 °C and H =60%). In Oat d press-cakes, tyramine and spermine content reduced after extrusion, while other biogenic amines did not show any significant change in the content. When comparing the Soy t, Soy d and Oat d press-cakes, oat d showed the lowest content of the biogenic amines. Many factors influence the formation of biogenic amines, including the press-cake's microbiology, physico-chemical properties, storage length, conditions, and the free amino acids in the substrate. Decarboxylation of amino acids present in the press-cakes undergoes protein denaturation during the process of extrusion which leads to the formation of biogenic amines. [100]. The amount of the biogenic amines was analysed in order to control its effect on press-cakes. In this study, the results obtained show a significant effect on some biogenic amines due to changes in extrusion parameters such as temperature and pressure. The amino acid analysis of the press-cakes before and after extrusion supports our results obtained for the biogenic amines (see table in **appendix 4** and **appendix 5**). Acrylamide, on the other hand, is generated at elevated temperatures during heat processing. This is largely due to the involvement of sugars such as glucose and fructose [101]. Oat d showed the maximum amount of acrylamide content, while soy t showed the lowest value of acrylamide when comparing the overall values of all press-cakes. However, the highest individual content was observed in Soy d at 110 °C and 60% humidity (76 µg/kg), while the lowest was detected at 110 °C temperature and 30% humidity (10 ug/kg) in soy t. The result of this study can be supported from the nutritional analysis presented in **table 1,** where the Oat d press-cakes had the highest amount of increase in sugar content compared to other press-cakes. Our results agreed with studies conducted by Richard Stardler et al. [102], which also showed an increase in acrylamide content in press-cakes as the temperature increased. The unsaturated fatty acids that are present in the products react to form fatty acid hydroperoxides with oxygen. Hydroperoxides are unstable and break down into different compounds that create off-flavours, resulting in a rancid, stale taste. The data revealed that the antinutrient content depends on the content of fat in the press-cakes samples. The lower the values of fat content, the lower the values of the breakdown of products in the samples. Peroxide content was detected in less than 20 Mekv/kg in Soy d press-cakes but did not show detection in Soy t or Oat d press-cakes. Similarly, free fatty acid content was present only in Soy d press-cakes (~2%). This occurred when fats present in Soy d press-cakes underwent the process of thermal oxidation during extrusion.

3.5. The effect of extrusion on the functional technological properties of plant origin press-cakes

3.5.1. The analysis of the effect of extrusion on water holding capacity

Water holding capacity (WHC) reflects the protein's ability to retain water and shape the protein gel network in meat analogues. The higher the WHC, the greater the juiciness in meat analogues [103]. The WHC of meat analogues was therefore investigated depending on the form of press-cakes before and after extrusion.

Table 4. Data on the effect of the extrusion on the water-holding capacity of the press-cakes

Press-cakes extrusion parameters		*WHC, g/g		Press-cakes extrusion parameters		*WHC, g/g
H (%)	**T (°C)**	**Soy t**	**Soy d**	**H (%)**	**T (°C)**	**Oat d**
Untreated press-cakes		$4.15 \pm 0.01^{a,A}$	$5.572 \pm 0.47^{a,A}$	Untreated press-cakes		$2.88 \pm 0.17^{a,A}$
30	110	$4.28 \pm 0.19^{b.B}$	$6.095 \pm 0.04^{n,B}$	30	110	$3.58 \pm 0.19^{b,B}$
	120	$4.57 \pm 0.15^{c,C}$	$6.135 \pm 0.17^{c,C}$		120	$3.96 \pm 0.33^{c,C}$
60	110	$4.81 \pm 0.10^{d,D}$	$6.658 \pm 0.12^{d,D}$	45	110	$4.39 \pm 0.19^{d,D}$
	120	$5.45 \pm 0.17^{e,E}$	$6.877 \pm 0.12^{e,E}$		120	$4.85 \pm 0.17^{e,E}$

$^{a-e}$ within the same column indicate significant differences for temperature, 2-way ANOVA, $p < 0.05$
$^{A-E}$ within the same column indicate significant differences for humidity, 2-way ANOVA, $p < 0.05$
* indicate that their interactions are significant for all values within the same column, 2-way ANOVA, $p > 0.05$

The untreated Oat d samples showed a water holding capacity of 2.88 g/g, which is lower than the extruded Oat d samples having the value of 3.58 g/g and increasing up to 4.85 g/g with increasing temperature and humidity (**table 4**). Similarly, untreated Soy t press-cakes and Soy d press-cakes showed a water holding capacity of 4.15 g/g and 5.57 g/g, respectively, while this increased after the process of extrusion up to 5.45 g/g and 6.88 g/g, respectively. The water holding capacity of the extruded press-cakes increases with increasing temperature and increasing humidity. This arises due to the ability of the protein in the press-cakes to form a gel network by hydrophobic interactions, which takes place due to increasing temperature. Our results correlate with the study made by Wi Gi Hyun et al. [104], who reported regarding the increasing water-holding capacities with an elevation of temperature. In the study, he has justified that when the hydrophobic groups are exposed and the protein structure is unfolded due to heating, hydrophobic interaction is induced. The highest water holding capacity was noted at an extruder temperature of 120 °C having a humidity of 60% for Soy and 45% for Oat. When compared to the study conducted by Wu Xufeng et al. [105], where the presscakes were fermented, the results are quite similar and lie within the same range. In our study, for the press-cake samples, the temperature and humidity independently showed significance (p<0.05), whereas their interactions did not have any significance (p>0.05).

3.5.2. The analysis of the effect of extrusion on oil binding capacity

Fat binding with food materials, particularly proteins and carbohydrates, affects the properties of texture and quality of food. Proteins' capacity to take in and retain lipids, as well as engage with lipids in emulsions and other food systems, is critical in formulations [106]. According to Rashid et al., [107], the oil binding capacity mechanism is primarily due to trapping of oil by capillary attraction. Moreover, the hydrophobic nature of proteins influences fat absorption. As a result, based on the amount of polar side chains of amino acids located on the surface of protein molecules, the oil binding ability of the samples can differ [108]. In addition, the oil binding capacity is often correlated with the size of the particle, the total charge density and the hydrophilic existence of the individual particles.

Table 5. Data on the effect of extrusion on oil-binding capacity changes in the press-cakes

Press-cakes extrusion parameters		***OBC, g/g**		**Press-cakes extrusion parameters**		***OBC, g/g**
H (%)	**T (°C)**	**Soy t**	**Soy d**	**H (%)**	**T (°C)**	**Oat d**
Untreated press-cakes		$1.71 \pm 0.18^{a,A}$	$1.99 \pm 0.23^{a,A}$	Untreated press-cakes		$1.59 \pm 0.16^{a,A}$
30	110	$1.99 \pm 0.25^{b,B}$	$2.03 \pm 0.10^{b,B}$	30	110	$1.96 \pm 0.20^{b,B}$
	120	$2.42 \pm 0.15^{c,C}$	$2.48 \pm 0.12^{c,C}$		120	$2.40 \pm 0.21^{c,C}$
60	110	$2.62 \pm 0.17^{d,D}$	$2.65 \pm 0.18^{d,D}$	45	110	$2.59 \pm 0.15^{d,D}$
	120	$2.93 \pm 0.10^{e,E}$	$2.97 \pm 0.11^{e,E}$		120	$2.86 \pm 0.10^{e,E}$

$^{a\text{-}e}$ within the same column indicate significant differences for temperature, 2-way ANOVA, $p < 0.05$
$^{A\text{-}E}$ within the same column indicate significant differences for humidity, 2-way ANOVA, $p < 0.05$
* indicate that their interactions are significant for all values within the same column, 2-way ANOVA, $p > 0.05$

In this study, the oil binding capacity of untreated Oat d sample was 1.59 g/g while those of Soy t and Soy d press-cakes were 1.71 g/g and 1.99 g/g, respectively (**table 5**). The capacity of oil-binding increased when the press-cakes were extruded. After extrusion, the oil binding capacity increased from 1.96 g/g to 2.86 g/g for Oat d, 1.99 g/g to 2.93 g/g for Soy t and 2.03 g/g to 2.97 g/g for Soy d. This increase occurred as the temperature and humidity of the extrusion increased. This was reported due to the porosity of fibres present in press-cake samples, which gave rise to the ability to trap oil droplets. The temperature and humidity independently had a significant ($p<0.05$) effect on the oil binding capacity, unlike their interaction which did not have a significant effect ($p>0.05$). A high lipid retention capacity is necessary to prevent the creation of fat pockets, which, apart from being unappealing to the user, can also lead to the degradation of products [109].

3.5.3. The analysis of the effect of extrusion on bulk density

Bulk density takes into account the expansion of extruded material in all directions. The bulk density of the untreated Soy t, Soy d and Oat d was 0.49 g/ml, 0.63 g/ml and 0.49 g/ml, respectively (**table 6**). The bulk density of plant origin press-cakes increased after undergoing the extrusion process but gradually decreased with increasing temperature and humidity. The highest density was observed at an extruder temperature of 110 °C with a humidity of 30%, while the lowest value was recorded at an extruder temperature of 120 °C with a humidity of 45% and 60%. The values of bulk density did not decrease with significant differences. A lower density extrudate was created by an increase in barrel temperature. As depicted by Fletcher et al. [110], an increase in the temperature of the barrel would increase the extruder's level of water overheating and facilitate the production of bubbles and also a decrease in the viscosity of the melt, leading to a decrease in density, as observed in this work. In this study, for the press-cake samples, the temperature and humidity independently had a significant ($p<0.05$) effect on the bulk density, whereas their interactions did not have any ($p>0.05$).

It has been found that temperature and feed moisture are the key factors affecting the extruded density and lateral expansion [111].

Table 6. Data on the effect of extrusion on bulk density changes in the press-cakes

<table>
<tr><th colspan="2">Press-cakes extrusion parameters</th><th colspan="2">*BD, g/ml</th><th colspan="2">Press-cakes extrusion parameters</th><th>*BD, g/ml</th></tr>
<tr><th>H (%)</th><th>T (°C)</th><th>Soy t</th><th>Soy d</th><th>H (%)</th><th>T (°C)</th><th>Oat d</th></tr>
<tr><td colspan="2">Untreated press-cakes</td><td>0.49 ± 0.06^{a,A}</td><td>0.63 ± 0.07^{a,A}</td><td colspan="2">Untreated press-cakes</td><td>0.49 ± 0.09^{a,A}</td></tr>
<tr><td rowspan="2">30</td><td>110</td><td>0.80 ± 0.05^{e,E}</td><td>0.88 ± 0.11^{e,E}</td><td rowspan="2">30</td><td>110</td><td>0.73 ± 0.63^{e,E}</td></tr>
<tr><td>120</td><td>0.76 ± 0.18^{d,D}</td><td>0.85 ± 0.11^{d,D}</td><td>120</td><td>0.66 ± 0.74^{d,D}</td></tr>
<tr><td rowspan="2">60</td><td>110</td><td>0.72 ± 0.18^{c,C}</td><td>0.78 ± 0.11^{c,C}</td><td rowspan="2">45</td><td>110</td><td>0.60 ± 0.11^{c,C}</td></tr>
<tr><td>120</td><td>0.70 ± 0.20^{b,B}</td><td>0.75 ± 0.13^{b,B}</td><td>120</td><td>0.57 ± 0.98^{b,B}</td></tr>
<tr><td colspan="7">$^{a-e}$ within the same column indicate significant differences for temperature, 2-way ANOVA, $p < 0.05$
$^{A-E}$ within the same column indicate significant differences for humidity, 2-way ANOVA, $p < 0.05$
* indicate that their interactions are significant for all values within the same column, 2-way ANOVA, $p > 0.05$</td></tr>
</table>

Texturizing plant proteins and fibres into a shape that resembles a meat matrix can be done using an extruder. Plant proteins in press-cakes open, straighten, and position beside one another during extrusion cooking. Unfolding proteins creates new reactive areas and binding sites, allowing them to link tightly to one another. Decreased cooking shrinkage, better water binding, and improved textural characteristics are the advantages of the new structure. These are the functional properties that industries are actively working to improve in order to produce quality goods that customers enjoy. The best water-holding and oil binding capacity were demonstrated in Soy d press-cakes extruded at 120°C and 60% humidity having a protein content of 15.3%. On the other hand, the best bulk density was observed in Soy d press-cakes extruded at 110 °C and 30% humidity having a protein content of 26.1%.

3.6. Meat analogue matrices with the addition of plant origin press-cakes

Based on the proximity, anti-nutritional and functional technological parameters analysis of the press-cakes, meat analogues were produced in the form of patties with the addition of 3% and 6% amount of each extruded plant origin press-cakes. The base for the meat analogue matrix was formed from texturised soy protein. The recipe for each meat analogue can be observed in **appendix 8**.

3.7. The effect of extruded press-cakes addition on the nutritional value of the meat analogues

The proximate analysis for meat analogues has been presented in **appendix 9** and **appendix 10**. The amount of humidity of meat analogues lies between the range of 42.78 - 46.35%. This moistness arises due to the presence of water, oil and emulsion in the meat analogues matrix. The total fat content of meat analogues containing extruded Soy t, Soy d and Oat d press-cakes were between 19.6 - 22.6%, 19.7 - 22.3% and 23 - 23.8%, respectively. The lowest amount of fat (19.6%) was detected in meat analogue containing 6% of Soy d press-cakes, while the highest (23.8%) was observed in meat analogue containing 3% of Oat d press-cakes. In the overall comparison of meat analogues, the ones containing extruded Oat d press-cakes showed a more extensive content of fat. This occurred as high amount of monounsaturated fatty acids, namely oleic acid was present. From **appendix 14** and

appendix 15, the highest level of oleic acid was detected in meat analogues containing extruded oat press-cakes, while ω-6 linoleic acid and ω-3 alpha-linoleic acid content were more significant in meat analogues containing extruded Soy d press-cakes. The presence of monounsaturated fatty acids is beneficial for health, especially in improving the diet. There are some amounts of saturated palmitic acid content that was detected in the meat analogues. They were within the range of 4 - 5%. Oleic acid and ω-3 alpha-linoleic acid decreased with the increasing amount of extrudate, while ω-6 linoleic acid increased as the amount of extrudates increased. Similarly, the palmitic acid also increased with the increasing amount of extrudates. This result can be supported by observing the number of fatty acids from press-cakes which showed similar characteristics. Also, the increase in these particular saturated and unsaturated fatty acids was due to the addition of rapeseed oil to the matrix, which contains oleic acid, linoleic acid and palmitic acid. The addition of 3% and 6% extruded press-cakes to the meat analogue matrix showed minimal differences. At 110 °C extruded temperature as the amount of extruded press-cakes increased from 3% to 6%, the fat percentage decreased, but for extrusion temperature of 120 °C, the fat percentage increased with the increasing amount of press-cakes, depicting that fat binding capacity is high in meat analogues with 6% extruded press-cakes due to increasing of the protein content. On the basis of extrusion regimes, temperature showed no significant effect on the fat content, while an increase in the humidity of extruded press-cakes decreased the amount of fat present in the meat analogues.

The nitrogen content was in the range of 2.3 - 2.6 %. The protein content was between 14% - 17% for all meat analogues. Higher amounts of non-essential and conditional non-essential amino acids were present compared to essential amino acids meat analogues containing extruded soy t press-cakes (**appendix 11**). Similar results were observed for meat analogues containing extruded Soy d (**appendix 12**) and Oat d press-cakes (**appendix 13**). While the highest amount of essential acid present in meat analogues was isoleucine, there was a low amount of methionine. Glutamic acid dominated the presence of non-essential amino acids. In comparing meat analogues with different added extruded press-cakes, meat analogues containing extruded soy t showed a high presence of essential and non-essential amino acids. An overall comparison of extruded press-cakes, Soy d, showed a high amount of protein content than Soy t and Oat d press-cakes. As the amount of extruded press-cakes added to the meat analogue matrix increased, the protein content also increased, depicting that adding more amount of press-cakes can indeed increase the amount of protein in the product. Based on extrusion regimes, meat analogues containing press-cakes extruded at 110 °C showed a lower amount of protein as compared to meat analogues containing press-cakes extruded at 120 °C temperature. However, meat analogues having press-cakes extruded at 30% humidity showed more content of protein than meat analogues having press-cakes extruded at 60% humidity. Proteins are an essential aspect in meat analogue production along with fats and fibres as it enhances water-binding and helps in achieving essential consistency [112]. The ash content was ~2% in the meat analogues. The increasing amount of different extruded plant origin press-cakes did not show a significant difference in ash content. There was little to no difference in minerals when either of the press-cakes was added in amounts of 3% or 6% in the meat analogue matrix. The total sugar content was between 0.4 - 3.5%. Meat analogues containing extruded Oat d press-cakes contained a higher amount of glucose (0.38 - 0.69%) than meat analogues with extruded soy press-cakes. The total sugar increased when the amount of added press-cakes extruded at 30% increased while the total sugar content decreased with adding a high amount of press-cakes extruded at 60% humidity. The salt content was negligible in all plant press-cakes at less than 0.03%

The presence of dietary fibres in meat analogues appeals to many consumers who are health conscious [113]. The amount of dietary fibres in 100 g of meat analogues was 5.2 - 8.3%. On comparing meat analogues, the ones containing extruded Soy d press-cakes showed higher levels of fibre. Increasing the amount of press-cakes in the matrix increased the content of dietary fibres. The addition of press-cakes extruded at increasing temperature increased the amount of dietary fibres, but the addition of press-cakes extruded at 60% humidity showed lower content of dietary fibres. Meat analogues should consist of high amount of moisture, protein, fat and fibre content as present in conventional meat burger for it to be termed as a successful alternative product. Based on the chemical analysis and comparing it to the traditional meat burger patties containing a high amount of chemical composition, the best and successful meat analogue matrices are the meat analogues containing 6% of soy t press-cakes extruded at 110 °C and 60% humidity, meat analogues with 6% Soy d press-cakes extruded at 110 °C and 60% humidity and meat analogues having 6% of Soy d press-cakes extruded at 120 °C and 60% humidity. These meat analogues show a chemical composition that resembles meat burger patties. The overall proximate analysis of meat analogues containing extruded plant origin press-cakes depicts that there is a high presence of proteins and unsaturated fatty acids but a lower amount of saturated fatty acids and sugars, making it an ideal product that has nutritional value making it ideal for consumers who prefer a healthy lifestyle.

3.8. The effect of extruded press-cakes addition on anti-nutritional substance content in the meat analogues

3.8.1. The analysis of the effect of the addition of the extruded press-cakes on trypsin inhibitor activity in the meat analogues

Meat analogue samples showed trypsin inhibitor activity in the range of 0.44– 0.85 mg, 0.44 – 1 mg and 0.14 – 0.58 mg containing extruded Soy t, Soy d and Oat d press-cakes (**table 7**). This occurred due to the presence of texturised soy protein in the meat analogue matrix, which has a higher content of trypsin inhibitor.

Table 7. Data of trypsin inhibitor activity in the meat analogues containing extruded press-cakes

Meat analogues	TIA (mg / g dm)	Meat analogues	TIA (mg / g dm)	Meat analogues	TIA (mg / g dm)
MT-110-30-3	0.53±0.03	MD-110-30-3	0.63±0.03	MO-110-30-3	0.24±0.03
MT-110-30-6	0.85±0.04	MD-110-30-6	1.00±0.03	MO-110-30-6	0.28±0.06
MT-120-30-3	0.77±0.05	MD-120-30-3	0.46±0.04	MO-120-30-3	0.58±0.05
MT-120-30-6	0.61±0.02	MD-120-30-6	0.63±0.01	MO-120-30-6	0.32±0.05
MT-110-60-3	0.50±0.08	MD-110-60-3	0.71±0.06	MO-110-45-3	0.54±0.01
MT-110-60-6	0.44±0.02	MD-110-60-6	0.44±0.04	MO-110-45-6	0.50±0.04
MT-120-60-3	0.53±0.04	MD-120-60-3	0.56±0.01	MO-120-45-3	0.14±0.06
MT-120-60-6	0.51±0.04	MD-120-60-6	0.53±0.08	MO-120-45-6	0.51±0.02
All value within the same column indicates significant differences for three factors(temperature, humidity and amount) independently, 3-way ANOVA, $p < 0.05$; *The interaction did not show a significant difference ($p>0.05$)					

As the amount of extruded press-cakes added to the meat analogue matrix increased, there was not much difference in the trypsin inhibitor activity as adding 6% extruded press-cakes in meat analogues increased as well as decreased the trypsin inhibitor activity. The highest level of trypsin inhibitor activity was 1 mg / g of dry matter, which contained 6% of extruded Soy d press-cakes, while low trypsin inhibitor activity was found in meat analogue containing 3% of extruded Oat d press-cakes (0.14 mg / g of dry matter). On comparing the meat analogues having different plant origin, press-cakes added, a high range of inhibition was observed in extruded Oat d meat analogues compared to the inhibition in Soy t and Soy d containing meat analogues. The samples showed statistical significance to all parameters as $p<0.05$. The remaining trypsin can be lowered during the cooking of meat analogues at high temperatures to inactivate heat-sensitive factors such as trypsin inhibitors [114].

3.8.2. The analysis of the effect of the addition of the extruded press-cakes on biogenic amines, acrylamide and free-fatty acid content in the meat analogues

The amount of biogenic amines present in meat analogues with extruded plant origin press-cakes are presented in **appendix 16.** Among the detected biogenic amines, histamine levels were high in the meat analogues. This occurred due to the soy protein texturate in the base matrix having a 20 mg/kg histamine content. The lowest histamine level was observed in meat analogues containing 6% of extruded Soy t press-cakes, while the highest was observed in meat analogues with 3% extruded Soy t press-cakes. Meat analogues press-cakes extruded at 120 °C and 60% humidity showed a lower amount of histamine than those containing press-cakes extruded at 110 °C at 30% humidity. This shows that their interaction shows significant differences. Putrescine was detected in amounts lower than 5 mg/kg. Meat analogues containing extruded Oat d press-cakes showed lower than 5 mg/kg of cadaverine, while those containing extruded Soy d press-cakes showed lower content <5 mg/kg of tyramine content. The cadaverine content lowered with the addition of press-cakes extruded at high temperature and humidity in the meat analogue matrix. The spermine content in all meat analogues was within the range of 11 - 22 mg/kg and did not show any significant difference. These results can be supported when observing the amino acid profile of meat analogues in the **appendix 11, appendix 12** and **appendix 13**. The presence and amounts of histamine, lysine, arginine, tyrosine and methionine correlate to the respective biogenic amine formation due to the decarboxylation process. The formation of biogenic amines can also be affected by the physicochemical properties, amount of moisture, storage time and extrusion cooking at different regimes. The biogenic amines analysis for meat analogues was carried out to check their levels in the product, which may cause allergic reactions such as trouble breathing, skin irritation, rash, nausea, headache, and hypertension in consumers. These biogenic amines can be prevented using raw materials of high quality, good manufacturing practice and freezing [115]. Acrylamide content in meat analogues was within the range of 5– 47 ug/kg. High levels were observed in meat analogues containing extruded Soy t press-cakes while the lowest in meat analogues containing extruded Soy d press-cakes. The acrylamide content increased with the addition of press-cakes extruded at 120 °C. The acrylamide content is produced at high temperatures due to the decarboxylation process of the amino acid, asparagine which is facilitated as the result of the reaction between some carbonyl groups and reducing sugars present in meat analogues [116]. This effect can be lowered when cooking the meat analogues at low temperatures [117].

Peroxide content was detected in less than 7 Mekv/kg, where the lowest was detected in meat analogues containing extruded Oat d press-cakes while the highest in meat analogues containing extruded soy t press-cakes. Peroxide values are analysed in order to observe the amount of hydroperoxides produced. Due to their unstable nature, they decompose quickly, producing several secondary compounds such as hydrocarbons, ketones, esters, aldehydes, alcohols, and acids, which create off-flavours and odours in meat analogues [118]. Free fatty acids, on the other hand, are produced by the degradation of enzymes of lipids. The free fatty acid analysis provides insight into lipid stability during storage [119]. The free fatty acid content of meat analogues was relatively low, up to 1.6 %. The content increased as the amount of extruded press-cakes added to the meat analogues increased. Similarly, there was an increase in the amount of free fatty acid content when the meat analogues contained press-cakes extruded at 120°C and 60% humidity. During frozen storage, lipolytic enzyme activity and lipid autooxidation may have been partially inhibited, resulting in lower free fatty acid values. Based on analysis of biogenic amines, acrylamide and free fatty acids, it can be said that the amount of biogenic amines and acrylamide levels in meat analogues were lower than the limit set by EFSA as allowable intake of these compounds by consumers. The low peroxide and free fatty acids contents depicted that a low amount of lipid oxidation took place but did not induce rancidity in meat analogues. The overall analysis of biogenic amines, acrylamide and free fatty acids suggest that successful meat analogue which lowered the antinutrients was those that contained 6% of Soy d press-cakes extruded at 60% humidity and temperature of 110 °C and 120 °C. The addition of different amounts of press-cakes extruded at different humidity and temperature lowered the biogenic amines and free fatty acids but increased the acrylamide. These characteristics make the meat analogues with extruded plant origin press-cakes a suitable product for safe consumption.

3.9. The analysis of the effect of the addition of the extruded press-cakes on functional technological properties of the meat analogues

Water holding capacity is a significant factor because it impacts the quality and output of fresh meat and its products. WHC reflects the capacity of a protein to retain water and shape a protein gel network in meat analogues. The higher the water-holding capacity in meat analogues, the more the juiciness [120]. **Table 8** shows the effect of extruded plant origin press-cakes on the WHC of uncooked meat analogues. There was an increase in the WHC of meat analogues as temperature and humidity, as well as the amount of press-cakes added to the meat analogue matrix increased. The maximum level of WHC was observed in meat analogue, which consisted of 6% of Soy d press-cakes (1.738 g/g), which was extruded at a temperature of 120 °C with humidity of 60%. Overall, the meat analogues with Soy t, Soy d and Oat d were observed to be between 1.288– 1.685 g/g, 1.296– 1.819 g/g and 1.03 g/g – 1.172 g/g, respectively. The differences in value are not high as the temperature and humidity are independently significant ($p<0.05$), but the amount and the interactions are not ($p>0.05$).

Table 8. Data of functional technological properties of meat analogues containing extruded press-cakes

Meat analogues	WHC*, g/g	OBC*, g/g	BD*, g/ml	Cooking Loss, %
MA-C	1.17 ± 0.01	1.20 ± 0.02	0.96 ± 0.02	3.88
MT-110-30-3	1.22 ± 0.01	1.25 ± 0.01	1.01 ± 0.02	3.61
MT-110-30-6	1.38 ± 0.01	1.40 ± 0.02	1.02 ± 0.02	3.62
MT-120-30-3	1.39 ± 0.01	1.40± 0.02	1.03 ± 0.01	4.06
MT-120-30-6	1.47 ± 0.01	1.44 ± 0.01	1.03 ± 0.02	2.92
MT-110-60-3	1.54 ± 0.02	1.51 ± 0.01	1.05 ± 0.03	3.64
MT-110-60-6	1.56 ± 0.02	1.51 ± 0.01	1.05 ± 0.03	3.18
MT-120-60-3	1.66 ± 0.02	1.58 ± 0.01	1.05 ± 0.03	3.08
MT-120-60-6	1.69 ± 0.01	1.64 ± 0.02	1.06 ± 0.03	2.97
MD-110-30-3	1.30 ± 0.02	1.40 ± 0.01	1.02 ± 0.02	3.60
MD-110-30-6	1.36 ± 0.02	1.43 ± 0.01	1.03 ± 0.02	2.78
MD-120-30-3	1.39 ± 0.01	1.53 ± 0.01	1.05 ± 0.02	2.77
MD-120-30-6	1.45 ± 0.02	1.54 ± 0.02	1.05 ± 0.02	4.96
MD-110-60-3	1.46 ± 0.01	1.57 ± 0.01	1.07 ± 0.02	2.43
MD-110-60-6	1.52 ± 0.01	1.60 ± 0.01	1.08 ± 0.02	2.02
MD-120-60-3	1.74 ± 0.02	1.61 ± 0.01	1.08 ± 0.01	3.39
MD-120-60-6	1.82 ± 0.01	1.69 ± 0.03	1.09 ± 0.02	2.58
MO-110-30-3	1.03 ± 0.04	1.29 ± 0.05	0.98 ± 0.02	3.89
MO-110-30-6	1.04 ± 0.03	1.41 ± 0.06	0.99 ± 0.01	4.19
MO-120-30-3	1.10 ± 0.02	1.42 ± 0.05	0.99 ± 0.01	4.38
MO-120-30-6	1.13 ± 0.05	1.52 ± 0.10	0.99 ± 0.04	4.07
MO-110-45-3	1.07 ± 0.03	1.69 ± 0.09	0.99 ± 0.03	4.01
MO-110-45-6	1.09 ± 0.03	1.71 ± 0.04	1.00± 0.04	4.02
MO-120-45-3	1.16 ± 0.04	1.86 ± 0.02	1.01 ± 0.04	4.47
MO-120-45-6	1.17 ± 0.05	1.91 ± 0.03	1.02 ± 0.02	3.42

All value within the same column indicates significant differences for three factors(temperature, humidity and amount) independently, three-way ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Oil binding capacity is a functional property of meat analogues that is influenced by pore size and molecule charges. High OBC implies that proteins have a higher hydrophobic character, causing fat to link to protein through capillary attraction physically. As a result of exposing non-polar amino acids and increasing hydrophobicity, the protein increases oil absorption. OBC has an effect on shelf life. As a result, meat analogue with the highest OBC is preferred [121]. Similar to the results of water holding capacity, the meat analogues increased after adding the extruded plant origin press-cakes, which were extruded at high temperature and humidity. The meat analogues with Oat d, Soy t and Soy d were between 1.25 – 1.91 g/g, 1.22– 1.64 g/g and 1.40– 1.69 g/g, respectively. The differences in value are not high as each factor is independently significant ($p<0.05$), but the interactions are not significant ($p>0.05$). The bulk density of meat analogues with extruded plant origin press-cakes increased with increasing amounts of extruded press-cakes. The difference between the bulk density values of meat analogues is not high as the amount, and the interactions are not significant ($p>0.05$). The highest bulk density was observed at extrudate temperature of 120 °C having 45% or 60% humidity when 6% extruded press-cakes were added to the meat analogue matrix, while the lowest was observed at extrudate temperature of 110 °C with 30% humidity when 3% of the extrudate was added to the meat analogue matrix. This increase in bulk density occurred due to other ingredients present in the meat analogue matrix whose variables have not been considered. Cooking loss is an indicator that shows how much meat analogues shrinks during cooking and is used to assess the quality in terms of juiciness and yield of the finished product. In general, preparation parameters such as constituents of composite materials have an impact on the cooking loss of processed products [122]. From **table 8,** it can be noted that the cooking loss is within the range of 2.5 – 5% for the meat analogues. The addition of press-cakes to the meat analogues did not affect the cooking loss as there was no significant difference. An overall analysis of the functional technological properties of the meat analogues depicts that the meat analogue containing 6% of press-cakes extruded at a temperature of 120 °C and humidity of 45% or 60% showed best results, especially the meat analogue with extruded Soy d press-cakes which showed maximum water-holding capacity, oil-binding capacity and bulk density than meat analogues with other extruded press-cakes.

3.10. The analysis of the effect of the addition of the extruded press-cakes on the texture and colour of meat analogues

3.10.1. The analysis of the effect of the addition of the extruded press-cakes on colour changes in the meat analogues

The colour analysis found that samples cooked with similar parameters, i.e. baking time and baking temperature, showed similar *L* and *a* values, which is a distinctive colour parameter for products made from meat, implying that this method can be applied to produce meat analogues with similar appearances to their meat equivalents. One disadvantage of using plant proteins is that the colour of meat analogues may fade when exposed to air or light for long time, resulting in unappealing products [123]. In our samples, the brightness of the cooked meat analogues was lower than the control, which does not contain any added press-cake samples. This is due to the addition of extruded press-cake samples, which lowered the amount of brightness. The brightness and yellowness of the cooked meat analogues containing extruded press-cakes are very close to the brightness and yellowness of the cooked meat patties. The meat analogue samples containing extruded press-cakes have similar redness to that of meat patties. The brightness values of meat analogues with Soy t, Soy d and Oat d press-cakes are between 43 – 54, while redness and yellowness range from 7-11 and 16 – 24, respectively (**appendix 17**). All values of colour measurements show a significant difference as $p<0.05$. The result of the colour measurement shows a positive effect of the addition of extruded press-cakes to meat analogues, making it an excellent alternative to meat patties.

3.10.2. The analysis of the effect of the addition of the extruded press-cakes on texture changes in the cooked meat analogues

Texture profile analysis is useful as the textural characteristics derived from TPA correlates with the sensory assessment of textural characteristics [124]. The capability to retain fat, their oil and water-holding capacity, as well as their gelling and emulsifying properties, which can be measured via texture analysis, are all factors to consider [124]. The sensory characteristics and rheological properties of different foods have been compared using parameters found in texture profile analysis, such as hardness, adhesiveness, cohesiveness, springiness and gumminess [125]. **Appendix 18** depicts the textural parameters on the addition of different extruded plant origin press-cakes. The most commonly evaluated parameter for texture analyses is hardness or firmness, which is the force required to achieve a deformation. This texture property is crucial for meat analogue products such that they are not deformed during the process of cooking [126]. The hardness decreased after the addition of extruded press-cakes. The lowest measure of hardness was noted when 6% of extruded press-cakes were added to the meat analogues, while the highest was observed at 110 °C with 30% humidity and 3% added oat presscakes. Hardness for meat analogues with extruded soy t and d press-cakes were between 9 – 30 N and 9 – 24 N, respectively. The hardness increased as the amount of extruded press-cakes in the meat analogues increased. The firmness detected occurred due to the use of rapeseed oil in the matrix, which induces protein-protein interaction. Wi Gi Hyun et al. [104] findings matched our observations, in which rapeseed oil formed a dense protein gel network of soy protein isolate matrix due to hydrophobic interaction between the oil globule and the hydrophobic peptides in the protein. The necessary work for the prevailing attraction force between the surface of foodstuffs and different materials coming into contact is called adhesiveness or stickiness. In fact, the force needed to separate the material that sticks to the teeth while eating is known as adhesiveness [127]. Meat analogue samples show the adhesiveness to be around zero joules. These values are significant ($p<0.05$). Cohesiveness, also known as consistency, is an important textural parameter that indicates consumer acceptance. The forces which keep the product intact, are defined as cohesiveness, and it is expressed as the force content that can cause a material to deform until it breaks [127]. The cohesiveness of all press-cake samples are in the range of 0.70 – 0.77 and showed significance when statistically analysed ($p<0.05$). Heat treatment, interaction of proteins, elasticity, and the degree of protein denaturation are all factors that influence springiness [124]. Meat analogues with extruded Oat d press-cakes had a value of springiness between 2.5 mm – 3.05 mm, while the springiness of the meat analogues containing extruded Soy t and Soy d press-cakes had values between 1.5– 5.8 mm and 2.4– 2.9 mm, respectively. All values are statistically significant ($p<0.05$). Gumminess lowered with the addition of extruded press-cakes to meat analogue matrix. It can be noted that the gumminess of the meat analogues with the addition of 6% of extruded Soy t press-cakes was lower while meat analogues with the addition of 3% of extruded Oat d press-cakes showed a high level of gumminess close to that of the control sample with no added press-cakes. All values were statistically significant ($p<0.05$). The texture profile analysis depicts that the addition of extruded press-cakes to the meat analogue matrix did show some significant effect and improved its hardness, springiness and gumminess as compared to the control meat analogue. The results of the texture profile analysis show similar characteristics of meat analogues with extruded press-cakes to that of meat patties. The texture of meat analogues containing 6% of Soy d press-cakes extruded at 120 °C temperature, and 60% humidity and 32% texturised soy protein as the base matrix resembled the closest to the texture of meat patties.

3.11. The analysis of the effect of the addition of the extruded press-cakes on sensory properties of the meat analogues

Prior to consumption, the product's appearance is critical for attracting consumers and establishing expectations. As a result, it is necessary to provide good sensory qualities that can be experienced before and after consumption of the product [128]. To create positive expectations, meat analogues should have a similar overall appearance to familiar meat products. The recreation of the distinctive appearance, taste, flavour, aroma, texture, mouthfeel, and moistness of conventional meat products is another challenge for meat analogues [123]. **Fig.10** shows the appearance of the baked meat analogues with and without the addition of extruded press-cakes. Meat analogues containing extruded press-cakes appear to be brighter than the meat analogue without press-cakes. This observation correlates our results from the colour measurements of the extruded press-cakes, and it can be stated that the addition of extruded press-cakes did show some significant effect on the appearance of meat analogues.

(a) (b) (c) (d)

Fig. 10. The appearance of the meat analogues (a) without the addition of the extruded press-cakes (b) with the addition of the extruded Soy t press-cake (c) with the addition of the extruded Soy d press-cake and (d) with extruded Oat d press-cake

Consumer acceptance for texture can be predicted using instrumentation together with sensory assessment. In order to create vegetarian meat analogues, the right protein source must be chosen. Food additives, in addition to choosing a source of plant protein, is a way to enhance the texture of meat analogues. Hydrocolloids are commonly used in meat analogues to compensate for the loss of textural quality that occurs when part of the fat and salt content is reduced. Finally, the texture and mouthfeel properties of meat analogues can be influenced by processing methods. Lin et al. [123] investigated the effects of moisture content and cooking temperature on the properties of a texturised soy protein-based meat analogues. Six researchers conducted a qualitative descriptive analysis of the product, rating different sensory parameters. There was no real distinction in scores. The panellists' scoring of each sensory attributes for each sample with added press-cakes along with the control sample can be observed from their spider diagram shown in **Appendix 23** . The results of detailed sensory analysis (**appendix 19, appendix 20** and **appendix 21**) depicted the significant effect of the addition of the extruded plant origin press-cakes on the sensory characteristics of meat analogues such as odour, the intensity of taste, acidity, bitterness, nutty flavour and aftertaste. Meat analogues with extruded Soy t press-cakes showed high odour intensity in comparison with meat analogues having other extruded press-cakes. The acidity decreased when the extruded press-cakes were added to the meat analogue matrix. Similarly, astringency also lowered with the addition of extruded press-cakes to the meat analogue matrix. The acceptability of overall appearance, odour, taste and texture increased after adding the extruded press-cakes. High acceptability of appearance, odour and texture was observed in meat analogues containing extruded Soy t press-cakes while high acceptability of texture was observed in meat analogues with extruded Soy d press-cakes. The overall acceptability in **appendix 22** depicted that meat analogues with extruded soy press-cakes were highly received by the sensory panellists. On overcall comparison of the meat analogues with extruded press-cakes, meat analogue made by adding 6% of Soy d press-cakes extruded at 120 °C, and 60% showed the highest scores followed by meat analogue made by adding 6% of Oat d press-cakes extruded at 120 °C and 45% humidity, suggesting capability of both these meat analogue matrices to be well accepted by the consumers if launched in the market.

CONCLUSION

1. In the current study, the inclusion of soy and oat press-cakes, as a by-product of plant-based product manufacturing, in meat analogues were examined for their chemical, functional and sensory properties. These plants can be grown and processed in Lithuania, having useful content of proteins and carbohydrates (after the main manufacturing processes) left with the potential to utilise them into the new plant origin product matrices as evidence for sustainable use of raw materials.

2. The low moisture extrusion process has been applied to increase the functional properties and nutritional value of the soy and oat press-cakes. To detect the optimal extrusion parameters and to assure quality and functional properties of the extruded press-cakes, a pre-testing of extrusion process was conducted at various temperatures and humidity.

3. The best four extrusion models were designed and validated at temperatures (110 °C and 120 °C) and humidities (30% and 60% for soy press-cakes and 30% and 45% for oat press-cakes).

4. The analysis of extruded Soy t, Soy d and Oat d press-cakes revealed that the soy press-cakes were more resistant to the extrusion process than the oat ones. Lower protein losses were obtained by extruding the press-cakes at 30% humidity regardless of the temperature. Trypsin inhibitor content reduced up to 60% when the press-cakes were extruded at a temperature of 110 °C or 120 °C with 60% humidity. In terms of biogenic amines, lowest amount of histamine content was detected in extruded Oat d press-cakes. The amount of cadaverine and tyramine was negligible while the putrescine content was between 5 mg/kg – 29 mg/kg. The amount of acrylamide increased with increasing temperature. Peroxide values and free fatty acid content were not detectable for extruded Soy t and oat press-cakes, while extruded Soy d press-cakes showed more than 20 Mekv/kg of peroxide value and 1.8%-2.8% of free fatty acid content. The functional technological properties increased after extrusion. Press-cakes extruded at 120 °C, and 45 or 60% humidity showed the maximum water-holding capacity (Soy t – 5.45 g/g; Soy d – 6.88 g/g; Oat d – 4.85 g/g) and oil-binding capacity (Soy t – 2.93 g/g; Soy d – 2.97 g/g; Oat d – 2.86 g/g) while those extruded at 110 °C and 30% humidity showed maximum bulk-density (Soy t – 0.80 g/ml; Soy d – 0.88 g/ml; Oat d – 0.73 g/ml).

5. The best functional properties and nutritionally rich extruded press-cakes were included in meat analogues matrices. Based on chemical analysis, anti-nutritional effect and functional technological properties, meat analogue matrices with texturised soy base with inclusion of 3% or 6% of the extruded press-cakes were designed. The analysis of chemical composition depicted that meat analogues containing 6% Soy t and Soy d press-cakes extruded at 110 °C or 120 °C and 60% humidity showed the best results. In terms of anti-nutritional effect, 6% of Soy d press-cakes extruded at 60% humidity, and temperature of 110 °C and 120 °C showed a low amount of anti-nutrients. The highest functional technological properties were noticed in 6% of Soy d press-cakes extruded at 60% humidity and temperature of 110 °C and 120 °C (WHC – 1.82 g/g; OBC – 1.69 g/g; BD – 1.09 g/ml; CL – 2.58%). A matrix modification is recommended when adding more than 6% of extruded press-cakes to the meat analogues. According to the results of the texture and sensory evaluation, a meat analogue with 6% press-cakes extruded at 60% humidity and 120 °C received the highest acceptability scores.

6. The overall results show that the addition of extruded press-cakes to the meat analogue matrix did impact its chemical, functional and sensory properties and meat analogues having 6% of press-cakes extruded at 60% humidity with an extrusion temperature of 110 °C or 120 °C would be most suitable for further development.

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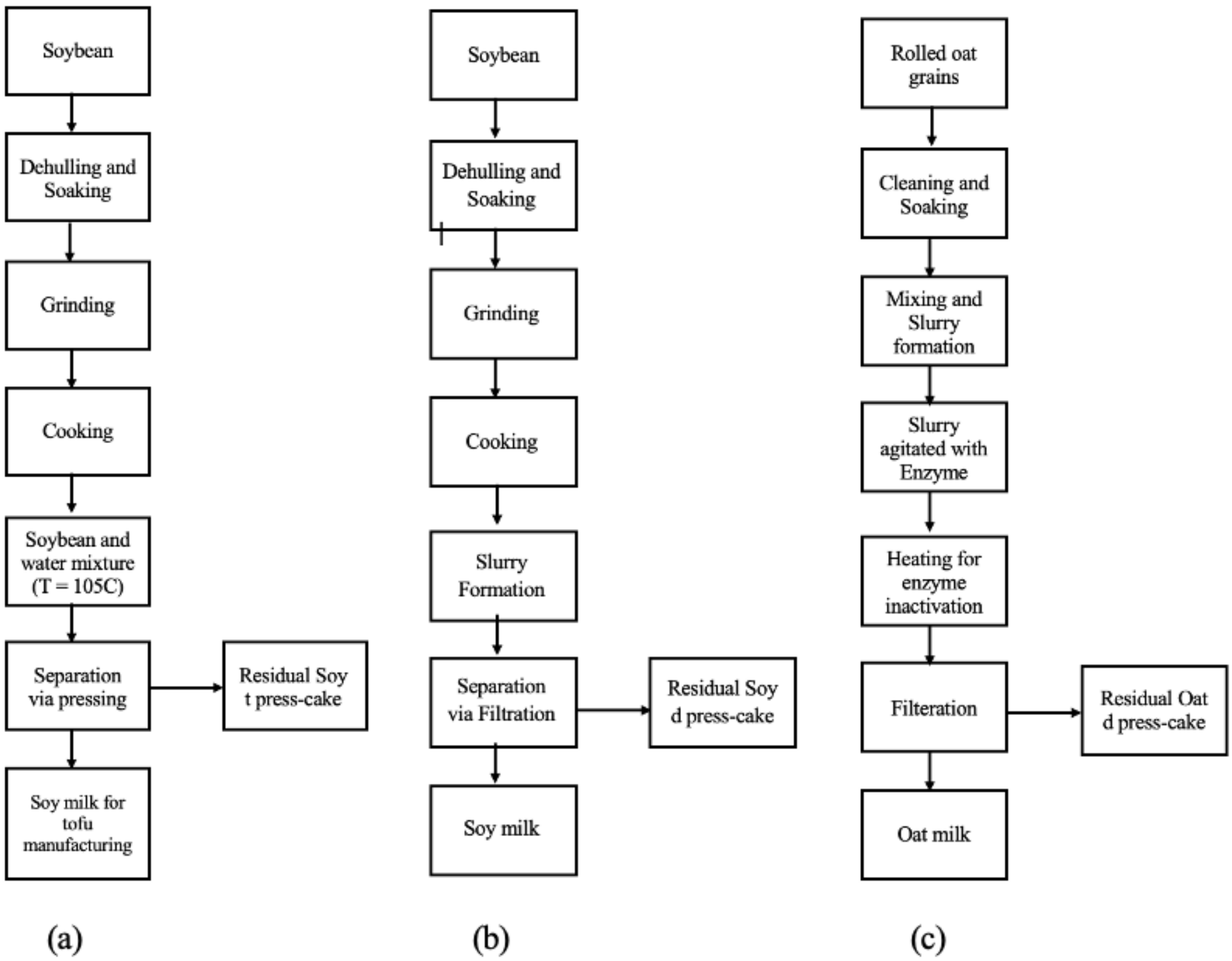
LIST OF APPENDICES

Appendix 1. Stages of (a) Soy t (b) Soy d and (c) Oat d press-cakes production process



Appendix 2. Parameters of the extrusion process for the press-cakes

Sample	Humidity content, % (before experiment)	T_{max} of extrusion, °C	Temperature in zones (1-11), °C										Screw speed, rpm	Torque, %	P 1, bar	P 2, bar	Throughput, kg/h		Moisture content, % (after extrusion)	SME, W*h/kg
			1	2	3	4	5	6	7	8	9	10	11							
Soy t	28	110	18	65	85	95	99.6	105	110	110	110	110	111	100.0	6.0	14.4	13.4	3.4	25	38.5
Soy t	28	120	18	65	85	95	105	110	120	119	120	120	120	100.0	4.2	11.0	10.8	2.6	23	35.3
Soy t	60	110	18	64	84	95	100	104	110	110	110	110	111	100.0	4.0	6.3	5.4	8.5	57	10.2
Soy t	60	120	18	65	85	95	105	110	120	120	120	120	117	100.0	4.0	7.6	7.0	4.1	56	21.2
Soy d	31	110	19	64	85	95	100	106	110	110	110	110	111	99.9	12.0	21.7	20.0	10.7	23	24.2
Soy d	31	120	18	64	85	95	105	110	120	120	120	120	120	100.0	4.7	5.9	5.2	12.7	25	7.9
Soy d	60	110	19	64	85	95	100	105	110	110	110	110	110	100.0	4.7	6.4	5.6	13.1	55	7.7
Soy d	60	120	18	65	85	95	105	110	120	120	120	120	120	100.0	5.0	5.9	5.2	14.1	56	7.7
Oat d	28	110	19	65	85	95	100	105	110	110	105	110	112	100.0	6.3	28.5	26.3	13.6	25	9.9
Oat d	28	120	18	65	85	95	105	110	120	119	113	120	119	100.0	23.5	25.8	23.9	13.5	24	37.8
Oat d	45	110	19	65	85	95	100	105	110	120	119	110	114	99.9	8.0	8.6	7.5	16.1	41	10.8
Oat d	45	120	19	65	85	95	105	110	120	120	110	120	117	100.0	7.3	8.9	7.8	15.3	40	10.3

Appendix 3. Colour measurements of non-extruded (untreated) and the extruded press-cakes

Extruded Soy t press-cakes				
Humidity (%)	**Temperature (°C)**	**L**	**a**	**b**
Untreated		68.65 ± 0.37^{b,B}	1.47 ± 0.28^{c,C}	31.50 ± 0.34^{d,D}
30	110	71.37 ± 0.41^{d,D}	2.64 ± 0.32^{e,E}	33.25 ± 0.17^{e,E}
	120	79.39 ± 0.43^{e,E}	2.54 ± 0.29^{d,D}	30.36 ± 0.22^{c,C}
60	110	70.51 ± 0.42^{c,C}	-0.26 ± 0.07^{a,A}	24.44 ± 0.27^{b,B}
	120	65.17 ± 0.08^{a,A}	0.41 ± 0.27^{b,B}	22.46 ± 0.14^{a,A}
Extruded Soy d press-cakes				
Humidity (%)	**Temperature (°C)**	**L**	**a**	**b**
Untreated		83.49 ± 0.46^{e,E}	1.00± 0.07^{a,A}	21.64 ± 0.29^{b,B}
30	110	59.90 ± 0.08^{a,A}	3.68 ± 0.44^{d,D}	22.43 ± 0.05^{d,D}
	120	65.41 ± 0.22^{b,B}	4.40 ± 0.29^{e,E}	25.45 ± 0.35^{e,E}
60	110	76.65 ± 0.29^{c,C}	2.52 ± 0.14^{b,B}	20.84 ± 0.06^{a,A}
	120	77.13 ± 0.89^{d,D}	2.66 ± 0.14^{c,C}	22.34 ± 0.40^{c,C}
Extruded Oat d press-cakes				
Humidity (%)	**Temperature (°C)**	**L**	**a**	**b**
Untreated		62.45 ± 0.04^{e,E}	6.03 ± 0.02^{a,A}	22.76 ± 0.01^{b,B}
30	110	57.66 ± 0.03^{d,D}	7.66 ± 0.03^{b,B}	25.09 ± 0.00^{d,D}
	120	50.93 ± 0.01^{b,B}	9.13 ± 0.05^{d,D}	24.86 ± 0.03^{c,C}
45	110	55.85 ± 0.04^{c,C}	8.64 ± 0.03^{c,C}	27.36 ± 0.20^{e,E}
	120	50.44 ± 0.03^{a,A}	9.35 ± 0.03^{e,E}	22.25 ± 0.02^{a,A}

$^{a-e}$ within the same column indicate significant difference for temperature, 2-way ANOVA, $p < 0.05$
$^{A-E}$ within the same column indicate significant differences for humidity, 2-way ANOVA, $p < 0.05$
The interactions are significant for all values within the same column, 2-way ANOVA, $p < 0.05$

Appendix 4. Amino acid analysis of untreated and extruded soy press-cakes

Amino Acid Content, g/100g	Untreated	Extruded Soy t press-cakes, humidity/temperature				Untreated	Extruded Soy d press-cakes, humidity/temperature			
		30%		60%			30%		60%	
		110 ^0C	120 ^0C	110 ^0C	120 ^0C		110 ^0C	120 ^0C	110 ^0C	120 ^0C
Aspartic Acid	4.18±0.03	3.48±0.04	3.35±0.03	2.04±0.05	2.12±0.03	3.10±0.01	3.58±0.02	3.10±0.03	3.58±0.09	3.10±0.03
Glutamic Acid*	5.94±0.05	4.86±0.02	4.68±0.04	2.81±0.03	2.86±0.06	4.60±0.08	4.88±0.09	4.60±0.07	4.88±0.04	4.60±0.05
Serine	1.75±0.02	1.29±0.06	1.24±0.09	0.72±0.04	0.79±0.02	1.39±0.07	1.36±0.08	1.390.04±	1.36±0.03	1.39±0.07
Glycine	1.21±0.01	1.11±0.03	1.03±0.08	0.67±0.04	0.70±0.03	1.08±0.07	1.15±0.07	1.00.068±	1.15±0.05	1.08±0.09
Histidine	0.58±0.08	0.82±0.04	0.81±0.04	0.53±0.05	0.54±0.04	0.66±0.10	0.85±0.03	0.66±0.08	0.85±0.04	0.66±0.09
Threonine	1.65±0.03	1.18±0.06	1.16±0.05	0.76±0.07	0.76±0.06	1.12±0.13	1.24±0.04	1.120.03±	1.24±0.03	1.12±0.03
Alanine	1.43±0.04	1.17±0.07	1.14±0.06	0.70±0.02	0.73±0.09	1.15±0.04	1.24±0.04	1.15±0.02	1.24±0.04	1.15±0.10
Arginine	2.44±0.06	1.95±0.07	1.91±0.03	1.23±0.05	1.26±0.08	1.74±0.04	1.99±0.06	1.74±0.05	1.99±0.04	1.74±0.01
Proline	3.61±0.03	1.49±0.03	1.73±0.05	0.95±0.03	0.80±0.03	0.99±0.03	2.01±0.06	0.99±0.09	2.01±0.07	0.99±0.02
Cysteine	0.92±0.04	0.81±0.05	0.84±0.06	0.54±0.05	0.58±0.04	0.00±0.02	0.84±0.09	0.00±0.04	0.84±0.09	0.00±0.01
Tyrosine	1.15±0.04	1.06±0.02	1.05±0.04	0.71±0.04	0.74±0.05	0.77±0.06	1.09±0.07	0.77±0.05	1.09±0.05	0.77±0.10
Valine	1.31±0.03	1.36±0.04	1.42±0.03	0.90±0.01	1.13±0.06	1.29±0.03	1.52±0.06	1.29±0.02	1.52±0.07	1.29±0.05
Methionine	0.91±0.11	0.58±0.05	0.38±0.05	0.36±0.04	0.28±0.01	0.25±0.05	0.42±0.03	0.25±0.04	0.42±0.09	0.25±0.04
Lysine*	4.22±0.03	2.23±0.02	2.07±0.07	1.42±0.02	1.65±0.07	1.26±0.07	2.21±0.02	1.26±0.05	2.21±0.09	1.26±0.06
Isoleucine*	2.63±0.06	2.00±0.04	1.93±0.06	1.16±0.07	1.19±0.04	2.11±0.03	2.12±0.04	2.11±0.07	2.12±0.10	2.11±0.09
Leucine	1.62±0.04	1.49±0.05	1.48±0.03	0.91±0.08	0.93±0.06	1.32±0.05	1.42±0.07	1.32±0.04	1.42±0.01	1.32±0.06
Phenylalanine	1.80±0.07	1.25±0.02	1.24±0.04	0.79±0.09	0.81±0.02	1.46±0.08	1.31±0.012	1.46±0.01	1.31±0.03	1.46±0.04
Tryptophan	2.33±0.01	2.19±0.01	2.11±0.06	1.17±0.10	1.16±0.04	1.76±0.07	2.30±0.08	1.76±0.03	2.30±0.08	1.76±0.07

All values within the same row indicates significant differences for two factors (temperature and humidity), 2-way ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 5. Amino acid analysis of untreated and extruded oat d press-cakes

Amino Acid Content, g/100g	Untreated	Extruded Oat d press-cakes, humidity/temperature			
		30%		45%	
		110 ^{0}C	120 ^{0}C	110 ^{0}C	120 ^{0}C
Aspartic Acid	6.51±0.07	2.11±0.02	2.33±0.05	1.75±0.08	1.74±0.06
Glutamic Acid*	13.00±0.08	4.45±0.08	5.39±0.01	3.77±0.05	3.96±0.03
Serine	3.51±0.05	1.10±0.09	1.23±0.05	0.91±0.03	0.93±0.04
Glycine	2.91±0.04	1.08±0.05	1.18±0.06	0.88±0.05	0.89±0.07
Histidine	1.64±0.01	0.45±0.06	0.50±0.04	0.39±0.09	0.38±0.03
Threonine	2.54±0.03	0.81±0.05	0.90±0.02	0.66±0.08	0.67±0.08
Alanine	3.88±0.04	1.13±0.04	1.24±0.03	0.92±0.096	0.94±0.02
Arginine	5.04±0.05	1.11±0.09	1.22±0.04	0.83±0.03	0.84±0.03
Proline	3.22±0.06	2.44±0.09	0.95±0.06	1.45±0.05	1.27±0.04
Cysteine	0.13±0.09	0.00±0.01	0.00±0.01	0.00±0.03	0.00±0.01
Tyrosine	3.25±0.09	0.75±0.03	0.82±0.04	0.61±0.05	0.63±0.04
Valine	4.25±0.10	1.18±0.05	1.35±0.09	1.02±0.08	1.03±0.08
Methionine	1.71±0.04	0.21±0.06	0.19±0.03	0.11±0.05	0.11±0.07
Lysine*	1.97±0.01	0.72±0.08	0.78±0.04	0.61±0.02	0.59±0.01
Isoleucine*	5.82±0.10	1.74±0.07	1.89±0.05	1.38±0.01	1.41±0.05
Leucine	1.39±0.03	0.71±0.07	0.81±0.07	0.54±0.04	0.54±0.03
Phenylalanine	3.10±0.07	1.54±0.06	1.65±0.01	1.22±0.06	1.24±0.08
Tryptophan	4.66±0.09	1.68±0.05	1.83±0.05	1.34±0.03	1.37±0.02

All values within the same row indicates significant differences for two factors (temperature and humidity), 2-way ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 6. Fatty acid analysis of extruded plant press-cakes

Fatty Acid composition, %	Extruded Soy t press-cakes, humidity/temperature				Extruded Soy d press-cakes, humidity/temperature				Extruded Oat d press-cakes, humidity/temperature			
	30%		60%		30%		60%		30%		45%	
	110 °C	120 °C	110 °C	120 °C	110 °C	120 °C	110 °C	120 °C	110 °C	120 °C	110 °C	120 °C
Myristic acid	0.00±0.00	0.19±0.01	0.43±0.09	0.19±0.07	0.18±0.06	0.00±0.08	0.00±0.04	0.48±0.03	0.46±0.09	0.20±0.02	0.41±0.01	0.22±0.03
Palmitic acid	9.95±0.01	10.50±0.03	12.92±0.07	13.43±0.04	11.87±0.04	10.62±0.09	11.23±0.09	14.55±0.01	15.76±0.08	15.44±0.03	15.51±0.03	14.92±0.05
Palmitoleic acid	0.16±0.07	0.16±0.03	0.63±0.05	0.82±0.03	0.19±0.04	0.22±0.01	0.00±0.07	0.77±0.09	0.33±0.10	0.24±0.03	0.38±0.07	0.29±0.08
Stearic acid*	3.90±0.03	3.71±0.01	5.29±0.01	4.68±0.02	4.20±0.04	4.05±0.03	4.18±0.02	6.64±0.01	1.53±0.06	1.23±0.09	1.32±0.07	1.36±0.01
Oleic acid	18.59±0.04	19.14±0.01	21.50±0.09	27.39±0.01	24.39±0.03	23.89±0.03	24.36±0.04	29.04±0.03	39.82±0.11	39.71±0.13	39.42±0.09	42.12±0.07
ω-6 Linoleic acid	57.49±0.09	56.59±0.1	49.85±0.11	46.06±0.13	51.75±0.09	53.32±0.07	52.86±0.05	42.39±0.03	38.96±0.03	40.54±0.04	39.08±0.05	38.56±0.03
ω-3 alpha-Linoleic*	9.01±0.03	8.83±0.10	8.82±0.09	7.43±0.08	6.32±0.04	6.59±0.01	6.86±0.03	5.25±0.04	1.25±0.05	1.16±0.05	1.23±0.09	1.09±0.09
Arachidic acid*	0.36±0.01	0.31±0.01	0.00±0.00	0.00±0.00	0.28±0.04	0.27±0.03	0.28±0.03	0.39±0.09	0.10±0.01	0.09±0.01	0.20±0.02	0.10±0.04
Eicosenoic acid*	0.15±0.09	0.12±0.03	0.00±0.00	0.00±0.00	0.18±0.09	0.17±0.10	0.00±0.00	0.32±0.07	0.80±0.04	0.76±0.03	1.13±0.03	0.76±0.01
Behenic acid	0.29±0.07	0.28±0.01	0.00±0.00	0.00±0.00	0.27±0.04	0.27±0.07	0.00±0.00	0.18±0.04	0.06±0.00	0.05±0.00	0.04±0.00	0.04±0.00
Erucic acid*	0.00±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.00	0.05±0.00	0.67±0.02	0.05±0.00
Lignoceric acid*	0.00±0.00	0.11±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.18±0.05	0.00±0.00	0.00±0.00	0.05±0.00	0.06±0.00	0.05±0.00	0.05±0.00
Nervonic acid*	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.06±0.01	0.06±0.01	0.09±0.02	0.05±0.01

All values within the same row indicates significant differences for two factors (temperature and humidity), 2-way ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 7. Data on the effect of the extrusion on the antinutrients composition changes of the press-cakes

Press-cakes extrusion parameters		Biogenic amines, mg/kg					Acrylamide*, µg/kg	Peroxide Value, Mekv/kg	Free fatty acid content, %	Fatty acid content, mg KOH/g
H (%)	T (°C)	HIS	CAD	PUT	TYR	SPE				
Soy t press-cakes										
Untreated		<5	<5	30±0.71^{d,D}	<5	47±0.69^{d,D}	nd	nd	nd	nd
30	110	9±0.57	<5	19±0.33^{a,A}	<5	49±0.43^{d,E}	10±0.51^{a,A}	nd	nd	nd
	120	7±0.49	<5	19±0.34^{b,B}	<5	44±0.42^{c,C}	12±0.49^{b,B}	nd	nd	nd
60	110	<5	<5	11±0.69^{c,C}	<5	27±0.20^{a,A}	13±0.20^{c,C}	nd	nd	nd
	120	21±0.99	<5	7±0.72^{e,E}	<5	35±0.17^{b,B}	15±0.79^{d,D}	nd	nd	nd
Soy d press-cakes										
Untreated		<5	<5	10±0.94^{c,C}	<5	70±0.27^{c,C}	nd	nd	nd	nd
30	110	45±0.61	<5	8±0.63^{b,B}	<5	83±0.29^{d,D}	28±0.41^{b,B}	>20	1.9±0.09^{b,B}	3.7±0.03^{b,B}
	120	46±0.43	<5	4±0.23^{a,A}	<5	85±0.66^{e,E}	31±0.26^{c,C}	>20	1.8±0.04^{a,A}	3.5±0.01^{a,A}
60	110	8±0.23	6	29±0.21^{e,E}	<5	42±0.66^{a,A}	76±0.53^{d,D}	>20	nd	nd
	120	<5	<5	16±0.17^{d,D}	<5	45±0.48^{b,B}	27±0.74^{a,A}	>20	2.8±0.41^{c,C}	5.6±0.04^{c,C}
Oat d press-cakes										
Untreated		<5	<5	8±0.32^{b,B}	<5	18±0.11	nd	nd	nd	nd
30	110	<5	<5	12±0.12^{d,D}	nd	nd	41±0.56^{b,B}	nd	nd	nd
	120	<5	<5	9±0.47^{c,C}	nd	nd	49±0.73^{d,D}	nd	nd	nd
45	110	<5	<5	8±0.36^{b,B}	nd	nd	37±0.82^{a,A}	nd	nd	nd
	120	<5	<5	7±0.58^{a,A}	nd	nd	45±0.45^{c,C}	nd	nd	nd

$^{a-e}$ within the same column indicate significant differences for temperature, 2-way ANOVA, $p < 0.05$
$^{A-E}$ within the same column indicate significant differences for humidity, 2-way ANOVA, $p < 0.05$
* indicate that their interactions are significant for all values within the same column, 2-way ANOVA, $p > 0.05$

nd – not detected

Appendix 8. Data of the meat analogue matrices with the addition of the extruded press-cakes (only main ingredients are shown in the recipe)

Meat analogues	Soy protein isolate, %	Water, %	Industrial emulsion, %	Oil, %	Amount of press-cakes, %	Added press-cakes
MA-C	34.2	34.2	17.1	12.3	0	None
MT-110-30-3	33.2	33.2	16.6	11.9	3	Soy t
MT-110-30-6	32.1	32.1	16.1	11.6	6	Soy t
MT-120-30-3	33.2	33.2	16.6	11.9	3	Soy t
MT-120-30-6	32.1	32.1	16.1	11.6	6	Soy t
MT-110-60-3	33.2	33.2	16.6	11.9	3	Soy t
MT-110-60-6	32.1	32.1	16.1	11.6	6	Soy t
MT-120-60-3	33.2	33.2	16.6	11.9	3	Soy t
MT-120-60-6	32.1	32.1	16.1	11.6	6	Soy t
MD-110-30-3	33.2	33.2	16.6	11.9	3	Soy d
MD-110-30-6	32.1	32.1	16.1	11.6	6	Soy d
MD-120-30-3	33.2	33.2	16.6	11.9	3	Soy d
MD-120-30-6	32.1	32.1	16.1	11.6	6	Soy d
MD-110-60-3	33.2	33.2	16.6	11.9	3	Soy d
MD-110-60-6	32.1	32.1	16.1	11.6	6	Soy d
MD-120-60-3	33.2	33.2	16.6	11.9	3	Soy d
MD-120-60-6	32.1	32.1	16.1	11.6	6	Soy d
MO-110-30-3	33.2	33.2	16.6	11.9	3	Oat d
MO-110-30-6	32.1	32.1	16.1	11.6	6	Oat d
MO-120-30-3	33.2	33.2	16.6	11.9	3	Oat d
MO-120-30-6	32.1	32.1	16.1	11.6	6	Oat d
MO-110-45-3	33.2	33.2	16.6	11.9	3	Oat d
MO-110-45-6	32.1	32.1	16.1	11.6	6	Oat d
MO-120-45-3	33.2	33.2	16.6	11.9	3	Oat d
MO-120-45-6	32.1	32.1	16.1	11.6	6	Oat d

MA-C- Meat analogue control; MT – meat analogue with Soy t press-cakes; MD – Meat analogue with Soy d press-cakes; MO – Meat analogue with Oat d press-cakes

Appendix 9. Data of the effect of added extruded press-cakes samples on the chemical composition of the meat analogues (1)

Indicator, %	Humidity*	Fat content	Saturated Fatty acid*	Mono-unsaturated fatty acid*	Poly-unsaturated fatty acid	Trans fatty acid	Nitrogen *	Protein
MT-110-30-3	44.62±0.13	22.5±0.13	1.69±0.09	13.42±0.07	7.38±0.09	0.01±0.00	2.58±0.09	15.1±0.03
MT-110-30-6	43.80±0.13	21.9±0.06	1.68±0.07	12.86±0.06	7.36±0.06	0.00±0.00	2.50±0.03	15.6±0.04
MT-120-30-3	44.59±0.11	22.5±0.11	1.67±0.03	13.46±0.03	7.36±0.04	0.01±0.00	2.43±0.01	15.2±0.06
MT-120-30-6	43.54±0.07	22.6±0.07	1.89±0.04	13.27±0.01	7.42±0.03	0.01±0.00	2.58±0.07	16.1±0.07
MT-110-60-3	45.45±0.09	21.2±0.09	1.57±0.09	13.09±0.09	7.03±0.03	0.01±0.00	2.38±0.06	14.9±0.09
MT-110-60-6	46.11±0.09	19.6±0.01	1.51±0.09	11.57±0.01	6.52±0.06	0.00±0.00	2.63±0.01	16.3±0.09
MT-120-60-3	45.96±0.07	21.5±0.09	1.56±0.03	12.94±0.09	6.99±0.03	0.01±0.00	24.10±0.07	15.1±0.11
MT-120-60-6	45.96±0.07	22.4±0.13	1.73±0.09	13.42±0.07	7.24±0.06	0.00±0.00	23.70±0.09	14.8±0.13
MD-110-30-3	45.00±0.07	22.2±0.03	1.67±0.06	13.21±0.09	7.32±0.31	0.01±0.00	2.37±0.00	14.8±0.00
MD-110-30-6	44.11±0.11	20.0±0.07	1.58±0.01	11.56±0.04	6.86±0.27	0.01±0.00	2.66±0.03	16.6±0.01
MD-120-30-3	44.84±0.11	22.3±0.13	1.72±0.03	13.25±0.03	7.33±0.03	0.01±0.00	2.39±0.00	14.9±0.03
MD-120-30-6	43.98±0.03	19.8±0.09	1.79±0.07	11.36±0.03	6.64±0.08	0.00±0.00	2.60±0.00	16.3±0.04
MD-110-60-3	45.77±0.07	22.3±0.03	1.64±0.04	13.36±0.01	7.29±0.06	0.00±0.00	2.36±0.07	14.8±0.06
MD-110-60-6	46.23±0.09	19.6±0.01	1.49±0.06	11.42±0.06	6.68±0.04	0.00±0.00	2.62±0.06	16.4±0.07
MD-120-60-3	45.83±0.04	21.4±0.09	1.57±0.07	12.80±0.09	7.02±0.07	0.00±0.00	2.38±0.09	14.9±0.09
MD-120-60-6	46.35±0.07	19.7±0.13	1.47±0.01	11.51±0.09	6.71±0.07	0.01±0.00	2.60±0.04	16.3±0.09
MO-110-30-3	43.26±0.07	23.3±0.09	1.75±0.04	14.23±0.09	7.32±0.01	0.00±0.00	2.51±0.04	15.7±0.11
MO-110-30-6	42.74±0.04	23.6±0.13	1.77±0.03	14.56±0.06	7.27±0.07	0.01±0.00	2.53±0.03	15.8±0.13
MO-120-30-3	43.57±0.08	23.8±0.01	1.75±0.01	14.71±0.03	7.34±0.00	0.00±0.00	2.48±0.03	15.5±0.00
MO-120-30-6	43.12±0.07	23.5±0.13	1.81±0.11	14.48±0.06	7.20±0.08	0.01±0.00	2.43±0.06	15.2±0.01
MO-110-45-3	43.79±0.07	23.5±0.09	1.72±0.03	14.50±0.07	7.27±0.09	0.01±0.00	2.34±0.04	14.6±0.03
MO-110-45-6	43.88±0.04	23.3±0.03	1.69±0.04	14.41±0.07	7.19±0.01	0.01±0.00	2.45±0.4	15.3±0.04
MO-120-45-3	44.39±0.09	23.6±0.07	1.72±0.09	14.63±0.06	7.24±0.06	0.01±0.00	2.49±0.11	15.6±0.06
MO-120-45-6	43.91±0.09	23.0±0.03	1.68±0.09	14.19±0.07	7.13±0.06	0.00±0.00	2.55±0.07	15.9±0.07

All values within the same column indicates significant differences for three factors (temperature, humidity and amount) independently, 3-way ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 10. Data of the effect of added extruded press-cakes samples on the chemical composition of the meat analogues (2)

Indicator, %	Ash *	Sucrose	Glucose	Fructose*	Total Sugar	Salt	Fibre*
MT-110-30-3	2.04±0.04	2.76±0.03	<0.2	0.36±0.03	3.12±0.01	<0.03	6.8±0.01
MT-110-30-6	2.05±0.04	2.84±0.07	<0.2	0.37±0.04	3.21±0.09	<0.03	7.8±0.03
MT-120-30-3	2.01±0.03	2.69±0.13	<0.2	0.35±0.01	3.04±0.05	<0.03	6.7±0.04
MT-120-30-6	2.04±0.00	2.76±0.07	<0.2	0.36±0.01	3.12±0.01	<0.03	7.8±0.06
MT-110-60-3	1.98±0.04	2.78±0.09	<0.2	0.34±0.02	3.12±0.03	<0.03	5.2±0.07
MT-110-60-6	2.15±0.01	0.23±0.00	<0.2	0.74±0.09	0.94±0.03	<0.03	7.6±0.09
MT-120-60-3	1.99±0.03	3.41±0.09	<0.2	0.41±0.03	3.82±0.11	<0.03	7.0±0.09
MT-120-60-6	1.96±0.03	0.19±0.01	<0.2	0.44±0.08	0.44±0.04	<0.03	7.6±0.11
MD-110-30-3	2.00±0.13	2.91±0.11	<0.2	0.36±0.07	3.27±0.05	<0.03	6.6±0.13
MD-110-30-6	2.12±0.03	1.87±0.09	<0.2	0.62±0.01	2.49±0.08	<0.03	8.3±0.01
MD-120-30-3	1.93±0.03	2.86±0.07	<0.2	0.38±0.02	3.24±0.01	<0.03	7.7±0.03
MD-120-30-6	2.15±0.04	2.06±0.63	<0.2	0.53±0.02	2.59±0.02	<0.03	7.5±0.04
MD-110-60-3	1.95±0.03	2.78±0.44	<0.2	0.37±0.08	3.15±0.04	<0.03	6.7±0.06
MD-110-60-6	2.11±0.04	1.25±0.46	<0.2	0.71±0.05	1.96±0.09	<0.03	7.4±0.07
MD-120-60-3	1.98±0.04	2.95±0.56	<0.2	0.37±0..04	3.32±0.05	<0.03	6.3±0.09
MD-120-60-6	2.09±0.04	0.45±0.59	<0.2	0.72±0.03	2.17±0.04	<0.03	7.1±0.09
MO-110-30-3	2.01±0.09	2.50±0.06	0.53±0.04	0.40±0.04	3.43±0.07	<0.03	6.6±0.11
MO-110-30-6	2.08±0.09	2.56±0.07	0.69±0.03	0.42±0.07	3.70±0.05	<0.03	8.1±0.13
MO-120-30-3	1.88±0.01	2.58±0.03	0.57±0.15	0.41±0.01	3.56±0.06	<0.03	7.1±0.00
MO-120-30-6	2.07±0.09	2.57±0.09	0.65±0.07	0.43±0.04	3.65±0.03	<0.03	8.0±0.01
MO-110-45-3	2.02±0.04	2.64±0.01	0.38±0.09	0.39±0.03	3.41±0.05	<0.03	6.2±0.01
MO-110-45-6	2.03±0.00	2.62±0.04	0.59±0.02	0.39±0.01	3.60±0.01	<0.03	7.2±0.03
MO-120-45-3	2.04±0.07	2.69±0.04	0.45±0.08	0.38±0.05	3.52±0.04	<0.03	5.4±0.04
MO-120-45-6	2.04±0.06	2.64±0.04	0.64±0.01	0.39±0.06	3.70±0.01	<0.03	7.4±0.06

All values within the same column indicates significant differences for three factors (temperature, humidity and amount) independently, 3-way ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 11. Amino acid analysis of meat analogues containing extruded Soy t press-cakes

Amino Acid Content, g/100g	MT-110-30-3	MT-110-30-6	MT-120-30-3	MT-120-30-6	MT-110-60-3	MT-110-60-6	MT-120-60-3	MT-120-60-6
Aspartic Acid	1.76±0.07	1.92±0.01	1.78±0.07	1.69±0.07	1.75±0.01	2.23±0.03	1.81±0.07	1.81±0.09
Glutamic Acid*	2.72±0.01	3.08±0.09	2.93±0.01	2.82±0.04	2.89±0.04	3.03±0.06	2.94±0.06	2.87±0.03
Serine	1.76±0.03	1.00±0.04	0.98±0.04	0.95±0.06	0.95±0.09	2.34±0.09	0.98±0.07	0.92±0.01
Glycine*	0.95±0.02	0.71±0.03	0.70±0.05	0.67±0.05	0.69±0.09	1.17±0.09	0.7±0.01	0.67±0.11
Histidine*	0.52±0.04	0.36±0.05	0.32±0.04	0.35±0.04	0.31±0.03	0.61±0.01	0.35±0.07	0.35±0.09
Threonine	1.01±0.01	0.92±0.08	0.90±0.04	0.87±0.03	0.86±0.03	1.21±0.03	0.90±0.01	0.87±0.05
Alanine*	0.86±0.03	0.78±0.03	0.76±0.04	0.73±0.05	0.75±0.01	1.07±0.07	0.76±0.09	0.73±0.13
Arginine	1.12±0.02	1.28±0.03	1.24±0.03	1.20±0.05	1.22±0.08	1.27±0.06	1.22±0.09	1.18±0.09
Proline*	1.06±0.05	0.98±0.06	1.37±0.06	1.01±0.09	1.69±0.01	1.35±0.11	0.82±0.08	0.77±0.01
Cysteine	0.48±0.01	0.42±0.03	0.44±0.06	0.36±0.04	0.40±0.09	0.50±0.07	0.40±0.03	0.38±0.03
Tyrosine	0.78±0.02	0.64±0.03	0.62±0.05	0.59±0.05	0.62±0.08	0.85±0.05	0.61±0.04	0.59±0.09
Valine*	0.80±0.05	0.85±0.04	0.78±0.04	0.79±0.01	0.72±0.07	0.96±0.06	0.68±0.04	0.70±0.07
Methionine*	0.20±0.01	1.08±0.06	0.12±0.01	0.02±0.03	0.15±0.01	0.17±0.08	0.18±0.06	0.16±0.05
Lysine*	0.70±0.04	1.01±0.07	0.85±0.03	0.70±0.07	0.80±0.02	1.04±0.05	1.88±0.08	1.00±0.05
Isoleucine*	1.48±0.07	1.34±0.09	1.32±0.03	1.23±0.09	1.27±0.03	1.54±0.01	1.28±0.09	1.22±0.06
Leucine	1.15±0.09	1.03±0.01	0.99±0.01	0.98±0.03	1.02±0.03	1.20±0.02	1.04±0.02	0.95±0.05
Phenylalanine*	1.00±0.07	0.98±0.09	0.97±0.07	0.89±0.04	0.93±0.01	1.09±0.02	0.92±0.01	0.90±0.03
Tryptophan	1.07±0.01	1.06±0.04	1.01±0.01	1.06±0.08	0.99±0.04	1.15±0.07	1.01±0.01	0.97±0.03

All values within the same row indicates significant differences for three factors (temperature, humidity and amount), three-way factorial ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 12. Amino acid analysis of meat analogues containing extruded Soy d press-cakes

Amino Acid Content, g/100g	MD-110-30-3	MD-110-30-6	MD-120-30-3	MD-120-30-6	MD-110-60-3	MD-110-60-6	MD-120-60-3	MD-120-60-6
Aspartic Acid	2.16±0.07	2.34±0.03	2.06±0.04	2.27±0.04	2.22±0.04	2.17±0.01	1.80±0.09	2.19±0.11
Glutamic Acid*	3.24±0.04	3.50±0.04	3.13±0.05	3.45±0.03	3.34±0.03	3.31±0.09	2.78±0.03	3.25±0.04
Serine	0.90±0.03	0.97±0.05	0.87±0.05	0.94±0.04	0.91±0.02	0.89±0.07	0.77±0.04	0.90±0.04
Glycine*	0.74±0.07	0.79±0.05	0.71±0.09	0.78±0.04	0.74±0.02	0.74±0.04	0.64±0.05	0.73±0.09
Histidine*	0.30±0.09	0.39±0.03	0.33±0.07	0.36±0.04	0.36±0.09	0.37±0.03	0.34±0.06	0.37±0.08
Threonine	0.74±0.01	0.79±0.01	0.71±0.08	0.78±0.05	0.75±0.01	0.75±0.03	0.63±0.08	0.74±0.01
Alanine*	0.79±0.01	0.85±0.09	0.75±0.02	0.83±0.02	0.80±0.08	0.79±0.02	0.68±0.06	0.79±0.03
Arginine	1.24±0.07	1.35±0.09	1.20±0.03	1.33±0.01	1.28±1.13	1.26±0.02	1.08±0.05	1.25±0.04
Proline*	0.98±0.04	1.15±0.08	1.14±0.04	1.09±0.02	0.88±0.05	0.82±0.05	0.58±0.05	1.22±0.06
Cysteine	0.46±0.03	0.50±0.07	0.46±0.05	0.52±0.04	0.45±0.09	0.49±0.06	0.37±0.03	0.43±0.07
Tyrosine	0.66±0.05	0.71±0.06	0.63±0.01	0.71±0.09	0.68±0.08	0.66±0.07	0.56±0.02	0.66±0.01
Valine*	0.81±0.07	0.88±0.01	0.79±0.07	0.99±0.08	0.83±0.07	0.81±0.08	0.68±0.02	0.82±0.02
Methionine*	0.31±0.06	0.35±0.02	0.33±0.09	0.23±0.06	0.34±0.02	0.33±0.01	0.29±0.01	0.32±0.08
Lysine*	1.25±0.08	1.45±0.03	1.20±0.03	1.28±0.11	1.31±0.03	1.30±0.09	0.79±0.09	1.10±0.03
Isoleucine*	1.37±0.09	1.48±0.04	1.36±0.03	1.47±0.01	1.35±0.04	1.39±0.03	1.17±0.08	1.36±0.05
Leucine	0.95±0.04	1.08±0.04	0.95±0.04	1.02±0.13	0.92±0.01	0.99±0.04	0.89±0.03	0.97±0.05
Phenylalanine*	1.05±0.01	1.13±0.01	1.00±0.05	1.09±0.06	1.03±0.03	1.05±0.05	0.87±0.05	1.02±0.01
Tryptophan	1.13±0.07	1.23±0.02	1.14±0.09	1.21±0.04	1.14±0.04	1.16±0.05	0.95±0.06	1.14±0.02

All values within the same row indicates significant differences for three factors (temperature, humidity and amount), three-way factorial ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 13. Amino acid analysis of meat analogues containing extruded Oat d press-cakes

Amino Acid Content, g/100g	MO-110-30-3	MO-110-30-6	MO-120-30-3	MO-120-30-6	MO-110-45-3	MO-110-45-6	MO-120-45-3	MO-120-45-6
Aspartic Acid	1.75±0.03	1.74±0.09	1.47±0.01	1.81±0.04	1.75±0.07	1.62±0.09	1.86±0.10	1.81±0.03
Glutamic Acid*	2.73±0.04	2.79±0.01	2.43±0.03	2.83±0.05	2.75±0.09	2.55±0.08	2.96±0.11	2.83±0.03
Serine	0.77±0.09	0.77±0.03	0.71±0.04	0.76±0.01	0.77±0.01	0.71±0.05	0.82±0.09	0.76±0.09
Glycine*	0.58±0.06	0.59±0.08	0.53±0.08	0.58±0.02	0.58±0.01	0.54±0.06	0.62±0.01	0.58±0.02
Histidine*	0.27±0.03	0.32±0.05	0.33±0.07	0.28±0.09	0.24±0.02	0.25±0.07	0.32±0.02	0.29±0.08
Threonine	0.61±0.07	0.63±0.06	0.56±0.05	0.63±0.08	0.62±0.04	0.59±0.04	0.66±0.03	0.63±0.04
Alanine*	0.67±0.08	0.64±0.03	0.56±0.05	0.64±0.07	0.62±0.03	0.58±0.01	0.67±0.01	0.63±0.06
Arginine	0.98±0.01	0.97±0.02	0.86±0.02	0.97±0.06	0.97±0.03	0.89±0.02	1.05±0.07	0.96±0.05
Proline*	0.80±0.02	0.00±0.03	0.00±0.03	0.28±0.06	0.48±0.05	0.53±0.02	0.00±0.09	0.83±0.07
Cysteine	1.75±0.01	1.22±0.01	1.47±0.04	1.32±0.07	1.31±0.07	1.24±0.03	1.56±0.05	1.50±0.03
Tyrosine	0.49±0.03	0.51±0.09	0.46±0.01	0.51±0.09	0.50±0.01	0.46±0.04	0.54±0.06	0.51±0.04
Valine*	1.08±0.04	0.97±0.07	0.96±0.09	0.99±0.03	1.02±0.09	0.93±0.04	1.09±0.07	1.05±0.05
Methionine*	0.04±0.04	0.00±0.04	0.00±0.07	0.05±0.02	0.01±0.08	0.00±0.06	0.04±0.02	0.02±0.03
Lysine*	0.63±0.09	0.45±0.03	0.53±0.06	0.71±0.04	0.42±0.01	0.47±0.07	0.66±0.03	0.78±0.05
Isoleucine*	1.09±0.08	1.03±0.02	0.95±0.05	1.08±0.05	1.02±0.04	0.96±0.09	1.12±0.04	1.05±0.02
Leucine	0.99±0.06	0.58±0.01	0.84±0.04	0.62±0.06	0.59±0.02	0.62±0.11	0.81±0.05	0.60±0.09
Phenylalanine*	1.07±0.07	0.78±0.04	0.89±0.03	0.79±0.06	0.78±0.03	0.72±0.13	0.96±0.03	0.78±0.01
Tryptophan	1.06±0.04	0.75±0.05	0.72±0.01	0.79±0.07	0.77±0.02	0.69±0.10	0.67±0.04	0.82±0.01
All values within the same row indicates significant differences for three factors (temperature, humidity and amount), three-way factorial ANOVA, $p < 0.05$; *The interaction did not show a significant difference ($p>0.05$)								

Appendix 14. Fatty acid analysis of meat analogues contaning plant press-cakes (1)

Fatty Acid composition, %	Myristic acid	Palmitic acid*	Palmitoleic acid*	Stearic acid	Oleic acid*	ω-6 Linoleic acid	ω-3 alpha-Linoleic*
MT-110-30-3	0.07±0.03	4.76±0.03	0.17±0.07	1.75±0.08	58.01±0.21	24.02±0.011	8.46±0.04
MT-110-30-6	0.05±0.03	4.78±0.04	0.17±0.04	1.8±0.02	57.12±0.15	24.70±0.01	8.56±0.06
MT-120-30-3	0.05±0.01	4.66±0.09	0.17±0.02	1.76±0.03	58.18±0.13	23.92±0.05	8.51±0.08
MT-120-30-6	0.17±0.07	5.19±0.08	0.19±0.05	1.93±0.04	57.13±0.11	24.11±0.06	8.39±0.09
MT-110-60-3	0.05±0.09	4.50±0.03	0.16±0.03	1.70±0.09	58.71±0.31	23.48±0.09	8.55±0.09
MT-110-60-6	0.05±0.11	4.85±0.09	0.16±0.01	1.84±0.08	57.52±0.10	24.67±0.09	8.47±0.05
MT-120-60-3	0.05±0.02	4.57±0.07	0.17±0.04	1.72±0.09	58.58±0.54	23.45±0.10	8.50±0.07
MT-120-60-6	0.06±0.03	4.78±0.07	0.17±0.06	1.83±0.01	58.34±0.16	23.58±0.17	8.49±0.01
MD-110-30-3	0.05±0.04	4.71±0.01	0.17±0.09	1.75±0.01	57.88±0.43	24.10±0.05	8.53±0.08
MD-110-30-6	0.00±0.03	4.98±0.01	0.18±0.08	1.83±0.04	56.18±0.57	25.53±0.07	8.43±0.07
MD-120-30-3	0.06±0.09	4.76±0.04	0.17±0.07	1.87±0.04	57.76±0.79	23.94±0.08	8.55±0.05
MD-120-30-6	0.11±0.09	5.85±0.02	0.17±0.04	2.00±0.07	55.82±0.23	24.98±0.03	8.22±0.07
MD-110-60-3	0.00±0.01	4.57±0.03	0.16±0.06	1.71±0.01	58.27±0.53	23.80±0.06	8.55±0.08
MD-110-60-6	0.03±0.12	4.84±0.04	0.16±0.03	1.78±0.03	56.73±0.65	25.23±0.08	8.58±0.05
MD-120-60-3	0.00±0.01	4.64±0.05	0.16±0.05	1.74±0.04	58.23±0.76	23.86±0.09	8.27±0.04
MD-120-60-6	0.04±0.01	4.84±0.03	0.16±0.07	1.78±0.02	56.96±0.73	25.17±0.10	8.58±0.03
MO-110-30-3	0.09±0.04	4.66±0.01	0.18±0.03	1.73±0.04	59.28±0.11	23.66±0.07	7.41±0.05
MO-110-30-6	0.10±0.03	4.68±0.01	0.19±0.05	1.68±0.06	59.87±0.41	22.90±0.07	7.57±0.07
MO-120-30-3	0.06±0.09	4.64±0.06	0.19±0.02	1.70±0.05	60.01±0.75	22.99±0.08	7.61±0.01
MO-120-30-6	0.12±0.02	4.81±0.03	0.20±0.03	1.73±0.09	59.83±0.37	22.75±0.06	7.56±0.08
MO-110-45-3	0.07±0.01	4.60±0.02	0.18±0.05	1.68±0.08	59.89±0.28	22.88±0.11	7.73±0.05
MO-110-45-6	0.06±0.04	4.57±0.01	0.19±0.01	1.66±0.01	60.06±0.15	22.90±0.13	7.60±0.08
MO-120-45-3	0.06±0.05	4.55±0.03	0.18±0.03	1.69±0.03	60.13±0.18	22.75±0.05	7.61±0.09
MO-120-45-6	0.06±0.04	4.62±0.01	0.18±0.02	1.68±0.05	59.89±0.20	22.97±0.09	7.65±0.08

All values within the same column indicates significant differences for three factors (temperature, humidity and amount), three-way factorial ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 15. Fatty acid analysis of meat analogues contaning plant press-cakes (2)

Fatty Acid composition, %	Arachidic acid*	Eicosenoic acid*	Behenic acid*	Erucic acid	Lignoceric acid*	Nervonic acid*
MT-110-30-3	0.45±0.08	1.21±0.04	0.27±0.04	0.07±0.01	0.10±0.09	0.12±0.01
MT-110-30-6	0.46±0.02	1.20±0.11	0.28±0.05	0.07±0.06	0.11±0.01	0.11±0.06
MT-120-30-3	0.46±0.04	1.23±0.05	0.27±0.06	0.07±0.05	0.11±0.03	0.12±0.04
MT-120-30-6	0.46±0.05	1.18±0.07	0.26±0.07	0.06±0.03	0.11±0.05	0.11±0.07
MT-110-60-3	0.44±0.02	1.220.09	0.27±0.09	0.06±0.03	0.11±0.05	0.12±0.07
MT-110-60-6	0.45±0.04	1.16±0.05	0.28±0.07	0.06±0.04	0.11±0.02	0.12±0.04
MT-120-60-3	0.45±0.01	1.24±0.04	0.27±0.05	0.05±0.05	0.11±0.05	0.11±0.09
MT-120-60-6	0.46±0.04	1.19±0.08	0.27±0.06	0.05±0.01	0.10±0.05	0.12±0.01
MD-110-30-3	0.44±0.03	1.18±0.06	0.26±0.08	0.10±0.02	0.09±0.06	0.11±0.02
MD-110-30-6	0.44±0.05	1.20±0.04	0.26±0.07	0.08±0.03	0.10±0.04	0.11±0.03
MD-120-30-3	0.44±0.02	1.19±0.03	0.26±0.07	0.00±0.02	0.10±0.07	0.11±0.04
MD-120-30-6	0.44±0.04	1.12±0.05	0.26±0.09	0.00±0.01	0.11±0.09	0.10±0.05
MD-110-60-3	0.45±0.05	1.21±0.05	0.26±0.10	0.00±0.01	0.00±0.01	0.11±0.06
MD-110-60-6	0.45±0.03	0.45±0.06	0.26±0.11	0.00±0.03	0.10±0.08	0.00±0.01
MD-120-60-3	0.46±0.02	1.21±0.08	0.00±0.05	0.00±0.02	0.00±0.02	0.11±0.03
MD-120-60-6	0.43±0.05	1.15±0.07	0.25±0.07	0.10±0.02	0.00±0.01	0.00±0.01
MO-110-30-3	0.47±0.04	1.25±0.01	0.29±0.08	0.15±0.03	0.13±0.01	0.11±0.03
MO-110-30-6	0.46±0.02	1.30±0.04	0.27±0.08	0.16±0.01	0.11±0.02	0.12±0.02
MO-120-30-3	0.48±0.04	1.25±0.05	0.29±0.09	0.13±0.03	0.12±0.02	0.12±0.03
MO-120-30-6	0.47±0.04	1.29±0.06	0.28±0.09	0.14±0.03	0.11±0.03	0.11±0.04
MO-110-45-3	0.47±0.02	1.29±0.02	0.28±0.05	0.13±0.04	0.12±0.04	0.12±0.02
MO-110-45-6	0.48±0.01	1.29±0.04	0.28±0.07	0.12±0.01	0.12±0.05	0.12±0.01
MO-120-45-3	0.48±0.05	1.31±0.05	0.28±0.07	0.15±0.04	0.13±0.07	0.11±0.03
MO-120-45-6	0.46±0.02	1.29±0.09	0.28±0.01	0.15±0.07	0.12±0.09	0.11±0.01

All values within the same column indicates significant differences for three factors (temperature, humidity and amount), three-way factorial ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 16. Data of biogenic amine, acrylamide and free fatty acid content in meat analogues containing extruded press-cakes

Meat analogues	Biogenic amine content, mg/kg					Acrylamide Content*, µg/kg	Peroxide Value*, Mekv/kg	Free fatty acid content*, %	Fatty acid content*, mg KOH/g
	HIS	CAD*	PUT	TYR*	SPE*				
MT-110-30-3	33±0.13	7±0.03	<5	<5	20±0.04	13±0.19	3.0±0.03	0.41±0.02	0.82±0.02
MT-110-30-6	41±0.34	8±0.01	<5	<5	19±0.03	19±0.31	3.3±0.02	0.44±0.03	0.87±0.04
MT-120-30-3	33±0.21	6±0.01	<5	<5	20±0.02	47±0.26	5.9±0.04	0.26±0.01	0.52±0.05
MT-120-30-6	36±0.35	6±0.04	<5	<5	20±0.07	10±0.04	6.2±0.01	0.26±0.03	0.52±0.03
MT-110-60-3	46±0.45	6±0.03	<5	<5	19±0.02	10±0.62	3.2±0.06	0.67±0.02	0.34±0.02
MT-110-60-6	21±0.26	7±0.01	<5	<5	15±0.08	14±0.54	3.3±0.01	0.58±0.08	0.29±0.07
MT-120-60-3	43±0.37	6±0.07	<5	<5	18±0.02	36±0.34	3.0±0.03	0.35±0.03	0.70±0.09
MT-120-60-6	20±0.75	7±0.08	<5	<5	11±0.04	18±0.26	3.1±0.04	0.71±0.05	1.42±0.05
MD-110-30-3	36±0.19	6±0.01	<5	<5	19±0.05	6±0.57	4.5±0.02	0.46±0.01	0.91±0.04
MD-110-30-6	31±0.16	5±0.03	<5	<5	20±0.06	6±0.43	5.4±0.04	0.99±0.03	1.96±0.07
MD-120-30-3	40±0.53	6±0.04	<5	<5	18±0.02	7±0.23	4.1±0.05	0.45±0.05	0.90±0.09
MD-120-30-6	31±0.27	6±0.04	<5	<5	22±0.05	5±0.15	4.1±0.03	1.61±0.02	3.21±0.05
MD-110-60-3	40±0.21	7±0.05	<5	<5	21±0.03	7±0.21	4.0±0.02	0.42±0.01	0.84±0.05
MD-110-60-6	30±0.37	6±0.04	<5	<5	20±0.02	4±0.18	3.9±0.01	0.76±0.04	1.52±0.04
MD-120-60-3	20±0.43	6±0.05	<5	<5	21±0.09	11±0.19	4.1±0.02	0.50±0.06	1.00±0.07
MD-120-60-6	22±0.83	7±0.01	<5	<5	19±0.04	11±0.26	3.8±0.04	1.42±0.08	2.83±0.06
MO-110-30-3	35±0.83	<5	<5	10±0.02	18±0.06	12±0.29	1.8±0.01	0.31±0.01	0.62±0.03
MO-110-30-6	36±0.27	<5	<5	10±0.01	20±0.03	14±0.32	2.4±0.03	0.38±0.05	0.76±0.04
MO-120-30-3	38±0.26	<5	<5	11±0.08	19±0.01	16±0.26	2.0±0.02	0.30±0.07	0.60±0.02
MO-120-30-6	30±0.71	<5	<5	10±0.04	20±0.10	16±0.42	2.3±0.04	0.43±0.05	0.85±0.05
MO-110-45-3	33±0.53	<5	<5	11±0.07	12±0.04	13±0.36	2.0±0.05	0.31±0.01	0.62±0,03
MO-110-45-6	35±0.41	<5	<5	11±0.03	19±0.05	14±0.23	2.2±0.01	0.43±0.04	0.85±0.03
MO-120-45-3	38±0.35	<5	<5	9±0.07	19±0.08	12±0.38	1.9±0.04	0.32±0.05	0.64±0.02
MO-120-45-6	31±0.50	<5	<5	11±0.02	17±0.09	13±0.25	2.5±0.01	0.38±0.01	0.75±0.04

All values within the same column indicates significant differences for three factors (temperature, humidity and amount), 3-way ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 17. Colour measurements of meat analogues containing plant press-cakes

Meat Analogues	L	a	b
MA-C	49.36 ± 0.20	9.37 ± 0.55	20.33 ± 0.32
MT-110-30-3	48.46 ± 0.25	9.30 ± 0.19	19.77 ± 0.15
MT-110-30-6	46.97 ± 0.01	7.66 ± 0.22	19.72 ± 0.40
MT-120-30-3	45.78 ± 0.01	8.47 ± 0.27	17.23 ± 0.21
MT-120-30-6	46.53 ± 0.01	8.29 ± 0.20	16.93 ± 0.54
MT-110-60-3	45.57 ± 0.02	9.57 ± 0.21	22.27 ± 0.39
MT-110-60-6	46.65 ± 0.02	8.28 ± 0.22	20.61 ± 0.47
MT-120-60-3	47.96 ± 0.02	9.30 ± 0.09	17.41 ± 0.25
MT-120-60-6	46.50 ± 0.01	10.39 ± 0.38	19.45 ± 0.34
MD-110-30-3	46.26 ± 0.19	9.43 ± 0.33	23.53 ± 0.26
MD-110-30-6	51.68 ± 0.28	8.21 ± 0.27	20.48 ± 0.21
MD-120-30-3	49.55 ± 0.51	8.52 ± 0.33	21.56 ± 0.22
MD-120-30-6	50.01 ± 0.65	8.21 ± 0.09	19.56 ± 0.24
MD-110-60-3	51.32 ± 0.36	7.28 ± 0.20	22.61 ± 0.39
MD-110-60-6	53.54 ± 0.34	7.17 ± 0.37	20.40 ± 0.22
MD-120-60-3	49.95 ± 0.07	8.93 ± 0.22	22.47 ± 0.62
MD-120-60-6	49.81 ± 0.59	8.15 ± 0.29	23.12 ± 0.15
MO-110-30-3	49.36 ± 0.20	9.37 ± 0.55	20.33 ± 0.32
MO-110-30-6	48.52 ± 0.40	8.36 ± 0.39	18.48 ± 0.23
MO-120-30-3	46.38 ± 0.64	8.34 ± 0.16	16.67 ± 0.79
MO-120-30-6	43.40 ± 0.23	9.77 ± 0.86	18.52 ± 0.24
MO-110-45-3	42.19 ± 0.07	9.75 ± 0.18	17.46 ± 0.40
MO-110-45-6	48.22 ± 0.25	8.61 ± 0.17	17.58 ± 0.43
MO-120-45-3	46.29 ± 0.41	8.86 ± 0.48	18.22 ± 0.15
MO-120-45-6	43.50 ± 0.31	9.17 ± 0.59	16.17 ± 0.16

All values within the same column indicates significant differences for three factors (temperature, humidity and amount), three-way ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 18. Data of texture in the meat analogues containing extruded press-cakes

Meat analogues	Hardness, N	Adhesiveness, J	Cohesiveness	Springiness, mm	Gumminess, N
MA-C	26.68 ± 0.06	-0.002 ± 0.00	0.76 ± 0.02	3.05 ± 0.02	6.46 ± 0.01
MT-110-30-3	15.17 ± 0.08	-0.003 ± 0.00	0.77 ± 0.03	2.45 ± 0.20	2.81 ± 0.03
MT-110-30-6	9.08 ± 0.14	-0.001 ± 0.00	0.75 ± 0.01	1.99 ± 0.02	2.61 ± 0.03
MT-120-30-3	30.93 ± 0.08	-0.005 ± 0.00	0.74 ± 0.01	5.77 ± 0.05	2.66 ± 0.02
MT-120-30-6	29.45 ± 0.20	-0.019 ± 0.00	0.72 ± 0.02	2.34 ± 0.03	1.82 ± 0.03
MT-110-60-3	21.82 ± 0.13	-0.001 ± 0.00	0.77 ± 0.03	5.04 ± 0.02	2.50 ± 0.03
MT-110-60-6	13.54 ± 0.21	-0.003 ± 0.00	0.70 ± 0.02	2.25 ± 0.02	2.79 ± 0.02
MT-120-60-3	10.10 ± 0.21	-0.004 ± 0.00	0.74 ± 0.02	1.54 ± 0.02	2.48 ± 0.02
MT-120-60-6	15.51 ± 0.25	-0.002 ± 0.00	0.77 ± 0.01	2.56 ± 0.03	2.13 ± 0.01
MD-110-30-3	10.05 ± 0.20	-0.002 ± 0.00	0.74 ± 0.02	2.69 ± 0.02	1.78 ± 0.01
MD-110-30-6	13.80 ± 0.11	-0.002 ± 0.00	0.77 ± 0.02	2.85 ± 0.02	3.11 ± 0.02
MD-120-30-3	12.85 ± 0.20	-0.001 ± 0.00	0.75 ± 0.01	2.86 ± 0.02	2.88 ± 0.01
MD-120-30-6	10.90 ± 0.13	-0.006 ± 0.00	0.71 ± 0.02	2.40 ± 0.02	0.99 ± 0.01
MD-110-60-3	17.69 ± 0.24	-0.002± 0.00	0.73 ± 0.02	2.75 ± 0.02	3.63 ± 0.02
MD-110-60-6	23.68 ± 0.27	-0.002 ± 0.00	0.75 ± 0.02	2.48 ± 0.02	4.63 ± 0.01
MD-120-60-3	11.15 ± 0.23	-0.003 ± 0.00	0.77 ± 0.02	2.55 ± 0.02	2.10 ± 0.02
MD-120-60-6	9.40 ± 0.11	0.000 ± 0.00	0.75 ± 0.02	2.76 ± 0.02	2.33 ± 0.02
MO-110-30-3	39.37 ± 0.16	-0.012 ± 0.00	0.77 ± 0.02	2.47 ± 0.01	5.99 ± 0.02
MO-110-30-6	10.49 ± 0.03	0.000 ± 0.00	0.76 ± 0.02	3.06 ± 0.03	2.70 ± 0.01
MO-120-30-3	11.81 ± 0.11	-0.003 ± 0.00	0.74 ± 0.02	2.68 ± 0.00	1.83 ± 0.02
MO-120-30-6	17.26 ± 0.14	-0.005 ± 0.00	0.72 ± 0.02	2.73 ± 0.02	2.98 ± 0.02
MO-110-45-3	33.49 ± 0.14	-0.005 ± 0.00	0.73 ± 0.01	2.73 ± 0.01	6.18 ± 0.02
MO-110-45-6	16.12 ± 0.17	0.000 ± 0.00	0.77 ± 0.02	3.01 ± 0.01	4.50 ± 0.03
MO-120-45-3	21.36 ± 0.10	-0.005 ± 0.00	0.73 ± 0.02	2.59 ± 0.01	3.53 ± 0.02
MO-120-45-6	6.38 ± 0.06	-0.001 ± 0.00	0.71 ± 0.02	2.71 ± 0.02	1.13 ± 0.01

All valuse within the same column indicates significant differences for three factors (temperature, humidity and amount), three-way factorial ANOVA, $p < 0.05$;
*The interaction did not show a significant difference ($p>0.05$)

Appendix 19. Average of sensory characteristics of meat analogues with extruded Soy t press-cakes

Sensory Characteristics	MA-C	MT-110-30-3	MT-110-30-6	MT-120-30-3	MT-120-30-6	MT-110-60-3	MT-110-60-6	MT-120-60-3	MT-120-60-6
Overall odour Intensity	12.0 a	11.0 a	11.6 a	12.0 a	12.0 a	11.6 a	11.6 a	11.8 a	11.8 a
Cardboard odour	9.2 a	7.2 a	7.2 a	7.4 a	7.4 a	7.2 a	7.3 a	7.3 a	7.3 a
Sweet odour	4.8 a	5.4 a	5.2 a	5.0 a	5.0 a	4.8 a	5.1 a	5.0 a	5.0 a
Acidic odour	3.6 a	2.4 a	2.2 a	2.4 a	2.4 a	2.2 a	2.3 a	2.3 a	2.3 a
Odour of plant origin	7.2 a	5.2 a	5.6 a	5.4 a	5.4 a	5.0 a	5.3 a	5.3 a	5.3 a
Off flavour	2.4 a	2.2 a	2.2 a	2.0 a	2.0 a	1.6 a	2.0 a	2.0 a	1.9 a
Hardness of mass	7.8 a	8.4 a	8.4 a	8.6 a	8.6 a	7.2 a	8.2 a	8.2 a	8.2 a
Firmness of fibres	8.3 b	6.5 a	5.5 a	6.0 a	6.0 a	7.0 a	6.2 a	6.1 a	6.3 a
Juiciness	5.8 b	5.2 a	3.6 a	4.0 a	4.0 a	5.2 a	4.4 a	4.2 a	4.4 a
Greasiness	3.2 a	3.0 a	2.8 a	3.2 a	3.2 a	3.4 a	3.1 a	3.1 a	3.2 a
Mouthcoating	4.2 a	5.2 a	4.4 a	4.8 a	4.8 a	5.4 a	4.9 a	4.9 a	5.0 a
Overall intensity of taste	9.2 a	9.4 a	9.0 a	9.2 a	9.2 a	9.8 a	9.3 a	9.3 a	9.4 a
Sweetness	6.2 a	6.0 a	6.0 a	6.2 a	6.2 a	6.8 a	6.2 a	6.3 a	6.3 a
Saltiness	2.8 a	2.0 a	1.8 a	2.2 a	2.2 a	2.0 a	2.0 a	2.0 a	2.1 a
Acidity	2.5 a	1.8 a	1.8 a	1.8 a	1.8 a	1.8 a	1.8 a	1.8 a	1.8 a
Bitterness	3.4 a	4.0 a	4.0 a	3.2 a	3.2 a	2.8 a	3.4 a	3.3 a	3.2 a
Astringency	3.0 a	3.2 a	4.0 a	3.2 a	3.2 a	2.8 a	3.3 a	3.3 a	3.2 a
Taste of nuts	4.4 a	6.0 a	6.8 b	7.0 b	7.0 b	7.0 b	6.8 b	6.9 b	6.9 b
Chalky taste	2.0 a	3.5 a	3.6 a	3.4 a	3.4 a	3.6 a	3.5a	3.5 a	3.5 a
Cardboard taste	5.8 b	3.4 a	3.4 a	3.3 a	3.3 a	3.5 a	3.4 a	3.4 a	3.3 a
Meaty taste	4.0 a	3.2 a	3.2 a	3.1 a	3.1 a	3.3 a	3.2 a	3.2 a	3.2 a
Off taste	2.4 a	3.0 a	3.1 a	2.9 a	2.9 a	3.2 a	3.0 a	3.0 a	3.0 a
Intensity of after taste	5.6 b	2.8 a	2.9 a	2.7 a	2.7 a	3.0 a	2.8 a	2.8 a	2.8 a
Duration of after taste in mouth	6.7 b	2.6 a	2.7 a	2.5 a	2.5 a	2.8 a	2.7 a	2.7 a	2.6 a

a,b Different alphabets in the same row indicate significant statistical differences, ($P<0.05$, LSD test)

Appendix 20. Average of sensory characteristics of meat analogues with extruded Soy d press-cakes

Sensory Characteristics	MA-C	MD-110-30-3	MD-110-30-6	MD-120-30-3	MD-120-30-6	MD-110-60-3	MD-110-60-6	MD-120-60-3	MD-120-60-6
Overall odour Intensity	12.0 [a]	10.6 [a]	10.6 [a]	10.2 [a]	10.2 [a]	10.2 [a]	10.2 [a]	10.0 [a]	10.0 [a]
Cardboard odour	9.2 [a]	9.0 [a]	8.2 [a]	7.6 [a]	7.8 [a]	7.6 [a]	7.8 [a]	7.4 [a]	8.4 [a]
Sweet odour	4.8 [a]	4.4 [a]	4.6 [a]	4.8 [a]	4.6 [a]	4.8 [a]	4.8 [a]	4.8 [a]	4.8 [a]
Acidic odour	3.6 [a]	3.4 [a]	3.0 [a]	2.4 [a]	2.6 [a]	2.6 [a]	2.4 [a]	2.2 [a]	2.2 [a]
Odour of plant origin	7.2 [a]	6.4 [a]	6.0 [a]	6.2 [a]	6.2 [a]	6.0 [a]	5.8 [a]	5.8 [a]	5.4 [a]
Off flavour	2.4 [a]	2.0 [a]	2.3 [a]	3.0 [a]	3.3 [a]	2.0 [a]	2.0 [a]	1.8 [a]	3.0 [a]
Hardness of mass	7.8 [a]	7.6 [a]	7.6 [a]	7.8 [a]	8.4 [a]	8.6 [a]	8.2 [a]	8.8 [a]	8.8 [a]
Firmness of fibres	8.3 [a]	8.0 [a]	8.0 [a]	8.8 [a]	8.8 [a]	8.8 [a]	8.0 [a]	8.3 [a]	8.5 [a]
Juiciness	5.8 [a]	5.2 [a]	5.4 [a]	5.4 [a]	5.8 [a]	6.2 [a]	6.2 [a]	6.4 [a]	6.6 [a]
Greasiness	3.2 [a]	3.0 [a]	3.2 [a]	3.4 [a]	3.4 [a]	3.4 [a]	3.4 [a]	3.6 [a]	3.6 [a]
Mouthcoating	4.2 [a]	4.0 [a]	4.2 [a]	4.4 [a]	4.6 [a]	4.8 [a]	4.2 [a]	4.4 [a]	4.4 [a]
Overall intensity of taste	9.2 [a]	9.6 [a]	9.6 [a]	9.8 [a]	9.4 [a]	10.4 [a]	10.6 [a]	10.8 [a]	10.8 [a]
Sweetness	6.2 [a]	6.4 [a]	6.2 [a]	6.6 [a]	6.6 [a]	7.2 [a]	6.4 [a]	6.6 [a]	6.2 [a]
Saltiness	2.8 [a]	2.6 [a]	2.6 [a]	2.6 [a]	2.6 [a]	2.8 [a]	2.6 [a]	2.8 [a]	2.8 [a]
Acidity	2.5 [a]	2.4 [a]	2.2 [a]	2.2 [a]	2.0 [a]	2.0 [a]	2.2 [a]	2.2 [a]	2.2 [a]
Bitterness	3.4 [a]	4.6 [a]	3.2 [a]	2.8 [a]	3.0 [a]	3.0 [a]	3.4 [a]	4.2 [a]	3.4 [a]
Astringency	3.0 [a]	4.4 [a]	3.4 [a]	3.2 [a]	3.4 [a]	3.6 [a]	4.0 [a]	4.6 [a]	3.8 [a]
Taste of nuts	4.4 [a]	5.6 [a]	5.8 [a]	6.4 [a]	6.8 [b]	7.0 [b]	7.2 [b]	6.8 [b]	6.0 [a]
Chalky taste	2.0 [a]	3.4 [a]	4.0 [a]	4.0 [a]	4.2 [b]	4.0 [a]	4.2 [b]	4.2 [b]	4.5 [a]
Cardboard taste	5.8 [a]	5.0 [a]	5.6 [a]	4.6 [a]	4.8 [a]	4.8 [a]	4.6 [a]	4.8 [a]	5.8 [a]
Meaty taste	4.0 [a]	4.7 [a]	4.7 [a]	5.0 [a]	5.0 [a]	5.0 [a]	5.0 [a]	5.0 [a]	5.3 [a]
Off taste	2.4 [a]	2.4 [a]	2.4 [a]	2.4 [a]	2.4 [a]	2.0 [a]	2.0 [a]	2.0 [a]	2.0 [a]
Intensity of after taste	5.6 [b]	6.2 [a]	6.6 [a]	6.4 [a]	6.4 [a]	6.4 [a]	6.2 [a]	6.2 [a]	6.0 [a]
Duration of after taste in mouth	6.7 [a]	4.8 [a]	5.0 [a]	6.0 [a]	6.3 [a]	6.5 [a]	6.8 [a]	7.3 [b]	7.5 [b]

[a,b] Different alphabets in the same row indicate significant statistical differences, ($P<0.05$, LSD test)

Appendix 21. Average of sensory characteristics of meat analogues with extruded Oat d press-cakes

Sensory Characteristics	MA-C	MO-110-30-3	MO-110-30-6	MO-120-30-3	MO-120-30-6	MO-110-45-3	MO-110-45-6	MO-120-45-3	MO-120-45-6
Overall odour Intensity	12 a	10.6 a	10.6 a	10.2 a	10.2 a	10.2 a	10.2 a	10.0 a	10.0 a
Cardboard odour	9.2 a	9.0 a	8.2 a	7.6 a	7.8 a	7.6 a	7.8 a	7.4 a	8.4 a
Sweet odour	4.8 a	4.4 a	4.6 a	4.8 a	4.6 a	4.8 a	4.8 a	4.8 a	4.8 a
Acidic odour	3.6 a	3.4 a	3.0 a	2.4 a	2.6 a	2.6 a	2.4 a	2.2 a	2.2 a
Odour of plant origin	7.2 a	6.4 a	6.0 a	6.2 a	6.2 a	6.0 a	5.8 a	5.8 a	5.4 a
Off flavour	2.4 a	2.0 a	2.3 a	3.0 a	3.3 a	2.0 a	2.0 a	1.8 a	3.0 a
Hardness of mass	7.8 a	7.6 a	7.6 a	7.8 a	8.4 a	8.6 a	8.2 a	8.8 a	8.8 a
Firmness of fibres	8.3 a	8.0 a	8.0 a	8.8 a	8.8 a	8.8 a	8.0 a	8.3 a	8.5 a
Juiciness	5.8 a	5.2 a	5.4 a	5.4 a	5.8 a	6.2 a	6.2 a	6.4 a	6.6 a
Greasiness	3.2 a	3.0 a	3.2 a	3.4 a	3.4 a	3.4 a	3.4 a	3.6 a	3.6 a
Mouthcoating	4.2 a	4.0 a	4.2 a	4.4 a	4.6 a	4.8 a	4.2 a	4.4 a	4.4 a
Overall intensity of taste	9.2 a	9.6 a	9.6 a	9.8 a	9.4 a	10.4 a	10.6 a	10.8 a	10.8 a
Sweetness	6.2 a	6.4 a	6.2 a	6.6 a	6.6 a	7.2 a	6.4 a	6.6 a	6.2 a
Saltiness	2.8 a	2.6 a	2.6 a	2.6 a	2.6 a	2.8 a	2.6 a	2.8 a	2.8 a
Acidity	2.5 a	2.4 a	2.2 a	2.2 a	2.0 a	2.0 a	2.2 a	2.2 a	2.2 a
Bitterness	3.4 a	4.6 a	3.2 a	2.8 a	3.0 a	3 a	3.4 a	4.2 a	3.4 a
Astringency	3.0 a	4.4 a	3.4 a	3.2 a	3.4 a	3.6 a	4.0 a	4.6 a	3.8 a
Taste of nuts	4.4 a	5.6 a	5.8 a	6.4 a	6.8 b	7.0 b	7.2 b	6.8 b	6.0 a
Chalky taste	2.0 a	3.4 a	4.0 a	4.0 a	4.2 b	4.0 a	4.2 b	4.2 b	4.5 b
Cardboard taste	5.8 a	5.0 a	5.6 a	4.6 a	4.8 a	4.8 a	4.6 a	4.8 a	5.8 a
Meaty taste	4.0 a	4.7 a	4.7 a	5.0 a	5.0 a	5.0 a	5.0 a	5.0 a	5.3 a
Off taste	2.4 a	2.4 a	2.4 a	2.4 a	2.4 a	2.0 a	2.0 a	2.0 a	2.0 a
Intensity of after taste	5.6 b	6.2 a	6.6 a	6.4 a	6.4 a	6.4 a	6.2 a	6.2 a	6.0 a
Duration of after taste in mouth	6.7 a	4.8 a	5.0 a	6.0 a	6.3 a	6.5 a	6.8 a	7.3 b	7.5 b

[a,b] Different alphabets in the same row indicate significant statistical differences, ($P<0.05$, LSD test)

Appendix 22. Overall acceptability of meat analogues containing the plant origin press-cakes

Meat Analogues	Appearance	Odour	Taste	Texture	Overall
MA-C	9.0±2.65 [a]	7.6 ± 1.67 [a]	8.2 ± 1.79 [b]	8.4 ± 1.67 [b]	8.2 ± 1.30 [a]
MT-110-30-3	9.6±2.19 [a]	10.0 ± 0.71 [b]	8.2 ± 2.68 [b]	10.0± 1.58 [b]	9.4 ± 1.14 [b]
MT-110-30-6	9.4±3.13 [a]	9.2 ± 1.79 [b]	7.4 ± 2.60 [a]	9.2 ± 2.68 [b]	9.5 ± 1.73 [b]
MT-120-30-3	9.6±3.21 [a]	9.4 ± 1.95 [b]	8.4 ± 1.95 [b]	9.2 ± 2.78 [b]	9.8 ± 1.92 [b]
MT-120-30-6	9.0±2.55 [a]	9.4 ± 2.70 [b]	7.6 ± 3.05 [a]	8.8 ±2.86 [b]	10.2 ± 1.10 [b]
MT-110-60-3	9.2±1.30 [a]	9.6 ± 1.67 [b]	8.8 ± 1.64 [b]	9.2 ±2.17 [b]	8.6 ± 2.19 [a]
MT-110-60-6	10.4±2.51 [a]	9.8 ± 1.92 [b]	9.2 ± 1.79 [c]	9.8 ±2.28 [b]	8.8 ± 2.28 [a]
MT-120-60-3	11.0±1.73 [b]	9.8 ± 0.45 [b]	10.0 ± 0.71 [c]	10.6 ± 1.67 [c]	9.8 ± 1.92 [b]
MT-120-60-6	11.6±1.67 [b]	10.2 ± 0.45 [b]	10.4 ±1.14 [c]	10.6 ± 1.67 [c]	11.2 ±1.92 [c]
MD-110-30-3	8.4±2.07[a]	6.6 ± 1.14 [a]	7.0± 1.87 [a]	6.8 ± 1.92 [a]	7.0 ±1.23 [a]
MD-110-30-6	9.0±1.00[a]	7.6 ± 0.55 [a]	7.6 ± 1.14 [a]	8.2 ± 1.9 [a]	8.2 ± 0.45 [a]
MD-120-30-3	9.2± 0.84 [a]	6.8 ± 2.17 [a]	7.2 ± 2.95 [a]	7.4 ± 2.97 [a]	7.2 ± 2.39 [a]
MD-120-30-6	8.6± 1.67 [a]	7.4 ± 0.89 [a]	7.8 ± 2.05 [a]	8.0 ± 2.00 [a]	7.8 ± 1.10 [a]
MD-110-60-3	8.8±1.79 [a]	7.2 ± 0.84 [a]	8.4 ± 1.67 [b]	8.2 ± 2.05 [a]	8.2 ± 1.48 [a]
MD-110-60-6	8.8±1.79 [a]	7.2 ± 0.84 [a]	8.4 ± 1.67 [b]	8.2 ± 2.05 [a]	8.6 ± 1.82 [a]
MD-120-60-3	8.8±1.79 [a]	7.2 ± 1.30 [a]	8.2 ± 1.48 [b]	8.4 ± 2.19 [b]	8.4 ± 2.30 [a]
MD-120-60-6	8.8±1.79 [a]	7.0 ± 1.00 [a]	8.2 ± 1.48 [b]	8.2 ± 2.49 [a]	8.6 ± 2.12 [a]
MO-110-30-3	8.4±2.70 [a]	7.2 ± 1.92 [a]	6.0 ± 2.45 [a]	7.8 ± 2.95 [a]	7.4 ± 2.30 [a]
MO-110-30-6	8.4±2.70 [a]	7.2 ± 1.92 [a]	8.4 ± 1.82 [b]	6.2 ± 2.17 [a]	7.6 ± 2.19 [a]
MO-120-30-3	8.4±2.55 [a]	7.4 ± 2.30 [a]	8.0± 1.87 [a]	7.6 ± 2.07 [a]	8.2 ± 2.59 [a]
MO-120-30-6	8.4±2.55 [a]	7.6 ± 2.19 [a]	8.2 ± 1.92 [b]	7.8 ± 1.92 [a]	8.6 ± 2.19 [a]
MO-110-45-3	8.6±3.05 [a]	7.8 ± 2.59 [a]	7.2 ± 3.19 [a]	7.8 ± 1.92 [a]	7.6 ± 3.65 [a]
MO-110-45-6	9.5±3.51 [a]	8.2 ± 2.48 [a]	6.6 ± 2.51 [a]	8.0± 2.00 [a]	7.8 ± 2.93 [a]
MO-120-45-3	9.0±3.39 [a]	7.4 ± 2.07 [a]	7.33 ± 3.08 [a]	7.6 ± 1.67 [a]	7.6 ± 1.95 [a]
MO-120-45-6	10.8±2.78 [b]	9.0 ± 2.45 [b]	9.0± 3.00 [b]	9.4 ± 1.52	9.8 ± 2.49 [b]
[a,b,c] Different alphabets in the same row indicate significant statistical differences, (P<0.05, LSD test)					

Appendix 23. Graphical representation of each sensory characteristics for control (no added press-cakes) and extruded press-cakes containing meat analogues