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Flavoring of meat analogues produced by high moisture extrusion

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The environmental impact of the meat industry is already past the planet's carrying capacity and it is expected to double by the year 2050 by the growing population. 18% of the global greenhouse gas emissions come from the livestock sector and 40% of arable land is used to feed livestock instead of growing food for human consumption. The consumer attitudes towards plant-based meat alternatives needs to be changed and more viable plant-protein products that consumers accept need to be developed. This thesis focuses on flavouring of high moisture meat analogues produced from fava bean protein concentrate.
The thesis was done as a part of VTT's EXPRO project, which was financed by Business Finland. The aim was to create a good tasting product and be able to mask or reduce the unpleasant off-flavours associated with extrudates made from fava bean protein concentrate and to study how extrusion affects the sensory properties of flavour additives. The thesis also includes a literature review, the purpose of which was to find out what type of research has already been conducted about meat analogues.
The extrudates were flavoured with various flavour additives and maskers. More neutral tasting pea protein isolate was used as a reference sample in the small-scale sensory analyses. The descriptive sensory analysis was conducted on five differently flavoured fava bean protein concentrate extrudates. The study on how extrusion affects the sensory properties of flavour additives was done by creating plant-based patties that were either flavoured before or after extrusion with the same flavour additives. The descriptive sensory analysis was conducted the same way with same references for both the extrudates and plant-based patties. One session of lexicon creation and training was held for both before the descriptive sensory analysis with eight panellists. The descriptive sensory analysis with the extrudates was done twice.
The results for the plant-based patties were not statistically significant, but those for extrudates were. The results show that extrusion does not affect the sensory properties of flavour additives. The results of descriptive sensory analysis of the extrudates indicated that the most important flavour additive was a roast pork flavoured yeast extract. It had the largest impact on the sensory properties of extrudates. An extrudate with a mixture of spices, NaCl and the yeast extract had the largest impact on the sensory properties of the extrudate, which was concluded to be the best formulation.

Keywords	high moisture meat analogue, descriptive sensory analysis, flavouring, sensory properties

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Lihateollisuuden ympäristövaikutukset ovat jo ohittaneet planeetan kantokyvyn, ja väestönkasvun odotetaan kaksinkertaistuvan vuoteen 2050 mennessä. Maailmanlaajuisista kasvihuonekaasupäästöistä 18 prosenttia on karjatalouden tuottamaa ja pelloista 40 prosenttia käytetään rehun kasvatukseen sen sijaan, että niissä kasvatettaisiin ruokaa ihmisiä varten. Eläinperäisten proteiininlähteiden käytön vähentäminen on sekä kulttuurinen että teknologinen ongelma. Kuluttajien asenteita kasvipohjaisia proteiininlähteitä kohtaan on muutettava, ja on kehitettävä sellaisia kasviproteiinituotteita, joita kuluttaja haluavat. Tämä opinnäytetyö keskittyy härkäpapu proteiinikonsentraatista märkäekstruusiolla tuotettujen liha-analogien maustamiseen. Insinöörityö tehtiin VTT:lle osana Business Finlandin rahoittamaa EXPRO projektia. Tavoitteena oli luoda hyvänmakuinen tuote ja pystyä peittämään tai vähentämään härkäpapuproteiinikonsentraatin epämiellyttäviä sivumakuja ja tutkimaan, kuinka ekstruusio vaikuttaa mausteiden aistinvaraisiin ominaisuuksiin. Työ sisältää myös kirjallisuuskatsauksen, jonka tarkoituksena oli selvittää, millaisia tutkimuksia liha-analogien maustamisesta oli tehty.
Ekstrudaatit maustettiin erilaisilla mausteilla ja maskaajilla. Neutraalin makuista herneproteiini-isolaatista valmistettuja ekstrudaatteja, jotka olivat maustettu identtisesti, käytettiin vertailunäytteenä pienimuotoisissa aistinvaraisissa arvioinneissa. Kuvailevassa aistinvaraisessa arvioinnissa arvioitiin viittä eri tavalla maustettua härkäpapuproteiinikonsentraattiekstrudaattia. Ekstruusion vaikutusta mausteiden aistinvaraisiin ominaisuuksiin tutkittiin valmistamalla kasvispihvejä härkäpapu ekstrudaateista, jotka joko maustettiin ennen ekstruusiota tai sen jälkeen. Kuvaileva aistinvarainen arviointi toteutettiin samalla tavalla, käyttäen samoja referenssinäytteitä arvioitaessa ekstrudaatteja ja kasvispihvejä. Arvioijille pidettiin yksi sanaston luonti ja koulutuskerta ekstrudaateilla sekä kasvispihveillä ennen kuvailevaa aistinvaraista arviointia. Ekstrudaattien kuvaileva aistinvarainen arviointi toistettiin kahdesti, koska haluttiin varmistaa tulosten paikkansa pitävyys. Kasvispihvien tulokset eivät olleet tilastollisesti merkitseviä, mutta ekstrudaattien tulokset olivat.
Tulosten perusteella voidaan todeta, että ekstruusio ei vaikuta mausteiden aistinvaraisiin ominaisuuksiin. Ekstrudaattien kuvailevan aistinvaraisen arvioinnin tuloksista voidaan päätellä, että tärkein mauste oli paahdetun sianlihan makuinen hiivauute. Sillä oli yksinään suurin vaikutus ekstrudaattien aistinvaraisiin ominaisuuksiin. Mausteseoksella, NaCl:lla ja hiivauutteella saatiin suurin muutos ekstrudaattien aistinvaraisiin ominaisuuksiin, ja sen katsottiin olevan paras resepti.

Avainsanat	liha-analogi, kuvaileva aistinvarainen arviointi, maustaminen, aistinvaraiset ominaisuudet

Contents

List of Abbreviations

HMMA	High moisture meat analogue. A meat-like vegetable protein product, that typically has a moisture content of >40%.
HME	High moisture extrusion. A form of extrusion with a moisture content of >40%.
FBPC	Fava bean protein concentrate
PPI	Pea protein isolate
IFF	International flavours & fragrances. An American company specialized in fragrances, cosmetics, and flavours.

1 Introduction

To meet the world's protein demand in 2050, the dairy and meat production should double. The environmental impact of livestock is already beyond the planet's carrying capacity. More efficient feed conversion ratios and production methods will not alone solve the problems associated with the livestock industry. A cultural and behavioural fix is needed with technological changes. Humankind consumes too much meat already and as the population is increasing, so is the consumption of meat. The consumption of animal proteins is also a risk for future food security because current consumption it is not sustainable. In the future most of the protein humans consume must be coming from plant-based sources. (1)

The vegan diet is becoming increasingly popular, especially among young adults. In the United States the amount of people following vegan diet rose from 300 000 to 500 000 in 1997 and to between 2.5–6 million in 2012. In a study 5% of Israelis were following a vegan diet in 2014, 2% of survey's participants (n = 3618) in the United Kingdom identified themselves as vegans in 2007 and in India 31% of people follow a vegetarian diet, mostly for religious reasons. Vegans can be roughly, divided into two categories: health vegans and ethical vegans. Ethical vegans usually adhere to the vegan diet longer, eat more vitamins and consume more soy products. Health vegans consume more fruits and sweets. Vegan diets are not always healthy and are diverse. They can include healthy fats, legumes, nuts, and vegetables, but also foods high in sugar, unhealthy fats, and salt (2).

Ethical concerns were most often the reason for following the vegan diet (n = 201) compared to health reasons (n = 45) (2). Ethical reasons include mostly animal welfare but can also include the welfare of the planet and social problems. The welfare of animals goes beyond killing them for food, also considering how they are raised. Most animal farms maximize profits by doing the bare minimum required by law and not taking animal welfare into account. Especially chickens grow so large that they are in pain for the last 20% of their lives. Male chicks are slaughtered at birth because they cannot be used for egg production. Animals raised in tight spaces with other animals are in a risk of quick spreading diseases, which requires the use of antibiotics, and the risk of developing antibiotic resistance bacteria in these places is a real concern. In some countries the people working in the slaughterhouses are having to process the animals at such speed that there is a serious risk of injury or even death (3).

Livestock sector is responsible for 18% of the global greenhouse gas emissions. The 18% can be explained by considering the whole lifecycle of the animal and how they are fed. The emissions taken into account in the study were carbon dioxide from fossil fuels combustion, enteric fermentation by ruminants, methane from manure, deforestation and nitrous oxide from fertilizers used for land cultivation (4). The current amount of livestock is damaging the environment and should be halved to prevent from further ecological damage (1). 14% of the animal feed fed to livestock are edible for humans and 40% of the arable land is used to feed livestock. To get one kilogram of boneless meat 3.2 kilograms of human-edible food is used for monogastric livestock and 2,8kg for ruminants. 25% of the protein consumed globally are from animal sources and comprise 18% of total calories. Livestock are part of food security in some parts of the world and provide people with high-quality protein by converting feed from non-arable land to food for human consumption (5).

The aim of this project was to create a good tasting high moisture meat analogue (HMMA) from Nordic plant protein sources that can easily be used or developed other products. The main ingredient used for the project is fava bean protein concentrate, which has a strong beany, bitter and astringent aftertaste. Masking the aftertaste with natural ingredients is quite challenging, but it is important for creating a good tasting product. A HMMA with a neutral or very mild flavour would be a versatile product that could be flavoured easily to create a variety of products.

2 Traditional vegetable proteins

The production of traditional vegetable proteins, such as tofu, tempeh and seitan were invented out of need to replace meat in people’s diet for religious, health or financial reasons. These products have been consumed since ancient times in India, China, and

Indonesia (6). For example, tofu was mentioned in 965 CE in a Chinese document as the vice mayor’s mutton, because he could not afford meat and would instead buy tofu (7). The plant-based meat alternatives that were created to imitate meat were first introduced in the 1960s and new techniques have been invented since to Improve the texture and quality (6). In this chapter the most common plant-based protein products and meat alternatives are introduced.

2.1 Tofu

Tofu is a plant-based protein product that has been manufactured for 2000 years. The Chinese were the first to produce tofu and it is an integral part of their food culture. Tofu can be used in various dishes, depending on the type of tofu. Tofu can be categorized to momen tofu, which is a firm, soft tofu, and kinugoshi, which is a silken tofu. Firm tofu can be easily marinated, grilled, fried, or deep fried. Softer tofu can be used for soups or stews (8).

Tofu is made from soymilk. To produce soymilk, the soybeans are cleaned, soaked in water to rehydrate them and then grinded to break the structure of the soybeans. During the grinding water is added according to what type of tofu is being produced. The mixture is then heated to around 100 °C, which helps the separation. When the soymilk is separated from the solids, coagulants, like salt, acids, calcium chloride, magnesium chloride or slow acting calcium sulphate or magnesium sulphate are mixed in, which forms solid curds. Whether the curd is cut or not depends on what type of tofu is being produced. Firm or extra firm tofu is made by breaking or cutting the curds and pressing it in moulds to release water from it. The more water is released, the firmer tofu. Silken tofu’s curd is formed in the package therefore cutting the curd is not necessary (8).

2.2 Tempeh

Tempeh is a fermented soybean product, although other pulses can be used. Tempeh was discovered in Indonesia and is now equally popular in Malaysia. It is made by soaking soybeans in water overnight and then boiling them for 30 minutes. The beans are then dried and a piece of *Rhizopus oligosporus* is mixed with the soybeans. The soybeans are then wrapped traditionally in a banana leaf or nowadays, in a plastic wrap with some holes in it. The soybeans are left to ferment 24–48 hours in 30–38 °C, until a white mould has formed, that binds the soybeans together. Tempeh is a fresh product and needs to be dried, frozen, or blanched if it is stored for longer periods. Tempeh is used as a meat replacer and a source of vegetable protein. It needs to be cooked before consuming (9).

2.3 Seitan

Seitan is made from wheat gluten by washing wheat flour with water to remove all the starch. Gluten forms a chewy mass easily by hand. Seitan can be seasoned or marinated and then fried. Seitan has a high protein content and is a great meat replacer for people that can digest gluten (10).

2.4 Meat analogues

Meat is nutritionally fulfilling, flavourful, juicy, and chewy. It is an easy choice for nonvegetarians, but meat still has some negative impressions with it. Consumers are looking for a product that is convenient and nutritious. Meat analogues could provide consumers a nutritious, healthy, and convenient option for meat. Meat analogues can have a similar texture, colour, and appearance as meat, but contain only plant-based ingredients (11). Meat analogues can be split into two categories: textured vegetable proteins and high moisture meat analogues. Textured vegetable proteins are drier and are either sold as a dry product, that has a long shelf life or is marinated cold storage product. High moisture meat analogues have a high moisture content from production and have a similar shelf life as a meat product would have.

2.4.1 Textured vegetable proteins

Textured vegetable proteins are mostly produced by low moisture extrusion cooking. First textured vegetable protein products were developed in the 1960s to create vegan versions of meat-based foods like bacon and hamburgers. Soybeans are the most common ingredient used in textured vegetable proteins globally (12). Other ingredients can be used such as different legumes, oil seeds, mycoproteins and gluten. Textured vegetable proteins are easily seasoned, because they are soaked in a liquid to rehydrate them before consuming. Textured vegetable proteins can absorb three times their weight in water. The soaking liquid can contain different flavourings and oils (10).

The challenge is to obtain a product that has acceptable characteristics found in meat, such as flavour, colour, texture, mouthfeel, odour, and particle size. All these characteristics must be achieved by developing the product by precisely adding the right ingredients and using right techniques. Flavour and texture are the most difficult sensory properties to develop. A meat-like texture is hard to achieve through low moisture extrusion, because after the rehydration the meat analogue tends to have a spongy texture, instead of a springy texture of meat (10).

2.4.2 High moisture meat analogues

The market for plant-based protein products has been guided towards vegans and vegetarians who do not desire the taste of meat. Recently there has been a shift to try to produce meat alternatives that can imitate the texture, taste, and appearance of meat, to market plant-based meat alternatives to consumers that desire the taste of real meat. Flexitarian diet is gaining popularity, where a person would mostly eat a plant-based diet but would occasionally eat meat. Flexitarians enjoy the taste of meat, but for health or ethical reasons are consuming only small amounts of it. Companies like Impossible Foods and Beyond Meat are manufacturing products that have the appearance like raw meat and the burger patties have a “bleeding” effect when cut. These products have similar nutritional value as a traditional burger patty would have (12).

The most used ingredients in meat analogues are soya protein and wheat gluten. Soya is widely researched and has been in use for meat substitutes in the west for almost 40 years (13). Wheat gluten is easily available and very easy to modify to create a good texture and mouthfeel. The problem with soya bean is that it has been linked to deforestation and some consumers see soya bean as potentially harmful to their health. That creates an opportunity to use other ingredients that consumers already accept. Peas and other pulses are becoming more popular in meat analogues produced by high moisture extrusion (14). Also, the use of defatted peanut meal has been researched and could be a potential ingredient. Most of the 5 million tons of produced defatted peanut meal is used as animal feed (15). Sometimes oils and starch are added to the mixture to achieve a better texture.

Most of the ingredients used are either protein concentrates or protein isolates. Protein isolates have a more neutral colour and taste and contain more protein than protein concentrates, but the process also uses much more energy, making concentrates better environmentally. The plant protein concentrates also contain some carbohydrates, which are beneficial to the texture of the extrudate (16). The challenge of using pulse proteins is the bitter, beany and astringent off-flavour (17).

3 Literature review

3.1 Current applications of spices and flavour additives in the development of meat analogues

Meat analogues are plant-based protein products that try to resemble meat in flavour, texture, and appearance. A typical meat analogue contains water, plant-based protein, fat, flavourings, colouring agents, and binding agents. The flavour is a key factor in consumer acceptance of meat analogues. Some plant-based proteins used in meat analogues can have a strong off-taste or odour, which some consumers dislike.

Many techniques have been created to improve the texture of plant-based meat analogues, such as low moisture and high moisture extrusion, wet spinning, shear cell and 3D printing. There have been many studies conducted on the texture properties of meat analogues, and only a few studies on the flavour of meat analogues. The oldest study used for this literature review is from 2018 and the most recent from 2021. It shows that the flavouring of meat analogues has only recently started to be studied. The flavour properties of meat analogues need to be researched more, especially how to achieve a meat-like flavour and mask the off-taste of certain plant-based proteins. The markets for plant-based protein products are constantly growing because more people are following vegetarian or vegan diet each year, and eating less meat is a growing trend in the west.

3.1.1 Achieving a meat-like flavour in meat analogues

Achieving a meat-like savoury flavour in meat analogues is a challenge because the flavour compounds found in meat are formed through a complex process of decomposition, oxidation of lipids and fatty acids, Maillard reaction and other chemical reactions. There have been many studies focused on the volatile components that form during cooking of meat. (11). There are over 1000 volatile compounds that have been identified, which are produced during cooking of meat. The key flavour compounds are pyrazine, heterocyclic compounds and sulfhydryl compounds which are formed through the Maillard reaction (17). Aldehydes, furans, unsaturated ketones, aliphatic hydrocarbons, which are formed through the oxidation of fatty acids and thiols, sulphides and disulphides which are formed through the thermal degradation of thiamine(17,18). Sulphur-containing thiobenzines and furans are already used in meat analogues to produce a strong meat-like flavour (11,18).

Meat analogues made from plant-based proteins do not contain the compounds that would produce meat-like flavour. Using synthetic flavours, a meat-like flavour could be achieved in meat analogues, but there are many disadvantages. Synthetic flavourings can reduce the quality of the product and potentially generate harmful components. The flavour of a specific meat is usually related to lipids. That flavour is difficult to imitate using synthetic flavourings. Synthetic flavourings can also be destroyed easily when exposed to high temperature (17). The use of synthetic flavourings should be avoided because the popularity of more natural ingredients is rising amongst consumers, and synthetic flavourings could be viewed negatively (11).

Several studies show that creating meat-like flavour naturally can be achieved with hydrolyzed vegetable proteins, yeast extract, vegetable oils and natural spices, reducing sugars, amino acids, and vitamins (11,17). Flavours imitating beef and chicken can be produced from enzymatically hydrolyzed plant-base proteins (11,18). Alim et al. (19) identified 31 beef-like meaty aroma compounds from brewer’s yeast. Yeast extract generates peptides which have an impact on formation of beef-meaty aroma compounds through Maillard reaction. Reducing sugars like xylose, glucose, ribose, and fructose used together with amino acids like cystine, cysteine, lysine, proline, serine, methionine, and threonine can form new characteristic flavours during the Maillard reaction (11). Maillard-reacted beef bone hydrolysate (MRP) can be used in soy and gluten-based extrudates to provide them with meaty flavour (11). MRP is not suitable for vegetarians or vegans, therefore it could only be used for plant-based protein products for consumers that also eat meat products although there are ways to create beef-like aroma that is suitable for vegans. The use of animal-based products should be avoided in plant-based protein products if possible.

Vegetable oils can simulate the role of animal fats in flavour formation in meat analogues. Pressed canola oil, coconut oil, and sunflower oil contain similar fatty acids as animal lipids. These oils can be mixed in specific proportion and oxidized at a specific temperature to mimic the flavour of animal fats (17). Some volatiles found naturally in animal fat, such as some aldehydes, alcohols, sulphur compounds and hydrocarbons cannot be replaced by using vegetable oils (20). Lipid content that exceeds 15% has an adverse effect on the structure formation during extrusion. Fat in meat analogues plays an important role in the retention of volatile compounds (11,16,18).

Leghaemoglobin, short for legume haemoglobin, carries a molecule called heme. Heme is an iron containing molecule that is present in animals and plants. Heme is responsible of the meaty taste in meat and promotes “bloody” appearance (20)(21). The Leghaemoglobin as food additive is patented by Impossible Foods Inc. (US patent US9826772B2). Heme acts as a catalyst of the chemical reactions that can turn biomolecules into flavour molecules and odorants that are like meat. In meat myoglobin unfolds when it is cooked and exposes the heme cofactor that catalyses reactions which transforms vitamins, amino acids, nucleotides, and sugars found in animal muscle tissue to hundreds of aroma and flavour compounds. These compounds create a flavour profile distinctive to meat. Leghaemoglobin acts in a similar way when it is cooked and can create a meat-like flavour. Leghaemoglobin is created by inserting soy leghaemoglobin’s gene encoding into the genome of *Pichia pastoris*. *Pichia pastoris* is a yeast, that has been used for recombinant expression and is safe for human consumption. The yeast that has the gene encoding of leghaemoglobin is then fermented to get large amounts of leghaemoglobin reliably (22).

The use of spices such as oregano, black pepper, sage, clove and rosemary and flavour extracts can produce a complex meat flavour profile and mask off-flavours of meat analogues (20). Flavourings commonly used in meat analogues include garlic, paprika, onion powder, celery and many other spices and herbs (20). Salt is an important taste enhancer, but the current trend is to reduce the amount of sodium in food (11).

The most recent review on meat analogues (11) suggests that all the options above could be used for creating a meat-like flavour for meat analogues. Earlier studies and reviews, which some of them have been used as a source material also support the findings (16–20,22). However, the most recent review does not mention the use of yeast extracts, although all the other studies and reviews that have been focusing on flavouring of meat analogues suggest that yeast extract yields a meat-like aroma.

3.2 Effect of high moisture extrusion on spices and flavour additives

There have been many studies conducted on how low moisture extrusion affects the spices and flavourings. Low moisture extrusion has been used since 1930’s. Low moisture extrusion was first used to produce breakfast cereals. Since then it is being used to produce textured vegetable proteins, snack foods, pasta, confectionary products, and many other products (23). The use of high moisture extrusion is a much newer process and the effect on the sensory properties of flavourings and spices has not been studied as widely.

Yuliani et al.(24) studied the retention of volatiles on snack products with low moisture extrusion. The same problems are present with high moisture extrusion, with the loss of volatiles during the extrusion. The only difference is that the end product is cooled before exiting the extruder, which prevents it from expanding. At the exit die the snack products expand due to drop of pressure and some loss of flavour happens due to it. Pre-extrusion flavouring is desirable because the flavour is evenly distributed and helps to protect the flavours against oxidation. The downside of pre-extrusion flavouring is the harsh conditions that the flavourings are subjected to, such as high temperature, shear, and pressure during extrusion. Those conditions may change the sensory attributes of the flavourings. Adding the flavour additives and spices at the end of the extrusion barrel reduces the time the flavour additives and spices are subjected to high pressure, temperature and shear and can increase the retention of volatiles (24).

The loss of flavour during extrusion can be optimized by controlling the moisture content and choosing the right protein ingredients. Depending on the compounds used, during high temperature and pressure, chemical reactions may occur, which leads to loss of volatiles and change of flavour. The right microenvironment can help protect the volatile flavour substances. Guo et al. studied how material characteristics effects the structural characteristics and retention of volatiles of meat analogues. For the study they used soy protein concentrate and wheat gluten in different ratios with a mixture different of volatile compounds. The conclusion was that the higher the wheat gluten content and lower moisture content, better the retention of volatiles. Increasing wheat gluten and lowering the moisture content makes the meat analogues structure tighter and prevents breakage (25).

In 2014 a study on how pre-extrusion aromatization of soy protein isolate using flavour enhancers and volatile compounds effected the physical characteristic, sensory characteristic, and volatile retention of extrudates was conducted by Milani et al. In the study three liquid volatile compounds were used (ethyl butyrate, butyric acid and isovaleraldehyde) and two flavour enhancers (disodium 5-inosinate and monosodium glutamate monohydrate). For the study, soy protein isolate was mixed with water to achieve three fixed moisture contents of 30, 35 and 40%. The most desirable extrudate was achieved with 30% fixed moisture with 170 °C processing temperature. The extrudate had the best sensory acceptability, desirable physical characteristics and improved volatile retention (26). Guo et al. achieved a similar result in their study with 50, 60, 70 and 80% moisture contents. The extrudate with the lowest moisture and highest wheat gluten content produced the most desirable product with greater volatile retention (25). Even thou Milani et al. used lower moisture contents in their study, both studies reached the same conclusion: lower moisture content leads to better volatile retention.

Dried herbs and spices can contain high levels of *Salmonella*, *Bacillus cereus*, *Escherichia coli* and *Clostridium perfringens* (27). Many herbs and spices are grown and harvested in areas where the sanitary conditions are optimal for microbiological contamination (28). In 1993 a nationwide *salmonella* outbreak in Germany occurred, which was traced back to contaminated paprika powder that was used in potato chips. The temperature of the chips was 60 °C when the spices were applied, which is not enough to safely kill the *salmonella* bacteria (29). Generally bacterial spores are resistant to pressure, but high pressure with the combination of heat can sterilize the bacterial spores found commonly in herbs and spices (30). The advantage of high moisture extrusion is the combination of heat and pressure that will sterilize the product while it is being produced, and no post treatment is needed.

A study was conducted to determine how different pathogen inactivation methods affect the overall quality and sensory quality of spice and herbs. The spices and herbs were first irradiated, then subjected to ethylene oxide and after that heated with vacuum assisted steam at 82.22 °C in 5.2 bar of pressure. The vacuum assisted steam had no effect on oregano or onion powder, but it resulted in loss of all sesquiterpenes, increase in monoterpene concentration and odour differences in black peppercorn. The cumin seed only had a visual difference after the treatment (31). In another study black peppercorns were heated in 110-150 °C for 15 or 30 minutes. Higher temperature showed consistent increase of acceptability in sensory analysis. 15 minutes in 150 °C got the best sensory score (8.8). Some changes could be detected in volatiles when the heated samples were analysed with gas chromatography, but the changes did not affect the pepper flavour (32).

3.3 Effect of spices and flavour additives on sensory properties and texture of plant-based meat products with different processing methods

The sensory acceptance of meat analogues is mostly determined by flavour and visual appearance. The flavourings used must depend on the protein source and final product formulation. Some protein ingredients have a bitter, grassy, beany or astringent flavours, that need to be masked or some other practices needs to be used to remove undesirable flavours. These practices include fermentation, defatting and removal and deactivation of lipoxygenases (11).

Katayama and Wilson (33) studied what was the most acceptable concentration of vegan chicken flavouring in textured soy protein meat analogues. The flavours were added to four different shapes of textured soy protein meat analogues: shred, bit, narrow strip, and wide strip. The flavour was either added as a powder or in a liquid form at 3% or 4% concentration to 80 °C water and then soaked for 3 hours. Then the extra water was drained and then either fried in soybean oil at 191 °C for 4 minutes or baked at 160 °C for 8 minutes.

The fried version of the textured soy protein meat analogue with 4% chicken flavour was the most popular amongst the 125 consumers that tried the products. The fried version had three times the amount of oil than the baked version, which suggest that higher fat content may increase consumer acceptance (33).

Milani et al. (26) extruded soy protein isolate with different extrusion conditions and moisture and concluded that adding volatile compounds and flavour enhancers increases extrudates density under all conditions. Green tea flour has great antioxidant functionalities and has a potential to improve the microstructure and texture of meat analogues to imitate meat more closely. The study suggests that the antioxidant properties are the cause of the better microstructure in meat analogues (34).

Edible gum, like pectin and carrageenan can increase water absorption, provide better viscoelasticity, improve elasticity, strength, and fibrous structure. Starch can enhance product stability and improve sensory properties, like taste and colour. The starch content should not exceed 10%, because it can negatively affect fibre arrangement. Dietary fibres such as psyllium mucilloid, carrot fibre and methylcellulose can improve the nutritional quality of meat analogues, but also improve texture. Fibre has good water holding capacity and it can act as a lubricant. Fibre can help to reduce the amount of fat used in meat analogues by taking its place by having same functional characteristics (18).

3.4 Stability of flavour additives in plant-based meat during chilled storage

Most of the studies conducted on plant-based meats focused on microbial stability of the product, rather than sensory properties during storage (16,35,36). Most of the studies reviewed suggested that plant-based meats have a similar shelf life as similar meat products during cold and frozen storage. The studies also recommended that plant-based meats should be stored in a similar manner (11,16,35,36).

A study was conducted on high quality meat-like products called “likemeat”. The aim of the study was to create high-quality meat analogues that were either vegan or vegetarian. The purpose was to create a good quality base material with high moisture extrusion, that could be turned into different products by seasoning them and shaping them differently. At the end of the study a microbial and sensory study was conducted parallel to each other to detect any changes. Microbial stability was more important than sensory qualities. The microbial and sensory study were done with frozen (-20 °C) and cold storage (4 °C, 6 °C and 10 °C). Some of the samples were sterilized for the study. Without sterilizing the neutral products had approximately one week of shelf life and the sterilized neutral products had at least one month of shelf life when store at 4 °C. Flavoured “likemeat” products storage test at 6 °C showed that the sensory properties do not change even if the total microbial count rises. The herbs and marinades used did not improve shelf life of the products and can negatively influence it. A curry powder used for the study had high levels of bacteria, which affected the shelf life negatively. The sensory properties did not change when the flavoured products were frozen for 6 weeks (35). Since the microbial count rose too high for human consumption before any sensory properties changed, it can be concluded that flavour additives are stable at least for the period that the product is safe for human consumption. The frozen storage did not have any effect on sensory properties either, so flavoured high moisture meat analogues can be frozen without losing any sensory properties.

Modified atmosphere packaging can be used to retain flavour and prevent moisture loss for meat analogues. The packaging used for meat prevents the loss of moisture but allows the flow of oxygen so that it can interact with myoglobin to produce cherry colour compound oxymyoglobin. Similar type of packaging can benefit meat analogues by reducing the amount oxygen and increasing carbon dioxide to expand shelf life (16). How post-process handling and storage conditions effect the microbial safety and flavour change of meat alternatives should be further investigated (20).

3.5 Examples of descriptive sensory profiling of meat analogues

For the descriptive sensory profiling examples, 6 studies were reviewed: 3 studies on high moisture meat analogues (moisture content >40%) and 3 on low moisture meat analogues (moisture content <40%). Each study used extrusion to produce the samples for sensory evaluation. Different ingredients were used for the meat analogues in each study, but the sensory lexicons were very similar. The summary of the overviewed studies sensory protocols has been collected in Table 1.

Table 1. The number of attributes, the main discriminating factors and scales used in the sensory analyses reviewed.

Author	Main discriminating attributes	Number of sensory attributes	Scale
Omohimi et al. (37)	Hardness	9	9-point hedonic scale
Lin et al. (38)	Mushy, tough, and cohesive texture	7	*Not specified*
Grahl et al. (39)	Overall algae and musty and earthy taste	18	Unstructured line scale with verbal anchors.
De Angelis et al. (40)	Rancid, metallic, or chemical off-odours	24	0-10 structured scale
Kaleda et al. (41)	Sour taste and odour	19	0-10 with verbal scale anchors
de Boer et al. (42)	Bouillon, rye bread, soy sauce, and minced meat odours and oily and crispy mouthfeel	22	0-100

The three studies that focused on high moisture meat analogues were Omohimi et al., Grahl et al. and Lin, S., Huff, H.E., and Hsieh F. (37–39). Omohimi et al. used mucuna beans for their study. The most desirable product was produced with 47% feed moisture, 120 °C barrel temperature and a screw speed of 119 rpm. Grahl et al. used a mixture of soy and microalga spirulina (10-50%) with moisture content of 57-77% and a screw speed ranging from 600-1200 rpm, which had a little effect on the extrudate. Lin et al. used soy protein isolate with moisture content of 60-70% and cooking temperature of 138-160 °C. None of the studies used any flavour additives in their meat analogues.

Omohimi et al. used a 9-point hedonic scale for the descriptive sensory analysis. The test was conducted as a consumer study with the help of trained judges. The panel of ten judges had training two days before the sensory analysis on different tastes, like saltiness, sweetness and bitterness, and textures of meat analogues. The samples were analysed by 50 panellists, consisting of staff and students of a university. The attributes used for the sensory analysis were colour, texture, fibrousness, juiciness, firmness, chewiness, meatiness, hardness, and saltiness (37).

Study conducted by Lin et al. focused on the extrusion parameters with the aim to produce an extrudate with good texture. Before the sensory analysis four training sessions were held. The panellists who belonged to the department of food science or biological engineering were all trained. A total of 4 sessions of sensory analysis were held with two replications. The sensory analysis was focused on physical traits only. The terms used for the descriptive analysis were mushy, tough, moist, layered, springy, chewy, and cohesive (38).

A total of 12 trained panellists with a total of 18 hours of training were used in a study conducted by Grahl et al. (39) During the training panellists agreed on final list of descriptors, attributes, scale anchors, the chewing method, and other practices during the sensory analysis. Total of 18 attributes were chosen, that were divided under six descriptors: overall (overall, algae and musty), Appearance (colour, layered and moist), texture (elastic, firm, brittle and fibrous), flavour (overall, earthy and chicken), mouthfeel (soft, juicy and crumbly) and aftertaste (overall and umami). The scale anchors were line scales with varying verbal anchors, for example for juiciness the scale would be dry – juicy. The amount spirulina used in the extrudates made the most difference. The aftertaste, odour and flavour got more intense when the spirulina content in the extrudates increased. The increase in spirulina also made the samples softer and less elastic and fibrous. The moisture and spirulina content were the two factors that affected the samples the most (39). The study conducted by Grahl et al. was the most comprehensive study out of the studies that analysed high moisture meat analogues.

The similar descriptors between each study were hardness, chewiness, juiciness, and firmness. Lin et al. (38) only studied the physical traits of the extrudates and had one similar descriptor to Grahl et al., which was layered. Grahl et al. had four similar descriptors as a study conducted by Omohimi et al.; these were colour, texture, fibrousness, and meatiness/chicken flavour. Meatiness and chicken flavour are similar descriptors. Chicken flavour refers to specific type of meat and meatiness refers to any type of meat (37–39).

Three studies analysed extrudates or products that were produced by low-moisture extrusion. Two of the studies had a panel consisting of experts (40,41) and one study had trained amateur panel (42). Studies conducted by De Anegelis et al. (40) and Kaleda et al. were both published in 2020, and the study conducted by de Boer et al. (42) was published in 2006. The study conducted by Kaleda et al. (41) was used as a source material by De Angelis et al.

De Angelis et al. (40) used a panel consisting of seven experts that belonged to the sensory panel of Centre of Food and Fermentation Technologies Tallinn, Estonia. The purpose was to evaluate the sensory properties of meat analogues produced from oat protein and dry-fractionated pea protein. One training session was held before the sensory evaluation to identify descriptors and ranges, and to produce scale anchors for the descriptors. A total of 24 sensory attributes with scale anchors, distributed under 4 descriptors: appearance, odour, taste, and texture (40).

The sensory attributes chosen for appearance were colour and fibrousness. For odour the attributes were overall intensity of the sample (any odour), meat-like (pork or chicken-like odour), odours associated with cereals, legumes, sweetness, and off-odour intensity (rancid, metallic, chemical, etc.). The attributes chosen for taste were overall intensity of the taste, the taste of legumes and cereals, saltiness, umami, sweetness, bitterness, astringent, off-taste intensity, and aftertaste intensity. For texture the chosen attributes were hardness, springiness, chewiness, cohesiveness, moisture, and graininess. The samples were evaluated on an 11-point (0-10) structured scale (40).

Kaleda et al. (41) studied how fermentation and phytase treatment affects the sensory, physicochemical, and nutritional properties of meat analogues made from pea-oat protein blends. The trained panel consisted of eight members with at least two years of experience with sensory analysis. A single training session was conducted with each product before the sensory analysis to specify attributes. De Angelis et al. used the same descriptors in their study. The descriptors were texture, taste, appearance, and odour. The attributes used for appearance and texture were also the same. The attributes for odour were overall intensity, the odour associated with legumes and cereals, sour, and off-odour intensity. The attributes used for taste were overall intensity of the extrudate, the taste associated with legumes and cereals, sour, bitter, off-taste intensity, and after-taste intensity (41).

The same scale anchors were used for analysing taste as was used for odours. The taste attributes that the panel was analysing were taste associated with cereals, taste associated with legumes, saltiness, sweetness, umami (glutamate), bitterness (caffeine), astringent (dry mouth feel caused by tannins), off-taste intensity (non-characteristic taste, such as metallic, chemical, rancid, etc.) and aftertaste intensity after 5 seconds from swallowing the sample. The overall taste intensity had scale anchors of 0 no taste, 5 boiled chicken or pork and 10 very intense taste (40).

de Boer et al. studied how similar meat analogues differ in a descriptive analysis. 6 different minced meat substitutes were chosen for the descriptive analysis. 22 different attributes were chosen to describe the minced meat substitutes on a scale of 0-100. 12 attributes were related to flavour and smell and 10 for mouthfeel and appearance. The attributes for flavour were bitter, sweet, sour, salty and aftertaste and the attributes for smell were bouillon, rye bread, sour odour, seasoned, spicy, soy sauce and mincemeat. The appearance attribute was described with size. The mouthfeel attributes were granularity, compactness, oiliness, toughness, fibrous, elasticity, crispiness, dryness, and juiciness (42).

The sensory studies reviewed for this thesis were more focused on the texture of the meat analogues, rather than the flavouring. The sensory properties that were evaluated were mostly the unpleasant off-flavours or odours that were naturally present in the meat analogues. Because the thesis is focused on the flavouring of meat analogues, the texture is not important, unless some of the flavour additives change the texture of the meat analogues in a way that it is noticeable. The common thing between these studies were that the characteristic sensory properties of the meat analogues were included. For example, in the study conducted by Grahl et al. (39) spirulina was used that has a specific taste, which was included in the sensory evaluation (Table 1). The characteristic attributes of the ingredients and flavour additives together with off-flavours and odours should be evaluated in the descriptive sensory analysis.

4 Extrusion

Extrusion is a continuous processing system, which has many different unit operations that are combined as one. Extruders cook, convey, shear and mix food materials during the extrusion process. Extruders can have either one or two screws, which convey the materials and cause shearing. The speed of the screws can be altered, which affects the pressure and time the material spends inside the extrusion barrel. The materials are heated during the process by the extruder barrel and the shearing caused by the rotating screws, which forces the material particles to rub against each other. Different products require different cooking temperatures, which can be controlled by changing the temperature of the extruder barrel. The material is forced through a die opening, which is smaller than the diameter of the extruder barrel and that generates pressure inside the barrel. Depending on the product, there can be a cutter or a cooling die after the die opening. The shape of the product is determined by the geometry of the die exit (23).

Extrusion has been used for a long time for producing breakfast cereals and snack foods, such as puffed corn chips and since then increasing number of different products are being produced by extruding (23). Those products include pet foods, textured vegetable proteins, meat analogues, pasta, and baby foods (43). For snack foods high starch content is preferred because it helps the extrudate to expand as it exits the die (44). For snack foods nutritional value is not as important as for meat alternatives, such as textured vegetable proteins, whole muscle meat analogues or weaning foods. Different extrusion parameters can influence the nutritional value of the extrudates, such as protein denaturation and digestibility (43).

4.1 High moisture extrusion

High moisture extrusion is an extrusion process that has moisture content over 40%, because of the higher moisture content it uses less energy compared to low moisture extrusion (15). It uses twin-screws instead of one that is usually used for low moisture extrusion. The flour is mixed with water inside the barrel by co-rotating screws. After mixing, the mixture goes through heating and pressurizing. For the last phase the mixture is pushed through a narrow pipe, into a long cooling die, which is cooled by water from the outside, which reduces the mixtures temperature below boiling point and prevents it from expanding and breaking the texture (45). High moisture extrusion can be used to create meat-like fibrous and springy texture from vegetable proteins. It is the most used technique to create high quality meat alternatives that have similar texture as meat (14,15). The texturing happens in a long cooling die, where layers of aqueous protein melt of water-rich and protein-rich domains are formed during cooling (45). The largest advantage of high moisture extrusion is the continuous process compared to other techniques, like shear cell and electro spinning. Shear cell can be used to create a product with good structure, but the process must be stopped each time the ready product is collected, which is not favourable. Electrospinning can only be used for highly soluble proteins, which narrows the usability (16).

4.2 Low moisture extrusion

Low moisture extrusion has a moisture content lower than 40%. Low moisture extrusion is used to produce many different types of products, like snacks, textured vegetable proteins, sweets, pasta, and breakfast cereals. Because the mixture is dry, the process uses more energy than high moisture extrusion (15). The ingredients are fed to the extruder either premixed or through a mixer. The mixture is mixed and heated inside the barrel. For low moisture extrusion a single screw or a twin-screw can be used. The screw creates pressure inside the barrel. The mixture is then pushed through die hole, that can have a specific shape. If the mixture is heated to over 100 °C the water starts to boil after exiting the extruder, which causes the extrudate to expand (24). After exiting the extruder, the extrudate can be cut, shaped, fried, dried, shred or coated, depending on the product.

The flavouring of extrudates can be done before, after or during extrusion. post-extrusion flavouring is the easiest method of flavouring extrudates because the heat and pressure from the extrusion process will not affect the flavours through the loss of volatiles. However, the flavouring is just on the surface of the product and can stick to the package and to consumer’s fingers. Post-extrusion flavouring also needs oil as carrier, which increases the fat content of the product. In pre-extrusion flavouring, the flavourings are mixed with the ingredient before extrusion. This helps the flavourings spread more evenly, protects them against oxidation and less oil is needed, making the products less calorie dense. However, the flavourings are subjected to high pressure, temperature, and shear. That may cause thermal degradation and loss of flavour. Furthermore, as the extrudate exits the die, there is flashing of volatiles with water vapor, caused by sudden pressure drop. More than half of different volatiles are released during flashing of water vapor. Flavouring the extrudates during the extrusion process has a better flavour retention than pre-extrusion flavouring. The flavouring can be injected into the barrel at the right stage to minimize the loss of flavour. Some flavourings can be mixed pre-extrusion and the rest of the flavourings added during extrusion to protect the most heat sensitive volatiles (24).

5 Sensory analysis

Sensory analysis is a young scientific method of analysing different qualities of products reliably. Perceiving the products qualities happens through smell, touch, sight, taste and hearing. By analysing, evoking, measuring, and interpreting the responses to the product, a reliable method of testing can be achieved. The roots of modern sensory analysis dates to 1940s, when the United States military realized that nutritionally fulfilling food was not a guarantee for food acceptance by military personnel. US Army quartermasters of food and container institute supported a research which aim was to recognize the problems of identifying which foods were preferred more and which foods preferred less (46).

In the past food industries relied on one expert, such as wine tasters, dairy judges, brew masters, tea tasters and other experts in their field to evaluate the quality of their products. It was seen that the expert had gathered much experience in their field thus their analysis could be depended on to know what consumers were seeking in a product. These experts would often lead the production or make decisions that would affect the sensory qualities of the products. The issue with single authority is that their opinion would not necessarily represent the opinion of the consumers, also the expert might fall ill, retire, not be available or die, so it was not a very reliable method of analysis. Modern sensory evaluation is more reliable, than a single authority for several reasons: the opinion of many panellists outweighs the opinion of a single evaluator, panellists can always be replaced and with a large enough group and set standards and practices, reliable data could be received (46,47).

Human responses to different products and foods is a complex interpretation and sensory process. It is impossible to predict how consumers react to a certain product through an instrumental measures or predictions. Instrumental measurements cannot predict what human brain experiences prior to responding to sensory experience and how saliva might affect the flavour. Data gathered from human sensory analysis is the only way to know how consumers might react to a product. The data is collected throughout the product development process so that predictions can be made about how the product changed during the process. The changes in how consumers perceive and react to a product comes mainly from three sources: process, ingredients, and packaging. The goal of food development process is to improve the product's nutritional and sensory quality and microbial stability. Ingredients can be changed because the original ingredients availability is not guaranteed, a new ingredient can be more cost efficient or to improve quality. Packaging acts as a barrier between the product and the outside and it affects the products shelf life and how well it retains sensory qualities. The package can be designed in a way that it appeals to customers (47).

5.1 Principles of good practice

Principles of good practice are set of guidelines that ensure the data gathered from sensory analysis is scientifically valid. These guidelines cover the testing environment, test protocols, experiment design and panellist consideration (47).

5.1.1 Testing environment

The testing environment should be in an area with easy access, close to parking lot and preferably on the ground floor. The area should be designed in a way that the panellists do not have pass through the preparation area because seeing any clues regarding the products might bias their opinion. The sensory evaluation area should be free from outside noise or odours. Factories sensory evaluation area should not be next to a noisy area, such as delivery room or next to an area that might give off extra odours, such as smokehouse. Quiet and uninterrupted sensory analysis sessions increase the likelihood of success.

The evaluation area should be designed in a way that the panellists cannot influence each other. If booths are not available, some other barrier should be used to isolate the panellists. Plywood is an inexpensive material that can be used for building portable barriers. It is important that the panellists do not sit across each other. The ideal dimensions of the booths are 1 meter by 1 meter. If the booths are any smaller, it might affect the panellist's concentration. There should be enough space between the panellists, that they can move freely in the evaluation area without disturbing the other panellists. The number of booths in an evaluation are should be maximized, because the booth availability is usually a bottleneck in testing (48).

The illumination should be neutral, ideally between 300–700 lux with a dimmer switch and the possibility to change the light colour and intensity. The evaluation area should be comfortable so that the panellists will not be distracted. It should have a positive air pressure with great ventilation. The humidity should be between 50−55% and the temperature between 20−22 °C (48).

5.1.2 Panellist selection

The panel selection is an important aspect when designing a sensory study. Motivation is important and the panel members should have some type of incentive to take part in the study. It is only natural for the panellists to think of what they can gain from the study. The incentive can be monetary or a gift bag with company products for people who are not associated with the company. The participation of employees should always be voluntary, and the incentives also vary. The number of sensory studies a single employee participates should be limited to around 3 times a week so that the motivation can be maintained. Taking part in a sensory study be a social event and a break from regular work. Small company gifts can also be given to the employees or a larger gift like a paid company event for frequent participants. The employees should not be given monetary compensation for participating to the sensory studies. Also, it is important that management supports the employee's participation and does not view it as wasted company time. (48,49)

The sensory specialist is always responsible for the safety and health of the participants and sensory studies should not pose any risk. The sensory specialist should always disclose if there are any allergens or food additives present that may cause danger for the participants. If the results of the sensory study are published the identity of the panellists should be protected. (48,49)

Two to three times more people should be recruited for the screening sessions than is needed for the actual sensory analysis. This allows the sensory specialist to choose the top performers from the screening sessions to obtain the best results on the actual sensory study. Also, it is not unusual that some of the panellists might drop out for number of reasons. The sensory specialist should inform the people who will not be participating on the actual study in a way that it will not hurt their feelings and makes them feel appreciated. The training sessions depend on the type of study being conducted. For example, discrimination studies only require minimal training, but descriptive sensory analysis require more extensive training. The amount of training always depends on the type of study being conducted and how precise the results must be. The training should be planned in a way that the panellists stay motivated. The time used for training might demotivate some panellists and they might become less committed to the sensory study (48,49).

5.1.3 Sample preparation and experiment design

The sample's size, temperature, preparation and serving containers should always be standardized. If the sample sizes vary the panellists can easily distinguish between different samples and it might affect the outcome. The samples should always be stored and prepared at the same temperature. The panellists should not be able to touch the samples and notice a difference in temperature and thus distinguish the samples from each other. The preparation methods should always be the same and written down, so that the preparation will not affect the outcome between sensory evaluation sessions. The serving containers should be carefully selected to fulfil the purpose. Also, the way that the samples are presented is important, for example, when evaluating cereals with milk the cereals might get soggy after being in contact with milk for long periods of time. The panellists should have clear instructions when to pour the milk into the cereals to get the most accurate results (48).

The amount of sample served for should be carefully planned. The amount should be enough that the panellist can evaluate all the attributes, but not too much because sample preparation and cost can be a limiting factor in the sensory study. The sensory specialist should always think about how the sample is regularly eaten and if the samples need a carrier to get an accurate result. The panellists should be given clear instructions how to evaluate the samples. These instructions might be the order that the attributes are evaluated, how to evaluate each attribute, to drink and smell water between samples and whether to spit or swallow the samples. 3-digit randomization should be used for labelling the samples and they should be arranged in an order that each sample appears in the same spot the same number of times. If the samples are in the same order for every panellist, it will affect the results of the sensory study (48).

5.2 Analysing methods

The analysing method is always chosen to fit the purpose of the sensory study. The study might have a limiting factor like time or money. Descriptive analysis is the most reliable and precise method, but it is also the slowest and the most expensive (50,51). Acceptance testing is best fit for customer testing with large number of participants to find out the acceptance of single attribute or compare how different products are liked. It is a useful method for product development because the evaluators only know what is being evaluated (52). Discrimination testing can be used to test whether consumers can perceive a difference between two different products. It is useful for testing whether cheaper ingredients or changes in formulation affect the product negatively. The samples should be very similar, or discrimination testing is not useful (53,54).

5.2.1 Acceptance testing

Acceptance testing is used to measure the sensory appeal and it can be done using just a single product. Acceptance testing provides information whether the product is liked or disliked. Also, it can be used to compare two similar products to study which one has more sensory appeal (52).

Hedonic scale is usually a 9-point, or 11-point scale used to measure liking. The scale ranges from *dislike extremely* to like extremely with a neutral like nor dislike in the middle. The hedonic scale is easy to use and implement and it has been studied to be effective and useful in hedonic assessments of beverages and food. However, it has been studied that consumers are less likely to choose extreme categories, such as *like extremely* or *dislike extremely* and the spacing between categories is not equal (52).

Just-about-right scale is used to study the intensity and liking of specific attributes. It is a combination of hedonic and intensity judgment. The end anchors are chosen to represent the attribute that is being assessed. For example, if the level of salt was being studied the end anchor would be “much too salty” and “not salty enough” with a middle point of “just about right”. The JAR scale has limitations to its usefulness. The consumers might not know what attribute on the sheet is referring to, the scale can only be used on simple attributes, changing one attribute might affect the other attributes, and it is difficult to indicate how much the product needs to be changed from the results (52).

5.2.2 Discrimination testing

Discrimination testing is used to identify differences between two products. After identifying the better product, the sensory professionals can move on to descriptive sensory analysis. There are many different discrimination methods, but all are used for single purpose; to find out if the products are different from one another. Discrimination testing is used when formulation or production method is changed to study whether the products are perceived as different. The products should be very similar or else the discrimination testing will not be useful (53).

Paired comparison test has two variations, same/different test, and two-alternative forced choice test. Both tests have a chance of ½ of guessing the correct answer. If the panellists cannot perceive any difference the average correct answers should be around ½. Two-alternative forced choice test is used when for samples that are only different by specific attribute. The sensory professionals must know which attribute is different before setting up two-alternative forced choice test. The panellists are presented with two samples and they need to taste both and circle the sample that is for example saltier. A n-alternative forced choice test is a variation of the two-alternative forced choice test where the panellists much rank three samples, for example from the saltiest to least salty. The Same/different test is used to study whether the samples are perceived different but not specify by how they are different. The panellists are presented with two samples and they need to circle whether they are both the same sample or not. Duo-trio test is usually used instead of same/different test (54).

Triangle test is when the panellists are presented three samples and need to choose which one of the samples is different from the other two. The chance of guessing the correct answer is 1/3 so when the average of correct answers is larger that means that the panellists can perceive a difference in the products. The standard A-not-A test includes a training phase where the panellists are presented two samples labelled "A" and "not-A" and they can taste the samples. During the evaluation the panellists are presented one sample at a time and asked which one the sample is. The alternate A-not-A test is conducted by asking the panellists to evaluate one sample first, which is then removed and then evaluate the second sample. After evaluating the second sample the panellists must choose if both samples were the same or whether there were any differences. This variation of A-not-A test is useful when the samples have subtle differences which are not relevant to the study because the panellists are only presented with one sample at a time so they cannot compare them (54).

The weakness of discrimination tests is that the sensory professionals can only find out if the samples are different from each other but not by how much. To get reliable results, there needs to be many judges because the chance of guessing the right answer is so high.

5.2.3 Descriptive sensory analysis

Descriptive sensory analysis is a very sophisticated method, which can provide a complete sensory description of an analysed product. It can be used to find differences and similarities between different products and provide information what attributes are important for acceptance. Only trained sensory panellists are used for descriptive analysis (50). The results are useful for product development to measure how close new ingredients or products are to the target. Descriptive analysis is quite expensive; therefore it is mostly used when major changes are made to products (51).

Descriptive sensory analysis always starts with screening of candidates. Screening should always be category specific; if the testing is done on sweet beverages, then the screening should be done with various sweet beverages. Not all subjects are qualified to evaluate every possible category, some participants might be qualified when evaluating dairy products, but not when evaluating meat products. The screening should always be done when evaluating new product. The subject must demonstrate an ability to notice differences in products at a level better than chance with products on the same category. Usually around 70% of potential candidates are chosen for the training session. It is recommended to train more subjects that is needed, because the subjects might lose interest or are not able to attend every session (50).

The purpose of training is to develop a language that is used for the descriptive analysis and get a consensus among the group what each descriptor means. The training is led by a panel leader whose only task is to lead the conversation and let the subjects create their own terminology. If there are not any readily available terminology for reference or the group is inexperienced the training can take up to 10 hours in a period of 5 days. Teaching existing descriptive language to the subjects unfamiliar with it can take more than 7 hours. Each of the subjects have their own words describing the attributes in the beginning, but it is important that at the end of the training the group has a common understanding of the terminology and are able to communicate using the generated language (50). The panel leader should have some reference samples ready to help the group define attributes. The references should be related to the attributes of the evaluated product or help perceive differences between samples (51). Each subject has different sensitivity to each attribute, and it is not possible to make the group agree to the same level of intensity, or else only one evaluator would be needed. Each training sessions should last around 90 minutes (50).

Sample serving, coding and preparation should be standardized. The samples should be evaluated by the panellists at least two times, preferably three. The panel performance should always be monitored to determine if further training is needed. All the samples should preferably be evaluated in a single session, but if there are too many samples or it is not possible for some other reason, a proper experimental plan should be followed (51).

6 Materials and methods

6.1 Instruments

The samples were produced using lab-scale Thermo Scientific Process 11 Hygienic Parallel Twin-screw Extruder (Karlsruhe, Germany) with a cooling die attached with dimensions of 4x20x250 mm (height x width x length). The flour feeder used to feed the flour to the extruder was Brabender Loss-in-weight Feeder DDW-MD0-MT-S-0,6 (Duisburg, Germany). The pump used for feeding liquid into the extruder was a Cole-Parmer Masterflex L/S® Standard Digital Drive, 0.1 to 600 rpm; 115/230 VAC 50/60 Hz with a Masterflex L/S® Easy-Load® II Pump Head for Precision Tubing, PPS Housing, SS Rotor pump head (Vernon Hills, Illinois, United States of America).

6.2 Materials/ingredients

Commercial fava bean protein concentrate, fava bean flour (P60) was obtained from Suomen Viljava (Helsinki, Finland). The fava bean flour had a composition of 54% of proteins, 4% of fats, 23% of carbohydrates which 12% of are fibers and the maximum moisture content of 15%. The commercial pea protein isolate had a composition of 79% proteins, 9% of fats, and 1% of fibers, Nutralys F85M was obtained from Roquette (Lestrem, France). The commercial vital wheat gluten, Reppal VWG was obtained from Lantmännen (Stockholm, Sweden). The faba bean protein concentrate, and pea protein isolate were stored at the temperature of -21 °C in their original paper sacks and the vital wheat gluten was stored at room temperature in a resealable plastic bag.

Table 2. shows different flavour additives or spice mixtures that were obtained from Lihel (Finland). The flavour additives were stored at room temperature in resealable plastic bags.

Table 2. Flavour additives and their compositions obtained from Lihel.

Flavour additive	Ingredients	Recommended usage
Base taste (FAbt)	Yeast extract and roast pork flavouring	26 g/kg
Spice mix (FAsm)	Black pepper, garlic, and coriander	8 g/kg
Spice mix + ginger (FAsmg)	Black pepper, garlic, coriander, and ginger	10.5 g/kg
All together (FAall)	Yeast extract, black pepper, garlic, coriander, and ginger	37 g/kg
Lemon peel powder	Lemon peel	5 g/kg
Lovage powder	Lovage	10 g/kg

Two different commercial masking agents were used in the small-scale sensory analyses: masker protein flavour powder (SC1015199) and masker plant protein flavour powder (SC972039). The masking agents were provided by International flavours & fragrances (IFF) (New York, United states).

6.3 Sample production for small scale sensory analyses

The fava bean protein concentrate samples were produced using high moisture extrusion. The amount of flour and water was calculated after subtracting the amount of flavour additives needed. The percentages of flour and water used were 52% and 48% respectively. The extrudates consist of flour, water, and flavour additive. The neutral samples did not contain any flavour additives. The barrel temperatures had to be slowly increased to prevent blockage. The extrusion was always started with the eight barrel temperatures zones set to 100/100/100/100/100/80/70/60 °C respectively. Last four temperature zones in the barrel were changed, while the first four always remained the same. When the extrusion was started a timer was turned on at the same time to observe how long the whole extrusion process took. This time could be used to observe how changing the extrusion parameters affected the product.

The extrusion parameters that were used for sample production were: 140/145/145/135/100/80/70/60 °C barrel temperature zones, 45 °C cooling die temperature, 310 rpm screw speed, 184 mL/h water feed and 0.2 kg/h flour feed. Fava bean protein concentrate extrudate with these parameters is soft, has some fibrils and is slightly dry. The fibrils are a microstructure in extrudates, and resemble the structure of fishbones (Figure 1). Achieving a good structure with fava bean protein concentrate is quite challenging, therefore a stable production that could be repeated was more important. When the extrusion parameters were reached, and the extrusion process was stable the samples were collected. Every sample was produced using the same extrusion parameters. The samples were first put in a resealable plastic bags, which were put in a vacuum bag, which was then vacuum sealed. The samples were stored at -20 °C. The extruder was washed after each sample, so there were not any flavour present from the other samples.

The PPI control samples were produced in a similar manner, but the extrusion parameters and the amount of flour and water were different. The extrusion parameters for the PPI samples were: 120/140/140/125/115/80/70/60 °C barrel temperature zones, 30 °C

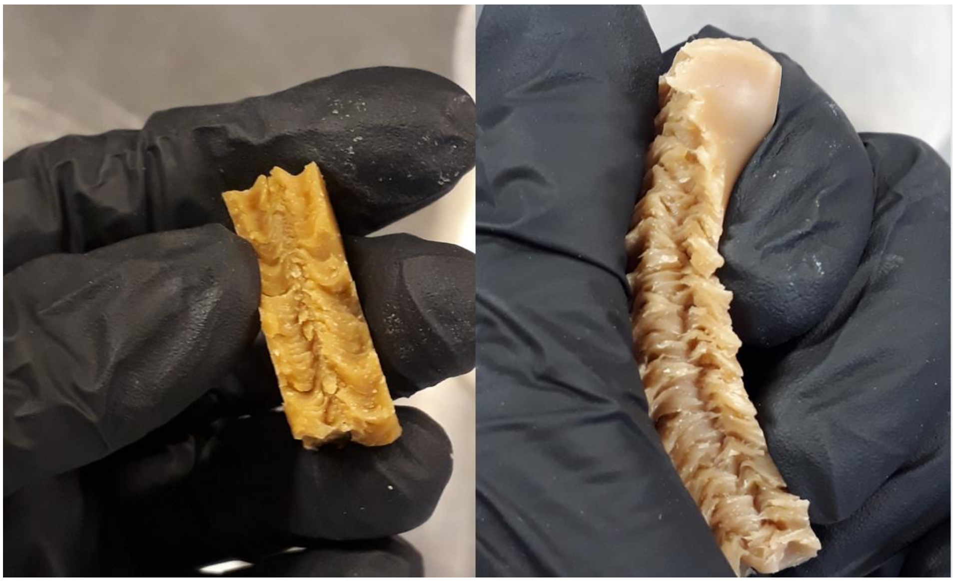

Figure 1. Pictures of fava bean protein concentrate (FBPC) extrudate (left) and pea protein isolate (PPI) extrudate (right) split lengthwise.

cooling die temperature, 300 rpm screw speed, 333 mL/h water feed and 0,3 kg/h flour feed. The PPI extrudate had a gummier texture and more fibrils than the FBPC extrudate (Figure 1). The percentage of flour and water after subtracting the flavour additives were 47% and 53% respectively. The amount of flavour additives in the samples ranged from 0,45% to 3,7%.

6.4 Small-scale sensory analyses

The small-scale sensory analysis panel always consisted of 4 members who all worked for VTT. The panellists received no training before the sensory analysis. Two of the panel members were familiar with the project and attended to every small-scale sensory analysis. The other two panellists changed in every session.

The samples were stored in -20 °C before the sensory evaluation and were taken out of the freezer to thaw at room temperature for 2 hours. After the samples had thawed completely, they were divided into plastic cups with a lid. Two 5cm long strips of sample were put into each plastic cup. The plastic cups were labelled with 3-digit number codes, which were randomly generated using excel and arranged in a different order for each panellist

Figure 2 The booth used for sensory analysis and a tray with the samples.

using a Latin square serving design so that the order of the samples would not affect the evaluation.

The plastic cups with the samples inside were divided into four trays and put into fridge. The samples were warmed in 40 °C oven for 10 minutes and let to cool for 30 minutes in room temperature before the sensory evaluation. Each tray consisted of 10 samples, a two-sided ballot (Appendix 1), a glass of water, notepad, a pencil, and an eraser (figure 2).

The sensory analysis was done in two parts. First the more neutral pea protein isolate control samples were evaluated, followed by a round table discussion about the samples. After that the fava bean protein concentrate samples used for the research were evaluated, followed by a round table discussion. The panellists were advised to first evaluate the odour and appearance by looking and smelling the samples and after that to evaluate the flavour, taste, and texture by tasting the samples. The panellists were asked to smell and drink water between each sample. The panellists were asked to evaluate total intensity of the odour and flavour, the intensity of off-odours and off-flavours, level of spices, texture, and overall liking of the samples. The panellists also had some empty space for open comments. The off-odour intensity and off-flavour intensity was evaluated on a scale from 0 to 10, where a score of 0 was the best. The intensity of flavour was evaluated on a just about right scale of 1-9, where 5 was “just about right”. The overall liking was evaluated on a scale of 0-10, where 10 was the best score. The ballot also contained a question about how much potential each sample had, which had a scale of -3 to 3 and some space for open comments after each sample.

6.5 Sample production for the descriptive sensory analysis

The descriptive sensory analysis was conducted in two parts: with the extrudates and plant-based patties made from the extrudates. The extrudates for the plant-based patties were produced with the same extrusion parameters that were used for the small-scale sensory analysis. The purpose of creating the plant-based patties were to analyse how extrusion affects the sensory properties of flavour additives. Half of the extrudates would be flavoured during extrusion and the other half would be flavoured after the extrusion by mixing the same amount of flavouring in the patty mixture than would be used in the extrudates. The extrudates (table 3) as such were used to analyse how flavour additives change the sensory properties of the extrudates. They were also produced with the same extrusion parameters, expect the water feed was increased to 200 mL/h.

Table 3. Flavourings used in the fava bean protein concentrate extrudates for the descriptive sensory analysis

Sample	Flavour additives
FBPC + NaCl	0.5% NaCl
FBPC, NaCl and masker	0.5% NaCl and 0.45% masker
FBPC, NaCl and base taste	0.5% NaCl and 2.6% base taste
FBPC, NaCl, base taste, spice mix and lemon peel	0.5% NaCl, 2.6% base taste, 0.8% spice mix and 0.125% lemon peel
FBPC, NaCl, base taste, spice mix, lemon peel and masker	0.5% NaCl, 2.6% base taste, 0.8% spice mix, 0.125% lemon peel and 0.45% masker

The plant-based patties were created by mixing frozen extrudates (54.1%) in a blender for one minute to make them break into small pieces. Water (40.5%) was then added and blended until the water was mixed with the extrudates, and then then the wheat gluten (5.4%) was added to the mixture and blended for 30 seconds. If the extrudates were not flavoured before the extrusion the flavour additives would be added with the wheat gluten. A total of eight different plant-based patties were made: four flavoured before extrusion and four after the extrusion (Table 4). 35g of the dough was used for each patty. The patties were shaped in the pan by pressing them lightly into a round patty with a diameter of 5cm. The patties were fried in a pan with rapeseed oil until cooked thoroughly, around 5 minutes flipping the patties halfway. After the patties had cooled, they were put into a resealable plastic bag, which was put into vacuum bag and sealed. The sealed bags were put into a freezer for storage.

Table 4. Flavourings used in the plant-base patties made from fava bean protein concentrate extrudates for the descriptive sensory analysis

Sample	Flavour additives
Base taste, spice mix and lemon peel	2.6% base taste, 0.8% spice mix and 0.125% lemon peel
base taste, spice mix, lemon peel and masker	2.6% base taste, 0.8% spice mix lemon 0.125% and 0.45% masker
NaCl, base taste, spice mix and lemon peel	0.5% NaCl, 2.6% base taste, 0.8 spice mix and 0.125% lemon peel
NaCl, base taste, spice mix, lemon peel and masker	0.5% NaCl, 2.6% base taste, 0.8 spice mix, 0.125% lemon peel and 0.45% masker

6.6 Lexicon creation for the descriptive sensory analysis

The panel training was started by creating a lexicon for the samples. To help with the lexicon creation some attributes that were commonly used in the previous sensory analyses were made into a list that was shared with the four panellists that attended the lexicon creation. The attributes shared with the panellists for flavours were beany, bitter, astringent, salty, umami, peppery/spicy, and meaty. For odour the attributes shared were beany, pungent, pork, meaty and herby. Also, references (Table 5) were used to help in identifying the attributes from the samples by smelling and tasting them.

Table 5. References used in the panel training

Reference and coding	**Ingredients**
Salty (S)	0.5% NaCl water mixture
Bitter (K)	0.05% caffeine water mixture
Beany (H)	2.5% fava bean protein concentrate flour water mixture
Umami (Y)	1.5% base taste flavour additive water mixture
Spice mix (M)	0.4% spice mix flavour additive and 0.06% lemon peel flavour additive water mixture

The lexicon creation for the extrudates was started with the odour and appearance. The panellists were asked to look at and smell one sample, and after that a round table discussion took place. During the round table discussion all the attributes were written down. The same procedure was done with every sample. After every sample's odour and appearance attributes had been written down a new round table discussion took place where the attributes that every panellist could agree on would be chosen for the training of the panellists. The lexicon creation for taste and flavour, and texture was done the same way, expect the panellists were asked to taste the samples instead of smelling them. Also, during the training the panellists smelled and tasted the references and could comment if they wanted more references, the intensity of a reference changed or to remove a reference. The lexicon creation for the plant-based patties was done in a similar manner, expect the appearance and texture was no longer evaluated, because from the results of the lexicon creation for the extrudates no attributes that would discriminate the samples from each other was found in appearance or texture. On the basis of the panellists' feedback during the lexicon creation, Reference M was made milder by reducing the amount of spice mix and lemon peel flavour additive to half, and Reference H was made stronger by doubling the amount of fava bean protein concentrate (table 5).

6.7 Panel training for the descriptive sensory analysis

Each panellist attended one training session for the extrudates and one training session for the plant-based patties before the descriptive sensory analysis. The training sessions for the extrudates and plant-based patties were held the same way. During the training the panellists went through the odour attributes first and then the taste and flavour attributes. A ballot was created from the results of the lexicon creation (Appendix 3) and the training went in the same order as the attributes are in the ballot. For each attribute the panellists were asked to first smell or taste the reference and then taste a sample that most likely has that attribute according to the lexicon creation. The panellists were asked to evaluate the reference's and the sample's attributes intensity on a 0-10 scale. It was important that the reference's intensity was on a level that the sample's attribute is represented realistically, for example if the sample had almost no bitterness and the reference sample would have been rated very low on the same attribute, there would not have been enough room to evaluate the attribute's intensity as low as it really was. The main goal of the training was to have the panellists agree on the intensity of the references, teach them to compare the sample's intensity to the reference's intensity, and teach them identify the attributes found in the lexicon creation. During the sensory evaluation the intensity of the reference was marked on a 0-10 line scale to the point that the panellists agreed on. The panellists were asked after each attribute to give their opinion on whether the instructions were written clearly enough and whether they thought that the attribute should be in the final descriptive sensory analysis ballot.

Because of the current restrictions the training had to be done in two parts for both the extrudates and plant-based patties with only four panellists present at a time. Agreeing on the intensities of the references with two separate groups was difficult, because the first group might have rated a reference sample much higher or lower than the second group. After the second group had tasted a reference for the first time, they were told how intense the first group rated it, and whether they agreed with the score the first group gave the reference. If there was a huge difference between the two groups, the second group was asked to try the reference again and asked if they still thought that their original opinion was valid or whether they would be willing to agree with the first group. If the two groups still were disagreeing, a compromise had to be made. Both groups agreed that reference K was not useful in the evaluation, because some people taste bitterness naturally much more sensitively than others, which meant that the panellists could not agree on the intensity of the reference. The intensities of the references were the same for both the extrudates and the plant-based patties because the same amount of flavour additives was used in both. This way the results could be compared.

During the training the ballot for the extrudates and plant-based patties were also modified (Appendix 3). The panellists could not find roast-like odour from the extrudates, therefore it was removed from the ballot. Also, meaty odour resembled meat broth the most in both the extrudates and plant-based patties, which was the final name for the attribute that the panellists would have to evaluate in the sensory analysis. The bitter taste in plant-base patties was split into two attributes: bitterness intensity and bitterness timing, which meant how intense the bitterness is and how long did it take for the bitterness to be at its most intense respectively.

6.8 Descriptive sensory analysis

The samples were served in plastic cups with random 3-digit code numbers. The sample size for the extrudates was a 5cm long strip of extrudate and for the plant-based patties it was a ¼ piece of plant-based patty that was 5cm in diameter. The sensory evaluation booths contained a computer, glass of water, notepad, pencil, an eraser, the sensory evaluation ballot and a tray with samples, references, a cream cracker for palate cleansing, a plastic fork, and a piece of paper towel (Figure 3). All but the reference S was covered with a lid to help them retain their odour. The panellists had to evaluate four different odour attributes (overall odour intensity, beany odour, spiciness, and meat broth), which were the same for both the plant-based patties and the extrudates. The extrudates had five flavour attributes (beany flavour, bitterness, saltiness, spiciness, and umami), that the panellists had to evaluate. The same attributes were used for the plant-based patties, expect the plant-based patties had one extra attribute that the panellists had to evaluate, which was bitterness timing. The panellists were asked to evaluate the odour attributes first and then flavour and taste attributes. They were also asked to smell and drink water between samples. The bitesize for the samples was around 1/5th of the sample's size.

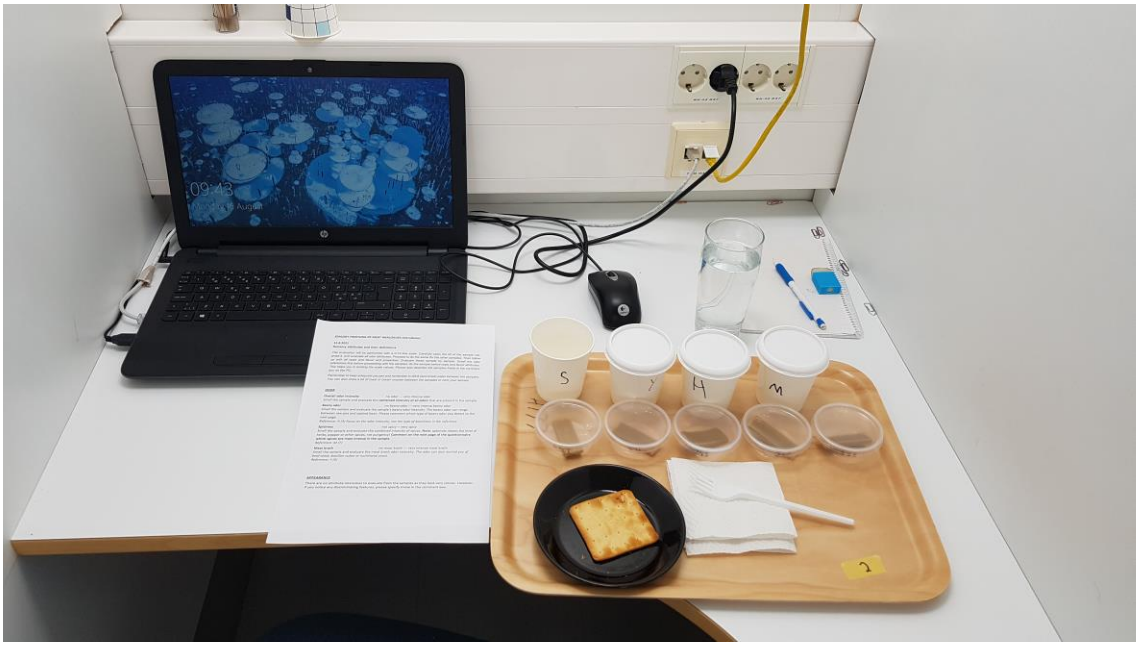

Figure 3 Sensory analysis booth with extrudate samples and references on a serving tray with a cream cracker as a palate cleanser.

The descriptive sensory analysis was done on a computer with Compusense Five Version 5.6 (Compusense Inc., Guelph, Ontario Canada) sensory analysis software. The trays had a number from 1 to 8, which the panellists had to enter to the software, which gave them the order to evaluate the samples in. The order of the extrudates (Table 3) was randomized by the Compusense software using Latin square serving design, which meant that every sample appeared in the same spot equal amount of times. There was a total of eight different plant-based patties (table 4), which was too many for one sensory analysis session. An incomplete block design was used, which meant that every panellist got six samples out of eight to evaluate. This meant that each sample got evaluated six times, instead of eight. A replicated complete block design descriptive sensory analysis with two sessions was done with the extrudates, but not with the plant-based patties.

6.9 Statistical analysis of sensory evaluation

The average and standard deviation was calculated from the results to observe the scores from the whole group and to see how spread out the scores were. IMB SPSS Statistic Version 26 (Armonk, New York, United States) was used for two-way mixed model ANOVA with samples as fixed factor and assessors as random factor and for Tukey’s honest significant difference test. The panel performance was checked with Panelcheck 1.4.2 software, following the proposed workflow. CAMO Software AS Unscrambler X Version 10.5.1 (Oslo, Norway) was used for principle component analysis.

7 Results and discussion

7.1 First small-scale sensory analysis

The purpose of the small-scale sensory analysis was to determine how well-liked each flavouring was, were the flavourings able to mask the off-odours and off-flavours of the meat analogues, the intensity of each flavouring, and how much potential for further development each flavouring had. Total of four different natural flavouring mixtures were used.

The fava bean protein concentrate used for the meat analogues has a bitter, beany and astringent flavour, therefore a more neutral tasting pea protein isolate control sample was used for each flavouring (table 6). The control sample was important for determining how big of a difference in flavour and odour there was between the more neutral pea protein isolate control sample and the strong-tasting fava bean protein concentrate when seasoned.

Table 6. Samples produced for the small-scale sensory analysis

Fava bean protein concentrate	Pea protein isolate	Flavourings used g/kg
Neutral	Neutral	-
Base taste	Base taste	26 g/kg
Spice mix	Spice mix	8 g/kg
Spice mix + ginger	Spice mix + ginger	10.5 g/kg
All together	All together	37 g/kg

The advantage of small-scale sensory analysis was that it was quick to setup, the panellists did not need any training and it was possible to determine whether we should continue using the same flavour additives, whether the amounts used should be changed, or whether the formulation should be completely changed. The downside of a small panel size was that the answers could vary considerably and not be accurate or might represent only a small group's opinion. In this case 2 of the panellists were working on the project and it was possible to discuss the results and make quick changes accordingly.

7.1.1 Results

The results of the small-scale sensory panel provided important data on how to continue with the research. The natural bitter, astringent and beany flavour of fava bean was not masked by the flavour additives, the control sample was more well-liked in every category, because of its more neutral taste and the intensity of some of the flavour additives was too high.

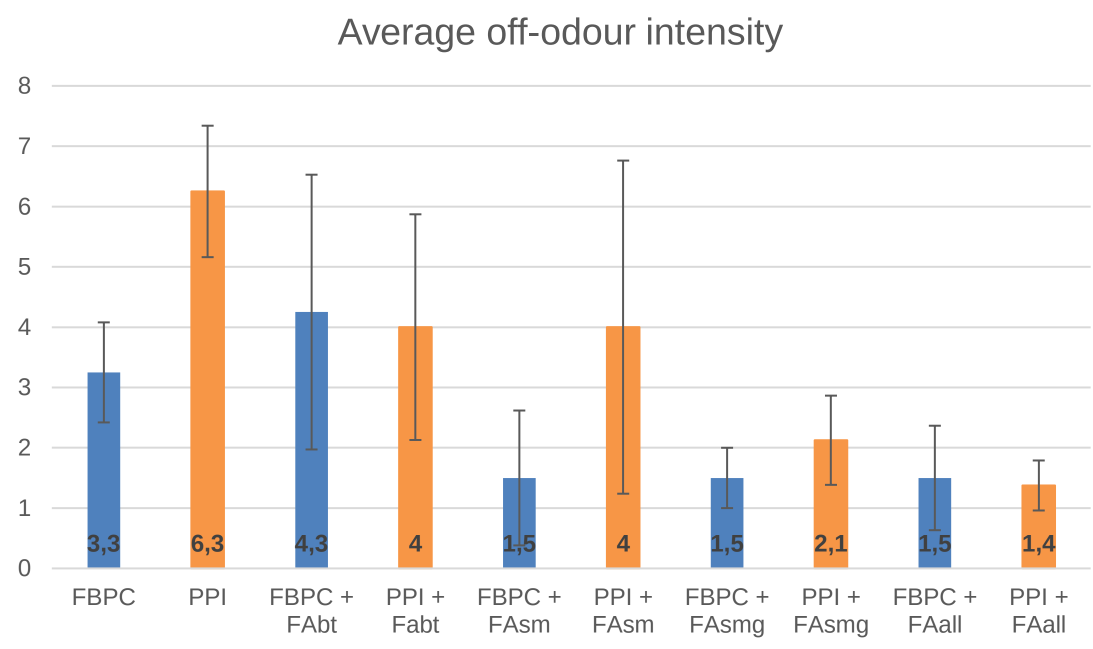

Figure 4 Average off-odour intensity of fava bean protein concentrate (FBPC) and pea protein isolate (PPI) extrudates with standard deviations (n=4). The full names of the flavour additives and what they are made of can be found from table 2.

The off-odour of neutral pea protein isolate sample was more intense. The fava bean protein concentrate has a more neutral odour, which can be seen from the results. The base taste alone might intensify the unpleasant odours of FBPC, while masking some of the PPI's off-odours and making the overall odour more pleasant, because of the base taste's mild meaty odour. The samples with spice mixture lowered the off-odour intensity of all the FBPC samples. The PPI samples that had spice mixture with ginger in them also got lower scores. The smell of ginger might be covering the unpleasant odours of PPI. It seems that the samples flavoured with all together -flavouring mixture had the lowest off-odour intensity on both the PPI and the FBPC (Figure 4). Open comments indicated that the FBPC samples had a meat broth -like odour in every sample, even on the ones that had no base taste -flavouring. The FBPC extrudates might have a natural odour that resembles meat broth.

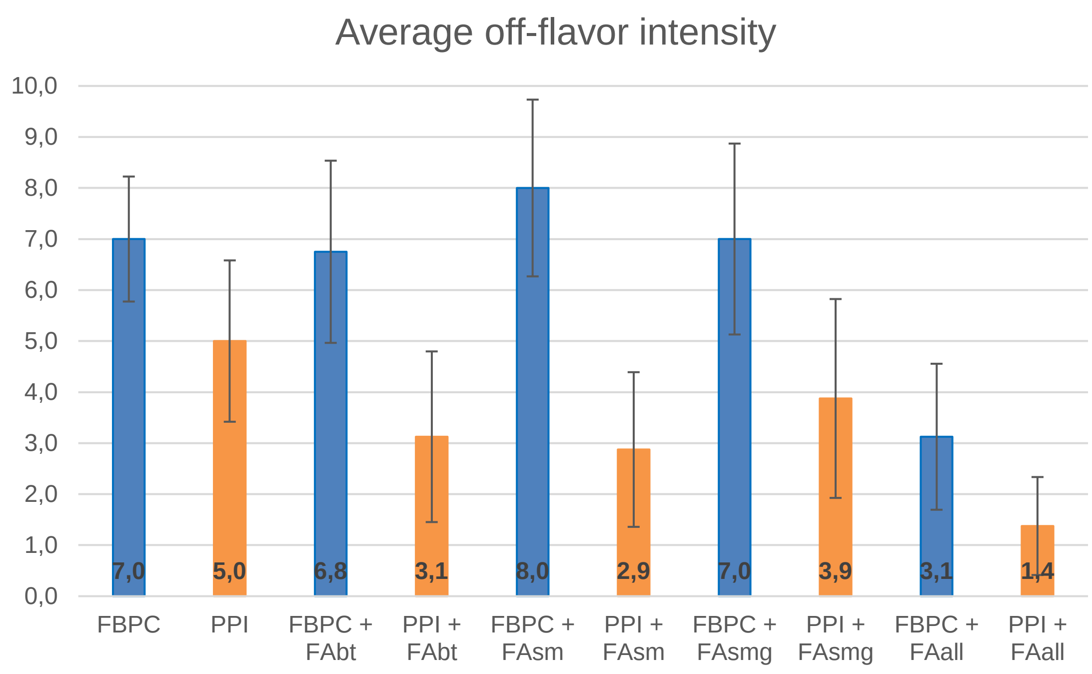

Figure 5 Average off-flavour intensity of fava bean protein concentrate (FBPC) and pea protein isolate (PPI) extrudates with standard deviations (n=4).

The average off-flavour was more intense in every FBPC sample compared to that of PPI regardless of the flavour additive (Figure 5). The FBPC has a very strong taste and it can be hard to mask. Some of the flavourings might have intensified some of the unpleasant flavours of the FBPC samples. Even if the panellists were not trained before the sensory analysis, it could also be that the overall flavour intensity was higher and the taste of the samples were unpleasant. Every FBPC sample was described as bitter and beany with astringent characteristics. Also, the level of spices was too intense in the samples that had the spice mixture. The more neutral tasting PPI benefited much more from the flavour additives. According to the comments of the panellists each PPI sample had an umami taste regardless of the flavour additive, which seems to be a characteristic taste of a PPI extrudate. Both samples had the best result with the all together -flavour additive (Figure 5), just like with the off-odour intensity (Figure 3).

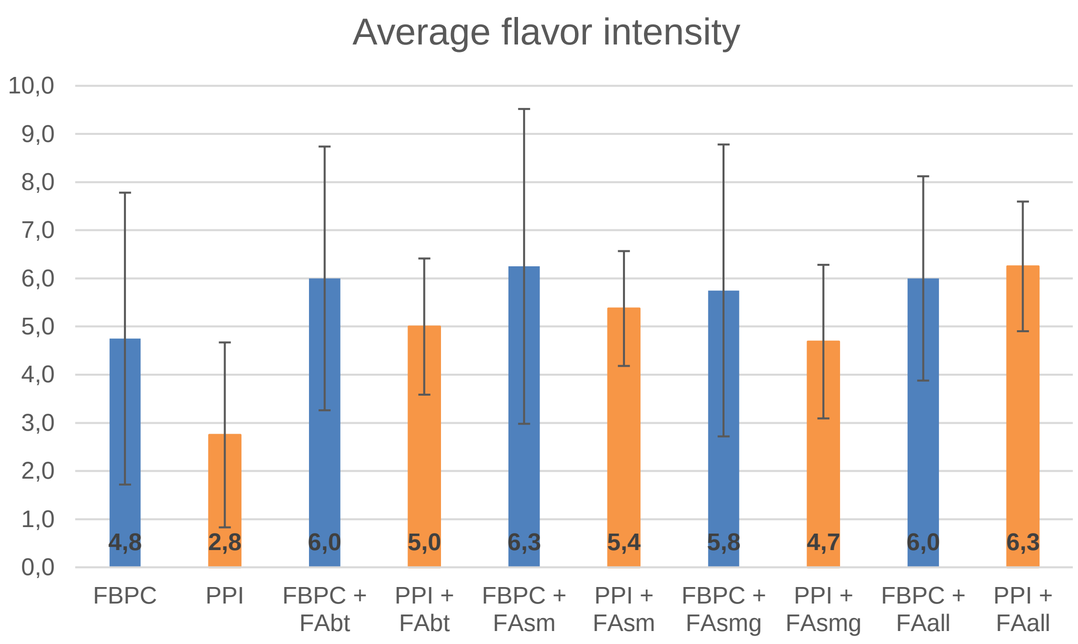

Figure 6 Average flavour intensity of fava bean protein concentrate (FBPC) and pea protein isolate (PPI) extrudates on a just about right scale with standard deviations. The target score is 5 (n=4)

The average flavour intensity of flavour additives was close to just about right with both the FBPC and PPI. The flavour intensity of seasoned FBPC samples was slightly too intense. The panellists thought that the flavour intensity of ginger and pepper was too high, and the overall taste was too spicy in the samples that had the spice mixture in them. The flavoured PPI samples were less intense, expect for the sample flavoured with all together flavouring which had a slightly more intense flavour than the similar FBPC sample. The PPI sample seasoned with all together -flavouring had a too meaty taste and the taste of ginger was too intense according to the panellists. Also, the taste of ginger was too intense on the PPI sample with the spice mixture with ginger according to the panellists (Figure 6).

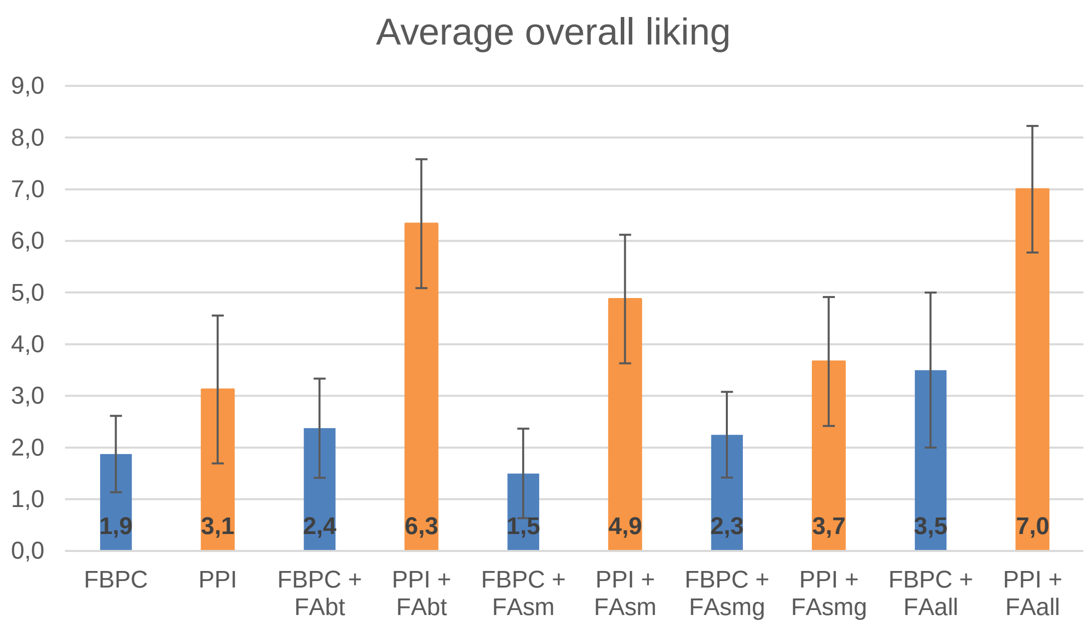

Figure 7 Average overall liking of fava bean protein concentrate (FBPC) and pea protein isolate (PPI) extrudates with standard deviation. The overall liking was evaluated on 1-9 scale (n=4).

The sensory result show that the PPI benefits from the flavour additives and was more well-liked than the FBPC samples. The neutral taste of PPI is easier to mask and the flavour additives are not only covering the off-flavours but enhancing the overall flavour. The FBPC samples were not very well-liked and received low scores from the sensory panel. The flavour additives improved liking in all but one FBPC sample, which had the spice mix with ginger. According to the results the flavour additives were not able to mask the characteristic beany, bitter and astringent off-flavours of fava bean. The PPI samples received higher scores than the FBPC samples in liking with every flavour additive. The most well-liked sample for both the FBPC and PPI was the all together -flavour additive, which shows that the base taste with a spice mixture is the appropriate way to continue (Figure 7).

7.1.2 Conclusions from the first small-scale sensory analysis

The amount of flavour additives used for seasoning the extrudates was close to just about right. The flavour of ginger and pepper was perceived to be too intense in the samples that had either the spice mixture or spice mixture with ginger. The main issue with the FBPC extrudates is the strong off-flavour, which affects the overall liking. The all together -flavour additive seems to be decreasing the intensity of off-flavours and increasing the overall liking. Flavouring the extrudates to increase overall liking does not seem to be the issue, because the overall liking of the more neutral tasting PPI samples was much higher. The off flavours need to be masked to increase the overall liking of the FBPC samples.

The results of small-scale sensory panel clearly showed that it was the right approach at this stage of the project. Similar small-scale sensory analysis can be done with new flavour additives or modified formulations and repeated if necessary. The second phase of the research takes much more preparation and time, so it was important that the samples were at an acceptable level before continuing.

7.2 Second small-scale sensory analysis

From the results of the first small-scale sensory analysis it was decided that the recipe was not ready yet and further experimentation was needed. The spice mix + ginger spice mixture had too intense taste, due to the ginger therefore it was no longer going to be used in the future samples. Lemon peel and lovage flavourings was received from Lihel, which might help with masking the unpleasant aftertaste and off-odours and replace the ginger. A masker (masker plant protein flavour powder SC972039) was also received from IFF, that was created especially for masking fava beans unpleasant aftertaste. The purpose of the second small scale sensory analysis was to study how well the masker works and whether the new flavourings improve the sensory properties of the samples.

The sensory evaluation ballot also needed some fine tuning. After analysing the results of the first small-scale sensory analysis we realized some questions were unnecessary and we were not getting all the information we needed with the current questions. The original ballot (Appendix 1) had a question about flavour intensity, which meant the flavour intensity of the whole sample. The question was changed to total flavour intensity and a new question “level of spices” was added. Also, “total odour intensity” question was added to the ballot. Potential for further development was removed from the ballot because overall liking was a good indicator whether the panellists thought the sample had potential or not, according to the results of the first sensory evaluation. Question about texture was changed so that the panellists only had to comment if a sample had a texture that was different from the other samples (Appendix 2). The sensory analysis was held in two parts on the same day, because there were too many samples for one session. The first session was held in the morning (Table 7) and the second session was held in the afternoon (Table 8). The panellists were the same for both sessions. The way that the sensory analysis was conducted, the amount of each sample was served and how the samples were prepared remained the same.

Table 7. First session's fava bean protein concentrate and pea protein isolate samples and the amount of flavouring used for each sample

FBPC	PPI	Flavourings used g/kg
Neutral	Neutral	None
Masker	Masker	4.5g of masker
Masker and base taste	Masker and base taste	4.5g of masker and 26g of base taste
Masker and spice mix	Masker and spice mix	4.5g of masker and 8g of spice mix
Masker and all together	Masker and all together	4.5g of masker and 37g of all together

Table 8. Second session's fava bean protein concentrate, and pea protein isolate samples and the amount of flavouring used for each sample

FBPC	PPI	Flavourings used g/kg
Spice mix, lovage and lemon peel	Spice mix, lovage and lemon peel	8g of spice mix, 5g of lovage and 2.5g of lemon peel
Spice mix, lovage, lemon peel and masker	Spice mix, lovage, lemon peel and masker	8g of spice mix, 5g of lovage, 2.5g of lemon peel and 4.5g of masker
Base taste, spice mix, lovage and lemon	Base taste, spice mix, lovage and lemon	26g of base taste, 8g of spice mix, 5g of lovage and 2.5g of lemon peel
Base taste, spice mix, lovage, lemon and masker	Base taste, spice mix, lovage, lemon and masker	26g of base taste, 8g of spice mix, 5g of lovage, 2.5g of lemon peel and 4.5g of masker

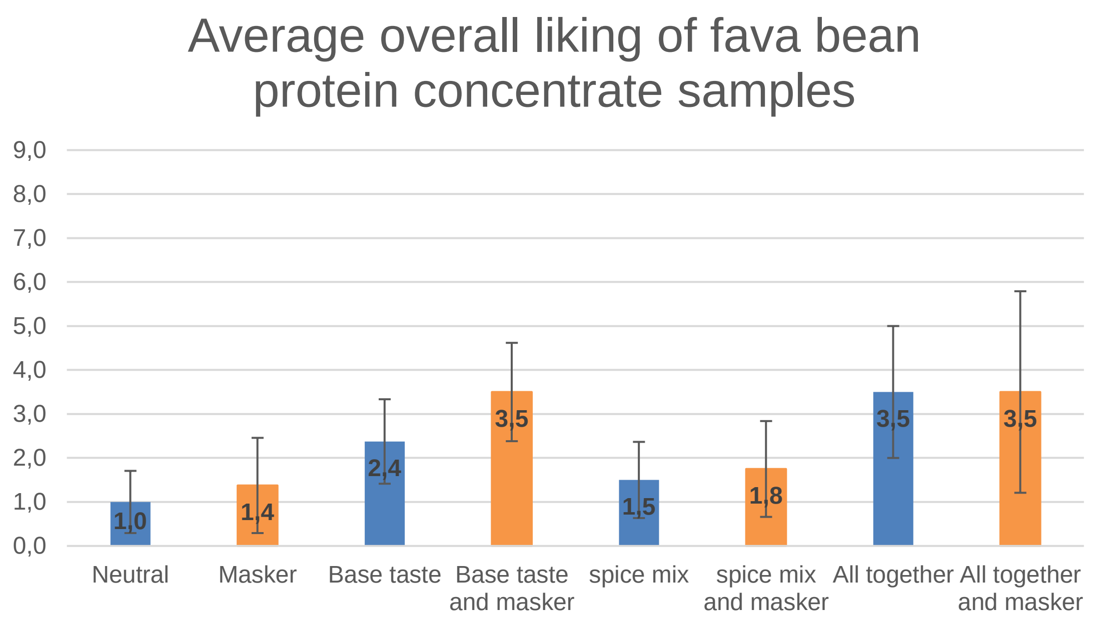

Figure 8. Average overall liking of fava bean protein concentrate extrudate (FBPC) with standard deviation between the same FBPC samples without (blue) and with masker (orange) (n=4).

The average overall liking of the base taste, spice mix and all together samples from the first small-scale sensory analysis (Figure 7) can be compared to the same samples with masker (Figure 8). The overall liking improved slightly when the samples had the masker included in the recipe. Only the samples with all together flavour additive had no change in overall liking whether the masker was included in the recipe or not.

Table 9. Average scores and standard deviations (in brackets) of fava bean protein concentrate samples with new flavour additives (n=4).

Sample (FBPC)	Off-odour intensity (0 - 10)	Off-flavour intensity (0 - 10)	Total odour intensity (0-10)	Total Flavour intensity (0-10)	Level of spices (1-9)	Overall liking (1 - 9)
Spice mix, lovage and lemon	1.7 (0.9)	5.7 (1.2)	3.8 (0.4)	5.8 (0.8)	4.3 (2.4)	1.5 (0.5)
Spice mix, lovage, lemon and masker	1.7 (0.9)	6.5 (0.7)	4,0 (1.2)	5.8 (0.4)	3.0 (1.2)	1.5 (0.5)
Spice mix, Base taste, lovage and lemon	1.9 (0.7)	6.0 (2.2)	5.6 (0.6)	7.3 (1.5)	4.0 (2.2)	3.4 (0.4)
Spice mix, base taste, lovage, lemon and masker	2.0 (0.8)	5.3 (0.9)	4.9 (1.4)	7.5 (0.9)	4.0 (1.9)	3.8 (0.4)

Lovage and lemon had little effect on the overall liking of the samples. Just like with the first small-scale sensory analysis, the samples with base taste flavour additive were the most well liked. The sample with spice mix, lovage and lemon with or without masker got an overall liking score of 1.5. The samples with spice mix, lovage, lemon and base taste got an overall liking score of 3.4 and with masker 3.8 (Table 9). It seems that the more heavily the samples are flavoured, the better the overall liking is with FBPC samples. The masker had little effect on the off flavour and off odour of the FBPC samples.

Table 10. Average scores and standard deviations (in brackets) of pea protein isolate samples with new flavour additives (n=4)

Sample (PPI)	Off-odour intensity (0 - 10)	Off-flavour intensity (0 - 10)	Total odour intensity (0-10)	Total Flavour intensity (0-10)	Level of spices (1-9)	Overall liking (1 - 9)
PPI neutral	4.5 (2.9)	3.0 (3.0)	4.8 (2.5)	1.6 (1.3)	1.1 (0.5)	4.0 (1.2)
PPI and masker	3.4 (1.6)	2.0 (1.2)	4.3 (1.5)	3.1 (0.5)	1.4 (0.6)	3.5 (1.1)
Spice mix, lovage and lemon	2.3 (2.1)	1.7 (1.2)	4.8 (0.4)	4.3 (1.3)	4.8 (1.9)	4.7 (1.1)
Spice mix, lovage, lemon and masker	0.7 (0.5)	1.5 (1.5)	7.0 (1.2)	5.3 (2.9)	6.3 (1.8)	4.8 (1.6)
Spice mix, Base taste, lovage and lemon	1.0 (0.8)	1.3 (0.9)	5.5 (1.8)	6.5 (0.5)	6.0 (0.7)	7.3 (1.1)
Spice mix, base taste, lovage, lemon and masker	1.0 (0.8)	0.7 (0.5)	6.0 (1.2)	7.3 (1.3)	6.0 (1.7)	7.9 (0.6)

The PPI control samples with the masker were slightly more well liked than the samples without the masker. Only the neutral PPI sample was evaluated to be better without the masker. Also, the masker lowered the off odour and off flavour of the PPI samples. Only one sample's off odour remained the same with and without masker (Table 10). The masker was originally made for masking fava bean protein concentrate's unpleasant flavours, but it was also improving the sensory properties of most PPI samples. The PPI most likely has same compounds that the masker was designed to cover, which are also found in fava bean and other pulses.

Since the results confirmed that the masker had a slight positive effect on the sensory properties of both the FBPC and PPI samples, it should be included in the future samples. Also, during the round table discussion the FBPC samples with lovage were said to have a "soapy" taste, which is why the lovage flavouring would not be used in the future samples. The lemon peel flavouring was slightly too strong, but the taste was pleasant, therefore the amount of lemon peel flavouring used in the future would be lowered to half.

7.3 Third small-scale sensory analysis

The aim of the third small-scale sensory analysis was to see how inclusion of salt affects the sensory properties of FBPC extrudates. Also, a new masker (masker protein flavour powder SC1015199) was going to be compared to the masker that was used in previous small-scale sensory analysis (masker plant protein flavour powder SC972039). During this sensory analysis PPI control samples were not used, because from the results of the two previous sensory analysis it could be determined that the effects of the flavour additives were going to be quite similar between the FBPC and PPI samples. The sensory analysis, how the samples were prepared, the amount of flavour additives used remained the same, and the same amount of both maskers were used (Table 11). 0.5% of NaCl by weight was used in half of the samples.

Table 11. Average scores and standard deviations (in brackets) of third small-scale sensory analysis of fava bean protein concentrate extrudates (n=4).

Sample	Off-odour intensity (0 - 10)	Off-flavour intensity (0 - 10)	Total odour intensity (0-10)	Total Flavour intensity (0-10)	Level of spices (1-9)	Overall liking (1 - 9)
FBPC neutral	3.9 (2.0)	5.5 (1.5)	4.5 (0.9)	5.3 (1.1)	1.5 (0.9)	3.3 (1.3)
FBPC and 0,5% NaCl	4.8 (1.1)	5.5 (1.1)	5.5 (1.1)	5.8 (0.8)	2.8 (1.9)	3.5 (0.9)
FBPC and SC972089 masker	3.8 (0.8)	5.8 (2.8)	4.5 (1.1)	6.3 (1.8)	1.3 (0.4)	2.5 (1.1)
FBPC, SC972089 masker and 0,5% NaCl	3.9 (1.2)	5.8 (1.9)	4.8 (1.5)	6.5 (1.8)	1.8 (0.8)	3.0 (0.7)
FBPC and SC1015199 masker	3.5 (1.1)	6.6 (1.1)	4.5 (0.5)	5.5 (1.7)	1.0 (0.0)	1.8 (0.4)
FBPC, SC1015199 masker and 0,5% NaCl	2.5 (1.5)	6.3 (1.3)	4.8 (1.3)	5.8 (1.3)	2.0 (1.0)	1.8 (0.8)
FBPC and base taste	1.5 (1.5)	5.5 (1.1)	4.8 (1.9)	5.5 (2.1)	4.3 (1.9)	3.8 (1.8)
FBPC, base taste and 0,5% NaCl	3.0 (1.9)	4.8 (1.1)	5.6 (1.4)	5.5 (1.1)	4.5 (2.2)	4.3 (1.3)

The addition of 0.5% NaCl in the sample increased overall liking in almost every sample and the off-flavour intensity was lower or the same, when compared to a similar sample without NaCl. Many plant-based protein products are high in NaCl and the trend has been to reduce the amount of NaCl in food products, because of the potential negative health effects. However, NaCl is cost effective and it had a positive effect on the sensory properties of the samples. The new masker received lower overall liking score and higher off-flavour score than the old masker. According to the results NaCl should be used in the samples along with the masker plant protein flavour powder (SC972039).

7.4 Descriptive sensory analysis of plant-based patties

The aim of the descriptive sensory analysis of plant-based patties was to study how extrusion affects the sensory properties of flavour additives. It also gave an opportunity to study whether the masker would mask the unpleasant beany and bitter sensory properties better after mixing it with the extrudates after extrusion. Because the plant-based patties were fried in oil it produced some extra flavours in the samples which might have had some effect on the sensory analysis. The plant-based patties had a roasted and slightly oily taste, which were not found on the extrudates.

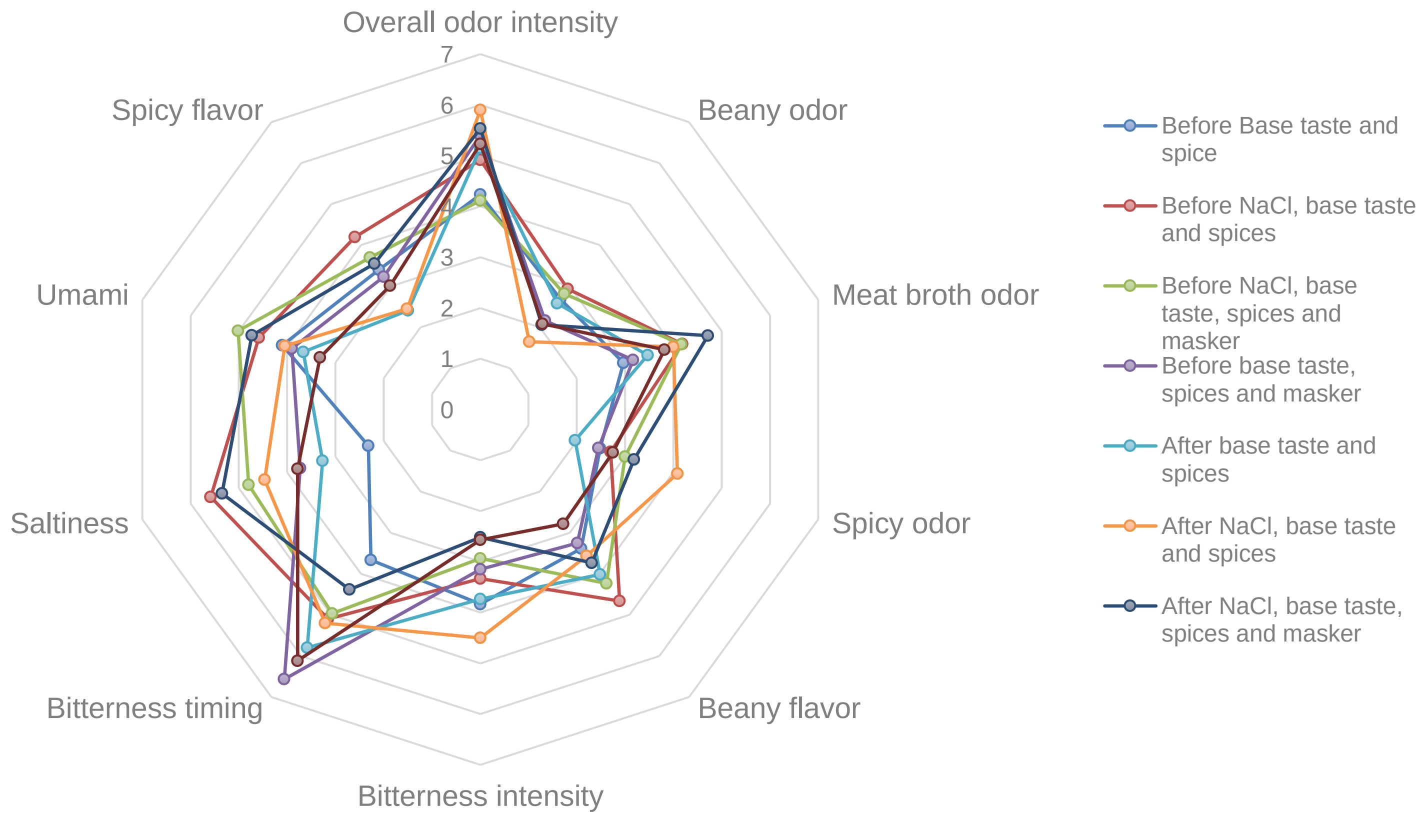

Figure 9 Spider plot made from the results of the descriptive sensory analysis of plant-based patties made from fava bean protein concentrate extrudates. The samples were evaluated on a 0-10 line scale, but the scale is zoomed to 0-7 to help in identifying the differences. Half of the samples were flavoured before extrusion (before) and half were flavoured after extrusion with the same flavouring (after). The samples were evaluated by 8 panellists who each evaluated 6 samples.

The results of the descriptive sensory analysis were not statistically significant. The p-values ranged from 0.087 to 0.92 depending on the attribute. The saltiness attribute had the lowest p-value and the beany odour had the highest, which was to be expected because saltiness was a quite easy attribute to evaluate and the beany odour one of the hardest, because the samples were flavoured. The samples (Table 3) were flavoured similarly. Every sample contained at least spice mix and base taste flavour additives, which gave the samples a similar flavour profile (Figure 9). Because the flavour profiles were so similar, the panellists had difficulties discriminating attributes between samples, which led to higher dispersion of the scores. The panellists were not able to discriminate whether the samples were flavoured before or after the extrusion. There were no systematic differences between the samples whether they were flavoured before or after extrusion (Table 12 and 13). The samples should have been flavoured more differently from each other so the panellists could have discriminated the samples from each other easier. According to the results extrusion does not affect the sensory properties of flavour additives. Also, the samples with the masker had no systematic difference when compared to the samples without masker. It would have been beneficial to have samples that would have had only the masker, to be able to study how well the masker works without any flavour additives present in the sample.

Table 12. Average results of odour attributes of descriptive sensory analysis of plant-based patties made from fava bean protein concentrate extrudates. Results not statistically significant. The samples were evaluated by 8 panellists who each evaluated 6 samples.

Sample	Flavoured before or after extrusion	Overall odour intensity	Beany odour	Meat broth odour	Spicy odour
Base taste and spice	Before	4.2	2.7	3.0	2.5
NaCl, base taste and spices	Before	4.9	2.9	4.2	2.7
NaCl, base taste, spices, and masker	Before	4.1	2.8	4.2	3.0
Base taste, spices, and masker	Before	5.4	2.2	3.2	2.5
Base taste and spices	After	5.2	2.6	3.5	2.0
NaCl, base taste and spices	After	5.9	1.7	4.0	4.1
NaCl, base taste, spices, and masker	After	5.5	2.1	4.7	3.2
Base taste, spices, and masker	After	5.2	2.1	3.8	2.8

Table 13. Average results of flavour attributes of descriptive sensory analysis of plant-based patties made from fava bean protein concentrate extrudates. Results not statistically significant. The samples were evaluated by 8 panellists who each evaluated 6 samples.

Sample	Flavoured before or after extrusion	Beany flavour	Bitterness intensity	Bitterness timing	Saltiness	Umami	Spicy flavour
Base taste and spice	Before	3.4	3.8	3.7	2.3	4.1	3.4
NaCl, base taste and spices	Before	4.7	3.3	5.1	5.6	4.6	4.2
NaCl, base taste, spices, and masker	Before	4.2	2.9	5.0	4.8	5.0	3.7
Base taste, spices, and masker	Before	3.3	3.2	6.6	3.7	3.9	3.2
Base taste and spices	After	4.0	3.7	5.8	3.3	3.7	2.4
NaCl, base taste and spices	After	3.6	4.5	5.2	4.5	4.1	2.5
NaCl, base taste, spices, and masker	After	3.7	2.5	4.4	5.4	4.7	3.6
Base taste, spices, and masker	After	2.8	2.6	6.1	3.8	3.3	3.0

7.5 Descriptive sensory analysis of extrudates

The results of the descriptive sensory analysis were statistically significant (Table 13). The panellists as a group could discriminate the attributes between samples well with two repetitions. With some attributes there were some panellists that did not agree with the group, but overall, the panellists were quite unanimous. The flavouring in the extrudates ranged from neutral to samples flavoured with spices and base taste flavour additive, which made the sensory evaluation much easier when compared to the sensory analysis of the plant-based patties.

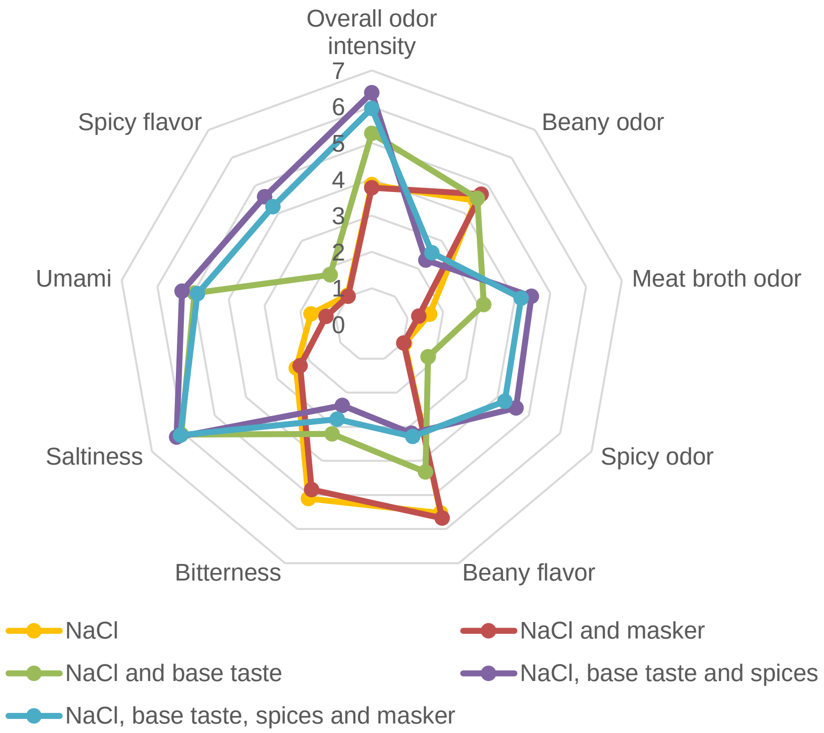

Figure 10 Spider plot of the fava bean protein concentrate extrudates descriptive sensory analysis results. The attributes were evaluated on a 0-10 line scale. The scale is zoomed to 0-7 to identify differences between samples better (n=8*2).

The extrudates could be split roughly into three groups according to the results of the descriptive sensory analysis (Figure 10). The samples with only NaCl and NaCl and masker would be one group. Both samples were higher in bitterness and beany flavour than the other three samples. The sample with NaCl and base taste flavour additive had similarities with both groups, but still had some differences to be put it into its own group. The sample with NaCl, base taste, and spices and the sample with NaCl, base taste, spices, and masker are very similar to each other and would be one group. The groups could be seen from post hoc analysis results (Table 14). The attributes of each sample were split into one or two groups according to the results. The groups can be seen more accurately with the post hoc analysis, while the spider plot gives a good overall look on the attributes of the samples.

Table 14. Picture of the fava bean protein concentrate extrudates sensory analysis results with two-way ANOVA, averages, standard deviations and post hoc analysis (Tukey's HSD) results (n=8*2).

		NaCl			NaCl and masker			NaCl and base taste			NaCl, base taste and spices			NaCl, base taste, spices, and masker		
Attribute	ANOVA p	Average	stdev	post hoc	Average	stdev	post hoc	Average	stdev	post hoc	Average	stdev	post hoc	Average	stdev	post hoc
Overall odour intensity	<0,001	3.9	2.1	c	3.8	1.6	c	5.3	1.5	b	6.4	1.0	a	6.0	1.4	ab
Beany odour	<0,001	4.5	2.1	a	4.7	1.5	a	4.5	1.7	a	2.3	1.8	b	2.6	1.7	b
Meat broth odour	<0,001	1.6	1.7	c	1.3	1.1	c	3.1	1.6	b	4.5	1.1	a	4.2	1.1	ab
Spicy odour	<0,001	1.0	1.3	b	1.0	1.2	b	1.8	1.7	b	4.6	1.7	a	4.2	2.0	a
Beany flavour	<0,001	5.5	1.3	a	5.7	1.3	a	4.3	1.7	b	3.2	1.8	b	3.3	1.7	b
Bitterness	<0,001	5.1	1.6	a	4.8	1.7	a	3.2	2.3	b	2.4	1.8	b	2.8	1.9	b
Saltiness	<0,001	2.4	1.9	b	2.3	1.8	b	6.1	1.6	a	6.2	1.1	a	6.1	1.5	a
Umami	<0,001	1.7	1.2	b	1.3	1.2	b	5.0	1.5	a	5.3	0.9	a	4.9	1.7	a
Spicy flavour	<0,001	1.3	1.8	c	1.1	1.6	c	2.5	2.3	b	5.5	1.3	a	5.4	1.3	a

According to the results the base taste, which consists of yeast extract and vegan roast pork flavouring is the most important flavour additive. It was also the most important flavour additive in the more neutral tasting pea protein isolate samples during the small-scale sensory analysis by increasing the overall liking the most by itself (Figure 7). The base taste flavour additive increases the umami taste and saltiness, while it decreases the beany and bitter flavour of the fava bean protein concentrate extrudates. The base taste's roast pork flavouring contained NaCl, which we got to our attention only after the sensory analysis. The increase in saltiness can be seen from the results (Table 13). The spices together with the base taste were able to lower the beany and bitter flavours more than the base taste alone. The spices also increased the meat broth odour of the samples and spicy flavour and odour. In the future it would be beneficial to have samples with only the spices so it would possible to study how the spices affect the sensory properties without the umami increasing base taste flavour additive. The masker had no effect on the negative sensory properties of the samples and overall, the attributes of the sample with NaCl and masker received almost the same scores as the attributes of the sample with only NaCl.

8 Conclusions

Flavouring meat analogues made from ingredients such as fava bean protein concentrate, which has naturally bitter, beany and astringent taste, is difficult. On the basis of the results meat analogue made from more neutral tasting ingredient, such as pea protein isolate is a much easier task. The combination of yeast extract with roast pork flavour, NaCl, plant protein flavour masker, and spice mixture with garlic, black pepper, coriander, and lemon was the most well liked combination of flavour additives. It can be seen from the results of the small-scale sensory analyses, that the same combination of flavour additives was the most well liked for both the FBPC and PPI extrudates. The descriptive sensory analysis results show that the same combination of flavour additives reduce the bitter and beany off-flavours of FBPC extrudate. The yeast extract with roast pork flavour alone reduced the negative sensory properties the most.

On the basis of the results of the descriptive sensory analysis of plant-based patties it can be said that extrusion does not affect the sensory properties of flavour additives. The panellists could not discriminate which sample was flavoured before or after extrusion. There were no systematic differences when the same samples that were flavoured at different times were compared to each other. There was some speculation that the extrusion might degrade the masker making it ineffective, but according to the results it had no systematic effect when the masker was added to the mixture.

The combination of flavour additives used in the sensory analyses can be used as a guide when flavouring meat analogues in the future. It is important to have an umami producing ingredient together with spices and NaCl. Still more research is needed on how to mask the unpleasant off-flavours of plant-based proteins so that more protein sources can be utilized.

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First small-scale sensory evaluation ballot

Sensory evaluation of flavoured meat analogues

Name ____ Date ___________

Welcome to the sensory evaluation of flavored meat analogues! In this session, we will compare samples with different flavorings and protein origins and discuss the next steps in the development. The goal for this sensory evaluation session is to find out the intensity of the flavorings and if the flavorings mask any off flavors or odors. The amount of flavorings used can be altered based on this session. The next step in development is to test if extrusion changes the sensory properties of the flavorings.

Your task is to go through the samples in a given order and in two parts: first, examine the odor and appearance of the samples (by looking and smelling), then the taste, flavor and texture (by tasting them). Write down attributes that describe the samples. Focus especially in attributes that discriminate the samples from each other. After everyone has gone through their samples individually, we will have a round-table discussion on the samples. Remember to smell and drink water between the samples.

Sample	**Off-odor intensity** (beany, bitter, astringent) 0= not perceivable, 10= very intense	**Off-flavor intensity** (beany, bitter, astringent) 0= Not perceivable, 10= Very intense	**Flavor intensity** 1= not intense enough, 5= just about right, 9= way too intense	**Texture** open comments OR specific attribute such as fibrils or gumminess etc.	**Overall liking** 1= Dislike very much, 5= Neither like nor dislike, 9= Like very much	**Potential for further development** ---...0...+++	**Open comments**
318							
924							
859							
722							
648							

Sensory evaluation of flavoured meat analogues

Name ____ Date ___________

Sample	**Off-odor intensity** (beany, bitter, astringent) 0= not perceivable, 10= very intense	**Off-flavor intensity** (beany, bitter, astringent) 0= Not perceivable, 10= Very intense	**Flavor intensity** 1= not intense enough, 5= just about right, 9= way too intense	**Texture** open comments OR specific attribute such as fibrils or gumminess etc.	**Overall liking** 1= Dislike very much, 5= Neither like nor dislike, 9= Like very much	**Potential for further development** ---...0...+++	**Open comments**
816							
927							
612							
173							
389							

Second small-scale sensory evaluation ballot

Sensory evaluation of flavoured meat analogues

Name ________________________________ Date __________

Welcome to the sensory evaluation of flavored meat analogues! In this session, we will compare samples with different flavorings and protein origins and discuss the next steps in the development. The goal for this sensory evaluation session is to find out the intensity of the flavorings and if the flavorings mask any off flavors or odors. The amount of flavorings used can be altered based on this session. The next step in development is to test if extrusion changes the sensory properties of the flavorings.

Your task is to go through the samples in a given order and in two parts: first, examine the odor and appearance of the samples (by looking and smelling), then the taste, flavor and texture (by tasting them). Write down attributes that describe the samples. Focus especially on attributes that discriminate the samples from each other. After everyone has gone through their samples individually, we will have a round-table discussion on the samples. Remember to smell and drink water between the samples.

Sample	**Off-odor intensity** (beany, bitter, astringent) 0= not perceivable, 10= very intense	**Off-flavor intensity** (beany, bitter, astringent) 0= Not perceivable, 10= Very intense	**Total odor intensity** 0= Not perceivable, 10= Very intense	**Total flavor intensity** 0= Not perceivable, 10= Very intense	**Level of spices** (Rate the level of spices) 1= not intense enough, 5= just about right, 9= way too intense	**Texture** Comment if sample differs in texture compared to other samples (tactile, mouthfeel)	**Overall liking** 1= Dislike very much, 5= Neither like nor dislike, 9= Like very much	**Open comments**

Sensory evaluation of flavoured meat analogues

Name ________________________________ Date __________

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The ballots used in the descriptive sensory analysis of extrudates and plant-based patties

SENSORY PROFILING OF MEAT ANALOGUES (extrudates)

12.8.2021
Sensory attributes and their definitions

The evaluation will be performed with a 0-10 line scale. Carefully open the lid of the sample vial, smell it, and evaluate all odor attributes. Proceed to do the same for the other samples. Then follow up with all taste and flavor and properties. Evaluate these sample by sample. Smell the odor references first before proceeding with the samples; do the sample before taste and flavor attributes. This helps you in binding the scale values. Please also describe the samples freely in the comment box on the PC.

Remember to keep adequate pauses and remember to drink (and smell) water between the samples. You can also chew a bit of toast or cream cracker between the samples to zero your senses.

ODOR

Overall odor intensity no odor --- very intense odor
Smell the sample and evaluate the **combined intensity of all odors** that are present in the sample.

Beany odor no beany odor --- very intense beany odor
Smell the sample and evaluate the sample's beany odor intensity. The beany odor can range between raw pea and cooked bean. Please comment which type of beany odor you detect to the next page.
Reference: H (4) Focus on the odor intensity, not the type of beaniness in the reference

Spiciness not spicy--- very spicy
Smell the sample and evaluate the combined intensity of spices. **Note**: spiciness means the level of herbs, pepper or other spices, not pungency! **Comment on the next page of the questionnaire which spices are most intense in the sample.**
Reference: M (7)

Meat broth no meat broth --- very intense meat broth
Smell the sample and evaluate the meat broth odor intensity. *The odor can also remind you of beef stock, bouillon cubes or nutritional yeast.*
Reference: Y (5)

APPEARANCE

There are no attribute intensities to evaluate from the samples as they look very similar. However, if you notice any discriminating features, please specify those in the comment box.

TASTE AND FLAVOR

Please note that the minimum bite size is one centimeter.

Beany flavor not beany --- very beany

Evaluate the intensity of beany flavor by tasting the sample. Comment on the type of beaniness on the next page.

*Reference: H (6) Note that the reference is both beany **and** bitter*

Bitterness no bitterness --- very bitter

Evaluate the sample's bitterness by tasting the sample.

Reference: H (5)

Saltiness not salty --- very salty

Evaluate the sample's saltiness by tasting the sample.

Reference: S (8)

Spiciness no spiciness --- very spicy

Evaluate the sample's spiciness by tasting the sample. **Note**: spiciness means the level of herbs, pepper or other spices, not pungency! **Comment on the next page of the questionnaire which spices are most intense in the sample.**

Reference: M (7)

Umami no umami --- very intense umami

Evaluate the sample's umami intensity by tasting the sample.

Reference: Y (6) → the umami in the reference is best perceived after some seconds from tasting

TEXTURE AND MOUTHFEEL

There are no attribute intensities to evaluate from the samples as they look very similar. However, if you notice any discriminating features, please specify those in a comment.

SENSORY PROFILING OF MEAT ANALOGUES (patties)

11.8.2021
Sensory attributes and their definitions

The evaluation will be performed with a 0-10 line scale. Carefully open the lid of the sample vial, smell it, and evaluate all odor attributes. Proceed to do the same for the other samples. Then follow up with all taste and flavor and properties. Evaluate these sample by sample. Smell the odor references first before proceeding with the samples; do the sample before taste and flavor attributes. This helps you in binding the scale values. Please also describe the samples freely in the comment box on the PC.

Remember to keep adequate pauses and remember to drink (and smell) water between the samples. You can also chew a bit of toast or cream cracker between the samples to zero your senses.

ODOR

Overall odor intensity no odor --- very intense odor
Smell the sample and evaluate the **combined intensity of all odors** that are present in the sample.

Beany odor no beany odor --- very intense beany odor
Smell the sample and evaluate the sample's beany odor intensity. The beany odor can range between raw pea and cooked bean. Please comment which type of beany odor you detect to the next page.
Reference: H (4) Focus on the odor intensity, not the type of beaniness in the reference

Spiciness not spicy--- very spicy
Smell the sample and evaluate the combined intensity of spices. **Note**: spiciness means the level of herbs, pepper or other spices, not pungency! **Comment on the next page of the questionnaire which spices are most intense in the sample.**
Reference: M (7)

Meat broth no meat broth --- very intense meat broth
Smell the sample and evaluate the meat broth odor intensity. *The odor can also remind you of beef stock, bouillon cubes or nutritional yeast.*
Reference: Y (5)

APPEARANCE

There are no attribute intensities to evaluate from the samples as they look very similar. However, if you notice any discriminating features, please specify those in the comment box.

TASTE AND FLAVOR

Please note that the minimum bite size is one centimeter.

Beany flavor not beany --- very beany

Evaluate the intensity of beany flavor by tasting the sample. Comment on the type of beaniness on the next page.

*Reference: H (6) Note that the reference is both beany **and** bitter*

Bitterness intensity no bitterness --- very bitter

Evaluate the sample's bitterness by tasting the sample.

Reference: H (5)

Bitterness intensity immediately --- after swallowing

Evaluate the timing of maximum bitterness by tasting the sample until swallowing.

Reference: H (5) → the maximum bitterness in the reference appears after some seconds

Saltiness not salty --- very salty

Evaluate the sample's saltiness by tasting the sample.

Reference: S (8)

Spiciness no spiciness --- very spicy

Evaluate the sample's spiciness by tasting the sample. **Note**: spiciness means the level of herbs, pepper or other spices, not pungency! **Comment on the next page of the questionnaire which spices are most intense in the sample.**

Reference: M (7)

Umami no umami --- very intense umami

Evaluate the sample's umami intensity by tasting the sample.

Reference: Y (6) → the umami in the reference is best perceived after some seconds from tasting

TEXTURE AND MOUTHFEEL

There are no attribute intensities to evaluate from the samples as they look very similar. However, if you notice any discriminating features, please specify those in a comment.