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Master's thesis

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Food Science and Technology

Functional and textural effects of partially replacing meat proteins by texturised pea and potato proteins in pork sausages

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Preface

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Abstract

Background: Meat, including pork, is a highly valued and nutritious protein source for humans. Unfortunately, the production of meat involves substantial greenhouse gas emissions. To reduce the impact on the environment, it is critical to develop meat alternatives in the food industry. The aim of this study was to develop pork sausages with meat proteins partially replaced by texturised pea and potato proteins and subsequently assess changes in functional and textural properties of these pork sausages.

Methods: Texturised vegetable protein products from pea and potato were obtained during extrusion cooking and used to replace 10%, 30%, or 50% meat proteins in emulsion-type pork sausages. The effect of protein texturisation were examined by Nuclear Magnetic Resonance spectroscopy and water-holding capacity measurements. The final sausages were investigated for changes in moisture loss, water distribution and mobility, chemical composition, texture, and sensory attributes.

Results: Texturisation of pea proteins caused a significant increase in the water holding capacity. Substituting meat proteins by texturised vegetable proteins in pork sausages resulted in a significant decrease in total moisture loss during processing and storage. Additionally, less water molecules were bound within the sausage gel network, resulting in a less firm and more gritty and juicy texture.

Conclusion: Partially replacing meat proteins by texturised pea and potato proteins in low-fat pork sausages caused changes in functionality and texture, which potentially can improve consumer acceptance. The results of this study highlight the feasibility and prospect of making pork sausages that can contribute to a reduction in meat consumption.

Abbreviations

CP/MAS	Cross-polarisation magic angle spinning
FAO	Food and Agriculture Organisation of the United Nation
kDa	Kilodalton
LF-NMR	Low-field Nuclear Magnetic Resonance
LVER	Linear viscoelastic region
MJ	Megajoule
NMR	Nuclear Magnetic Resonance
PC	Principal component
PCA	Principal component analysis
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PE	Pea protein
PP	Pea-potato protein
ppm	Parts per million
R1	Raw pea protein concentrate
R2	Raw potato protein concentration
RE	Reference
SD	Standard deviation
SME	Specific mechanical energy
SP/MAS	Single-pulse magic angle spinning
T1	Texturised pea protein product
T2	Texturised pea-potato protein product
TGA	Total glycoalkaloid
UNU	United Nations University
WHC	Water-holding capacity
WHO	World Health Organisation
w/w	Weight by weight

1. Introduction

The overall aim of this thesis was to assess how partial replacement of meat proteins by texturised pea and potato proteins affects functional and textural properties of emulsion-type pork sausages with low content of fat and salt to comply with the Nordic Keyhole nutrition label regulation. To provide a better platform for understanding the results presented later in this thesis, several topics will be presented in the introduction. Firstly, meat protein replacement will be introduced with focus on the challenges of developing new food products with reduced meat content. This will be followed by an introduction to pea and potato proteins and how these proteins can potentially substitute meat proteins. The basic theory of extrusion cooking and texturisation of vegetable proteins will then be described, followed by a short presentation of emulsion-type sausage production. Finally, an introduction to food product development with focus on functionality and texture will be given.

1.1 Meat protein replacement

The global meat consumption is extensively rising driven by world population growth (from around 7.4 billion in 2015 to estimated 8.9 billion in 2050) and increasing average individual incomes [1, 2]. The increase in meat consumption comes with an environmental cost as meat production is one of the primary sources of greenhouse gas emissions and thereby a big contributor to global warming. Meat produces more emissions per unit of energy compared with plant-based food products. Ruminant production usually leads to more emissions than that of non-ruminant mammals, such as pigs, and poultry production leads to less emissions than mammal production. Concerns about the major effects of emissions on the environment as well as on human health and the economics of the food system have led to a rapid increase in the development of meat alternatives in the food industry [3]. Furthermore, the growing public awareness of sustainable foods has resulted in a new consumer group of “flexitarians”, who consciously reduces meat consumption in their daily diets [4].

Developing new non-meat or reduced-meat products that are comparably nutritious and attractive for consumers in taste and texture as meat products have proven challenging [5]. The next section will describe some of the reasons why meat is a popular source of protein and can be difficult to replace.

1.1.1 Meat proteins and their functional properties

Meat is considered the highest quality protein source due to its nutritional characteristics and appreciated taste [6]. Meat proteins are highly nutritious as they contain all the essential amino acids with a composition profile that meets the adult essential amino acid requirements [7]. Furthermore, meat proteins, including sarcoplasmic (mostly globular), myofibrillar (fibrous), and stromal proteins (collagenous and reticular), are versatile and exhibit excellent functional properties, such as gelation, emulsification, and water-holding capacity (WHC) compared to proteins from plant sources [6].

In general, structure, size, and shape of proteins depend on both covalent bonds, such as disulphide bonds, and non-covalent interactions, such as hydrogen bonds, hydrophobic interactions, Van der Waals interactions, and electrostatic interactions. The presence of these molecular forces are involved in intermolecular interactions that determine physicochemical and functional properties of proteins [8]. Under suitable conditions, the structures of meat proteins can undergo structural changes and interactions to enable the functional characteristics. For example, gelation occurs as a result of matrix formation by extracted myofibrillar proteins and collagen protein-protein interactions. This stable gel network are able to immobilise fat, water, and other constituents. The excellent emulsification capability of some meat proteins, such as myofibrillar proteins, is attributed to their high length-to-diameter ratio and bipolar structural arrangement making it possible for their hydrophobic site to interact with fat and their hydrophilic site to interact with water. This realignment results in a reduction of surface tension of fat particles and the formation of a rigid protein membrane in fat emulsion. Another important functional property of meat proteins are their ability to bind, immobilise, and retain water in their network, also known as WHC, by hydrogen bonds. These functional properties contribute to the overall characteristics of meat and meat products, including texture, appearance, mouthfeel, juiciness, and physical stability during storage [6].

The favourable nutritional characteristics and functional properties of meat proteins have been very difficult to reproduce by any other food proteins or non-protein functional ingredients. Interestingly, vegetable proteins have lately become an attractive substitute for meat proteins to reduce the consumption of meat and other animal sources [5, 6]. In the next section, the potential and challenges of using vegetable proteins as meat protein replacement will briefly be introduced.

1.1.2 Vegetable proteins

There is an increased interest in using vegetable proteins as a substitute for meat proteins, because of their high protein delivery efficiencies in terms of energy used or greenhouse gas emitted [9]. In a study by González et al. (2011), protein delivery efficiencies of pork proteins delivered to Sweden were calculated to be 7.3 g protein per MJ and 25 g protein per kg CO_2 eq. The study showed that protein delivery efficiencies of vegetable proteins delivered to Sweden generally were higher than pork proteins, but the efficiencies increased with increasing protein content. For example, protein-rich pulses, such as peas, had protein delivery efficiencies of 70 g protein per MJ and 495 g protein per kg CO_2 eq., whereas tubers, such as potatoes, with low protein content had protein delivery efficiencies of 9.4 g protein per MJ and 89 g protein per kg CO_2 eq. [9].

There are some major issues of concern related to the direct use of vegetable proteins in meat products, such as antinutrients, off-flavours, and non-meat like textural properties [5]. These issues can be reduced by the use of low-moisture extrusion cooking, also called texturisation, which denatures and modifies vegetable proteins to resemble meat proteins [10]. Thus, texturised vegetable proteins have a potential as a replacement of meat proteins. During extraction or extrusion, processing conditions (i.e. temperature, pH, and ionic strength) highly influence protein functionality. For instance, heat treatment can cause the proteins to unfold, exposing buried hydrophobic groups, and promoting formation of covalent bonds between proteins. This results in new three-dimensional structures or aggregates of the proteins, which changes the ultimate protein functionality [11–14]. Furthermore, residual starch, fibre, and lipids in the protein material significantly contribute to product functionality [15]. The next section will elaborate on some of the mechanisms that govern protein structure and functionality in vegetable proteins.

1.1.2.1 Functional properties of vegetable proteins

Functional properties, such as solubility, water holding, fat absorption, emulsifying, foaming, and gelling, are related to the way vegetable proteins interact with major food constituents, such as water, other proteins, lipids, and carbohydrates, as well as with any minor constituents, such as salts, metal ions, acidulants, aroma compounds, and phenolic compounds. These properties influence the overall quality and sensory perception of foods [8, 16].

The most important functional properties of vegetable proteins in meat applications are high WHC, fat-absorption capacity, emulsification capacity and stability, and gelation ability [17]. WHC and fat-absorption capacity are measures of the amount of water and oil, respectively, bound per unit weight of protein material. These functional properties indicate the ability to prevent fluid leakage from the meat product during processing and storage [18]. High water solubility of a protein material is not a determinant of usefulness in meat systems. However, protein solubility, which is mediated by non-electrostatic and hydrophobic interactions, is closely associated with emulsification and gelation [14, 16]. Generally, higher solubility suggests that the extracted proteins are in a more native state [12].

Soy protein ingredients have since the 1960s been very popular as commercial products and been used for their nutritional and functional properties in many food categories including meat applications. Soy proteins are especially known for their excellent formation and stabilisation of emulsion, which are critical in many meat products. In addition, soy protein ingredients are commonly texturised to obtain meat-like products. However, other vegetable proteins also have the potential of becoming commercial products used in meat applications [19]. In this study, pea and potato proteins were texturised and used as partial meat protein replacement in pork sausages. In the next sections (1.2 and 1.3), the characteristics of pea proteins and potato proteins will be described. This will be followed by the elaboration of texturisation of vegetable proteins (section 1.4).

1.2 Pea proteins

Yellow field peas (*Pisum sativum L.*), referred to as peas throughout this thesis, are dried legume seeds also known as pulses. Peas are grown extensively all over the world and their ability to fix nitrogen is environmentally beneficial because it reduces the use of fertiliser in agriculture and minimises greenhouse gas production. The average protein content in peas is around 25%, however, protein-rich fractions (protein concentrates) with protein content of 45.8-63.4% can be prepared from dehulled peas with the milling technique called air classification [17, 20]. The remaining constituents in the fractions are starch, dietary fibre, other carbohydrates, and small amounts of lipids [21].

Air classified pea protein concentrate is attractive as a new food ingredient due to its low allergenicity, non-GMO status, and its content of fibre (about 2%), B-group vitamins, and minerals are well preserved. Furthermore, they have relatively low cost compared to animal-

derived proteins [12, 17, 22]. On the negative side, pea protein concentrates contain a number of antinutrients that lower the nutritional value of food by lowering the digestibility or bioavailability of nutrients. These antinutrients include protease inhibitors, lectins, saponins, polyphenols, phytate, and raffinose oligosaccharides [17]. However, with extensive heat and mechanical treatment, such as during extrusion, it is possible to effectively reduce these anti-nutritional compounds [21].

One of the challenges of using pea protein concentrate as meat protein replacement is that the proteins in peas are very different from meat proteins. Meat proteins consist of a versatile mixture of globular, fibrous, and collagenous proteins. In comparison, the predominant types of proteins in peas are globulins and albumins which account for 49-80% and 15-25%, respectively, of the total protein. In addition, smaller amounts of glutelins (11%) and prolamins (5%) are present. The albumins include the undesired protease inhibitors and lectins [20, 23]. The globulins are globular storage proteins that can be further classified based on their sedimentation coefficients into legumin (11S) and vicillin (7S). The ratio between these two globulins can vary from 1:1.3 to 1:4.2 between pea cultivars [24]. The pea legumin has a hexameric structure with a molecular weight range of 300-400 kDa. Each of the six subunit pairs have an acidic (high in glutamic acid) and a basic (high in alanine, valine, and leucine) subunit linked via a disulphide bond. Vicilin has a total molecular weight range of 150-190 kDa and constitutes of three subunits with no disulphide bond present. The vicilin fraction tend to have a higher variability than legumin and it can exhibit different surface properties and consequently different functionalities [12, 20].

Pea proteins, like other legumes, are deficient in the sulphur-containing essential amino acids methionine and cysteine [12]. The use of pea proteins as meat replacement can be challenging as it may reduce the nutritional value of a food product due to the lack of essential amino acids [20]. In addition, the amino acid composition of pea protein ingredients highly depends on cultivar genetics as well as the processing involved. This should be taken into account when promoting pea proteins for their nutritional value [13, 20].

As mentioned previously, high WHC of protein materials is important for their use in meat applications [12]. Generally, air classified pea protein concentrate has poor WHC, which limits its use in meat products [14, 21]. However, the WHC in pea proteins can be significantly improved as a result of thermal and mechanical energy during texturisation. In section 1.4.3, protein texturisation mechanisms will be further elaborated [21, 25].

1.3 Potato proteins

Potato tubers (*Solanum tuberosum*), referred to as potatoes throughout this thesis, are the world's fourth most important crop after rice, wheat, and corn. For many consumers, direct consumption of potatoes is part of their daily diet. Potatoes have a high content of starch (up to 80% of dry matter) and are therefore widely used as a raw material for the extraction of starch [26]. A side stream product of starch production is the potato juice, which contains approximately 1.5% (w/v) of soluble potato proteins [26, 27]. Recent developments have resulted in the recognition of extracted potato proteins as potential new food ingredients due to their unique functionalities and high nutritional quality [26, 28]. Hence, the potato juice from the starch production is a potential resource of large quantities of novel potato proteins for food applications [27].

The use of potato proteins in food applications as meat protein replacement can be challenging as the proteins of potato differ from meat. The soluble proteins in potato juice have been classified broadly into three groups: patatins (30-40%), protease inhibitors (40-50%), and other proteins (10-15%) [26, 29]. Patatins and protease inhibitors are well characterised, whereas limited information exists about the other proteins, which are considered to be enzymes involved in starch synthesis [26, 30]. One of these enzymes is polyphenol oxidase that can catalyse the reaction between the major phenolic compound, chlorogenic acid, and patatins or protease inhibitors causing the formation of a characteristic brown colour of potato protein concentrate [31].

Patatins, also known as tuberin, constitute a group of homologous storage glycoproteins that exist as dimers of 40-45 kDa subunits held together by non-covalent hydrophobic interactions. Patatins exhibit antioxidant activity and lipid acyl hydrolase activity, which suggest that they play a significant role in the plant defence. Patatins have relatively low denaturation temperature (around 55°C) and relatively low stability with a loss of structure at pH $\leq$ 4.5. Patatins are made of up to 366 amino acids, but the amino acid profile vary markedly between potato cultivars [26, 30, 32, 33]. Protease inhibitors are a heterogeneous group of storage proteins with molecular weights ranging from 5 to 25 kDa. The proteins vary according to chain length, amino acid composition, and inhibitory activities. Protease inhibitors are able to act on a variety of proteases and other enzymes, which has been hypothesised to help the breakdown of proteins during the developing stages of the tuber [32].

Potato proteins generally have a high nutritional value and high Protein Digestibility Corrected Amino Acid Score (PDCAAS) close to animal proteins [26]. PDCAAS is a measurement used for predicting dietary protein utilisation by multiplying the limiting amino acid score (i.e. the ratio of the first limiting amino acid in a gram of target food protein to the requirement value) by protein digestibility [34]. Potato proteins are nutritionally superior to most other plant and cereal proteins, because they contain a high proportion of the essential amino acid lysine and relatively high proportions of sulphur-containing essential amino acids, such as methionine and cysteine. In addition, potato proteins have very low allergenicity and may possess antioxidant activities and other health promoting properties [26, 32].

On the other hand, potato protease inhibitors are known for their anti-nutritional properties. Furthermore, potatoes contain the unwanted glycoalkaloids, which present a bitter taste and possible toxicological reactions, such as gastrointestinal disturbance and neurological disorders. During the recovering of potato proteins, the total glycoalkaloids (TGAs) need to be reduced to below 150 ppm to be safe for human consumption [26, 35].

Potato protein concentrates are traditionally prepared by precipitating the proteins with acidic heat treatment of the potato juice. This is followed by centrifugation and drying, resulting in a final concentrate with a high yield of minimum 85% crude protein [30]. However, thermal/acidic precipitation often leads to conformational changes and denaturation of the potato proteins. As described in the previous section, physicochemical and functional properties of protein materials highly affect the quality and sensory properties. Hence, the properties of the extracted potato proteins determine the usability as an ingredient in food applications. The effects of precipitation on the quality of potato proteins may vary, depending on the origin of protein, its denaturation degree, the content of other components, and processing conditions. However, generally the potato proteins obtained by thermal/acidic precipitation becomes highly unstable and insoluble with a great loss in functionality [28]. Other extraction techniques involving various combinations of ionic strength, pH, temperature, and solvents have been explored to retain the native and functional properties of potato proteins or modify them for enlarging their application. Recovery of potato proteins with desirable functional properties have shown to be a very costly process because it involves the separation technique chromatography [30, 35–38].

1.4 Texturisation of vegetable proteins

Proteins can be texturised by a technology known as extrusion cooking. Texturisation of proteins is the denaturation and restructuring of protein molecules into layered and cross-linked products that imitate the fibrous texture, functionality, and appearance of meat [39]. Extrusion cooking is a promising and cost-efficient technology, which popularity is steadily increasing in food processing. With the use of thermal and mechanical energies, extrusion cooking enables the use of components otherwise difficult to use in traditional food application [28]. The extrusion technology in food processing is very complex [40]. This section will give an overview of the primary principles of the technology and describe the proposed mechanisms behind texturisation of vegetable proteins.

1.4.1 Extrusion cooking

In food processing, extrusion cooking has gained in popularity due to its versatility, high productivity and lower processing costs compared to other similar processing methods. However, the additional energies used to transform food ingredients during extrusion will cause a negative impact on the environment [41].

The principle of extrusion cooking is that a raw food material is fed into an extruder barrel containing one or two screws that are used to convey the material along the barrel, while water is added. Further down the barrel, the volume becomes restricted causing a compression of the food material. The screws then knead the material and with a combination of high temperature, high pressure, and high shear, the material converts into a semisolid, plasticised mass. Finally, the mass is expelled through a restricted opening, the die, at the discharge end of the barrel. The extruded product is often further cooled down or dried before packaging. Hence, extrusion cooking is a continuous process that alters raw food ingredients with a combination of mixing, kneading, shearing, heating, cooling, shaping, and forming. Extrusion technology is able to make extruded food products of components otherwise considered inappropriate for human consumption [41]. For instance, besides converting protein material into new texturised and functional materials, the extreme extrusion conditions can remove bitter flavours and improve protein digestibility and nutritional quality of the materials [21]. The extrusion process can be either cold or hot with low or high moisture depending on the addition of heat and water, respectively [10, 41, 42]. The present study will focus on hot, low moisture extrusion cooking, where the food material is heated above 100°C and with a water

content of the extrudate below 35%. The concepts behind cold and high moisture extrusion will not be further described in this thesis.

Many factors influence the final quality of the extruded products. These can be related to the properties of ingredients, such as chemical composition and particle sizes, pre-extrusion conditions, extruder design, process conditions, and post-extrusion conditions [41]. In the following section, important food extruder design parameters and operating variables will be elaborated.

1.4.2 Food extruders

Food extruders exist in a wide variety of designs. The most commonly used designs in the food industry are twin-screw extruders that are co-rotating, intermeshing, and self-wiping. These extruders are able to process the most varied raw materials common in food products from a very low viscosity dough to a very high viscous mass [43].

Extrusion is a continuous process that operates under steady-state equilibrium conditions. An extruder can be divided into four sections: feed section, compression section, metering section, and die section. Figure 1.1 schematically shows the four sections of a twin-screw extruder system with nine heating zones, which resembles the one used in this study. In the feed section, the dry raw material is fed into the extruder at a constant feed rate with the use of a volumetric or gravimetric feeder. Before introducing extensive heating, pressuring, and shearing, water is added and mixed with the material to a dough-like consistency. It is essential that the process is kept constant as the product is conveyed forward, while removing any air. In the next section of the extruder, the compression section, the temperature increases and the screw profile changes to increase the pressure and mix and compress the material into a homogenous consistency of the extrudate. Additional compression of the extrudate occurs in the metering section where the deformation and restructuring of the raw material matrix to the finished product takes place. The residence time in this section is not more than 10-30 seconds. At the die section, the final product is pressed through a die hole to form a desired shape. The die design can be as simple as a single outlet hole to a complex section with various chambers and pathways [41, 43].

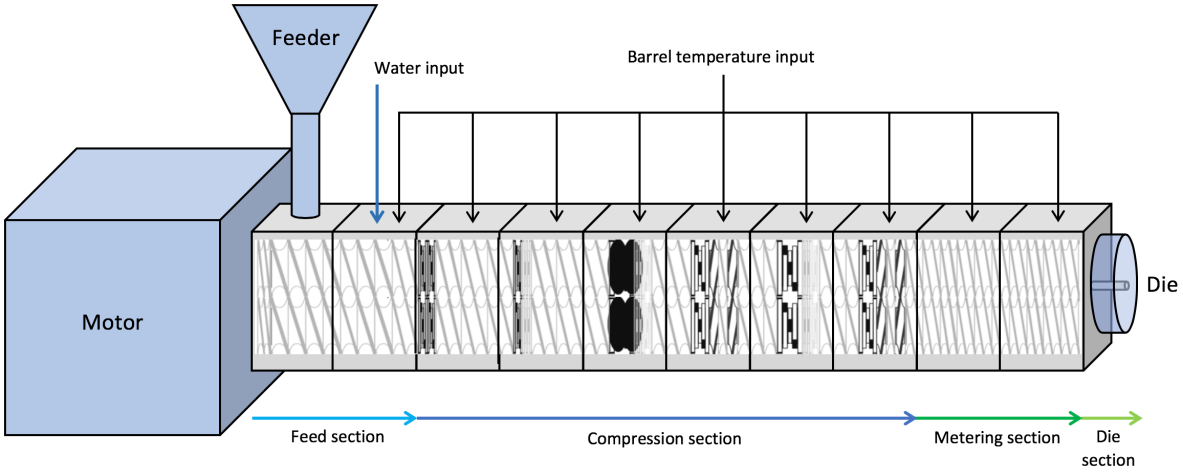

Figure 1.1: Schematic of twin-screw extruder system with nine heating zones (inspired by [44]).

The most important extruder operating variables are temperature and pressure in the barrel, diameter of the die, and the shear rate that is influenced by the internal design of the barrel, such as its length-to-diameter ratio and the geometry and speed of the screws. In co-rotating twin-screw extruders, the two screws are positioned adjacent to each other with the same direction of rotation. When the screws intermesh, a positive displacement pumping action happens moving the extrudate along the barrel. The flow pattern follows a "figure 8 profile" with a relatively uniform shear stress distribution around the screws. However, the screw configuration typically consists of a unique profile including clockwise or counter-clockwise rotating screws, mixing discs, paddles, and reverse screw elements, which creates a complex flow pattern with good mixing and heat transfer, large melting capacity, and good melt temperature control. Thus, the exact flow behaviour is not well understood, but the high process capability and flexibility of the extruder design result in a consistent food product quality [45].

An intermeshing, co-rotating twin-screw extruder is designed with a control panel that can monitor the specific mechanical energy (SME), die melt temperature, die pressure, and flowrate through the die. During extrusion, these operating parameters are maintained at predetermined values by controlling the material feedrate, screw speed, water input to the extruder, and the temperature profile of the extruder barrel. Furthermore, the control panel is able to protect the extruder from over pressurisation and hazardous conditions, such as inconsistency in feed rate, overtorque of the motor, over-the-limit pressure at the die, feed throat backup, temperature limit of the motor and the gearbox, cutter overtorque, or the backup or blockage of the takeaway system [41, 43]. The automated control, continuous operation, and high productivity and capability of extruders enables the production of new

food products with high product quality. However, the properties of the feed materials highly influence the conditions inside the extruder barrel and hence the structure and quality of the final extruded product [41]. In the next section, a short description of the molecular interactions among proteins and other constituents in the raw material during extrusion cooking will be given.

1.4.3 Protein texturisation mechanisms

Using low moisture extrusion, palatable texturised vegetable proteins can be obtained. The texturisation mechanisms are very complex and not fully understood. Several studies have been investigating the mechanisms of texturisation using soy and pea materials [39, 46–49]. These studies have suggested that the predominant texturisation mechanisms during extrusion are disulphide bonds, non-specific hydrophobic interactions, and electrostatic interactions. These mechanisms occur between the denatured proteins and other constituents in the hot continuous, viscoelastic melt when it enters the cooler die section. The cooling is essential to increase the viscosity and reduce the fluidity allowing a continuous realignment of the proteins in the direction of the flow and a resulting three-dimensional reorganisation of the molecules [50]. Addition of calcium chloride in the range 0.5-2.0% to the raw protein material has been known to increase the textural integrity of the final texturised product [51].
Extrusion cooking has been performed on pea protein materials with large differences in protein content, varying from 19% protein on a dry basis [25, 52, 53] to 87% protein on a dry basis [39, 54]. The protein-based materials constitute of additional amounts of starch, lipids, and other constituents, which are affected by extrusion and interact with the proteins. The physicochemical changes of the other constituents include: starch gelatinisation and degradation, lipid oxidation, degradation of vitamins, antinutrients, and phytochemicals, formation of flavours, and increase in mineral bioavailability and dietary fiber solubility. These constituents may affect the physical and sensory characteristics of the extrudate and hence the quality of the final product [10, 40, 41, 50].
Several studies have investigated the effects of low moisture extrusion conditions on the chemical, functional, and nutritional properties of pea protein materials [21, 25, 39, 49, 55, 56]. The studies revealed that the exact effects of extrusion processing varied greatly according to type of material (cultivars and extraction process) and extrusion conditions.

Generally, the formation of the texturised three-dimensional molecular structure during extrusion resulted in a lower protein solubility and a higher WHC. It was proposed that the increased WHC was the result from physical retention of water by capillary actions. The pea legumin seems to be more affected by texturisation than vicillin, as vicillin is deficient in disulphide bonding amino acids [21, 25, 39, 49]. Furthermore, extrusion improves the nutritional properties of pea protein materials by reducing protease inhibitor activities, reducing the level of antinutrients, and increasing protein digestibility. However, the amino acid profile may be affected by the extreme processing conditions. Especially, the concentration of lysine may significantly decrease during extrusion as it reacts with reducing sugars via Maillard reactions [21, 52, 56, 57]. Up until now, no studies have published any research on texturised potato proteins.

To summarise, texturisation alters globular vegetable proteins into fibrous structures that resemble the texture of meat tissues and have improved functional properties. The texturised products can potentially be used as meat replacement in products, such as emulsion-type pork sausages. In the next section, the production of emulsion-type pork sausages will be introduced.

1.5 Emulsion-type pork sausages

Emulsion-type pork sausages, such as frankfurters or hot-dogs sausages, are precooked, smoked/non-smoked ready-to-eat sausages that can be eaten cold or heated as part of a meal or on its own. The general steps of emulsion-type pork sausage processing are grinding of meat, chopping meat, addition of ice water, salts, spices, and fat, stuffing of meat batters, cooking, and packaging. During chopping, mechanical action and shear comminute meat into fine particles dispersed in a continuous water phase. Furthermore, the chopping brings salt, phosphate, and water into immediate contact with the myofibrillar system, which results in the swelling of myofibrils and partial solubilisation of myofibrillar proteins. These swollen and dissolved proteins can form a three-dimensional heat-stable network that may surround small emulsified fat particles preventing their cohesion to larger fat droplets. Upon heating, the meat proteins coagulate causing the formation of a stable gel network that immobilise fat, water, and other constituents, which give rise to the characteristic homogeneous texture typical of emulsion-type sausages. A failure to form the three-dimensional network and gel during processing can contribute to an excessive loss of water and fat. Thus, addition of NaCl, phosphates, and water plays a critical role in the structural changes of protein, the rheological

properties of meat batters, and the ultimate texture and sensory attributes of the pork sausages. Furthermore, fats are essential for texture, taste, flavour, and the physicochemical stability of the product [58–62].

Emulsion-type pork sausages are widely consumed at home or at food service industries all over the world. They often contain high amounts of fat (30%) with a relatively high degree of saturation of the fatty acids and salt (2-3%), which potentially can be harmful to consumers. Growing health awareness of consumers have resulted in demands for sausages with reduced fat and salt content. To comply with the Nordic Nutrition Recommendations and use the Nordic Keyhole label, fat and salt content of processed meat product, such as pork sausages, cannot exceed 10% and 2%, respectively. Reduction in fat and salt is a great challenge for the meat industry, because fat and salt highly affect the textural and sensory properties of sausages. Low fat emulsion-type sausages have been largely rejected by the consumers due to a less juicy, firmer, and more rubbery texture, darker colour, and overall less acceptable attributes than traditional sausages [59, 63–68].

Developing new emulsion-type pork sausages that will meet the goals of having reduced fat, salt and meat protein contents, as well as being accepted by the consumers, can seem ambitious and difficult. New product development usually involves multiple rounds of product evaluation and optimisation before launching the product [69]. The next section will further describe the stages involved in new product development of emulsion-type pork sausages with partially replaced meat proteins by texturised vegetable proteins.

1.6 Product development with focus on functionality and texture

A product development process often constitutes several stages for moving a product from the idea to launch and beyond. Within these stages, activities and tasks can be performed in different ways. Some activities are undertaken sequentially, while others in parallel or overlapping systems. The process often includes several iterations for making the optimal product [69]. The iterative product development process of the emulsion-type pork sausages with partially replaced meat proteins by texturised pea and potato proteins illustrated in Figure 1.2. In the upper half circle of the illustration, the process of altering raw protein materials of pea and potato to texturised protein materials is shown. The texturised products are then used for the production of pork sausage batters, which are further cooked to obtain the finished pork sausages, as demonstrated in the lower half circle.

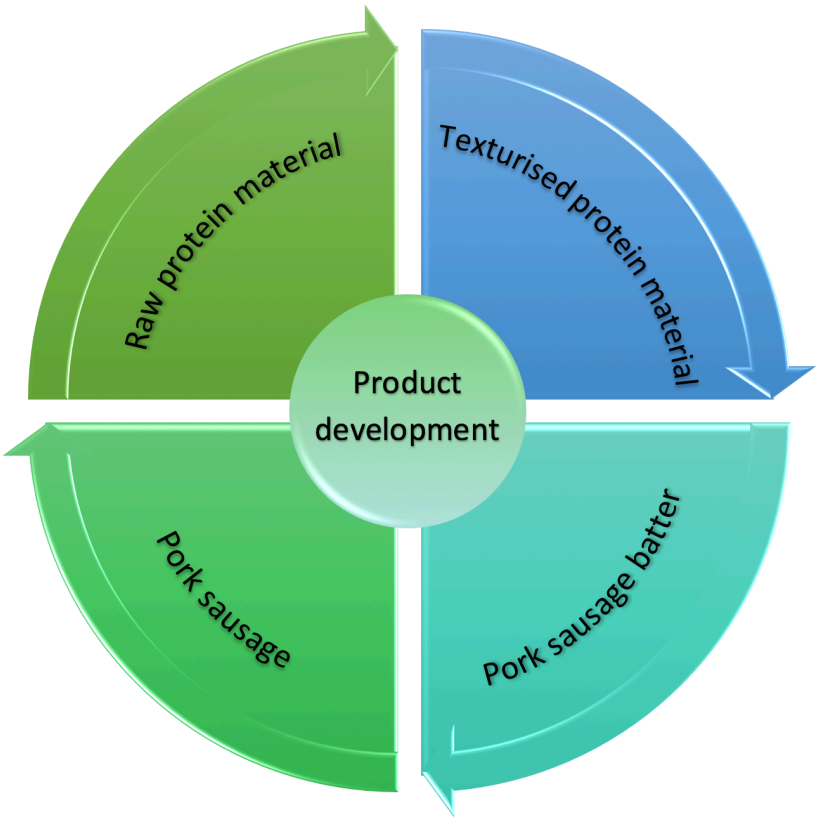

Figure 1.2: Illustration of the iterative process of pork sausage development.

During each stage of pork sausage development, functional and textural properties of the new product should be carefully assessed. These quality parameters are important for the final product. Functionality can be defined as physicochemical behaviours of proteins during processing and storage that affect the properties of the final product [6]. Texture is defined as the sensory and functional manifestation of the structural, mechanical, and surface properties of a food product, which can be detected through the senses of sight, hearing, touch, and kinesthetics. Texture is a multi-parameter attribute that derives from the structure of the food. In pork sausages, texture is the result from the complex protein network system created by the emulsification of swollen and dissolved myofibrillar proteins surrounding fat particles during chopping of the ingredients and the following heat-induced gelation causing a stable network that immobilise fat, water, and other constituents. Thus, functionality and texture are highly interrelated [6, 70].

To summarise, meat production is one of the primary sources of greenhouse gas emissions and greatly contributes to global warming. Consequently, there is a need to reduce the production and consumption of meat products. Developing new reduced-meat food products with comparable sensory attributes and nutritional characteristics as meat products have proven challenging. Vegetable proteins have become an attractive substitute for meat proteins due to the use of texturisation causing denaturation and layered restructuring of vegetable proteins into products that can imitate the fibrous texture, functionality, and appearance of meat. Substitution of meat proteins in products, such as emulsion-type pork sausages, may provoke unwanted emulsification changes of the complex meat batter structure or result in undesired gelling changes or reduction in water binding ability during cooking and storage, which will ultimately affect the quality and sensory properties of the sausages. Nevertheless, the functional and textural consequences of replacing meat proteins with texturised vegetable proteins in pork sausages are not known.

2. Research question, aim, and hypotheses

2.1 Research question

How does partial replacement of meat proteins by texturised vegetable proteins affect the functional and textural properties of low-fat and low-salt emulsion-type pork sausages?

2.2 Aim of study

The aim of this study was to assess the technological suitability of texturised vegetable proteins as replacement of meat proteins. We used texturised vegetable proteins from either pea only, or a combination of pea and potato, and studied the functional and textural changes of pork sausages in which 10%, 30%, and 50% of the meat proteins had been replaced by texturised pea and potato proteins. The pork sausages were produced as low-fat and low-salt emulsion-type sausages to comply with the Nordic Keyhole nutrition label regulation. Application of multiple methods to assess functionality and texture allowed us to study the effect of meat replacement on water binding ability, water distribution and mobility, firmness, and sensory attributes.

2.3 Hypotheses

When pea and pea-potato protein concentrates are texturised:

- The water-holding capacity increases.

When meat proteins are partially replaced by texturised pea or pea-potato proteins in emulsion-type pork sausages:

- The water binding ability during cooking, cooling, and heating, and the resulting water distribution and mobility and juiciness changes.
- The sensory and instrumental firmness changes.
- The sensory attributes cohesiveness, gumminess, grittiness, chewing time, and chewing residual changes.

3. Materials and methods

3.1 Raw and texturised pea and potato protein samples

In this section, materials and methods used for the texturisation and examination of protein materials are described. Table 3.1 details the abbreviations and a short description of the five different protein samples used in this project.

Table 3.1: Protein sample description.

Treatment	Sample	Protein material
Raw	**RE**	Reference of pork sausage batter with 100% meat proteins
	R1	AMN pea protein concentrate
	R2	KMC potato protein concentrate
Texturised	**T1**	AMN pea protein concentrate
	T2	3:1 mix of AMN pea protein concentrate and KMC potato protein concentrate

3.1.1 Raw materials

Air-classified AMN protein concentrate 55 (AM Nutrition, Stavanger, Norway) from food grade spring type yellow peas was used in this project. According to the producer, the protein fraction contained approximately 11% water, and on a dry basis 55% protein, 3% fat, 2% fiber, 8% starch, and 34% other carbohydrates.

In addition, KMC potato protein concentrate (KMC, Brande, Denmark) was used in this project. The concentrate was obtained by thermal/acidic precipitation of potato juice from the side stream of starch production. In order to obtain food grade status, KMC had reduced the total glycoalkaloids to below 150 µg/g. The water content of the potato protein concentrate was approximately 11%. On a dry basis, the chemical composition was approximately 85% protein, 2% fat, 6% fiber, and 7% other carbohydrates.

3.1.2 Low moisture texturisation of pea and potato protein concentrates

A ZSK 27 Mv Plus (Coperion, Stuttgart, Germany) intermeshing, co-rotating twin-screw extruder with a KT20 gravimetric twin screw feeder (Coperion K-Tron, Stuttgart, Germany) was used for low moisture texturisation of the raw protein materials. The screw diameter of the extruder was 27 mm with a length/diameter ratio of 40:1. The screw profile is described in table 7.1 in Appendix. The die contained a cylindrical hole with a diameter of 3.3 mm. The extruder barrel consisted of nine heating zones (barrel zones 2-10 in table 7.2 in Appendix), which are cooled by water. Based on preliminary trials, the process parameters that resulted in the best texturised quality of the pea protein concentrate mix (80.7% w/w R1 (wet basis), 16.9% w/w water, 1.6% w/w sunflower oil, and 0.8% w/w $CaCl_2$ powder) and the 3:1 pea-potato protein concentrate mix (57.8% w/w R1 (wet basis), 19.3% w/w R2 (wet basis), 20.6% w/w water, 1.5% w/w sunflower oil, and 0.8% w/w $CaCl_2$ powder), respectively, were chosen. The protein composition of the 3:1 pea-potato protein concentrate mix was 66% pea proteins and 34% potato proteins due to different protein content in R1 and R2. The resulting process parameters of the texturisation are shown in table 7.2 in Appendix. The final texturised protein products, T1 and T2, were dried for 10 minutes at 135°C in a prototype belt dryer (Drying Mate A/S, Viby, Denmark).

3.1.3 Water-holding capacity of pea and potato protein samples

The method used to assess the WHC of R1, R2, T1, and T2 was adapted and modified from Alonso et al. (2000) [25]. In a test tube, one gram of raw or texturised protein sample was mixed and saturated with 20 ml of 2% NaCl solution with pH adjusted to ~5.8 by 0.1M HCl to resemble pork sausage batter environment [71]. The mixture was allowed to stand for 20 minutes at room temperature, then centrifuged at 2,000 × g for 10 minutes at 25°C. The liquid retained by the solid was determined by the difference in sample weight before and after hydration. WHC is expressed as g of water retained per g of dry sample. Each sample was analysed in quadruplicate.

3.1.4 Solid-state ^{13}C NMR spectroscopy of pea and potato protein samples

The solid samples of R1, R2, T1, T2, and RE were analysed by ^{13}C magic angle spinning (MAS) Nuclear Magnetic Resonance (NMR) spectroscopy using Bruker Avance 400 (9.4 T) NMR Spectrometer (Bruker, Rheinstetten, Germany). The NMR spectrometer was operating at Larmor frequencies of 400.13 and 100.63 MHz for ^{1}H and ^{13}C, respectively. The measurements were carried out at 294 K using a double-tuned cross-polarisation (CP) MAS probe equipped for 4 mm rotors employing a spin-rate of 9 kHz and rf-field strengths of 83 kHz for both ^{1}H and ^{13}C. Single-pulse (SP) MAS spectra were recorded using a recycle delay of 128 s and 600 scans, whereas CP/MAS spectra were recorded using a contact time of 1 ms, a recycle delay of 8 s and 1024 scans. The acquisition time was 49.2 ms during which ^{1}H decoupling (TPPM) was applied. All ^{13}C MAS NMR spectra were referenced to the carbonyl resonance of α-glycine at 176.5 ppm (external sample). NMR spectra were processed and analysed using Bruker BioSpin TopSpin software, version 4.0.3 (Bruker, Rheinstetten, Germany).

3.1.5 Liquid-state ^{1}H NMR spectroscopy of pea and potato protein samples

20 mg of each sample of R1, R2, T1, T2, and RE was saturated in water, then centrifuged at 10,000 × g for 5 minutes. For ^{1}H NMR Spectroscopy, the samples were prepared in 5 mm NMR sample tubes by mixing 495 µl supernatant with 55 µl of D_2O (containing 5.8 mM TSP-d4). The samples were analysed at a temperature of 298 K by using Bruker Avance DRX 500 (11.7 T) spectrometer (Bruker, Rheinstetten, Germany) operating at a Larmor frequency of 500.13 MHz for ^{1}H using a double tuned inverse detection BBI probe equipped with Z-gradients. One dimensional ^{1}H experiments were performed using pre-saturation followed by a composite 90° pulse (zgcppr) in order to achieve sufficient water suppression. For each sample 256 scans were acquired using a recycle delay of 5 s, a spectral width of 10 kHz and an acquisition time of 1.63 s. All ^{1}H NMR spectra were referenced to TSP-d4 at 0.0 ppm. NMR spectra were processed and analysed using Bruker BioSpin TopSpin software, version 4.0.3 (Bruker, Rheinstetten, Germany).

3.2 Pork sausage batters and pork sausages

In this section, materials and methods used to assess the functionality and texture of pork sausage batters and pork sausages with partial replaced meat proteins by pea and potato proteins are described. Table 3.2 show product abbreviations and protein composition of the pork sausages.

Table 3.2: Protein composition of the pork sausages.

Product	Name of non-smoked sausages	Name of smoked sausages	Pea protein	Potato protein	Meat protein
RE00	RE00N	RE00S			100%
PE10	PE10N	PE10S	10%		90%
PE30	PE30N	PE30S	30%		70%
PE50	PE50N	PE50S	50%		50%
PP10	PP10N	PP10S	6.6%	3.4%	90%
PP30	PP30N	PP30S	19.8%	10.2%	70%
PP50	PP50N	PP50S	33.0%	17.0%	50%

3.2.1 Pork sausage production and moisture loss

In this project, a pork sausage recipe complying with the Nordic Keyhole nutrition label regulation (maximum 2% salt and 10% fat content in meat sausages) was used as the basic recipe [72]. In table 3.3, the composition of the seven types of pork sausage batters are detailed. The recipes were calculated with knowledge of the average content of protein and fat in lean pork cuts and pork fat (see table 3.4) [71], lean meats ability of binding water (0.3% of its weight [73]), and the measurements of WHC, water content, and protein content of the texturised protein materials.

During batter and sausage preparation, grounded lean pork cuts were mixed together with T1 or T2 in a high speed bowl cutter (Kilia, Neumünster, Germany). The texturised protein materials had been saturated in ice water for minimum 20 min before mixing. T1 and T2 were not added to the reference batter. Ice water, NaCl, NO_2^-, PO_4^{3-}, Kryta Frankfurter mix (dextrose, pepper, paprika, nutmeg, stock, hydrolysed corn protein, ascorbic acid, tarragon, garlic, coriander, cumin, yeast extract, natural pepper extract, and celery seeds), and grounded pork fat were then added, and the mixtures was cut and emulsified to fine pork sausage batters.

The pork sausage batters were stuffed into natural lamb casings with a diameter of 18-22 mm on a VF50 vacuum filling stuffer (Handtmann, Germany) giving each sausage an approximate weight of 65 g. The raw pork sausages were divided into two heating treatments; a non-smoked and a smoked. These treatments were primary chosen to assess the effects of smoke on smell, flavour, and taste, which will not be covered in this project. Furthermore, the sausages were divided into batch A, B, and C according to upper, middle, and lower placement in the oven, respectively. Each batch was weighed before cooking.

The cooking process parameters of the Doleschal thermal system (Inject Star Maschinenbau, Hagenbrunn bei Wien, Austria) were: cooking at 80°C for 15 min, drying at 60°C for 10 min, smoking or cooking at 60°C for 10 min for smoked or non-smoked sausages, respectively, cooking at 80°C for at least 20 min or to the core temperature of the sausage reaches 75°C, ventilation at 50°C for 2 min, and finally cooling by water sprinkling for 8 min. The final pork sausages were weighed to calculate the cooking loss of each batch of the sausages. The sausages were further cooled overnight in a cold room at 5°C.

After cooling, the pork sausages were weighed again to calculate the cooling loss. The diameter of the finished sausages were between 19 and 24 mm. The pork sausages were vacuum packed with a vacuum machine (Röscher Matic, Germany) in 250 mm X 300 mm (60 micron) sous vide bags (Sealed Air, Charlotte, NC, USA) for sensory analysis and 200 mm X 500 mm X 0.090 mm vacuum bags (LogiCon Nordic, Kolding, Denmark) for the rest of the analyses. Furthermore, approximately 250 gram of non-cooked batters of each pork sausage batter type were vacuum packed in 200 mm X 500 mm X 0.090 mm vacuum bags (LogiCon Nordic, Kolding, Denmark). The finished pork sausages and pork sausage batters were stored at different temperatures and for a different period of time depending on method used for functionality and texture assessment. In table 3.5, the different storage conditions of the sausages and batters are detailed.

Table 3.3: Ingredient composition of the seven pork sausage batters.

Ingredients	RE00	PE10	PE30	PE50	PP10	PP30	PP50
Lean pork cut [% w/w]	72.4	62.6	44.1	26.8	63.1	45.2	28.1
Water – bound to lean pork [% w/w]	21.7	18.8	13.2	8.0	18.9	13.6	8.4
Pork fat [% w/w]	2.3	4.4	8.4	12.1	4.3	8.1	11.8
PO_4^{3-} [% w/w]	0.3	0.3	0.3	0.3	0.3	0.3	0.3
NaCl [% w/w]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
NO_2^- [% w/w]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Kryta Frankfurter mix [% w/w]	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Texturised pea protein [% w/w]		2.5	7.3	11.8			
Texturised pea-potato protein [% w/w]					2.4	7.2	11.7
Water – bound to texturised protein [% w/w]		8.1	23.4	37.6	7.6	22.3	36.4
Total [% w/w]	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 3.4: Protein and fat content of lean pork cut and pork fat. All measurements were expressed as the mean ± SD [71].

Product	Protein content [g/100 g]	Fat content [g/100 g]
Lean pork cut	18.4 ± 0.5	12.2 ± 1.5
Pork fat	10.6 ± 0.4	50.8 ± 2.3

Table 3.5: Storage conditions of pork sausage batters and pork sausages.

Method	Product	Storage temperature	Storage time
Rheological analysis	Pork sausage batters	-40°C	17 days
		+5°C	3-5 days (thawing)
LF-NMR	Pork sausage batters	-40°C	14 days
		+5°C	2 days (thawing)
LF-NMR	Pork sausages	+5°C	1 day
		+0°C	13 days
		+5°C	2 days
Chemical analysis	Pork sausages	+5°C	1 day
		+0°C	20 days
		-18°C	0-58 days
		+5°C	1 day (thawing)
Sensory texture analysis	Pork sausages	+5°C	1 day
		+0°C	4-6 days
Instrumental texture analysis	Pork sausages	+5°C	1 day
		+0°C	43 days
		+5°C	1 day

3.2.2 Rheological analysis of pork sausage batters

Rheological measurements of the seven different pork sausage batters in triplicates were carried out with Kinexus Pro+ rotational rheometer (Malvern Instruments, UK). The measured data were registered with rSpace for Kinexus Pro 1.3 software. The samples were measured using 40 mm diameter serrated parallel steel plate geometry with 1 mm gap. After trimming the samples, a cylindrical cover was placed over each sample in order to create a closed, saturated volume round the sample and to prevent evaporation of the sample. The temperature of the samples was 5°C (sausage preparation temperature) controlled with an accuracy of ±0.1°C, by Peltier system of the rheometer. After temperature equilibrium, an oscillation amplitude sweep test with a constant frequency at 1 Hz and a controlled shear stress starting at 0.1 Pa was performed. The linear viscoelastic region (LVER) was measured at low deformation, where elastic (storage) modulus, G' and viscous (loss) modulus, G" were constant. A set of triggers (5% increase or decrease over 5 points of G* (complex modulus), G', or G" and 10% increase or decrease over 5 points of phase angle) determined the ultimate disruption of batter structure, called the yield point, which was the end of LVER. From the amplitude sweep test, a stress within the LVER was calculated, which was used in a final frequency sweep test from 10-0.1 Hz. The viscoelastic properties of the pork batters were described in terms of several rheological parameters.

3.2.3 LF-NMR relaxometry of pork sausage batters and pork sausages

Low-field NMR (LF-NMR) measurements of the seven pork sausage batters and the 14 pork sausages were performed in triplicate on a Bruker mq20 minispec NMR analyser (Bruker, Billerica, MA, USA) with a 0.47 T permanent magnet equivalent to 20-MHz proton resonance frequency held at constant 40°C. Each sample was placed in sample tubes and kept at 40°C for at least 15 min before measurement. Spin-spin transverse (T_2) relaxation times were determined using the Carr-Purcell-Meiboom-Gill (CPMG) sequence for all measurements [74]. Each sample was run with 16 scans, a 30 s recycle delay, and 8,000 echo maxima were recorded with a π-pulse separation of 40 µs.

Transverse relaxation times, T_2, were determined from the CPMG curves by multiexponential fitting of the experimental data using a weighed sum of exponential decays written in MATLAB, version R2017b (MathWorks, Natick, MA, USA) according to the following equation [75]:

$$I(t) = \sum_{n=1}^{N} Mn \cdot e^{-t/T_2 n}$$

where N is the number of water components, T_{2i} is the relaxation rate for component *i* and M*i* is the corresponding weight of component *i*. From the M*i* values the relative abundance of component *i*, R*i*, can be defined as:

$$Ri = 100\% \cdot Mi / \left(\sum_{n=1}^{N} Mn \right)$$

Differences in NMR parameters, measurement temperatures, and types of sausages can cause variations in T_2 relaxation times [76]. However, Bertram and Andersen (2004) proposed that the fastest relaxing component sometimes reported in meat is referred to as T_{21}. T_{21} is characterised by a time constant between 0 and 10 ms, and it represents protein-associated water or water tightly bound to macromolecules. The major relaxation component, T_{22}, is characterised by a time constant of approximately 35-50 ms. This component is ascribed to water trapped within the protein-dense myofibrillar network and often represents 80-95% of the water in meat. The slower relaxing component, T_{23}, is characterised by a time constant of approximately 100-250 ms and represents 5-15% of the water in meat. T_{23} is ascribed to water located outside the myofibrillar protein network, i.e. extra-myofibrillar water, which is only held by capillary forces [77, 78].

3.2.4 Sensory texture analysis of pork sausages

A modified quantitative descriptive analysis was performed for the sensory texture evaluation of the 14 pork sausages by a trained sensory panel of 10 assessors. During two training sessions, the assessors were exposed to extremes of the products, in order to develop a consensus vocabulary describing the texture of the sausages. The final vocabulary consisted of the texture attributes: firmness, juiciness, cohesiveness, gumminess, grittiness, chewing time, and chewing residual. A 15 cm line scale was used for the evaluation of the texture attributes.

A trial evaluation and the final product evaluation was performed by each assessor in individual booths. The pork sausages were heated in water baths at 70°C to achieve a serving

temperature of approximately 60°C (59.0-65.4°C). The sausages were weighed before and after heating to calculate the heating loss. Each sausage sample was identified with a random three-digit code and standardised to the length of 7 cm (with no sausage ends) before being placed on a separate white ceramic plate. The evaluations were performed in a total of three blocks corresponding to assessment days. Hence, all sample assessments were performed in triplicates (batch A, B, and C). Within a block, all 14 different pork sausages were evaluated in a random order by the assessors. However, due to heating treatment of the sausages, the assessors evaluated the samples in the same order. The assessors were instructed to cleanse their mouth with water or sparkling water and cucumber or melon between the sample evaluations to reduce carry over between samples.

An assessor analysis was performed with PanelCheck software, version 1.4.2 to examine the performance of the sensory panel. PanelCheck was used after the trial evaluation as part of training and after final assessment session to evaluate the panel's consensus and capability of replicating and discriminating between samples.

3.2.5 Instrumental texture analysis of pork sausages

Texture measurements of the 14 different sausages were determined in quadruplicate for each batch A, B, and C (total 12 replicates) using a TA-TX plus 100 texture analyser (Stable Micro System, Surrey, UK) fitted with a 35 mm diameter cylindrical probe. The samples were stored in a 5°C refrigerator, but were analysed at room temperature shortly after removed from the refrigerator. The conditions were as follows: pre-test speed, 1.0 mm/s; test speed, 1.0 mm/s; post-test speed, 10.0 mm/s; trigger type, auto; and trigger force, 0.05 N. Each sample (diameter 17 mm and 25 mm height) was compressed at the distance of 20 mm. Firmness of the pork sausages was defined as the resistance force at the compression.

3.3 Chemical analyses of texturised protein samples and pork sausages

Chemical analyses were performed to measure the water and protein content of T1 and T2 and the water, protein, and fat content of the pork sausages (data from the chemistry laboratory at DMRI). Before analyses, the texturised protein samples were finely grounded, whereas six pork sausages (two from each batch) were blended to a homogeneous mixture.

Water, protein, and fat content were determined according to NMKL method 23 [79], a modified AOAC Official method 981.10 [80], and a modified ISO 1443-1973 method [81], respectively. These methods are briefly outlined below.

Gravimetric determination of the water content involved the drying of the samples in duplicate at 102-105°C in a drying oven until constant weight. The total weight loss indicated the moisture loss. The method had an uncertainty of ±0.7%, rel. on the double determination [79, 82].

The total nitrogen of the samples were determined in duplicates with a Kjeltec-Tecator system (Foss Analytical A/S Denmark). The samples were digested with concentrated H_2SO_4 and a catalyst mixture at 410°C. NaOH was added to liberate the NH_3 distilled into a receiver containing H_3BO_3 indicator. The absorbing solution was titrated with a 0.1 M HCl. The crude protein content was calculated as % nitrogen × 6.25. The method had an uncertainty of ±3.1%, rel. on the double determination [80, 83].

The fat content of the sausage samples was determined in duplicates with the use of Hydrotec™ 8000 hydrolysis system (Foss Analytical A/S Denmark) and Soxtec™ 8000 extraction system (Foss Analytical A/S Denmark). The sausage samples were boiled with HCl to free the occluded and bound lipid fractions. The resulting solid (the fat), was filtrated, dried, and extracted with light petroleum. This method had an uncertainty of ±0.37 g/100 g on the double determination [81, 84].

3.4 Statistical analysis

Statistical analysis was performed with the software R Studio, version 1.1.453 (R Studio, Boston, MA, USA). The data were analysed using least square means that were considered to be significantly different when $P < 0.05$. Data in tables is presented as mean values with ± standard deviation (SD). Multivariate statistical analysis in chemometrics was performed using the data analytical software, LatentiX, version 2.12 (LatentiX, Frederiksberg, Denmark). Principal component analysis (PCA) models were auto-scaled and random validated.

In this study, the effect of smoke on functional and textural properties of the pork sausages was statistically tested. No smoking effects were seen in moisture loss and water distribution and mobility. However, statistical analysis of the sensory texture results was inconsistent. The effect of smoke was small, but significant ($P < 0.05$) on firmness, chewing time, and chewing residual in pork sausages with texturised pea proteins. A small ($P < 0.05$) effect was also found on firmness and cohesiveness in pork sausages with texturised pea-potato proteins. Smoke had no effect on the other sensory attributes. A statistical analysis also revealed that smoke had a significant ($P < 0.001$) effect on instrumental firmness of the pork sausages with texturised pea proteins, whereas no effect was found on the pork sausages with texturised pea-potato proteins. In this study, the effect of smoke was considered minimal as the statistical results were inconsistent and each sausage type received the same temperature and length of heat treatment.

4. Results

This section will describe the results obtained in the thesis. The results can be divided into the three parts of the product development process: ingredient, semi-finished product, and finished product (Figure 4.1). Figure 4.1 details the applied methods and their outcomes that were used to assess functionality and texture of the ingredients and products.

The ingredient assessments includes the results from the WHC method, the water and protein analyses, and the liquid-state ^{1}H and solid-state ^{13}C NMR spectroscopy of the raw and texturised protein samples. The results were compared between the protein samples in the following way: R1 vs. R2, raw vs. texturised protein samples, and texturised protein samples vs. reference sample and each other (Figure 4.2).

The semi-finished product assessment included rheological and LF-NMR measurements of the pork sausage batters (Appendix 7.2). Generally, the rheological measurements revealed that the batter structure had non-Newtonian shear-thinning behaviour (decreased viscosity under shear strain, power law index, $n < 1$) and was solid at rest (phase angle, $\delta < 45°$) (Table 7.3 in Appendix). The data obtained by oscillation frequency sweep tests indicate elastic-dominant behaviours (i.e. solid-like, $\tan\delta < 1$) of the sausage batters (Figure 7.1 in Appendix). Furthermore, the rheological results revealed that the reference sausages had the highest complex viscosity, whereas pork sausages with 30% meat proteins replaced by texturised pea-potato proteins had the lowest complex viscosity. The complex viscosity values of pork sausages with 50% substituted meat proteins were closest to the complex viscosity of reference sausages followed by pork sausages with 10% substituted meat proteins (Figure 7.2) [85].
The LF-NMR relaxometry data showed that the reference sausages and pork sausages with 10-30% meat proteins replaced by texturised pea-potato proteins contained one water population, pork sausages with 10-50% substituted meat proteins by texturised pea proteins contained two water populations, whereas sausages with the highest content of texturised pea-potato proteins contained three water populations (Table 7.4 in Appendix). The results from rheology and LF-NMR relaxometry will not be further described in this section due to relatively high standard deviations between the replicates.

The finished product assessment involved analysis results of the cooked pork sausages. The methods included chemical composition, moisture loss, LF-NMR relaxometry, sensory texture, and instrumental texture measurements. Figure 4.2 outlines the result comparison of the samples. Results of pork sausages with 0-50% meat proteins replaced by texturised vegetable (pea or pea-potato) proteins were compared. Furthermore, results of pork sausages with pea proteins (replacing 10-50% meat proteins) were compared with pea-potato proteins (replacing 10-50% meat proteins).

In this section, results obtained from the ingredient assessment will initially be described. This will be followed by results from the finished product assessment. The section will end with results obtained by comparative analyses involving different assessment methods.

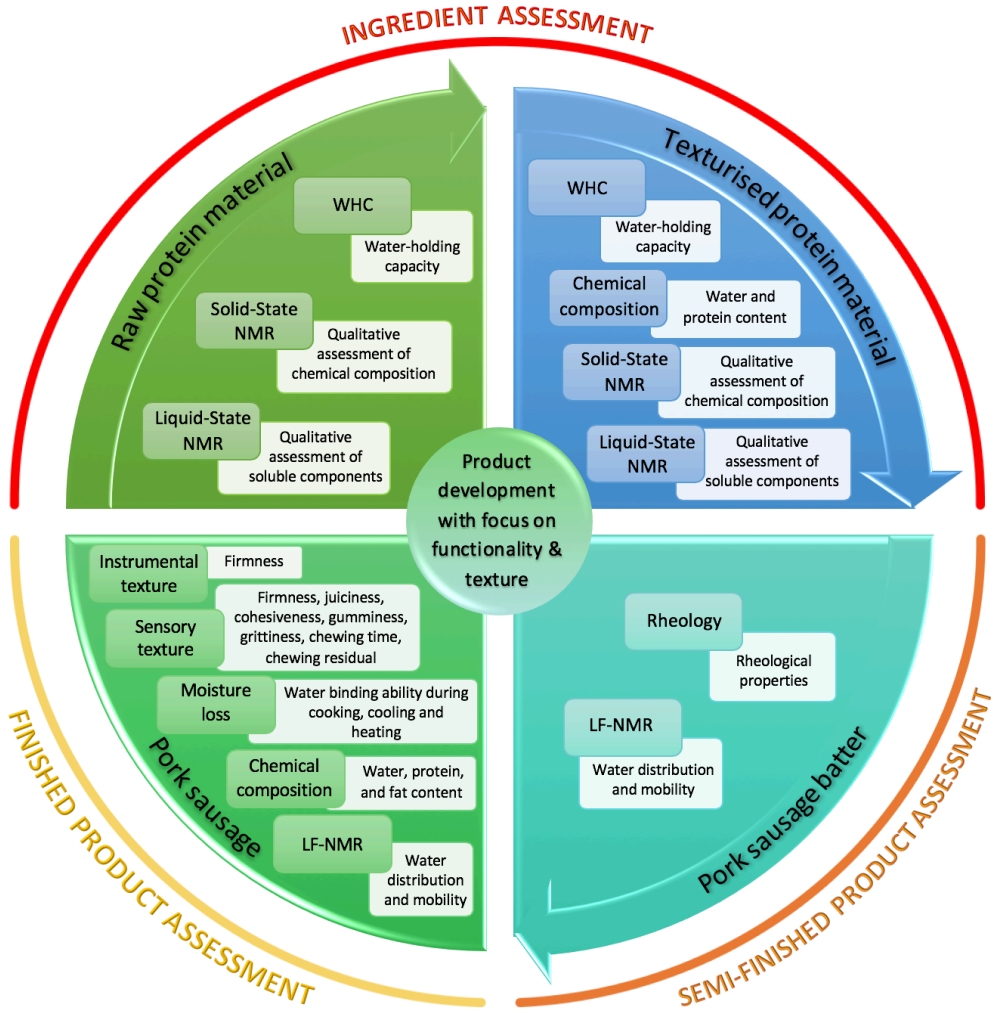

Figure 4.1: Illustration of the pork sausage development of this study with methods used to investigate functional and textural properties. Ingredient assessment (red) involved analyses of pea and potato protein samples. Semi-finished product assessment (orange) consisted of pork sausage batter analyses. Finished product assessment (yellow) involved analyses of the pork sausages.

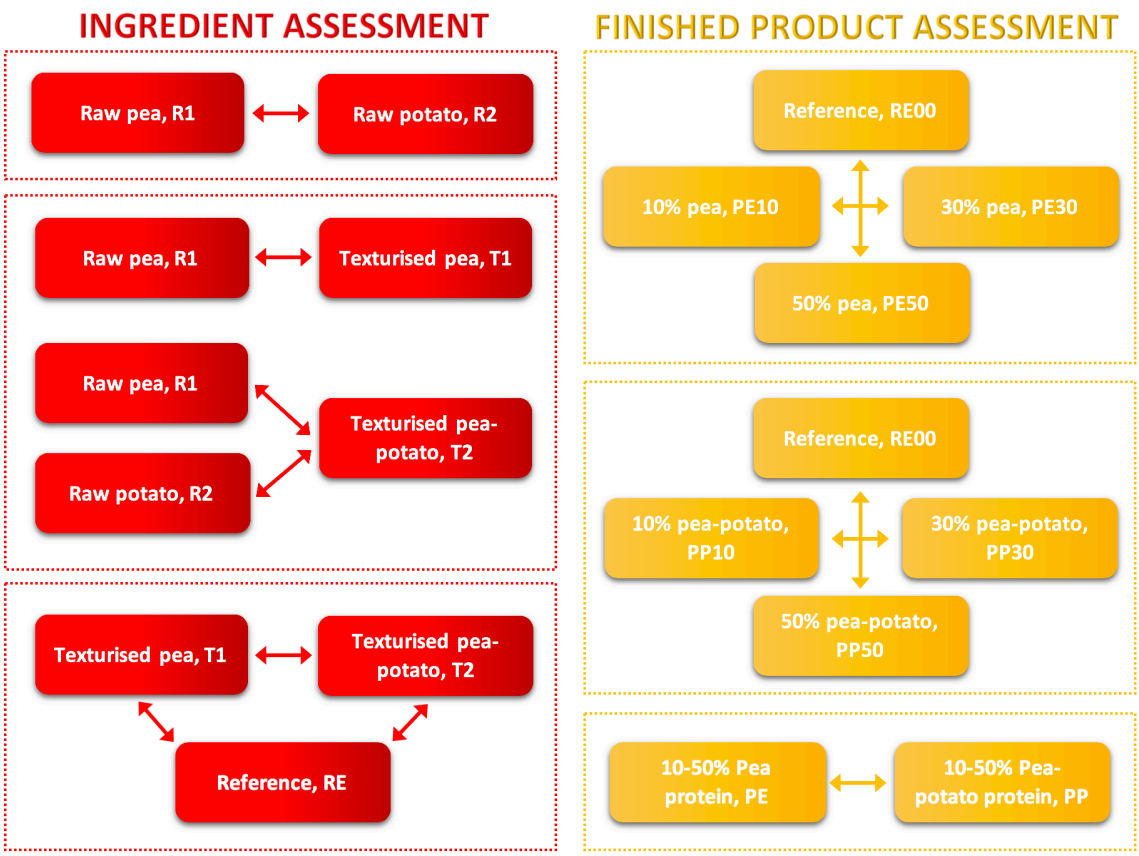

Figure 4.2: Comparison of the results during ingredient (red) and finished product (yellow) assessment.

4.1 Ingredient assessment

4.1.1 Water-holding capacity

To investigate how texturisation affected pea and potato protein materials' ability to bind and retain water in their network, WHC measurements were performed. The WHC data of the four protein samples (R1, R2, T1, and T2) are given in Figure 4.3.

The data reveal that the WHCs of the raw vegetable protein concentrates (R1 and R2) were significant different ($P < 0.001$). Figure 4.3 also illustrates that the WHC of the pea protein material increased significantly ($P < 0.001$), from 1.22 to 3.18 g water per g sample, by texturisation. The WHC of T2 was significantly higher ($P < 0.001$) than the WHC of R1. As the WHC of potato protein concentrate was high, no significant difference was observed between the WHC of T2 (3.12 g water per g sample) and R2 (3.08 g water per g sample). Hence, texturisation increased the WHC of pea protein concentrate but did not affect the WHC of potato protein concentrate. The WHC of the two texturised protein samples was not significant different.

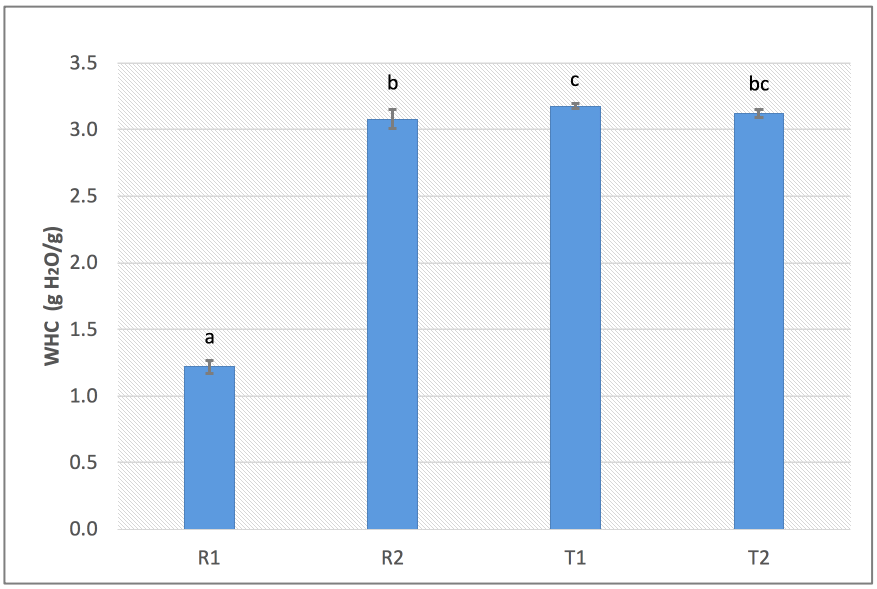

Figure 4.3: Effect of texturisation on raw pea protein concentrate (R1) and potato protein concentrate (R2) on WHC. Texturised products: pea protein concentrate mix (T1) and 3:1 pea-potato protein concentrate mix (T2). All measurements were expressed as the mean with error bars showing ± SD (n = 4). Different superscripts (a-c) above bars indicate significant differences ($P < 0.001$).

4.1.2 Solid-state ^{13}C NMR spectroscopy

Solid-state ^{13}C NMR spectroscopy was used to obtain compositional information of the protein samples (R1, R2, T1, T2, and RE). In Table 4.1, the ^{13}C chemical shift assignments observed in NMR spectra of protein materials are given [86–88]. The solid-state ^{13}C NMR spectra of the five different protein samples are presented in Figure 4.4. The CP/MAS experiments (Figure 4.4A) only detect the resonances originating from the immobile regions of the carbon sites in primarily polysaccharides and proteins, whereas the SP/MAS experiments (Figure 4.4B) detect all carbon signals quantitatively correct [87]. With linear combination of SP/MAS and CP/MAS spectra, a weighed difference spectrum (Figure 4.4C) can be obtained, displaying only resonances from carbon in the mobile regions, which in the present study only includes lipids [86].

CP/MAS NMR spectra can be divided into six regions based on chemical compound groups (Table 4.1). In Figure 4.4A, the CP/MAS NMR data indicate both similarities and differences between the composition of R1 and R2. In both spectra, a broad line shape covering the spectral range of 10-55 ppm originates from the aliphatic amino acid residues in the proteins. If any crystalline fat is present, it will also be detected in this region. In the range 55-80 ppm, the resonances from aliphatic amino acid residues overlap with resonances from polysaccharides. The CP/MAS spectra of R1 and R2 differentiate in this region, having broad, intense peaks at 72 and 55 ppm, respectively. The spectral region 80-110 ppm includes resonances from carbons in polysaccharides, which are most pronounced in R1. The spectrum of R1 contains several resonances in the range 90-110 ppm, which are characteristic for the anomeric carbons in the polysaccharides. Resonances with chemical shifts of 102-107 ppm indicate that the major polysaccharide components in R1 are cellulose and starch [89, 90]. These components were less pronounced in R2. The spectral region 110-165 ppm in CP/MAS spectra of R1 and R2 includes resonances from carbons in the aromatic side chains of amino acids with a resonance at 156-160 ppm characteristic for Cζ in either arginine or tyrosine. At 175 ppm, both spectra contain a broad, intense resonance from the carbonyl carbons in the peptide bonds of proteins.

In Figure 4.4A, the CP/MAS NMR data also reveal resonance differences as a result of texturisation. Generally, the CP/MAS spectra of R1 and T1 show the same peaks in the range 0-200 ppm, while the CP/MAS spectrum of T2 (combination of R1 and R2) reveals a combination of resonances from the spectra of R1 and R2. However, the peaks between the raw and texturised samples differentiate in their intensities. Hence, texturisation of the raw

protein concentrates alters the composition of polysaccharides and proteins, which can be caused by the denaturation and restructuring of the compounds during extreme extrusion conditions.

The CP/MAS spectra indicate many similarities in the polysaccharide and protein composition of T1 and T2 (Figure 4.4A). Although, the resonances from aliphatic amino acid residues and polysaccharides in the range 55-80 ppm differentiate between the two texturised samples corresponding to the contribution from the potato protein material to T2. In order to compare the CP/MAS data of RE with the texturised protein samples, the spectrum has been scaled up as pork sausage batter contains more water. The CP/MAS spectrum of RE reveals resonances in the spectral region 0-75 ppm, 126-140 ppm, and 175 ppm. These resonances primarily correspond to carbons in amino acids of proteins. Thus, in contrast to T1 and T2, RE contains only small amounts of carbohydrates.

Additional resonances from lipids are present in the SP/MAS spectra of the five protein samples and these resonances are highlighted in the weighed difference spectra of the samples (Figure 4.4C). All spectra show a narrow resonance at 14.7 ppm originating from the methyl groups of the fatty acids, followed by a series of narrow resonances in the range 20-35 ppm that originate from CH_2 groups in different environment in the fatty acid chain. However, these resonances are considerably lesser pronounced in the spectrum of R2 than the other spectra. Thus, R2 seems to only contain small quantities of lipids. The difference spectra of T1 and T2 reveal more pronounced resonances in the range 20-35 ppm than R1, which correspond to the addition of sunflower oil to the texturised materials. In the spectra of R1, T1, T2 and RE, two narrow resonances at 128.6 and 130.3 ppm originate from the unsaturated carbons in lipids and the resonance at 172.3 ppm originates from ester carbonyl carbon in triglycerides. The spectra of T1, T2, and RE have many similarities. However, the spectrum of RE differs from the other samples by differences in intensity of the two narrow resonances at 128.6 and 130.3 ppm. Hence, only small amounts of polyunsaturated fatty acids are present in the reference sample.

Table 4.1: Assignments of ^{13}C chemical shifts observed in NMR spectra of protein materials [86–88].

Compound	δ_{13C} [ppm]
CP/MAS spectrum – Proteins and polysaccharides:	
1) Aliphatic side chains, Cα and Cβ from the amino acids in proteins, and crystalline fat	0-80
2) Polysaccharides	55-110
- Anomeric carbons in polysaccharides	90-110
4) Carbons in the aromatic side chains of the amino acids in proteins	110-165
5) Cζ in either arginine or tyrosine	156-160
6) Carbonyl carbons in the peptide bonds (primarily) and esters and acid groups in the polysaccharides (broad, intense resonance)	175
Linear combination of the SP/MAS and CP/MAS spectra – Lipids:	
1) Methyl groups of fatty acids (narrow resonance)	14.7
2) CH_2 groups in different environments in the fatty acid chain (series of narrow resonances)	20-35
3) Glyceryl in triglycerides	62.5 and 69.7
4) Unsaturated carbons in lipids (two narrow resonances)	128.6 and 130.3
5) Ester carbonyl carbon in triglycerides	172.3

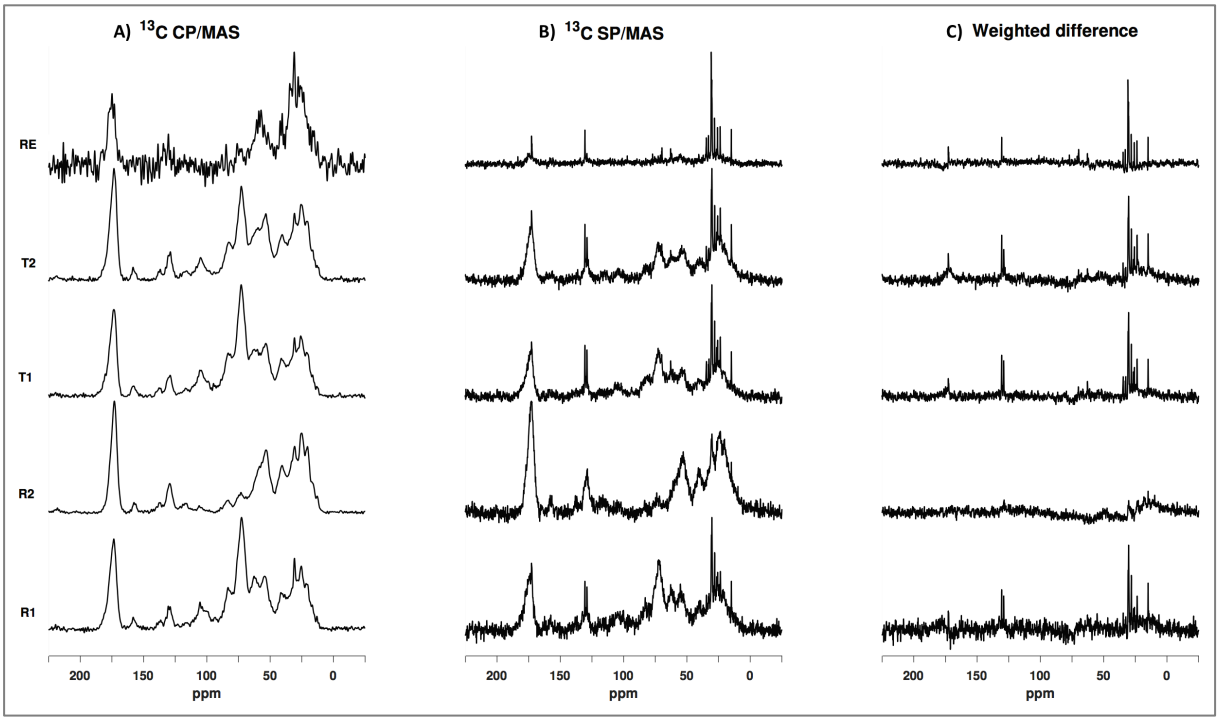

Figure 4.4: A) ^{13}C CP/MAS NMR, B) ^{13}C SP/MAS NMR, and C) linear combination of the SP/MAS NMR and CP/MAS NMR spectra (0-200 ppm) of raw pea protein sample (R1), raw potato protein sample (R2), texturised pea protein sample (T1), texturised pea-potato protein sample (T2), and reference sample without vegetable proteins (RE). All spectra are vertically scaled relative to the most intense resonance in the spectrum with the exception of the difference spectrum of R2.

4.1.3 Liquid-state ^{1}H NMR spectroscopy

Liquid-state ^{1}H NMR spectroscopy was used to obtain qualitative information of the soluble components in aqueous suspensions of the protein samples (R1, R2, T1, T2, and RE). Thus, only hydrogens from lipids, small carbohydrate, amino acids, or other small molecules are observed in ^{1}H NMR spectra (Figure 4.5). Generally, the spectral region 0.6-3.0 ppm contains resonances from aliphatic protons from lipids, amino acids and organic acids. The range 3.0-6.0 ppm contains resonances primarily from carbohydrates but also from protons in unsaturated lipids, glycerol backbones, and amino acids (α/β protons). In the higher range of 6.0-11.0 ppm, resonances from aromatic protons mainly from amino acids can be observed [86, 88, 91].

When comparing the ^{1}H NMR spectra of R1 and R2, significant differences are observed. In the total spectral region 0-11.0 ppm of R1, the resonances are much more pronounced than the spectrum of R2. R1 contains several soluble components. In the spectral region 2.5-2.8 of R1, an AB-system with four peaks from citrate can be seen. This organic acid is probably used as a preservative in the pea protein concentrate. The R1 spectrum is dominated by overlapping resonances from pyranosic carbohydrate protons in the range 3.0-4.5 ppm. Furthermore, distinctive resonances from anomeric protons in galactose and glucose units at 5.0 and 5.4 ppm, respectively, can be assessed. Finally, the higher ppm range of the R1 spectrum contains several resonances from aromatic protons originated from amino acids in soluble protein. In the R2 sample, only minor fractions are soluble.

The ^{1}H NMR spectra of R1 and the texturised samples have many similarities as resonances from soluble citrate, carbohydrates, and proteins can be observed. However, enlarging the spectra reveals small differences in the resonance intensities indicating that texturisation of the raw protein materials changes the content of soluble compounds.

Many similarities are observed in the ^{1}H NMR spectra of T1 and T2, whereas the ^{1}H NMR spectrum of RE differs considerably from these spectra. The spectrum of the reference sample reveals a resonance doublet at 1.30-1.35 ppm characteristic for lactate. Lactate is naturally occurring in meat as it is synthesised during post mortem glycolysis [77]. The 3.0-6.0 ppm range reveals only small amounts of soluble carbohydrates in the RE sample relative to T1 and T2. Finally, in the spectral region 5.5-8.5 ppm, the reference sample has more distinctive resonances from amino acids, such as histidine, than the texturised vegetable samples.

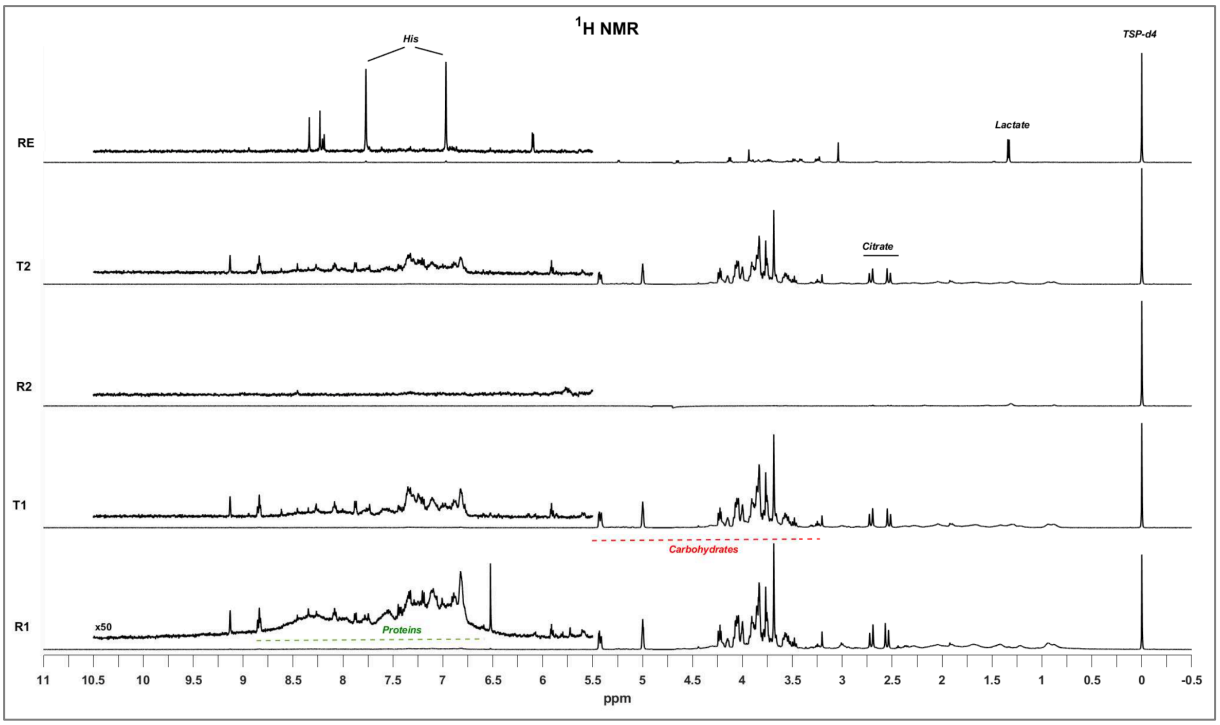

Figure 4.5: ^{1}H NMR spectra (0-11 ppm) of raw pea protein sample (R1), raw potato protein sample (R2), texturised pea protein sample (T1), texturised pea-potato protein sample (T2), and reference sample without vegetable proteins (RE). The spectral region 5.5-11 ppm is scaled by 50.

4.1.4 Water and protein content and essential amino acid composition

Chemical analyses were performed on the two texturised protein samples (T1 and T2) to measure the experimental water and protein content (Table 4.2). T1 contained 1.8 g water and 52.5 g protein per 100 g sample, whereas T2 contained 1.7 g water and 55.0 g protein per 100 g sample.

From the compositional data declared by the producers, the theoretical relative protein composition of T2 (3:1 pea-potato protein concentrate mix) was calculated to be 66% pea proteins and 34% potato proteins. With a water content of 1.7 g per 100 g sample, the protein content in T2 should have been about 59.4 g per 100 g sample. However, the experimental protein content was 55.0 g per 100 g sample (Table 4.2). No protein content alteration was observed after extrusion of exclusively pea protein concentrate. The lower protein content of T2 suggests that there is a reduction in potato proteins relative to pea proteins, as potato protein concentrate has a higher content of protein compared to pea protein concentrate (85% vs 55% on a dry basis, respectively). To further investigate the potato protein content in T2, the CP/MAS NMR data were analysed. The relative composition of pea and potato proteins was estimated from a weighed difference spectra of T2 and the weighed sum of T1 and R2 (Figure 4.6). These spectra revealed a relative composition of about 70% pea proteins and 30% potato proteins, which corresponds to the theoretical relative pea/potato protein composition (66%/34%). Thus, for the rest of the present study the relative pea/potato protein composition of T2 is considered to be 66%/34%, even though we recognise the inconsistency in results between theoretical and experimental protein analyses.

In Table 4.3, the essential amino acid composition of pork, R1, R2, and pea-potato protein mixture are presented. These compositions are compared with WHO/FAO/UNU adult essential amino acid requirements highlighting deficient values with red [7]. The data indicate that R1 has an insufficient content of nine out of 11 essential amino acids. Only the content of phenylalanine and tyrosine is above the requirements [22]. In contrast, R2 has a low phenylalanine and tyrosine content, while the content of the nine other amino acids are above the requirements [92]. The essential amino acid composition of T1 corresponds to R1, whereas the composition of T2 highly depends on the relatively composition of pea and potato proteins. Hence, the higher content of potato proteins relative to pea proteins, the higher amino acid values of essential amino acids with the exception of phenylalanine and tyrosine. Only pork meets the adult essential amino acid requirements [93].

Table 4.2: Compositional information of texturised pea protein sample (T1) and texturised pea-potato protein sample (T2). Water and protein content was experimentally measured and expressed as the mean (n = 2).

Product	Water content [g/100 g]	Protein content [g/100 g]
T1	1.8	52.5
T2	1.7	55.0

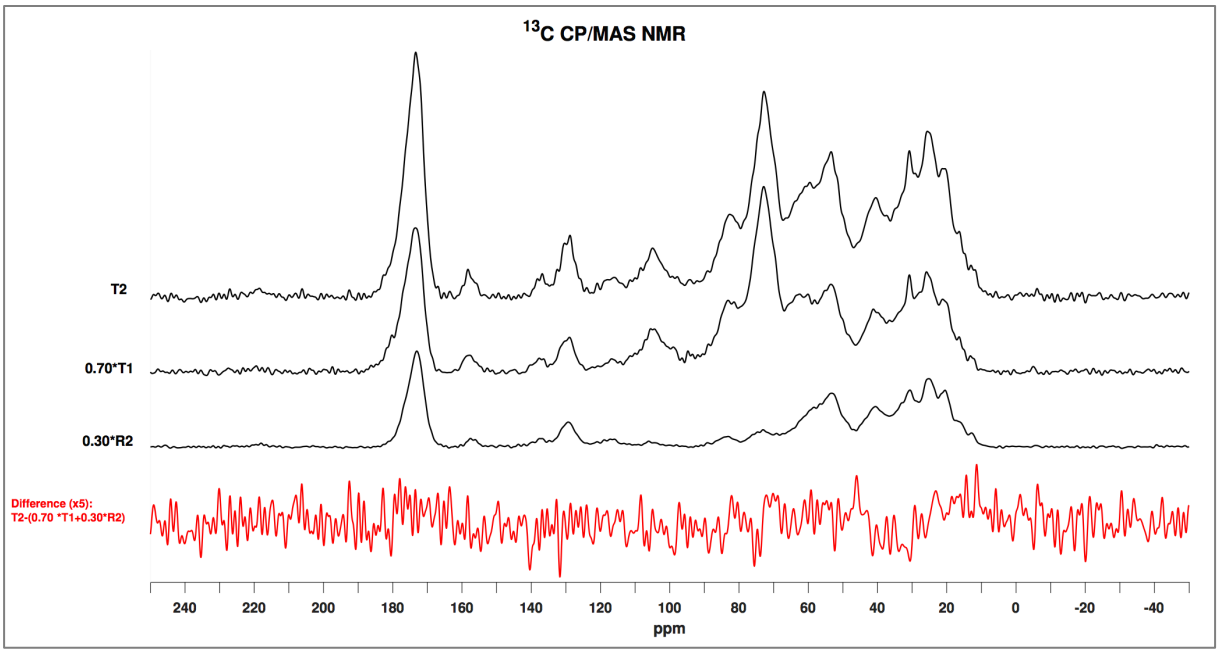

Figure 4.6: ^{13}C NMR spectra (-50-250 ppm) of texturised pea-potato protein sample (T2), texturised pea protein sample (T1) scaled by 0.70, and raw potato protein sample (R2) scaled by 0.30. The red spectrum is a linear combination of the ^{13}C NMR spectra of T2 and the weighed sum of T1 and R2 (T2 - (0.70 · T1 + 0.30 · T2)). The difference spectrum is scaled by 5 to visualise that only noise is detectable.

Table 4.3: WHO/FAO/UNU adult essential amino acid requirements and the essential amino acid composition of pork, pea protein concentrate (R1), potato protein concentrate (R2), and pea-potato protein mixtures with different protein composition. Amino acid values lower than the requirements are highlighted with red.

Essential amino acids	WHO/FAO/-UNU 2007[a] [mg/g protein]	Pork[b] [mg/g protein]	AMN pea protein concentrate[c] [mg/g protein]	KMC potato protein concentrate[d] [mg/g protein]	86% pea protein + 14% potato protein [mg/g protein]
Histidine	**15.0**	32.0	13.0	21.0	14.6
Isoleucine	**30.0**	49.0	21.5	54.0	28.0
Leucine	**59.0**	75.0	37.5	98.0	49.7
Lysine	**45.0**	78.0	37.5	79.0	45.8
Methionine + Cystine	**22.0**	38.0	10.3	36.0	15.5
Phenylalanine + Tyrosine	**38.0**	71.0	41.4	11.6	35.4
Threonine	**23.0**	51.0	18.5	57.0	26.2
Tryptophan	**6.0**	14.0	4.9	14.0	6.7
Valine	**39.0**	50.0	23.5	63.0	31.4

[a] [7], [b] [93], [c] [22], [d] [92]

4.2 Finished product assessment

4.2.1 Water, protein, and fat content and essential amino acid composition

Chemical analyses were performed to assess the content of water, protein, and fat in each type of the 14 different pork sausages (Table 4.4). This was done to be able to compare variations in the chemical composition with the functionality and texture results of the sausages. The data reveal that the water, protein, and fat content vary with 4.2 g, 2.7 g, and 1.8 g per 100 g sample, respectively (Table 4.4). In Figure 4.7, the visual differences of the pork sausages are shown.

Table 4.4: Water, protein, and fat content of the 14 pork sausage types. All measurements were expressed as the mean (n = 2).

Product	Water content [g/100 g]	Protein content [g/100 g]	Fat content [g/100 g]
RE00N	71.8	16.6	8.2
RE00S	71.0	17.3	8.4
PE10N	70.7	15.9	9.1
PE10S	71.1	16.0	8.9
PE30N	70.6	16.1	7.6
PE30S	70.0	16.1	7.7
PE50N	67.9	15.2	9.3
PE50S	67.7	14.6	9.4
PP10N	72.3	15.9	7.8
PP10S	71.7	15.7	8.0
PP30N	69.6	16.0[a]	8.8
PP30S	68.9	16.3	8.7
PP50N	67.6	16.0	9.0
PP50S	67.7	16.0	9.0

[a] too high SD between the duplicates in two analyses. The measurement was expressed as the mean (n = 4).

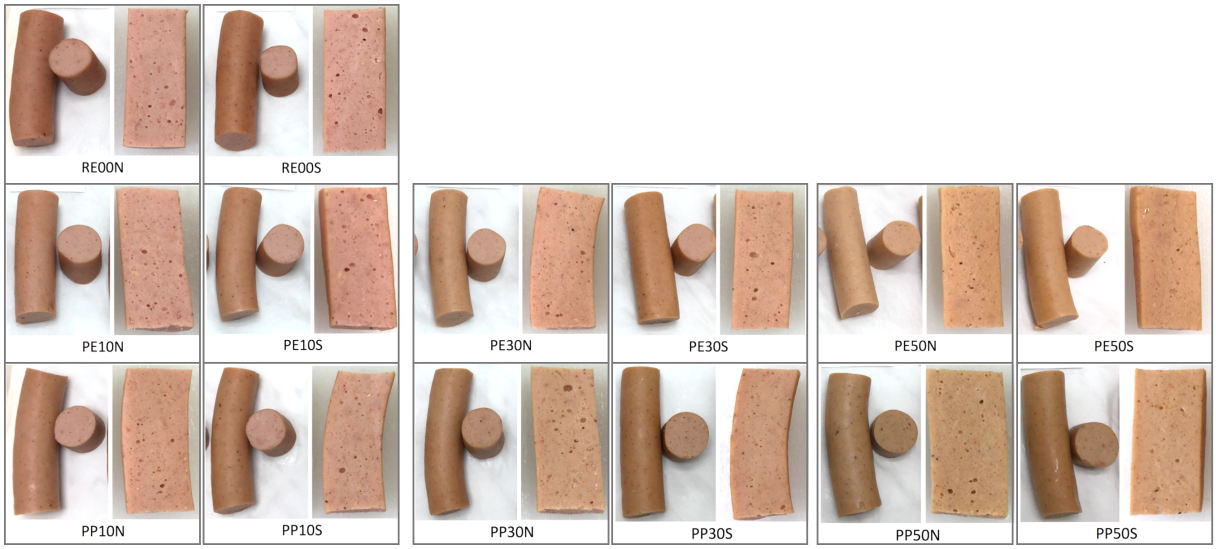

Figure 4.7: Pictures of the 14 pork sausage types with meat proteins replaced by texturised pea proteins (PE) or texturised pea-potato proteins (PP) in different concentrations (10-50%) and cooked with different smoking treatments (non-smoked or smoked). The reference sausages (RE) contained 0% texturised vegetable proteins.

The essential amino acid composition of each pork sausage depends on the relative composition of pork, pea, and potato proteins. Table 4.5 displays the theoretical content of the essential amino acids per g of protein in the pork sausages with 10-50% of the meat proteins replaced by texturised pea or pea-potato proteins. These amino acid values are compared with WHO/FAO/UNU adult essential amino acid requirements [7]. The sausage type with the highest concentration of pea proteins is deficient in the amino acids leucine and valine (highlighted in red). The theoretical essential amino acid contents in the other pork sausage types seem to be above the requirements.

Table 4.5: WHO/FAO/UNU adult essential amino acid requirements and amino acid composition in pork sausages with partially (10-50%) replaced meat proteins by texturised pea proteins (PE) or texturised pea-potato proteins (PP). Amino acid values lower than the requirements are highlighted with red.

Essential amino acids	WHO/FAO/-UNU 2007[a] [mg/g protein]	PE10 [mg/g protein]	PE30 [mg/g protein]	PE50 [mg/g protein]	PP10 [mg/g protein]	PP30 [mg/g protein]	PP50 [mg/g protein]
Histidine	15.0	30.1	26.3	22.5	30.5	27.4	24.3
Isoleucine	30.0	46.3	40.8	35.3	47.7	45.1	42.5
Leucine	59.0	71.3	63.8	56.3	73.9	71.8	69.7
Lysine	45.0	74.0	65.9	57.8	75.8	71.4	66.9
Methionine + Cystine	22.0	35.2	29.7	24.2	36.4	33.1	29.8
Phenylalanine + Tyrosine	25.0	68.0	62.1	56.2	66-7	58.2	49.6
Threonine	23.0	47.8	41.3	34.8	49.5	46.4	43.3
Tryptophan	6.0	13.1	11.3	9.5	13.5	12.5	11.5
Valine	39.0	47.4	42.1	36.8	49.1	47.3	45-5

[a] [7]

4.2.2 Moisture loss

The pork sausages ability to bind, immobilise, and retain water and fat in its gel network was measured as moisture loss during cooking, cooling, and pre-consumption heating. These measurements may indicate the functional properties of the proteins in the sausages [65]. Figure 4.8 illustrates the moisture losses of pork sausages with 0-50% meat proteins replaced by texturised pea (Figure 4.8A) or pea-potato (Figure 4.8B) proteins. Furthermore, the average moisture loss of the two protein types are presented in Figure 4.8C.

The data indicate that pork sausages without texturised proteins had significantly higher ($P < 0.01$) total moisture loss than sausages containing texturised proteins (Figure 4.8A,B). The high total moisture loss of the reference samples was especially because of a significantly higher ($P < 0.001$) moisture loss during cooking (green part of the bars). No significant differences in total moisture loss can been seen between the sausages with 10-50% meat proteins replaced by either pea or pea-potato proteins. Finally, Figure 4.8C reveals no significant differences in total moisture loss between pork sausages containing pea or pea-potato proteins. In addition, no interaction between protein type and concentration was observed.

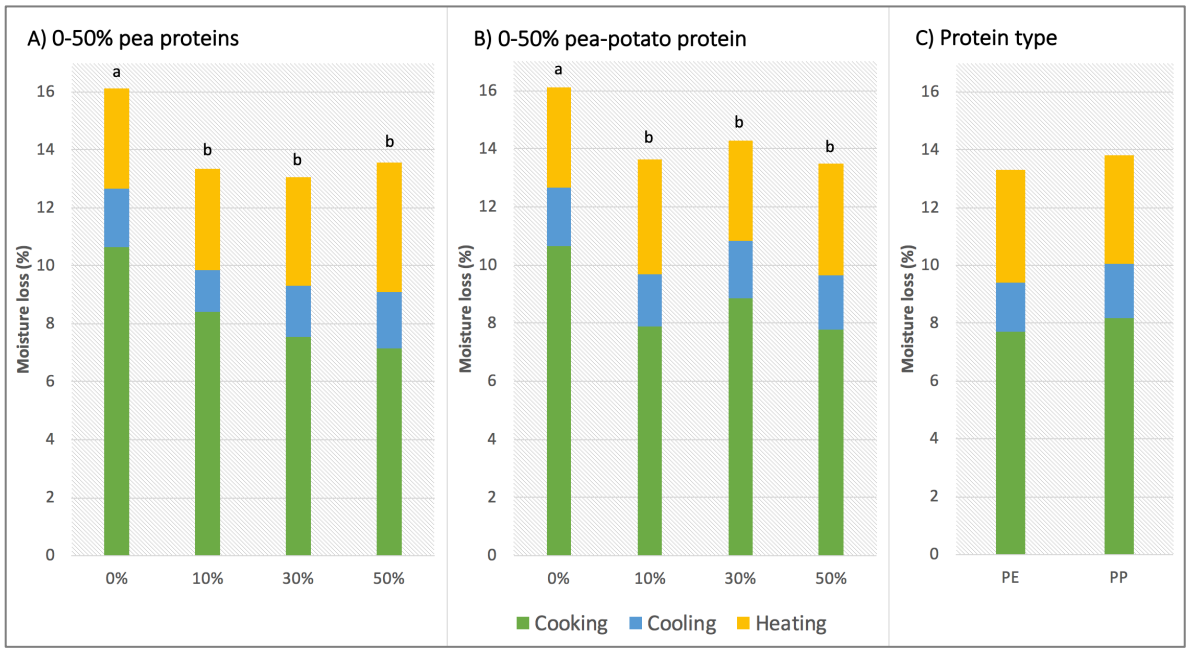

Figure 4.8: Effects of A) partially replacing meat proteins by texturised pea proteins (PE), B) partially replacing meat proteins by texturised pea-potato proteins (PP), and C) protein type on moisture loss of pork sausages during cooking, cooling, and heating. The reference sausages contained 0% texturised vegetable proteins. All measurements were expressed as the mean. Different superscripts (a-b) above bars indicate significant differences ($P < 0.01$).

4.2.3 LF-NMR relaxometry

LF-NMR relaxometry was performed to examine water distribution and mobility of the different pork sausages. Up to three peaks were identified in the sausages through the multi-exponential fitting of a T_2 distribution, which was used to assess relaxation times and relative abundance of hydrogen protons. The peaks are thought to be directly related to three water components, T_{21}, T_{22}, and T_{23}, in meat emulsions. Generally, the higher relaxation time, the looser the water is bound in the network [76, 77]. The results from the LF-NMR relaxometry of the pork sausages with meat proteins partially replaced by texturised pea or pea-potato proteins are presented in Figure 4.9A and Figure 4.9B, respectively. Figure 4.10 shows the effect of protein type on T_2 relaxation times and relative abundance in pork sausages.

The major relaxation component, T_{22}, is ascribed to water trapped within the protein-dense network of sausages [77]. For pork sausages with 100% meat proteins, this component had a time constant of 49.0 ms and represented 82.1% of the water in the meat. The data in Figure 4.9 indicate that the relative abundance of T_{22} reduces significantly ($P < 0.001$) as the meat proteins are substituted by texturised pea or pea-potato proteins in the pork sausages. Thus, the higher concentration of texturised vegetable proteins in the sausages, the lower relative abundances of T_{22}. Interestingly, the data show that T_{22} relaxation times of pork sausages with 50% substituted meat proteins were not significantly different from the reference sausages, whereas T_{22} relaxation times of pork sausages with 10-30% meat proteins replaced by texturised vegetable proteins were significantly lower ($P < 0.001$) than the reference sausages.

The relative abundance of the relaxation component, T_{23}, representing the loosely associated water in the sausage matrix [77], was 17.9% in pork sausages without texturised vegetable proteins. Pork sausages with 50% meat proteins replaced by texturised pea or pea-potato proteins had relative abundance of T_{23} that was not significantly different from the reference, while the relative abundance of T_{23} in pork sausages with 10-30% substituted meat proteins was significantly higher ($P < 0.001$) than the reference. These pork sausages therefore contained a larger amount of loosely bound water. The T_{23} time constant was 191.2 ms for pork sausages without texturised proteins. In figure 4.9, the relaxation time data of pork sausages with substituted meat proteins reveal differences between pea and pea-potato protein substitution. T_{23} relaxation times were not significantly different between reference sausages and sausages with 10% or 50% meat proteins replaced by texturised pea or pea-potato proteins, respectively. Generally, T_{23} time constants of pork sausages with 30% substituted meat proteins were significantly lower ($P < 0.001$) than the other pork sausages.

The relaxation component, T_{21}, has very short relaxation times and represents water tightly bound to macromolecules [77]. The results show that only pork sausages with the highest concentration of texturised vegetable proteins had a component with very short relaxation times (8.3-9.4 ms) that represented 11.5-14.4% of the water in the meat (Figure 4.9).

In Figure 4.10, the data show that the pork sausages with pea proteins had significantly higher ($P < 0.001$) relaxation time and relative abundance of T_{21} and significantly lower ($P < 0.001$) relaxation time and relative abundance of T_{22} than sausages with pea-potato proteins. Furthermore, the sausages containing pea proteins had significantly lower ($P < 0.01$) relaxation time of T_{23} than sausages containing pea-potato proteins, whereas the protein type had no effect on the relative abundance of T_{23}. Hence, pork sausages with texturised pea-potato proteins generally containing more loosely bounded water molecules in their network than sausages with texturised pea proteins. Statistical analyses of the LF-NMR data also revealed strong interactions ($P < 0.001$) between protein type and concentration for each the relaxation components. The effect of texturised protein concentration on water distribution and mobility in pork sausages is therefore dependent on protein type.

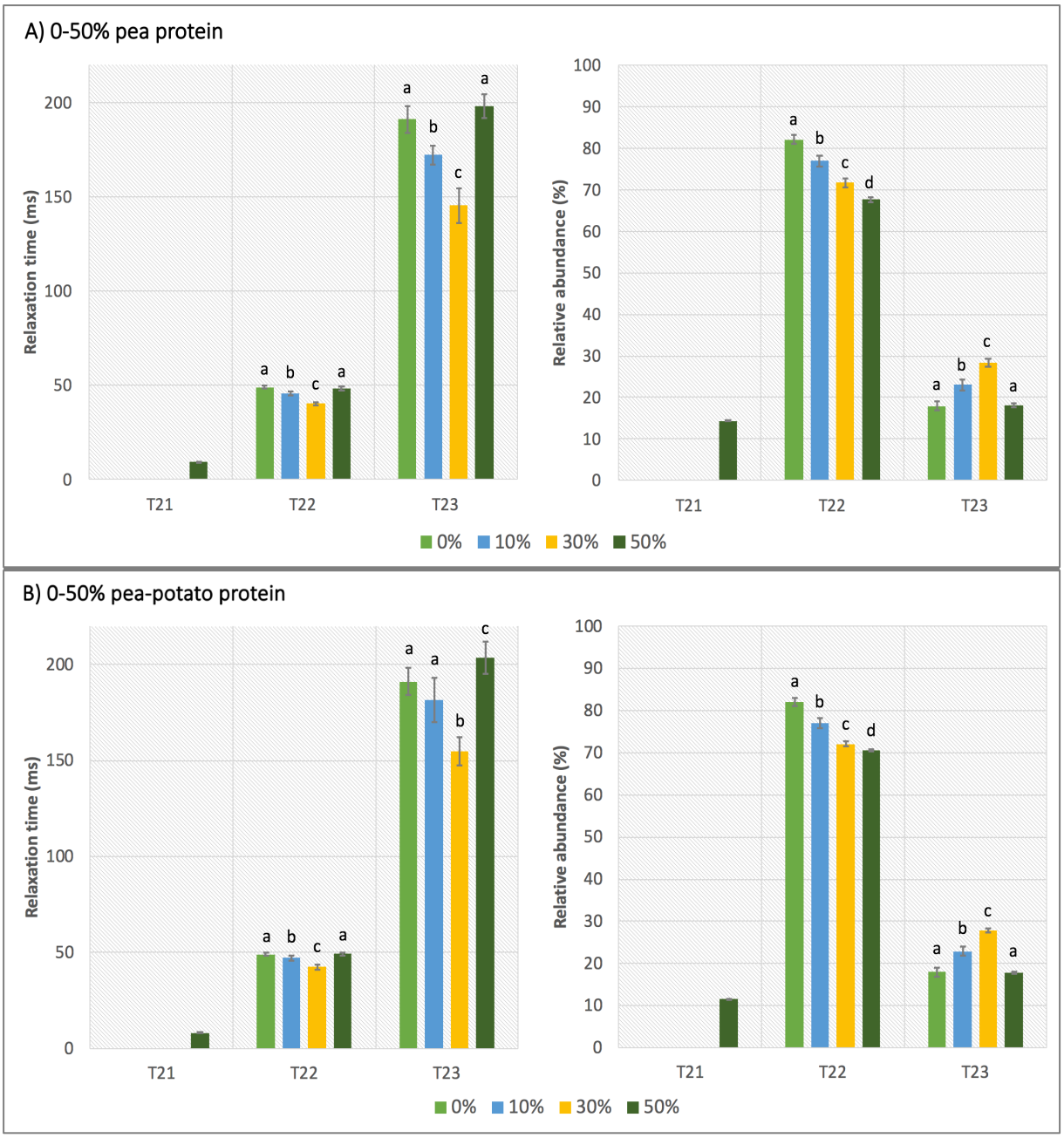

Figure 4.9: Effects of A) partially replacing meat proteins by texturised pea proteins and B) partially replacing meat proteins by texturised pea-potato proteins on T_2 relaxation times and relative abundance in pork sausages. The reference sausages contained 0% texturised vegetable proteins. All measurements were expressed as the mean with error bars showing ± SD (n = 6). Different superscripts (a-d) above bars indicate significant differences ($P < 0.05$) between means of the same relaxing component (T_{21}, T_{22}, or T_{23}).

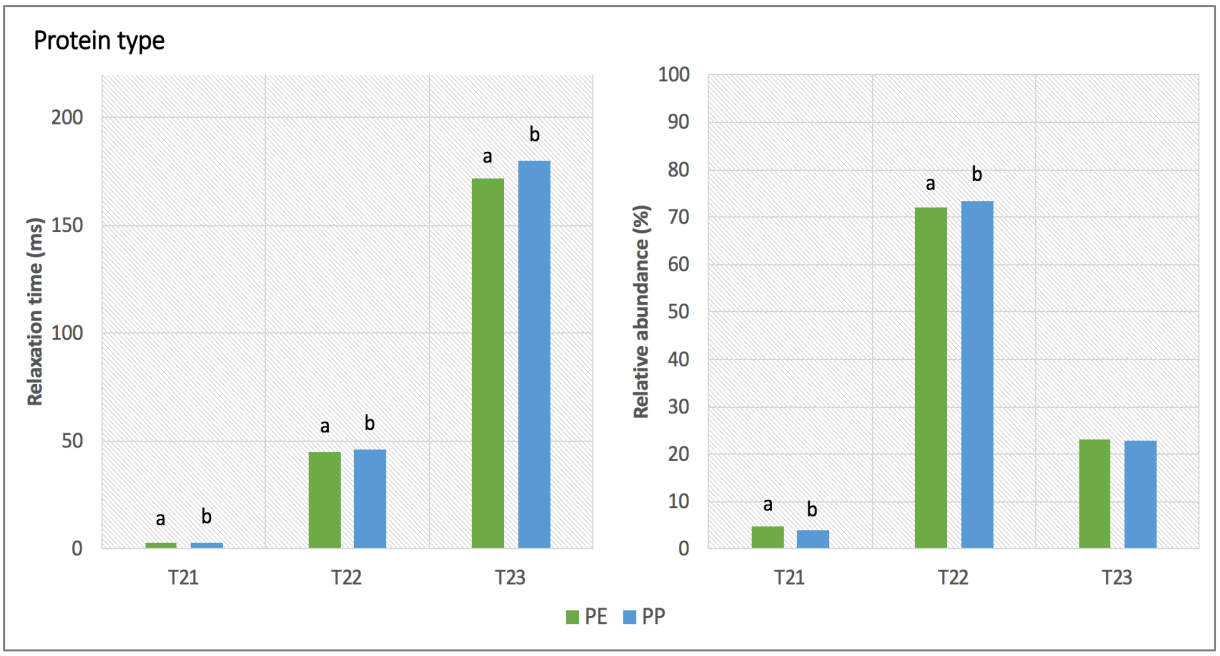

Figure 4.10: Effect of protein type on T_2 relaxation times and relative abundance in pork sausages. All measurements were expressed as the mean (n = 18). Different superscripts (a-b) above bars indicate significant differences ($P < 0.01$) between means of the same relaxing component (T_{21}, T_{22}, or T_{23}).

4.2.4 Sensory texture

During a sensory texture analysis of the different pork sausages, the properties of firmness, juiciness, cohesiveness, gumminess, grittiness, chewing time, and chewing residual were assessed by a trained sensory panel. In figure 4.11, the results from the sensory analysis of pork sausages with 0-50% meat proteins replaced by texturised pea (Figure 4.11A) or pea-potato (Figure 4.11B) proteins are presented. Figure 4.12 shows the effect of protein type on the sensory texture of the sausages.

The data in Figure 4.11 show no significant differences in all texture attributes of pork sausages with 0-10% meat proteins replaced by texturised vegetable proteins. Pork sausages with different concentrations of texturised pea or pea-potato proteins show the same result pattern with the highest concentration (50% substituted meat proteins) being most different from the reference sausages (0%). Sausages with 30% substituted meat proteins were second most different and sausages with 10% substituted meat proteins least different from the reference sausages.

Pork sausages with the highest concentration of texturised vegetable proteins had significantly lower ($P < 0.05$) firmness, cohesiveness, gumminess, chewing time, and chewing residual, but significantly higher ($P < 0.001$) grittiness than the other pork sausages. The juiciness of pork sausages with 50% substituted meat proteins was only significantly higher ($P < 0.05$) than pork sausages without texturised proteins.

Pork sausages with 30% meat proteins replaced by texturised pea proteins had significantly lower ($P < 0.05$) firmness, cohesiveness, gumminess, and chewing residual than the reference sausages, but was not significantly different from sausages with 10% substituted meat proteins. However, pork sausages with 30% meat proteins replaced by texturised pea-potato proteins had significantly lower ($P < 0.05$) firmness, cohesiveness, gumminess, chewing time, and chewing residual, but significantly higher ($P < 0.01$) grittiness than sausages with 0-10% substituted meat proteins.

Figure 4.12 indicate that pork sausages with pea or pea-potato proteins were not significantly different in firmness, juiciness, gumminess, and chewing residual. However, pork sausages with pea proteins had significantly higher ($P < 0.05$) cohesiveness and chewing time and significantly lower ($P < 0.01$) grittiness than sausages with pea-potato proteins. Interaction effects were observed between protein type and concentration for all attributes with the exception of juiciness. Texture changes between sausages with different texturised protein concentration are therefore significantly different ($P < 0.01$) depending on protein type.

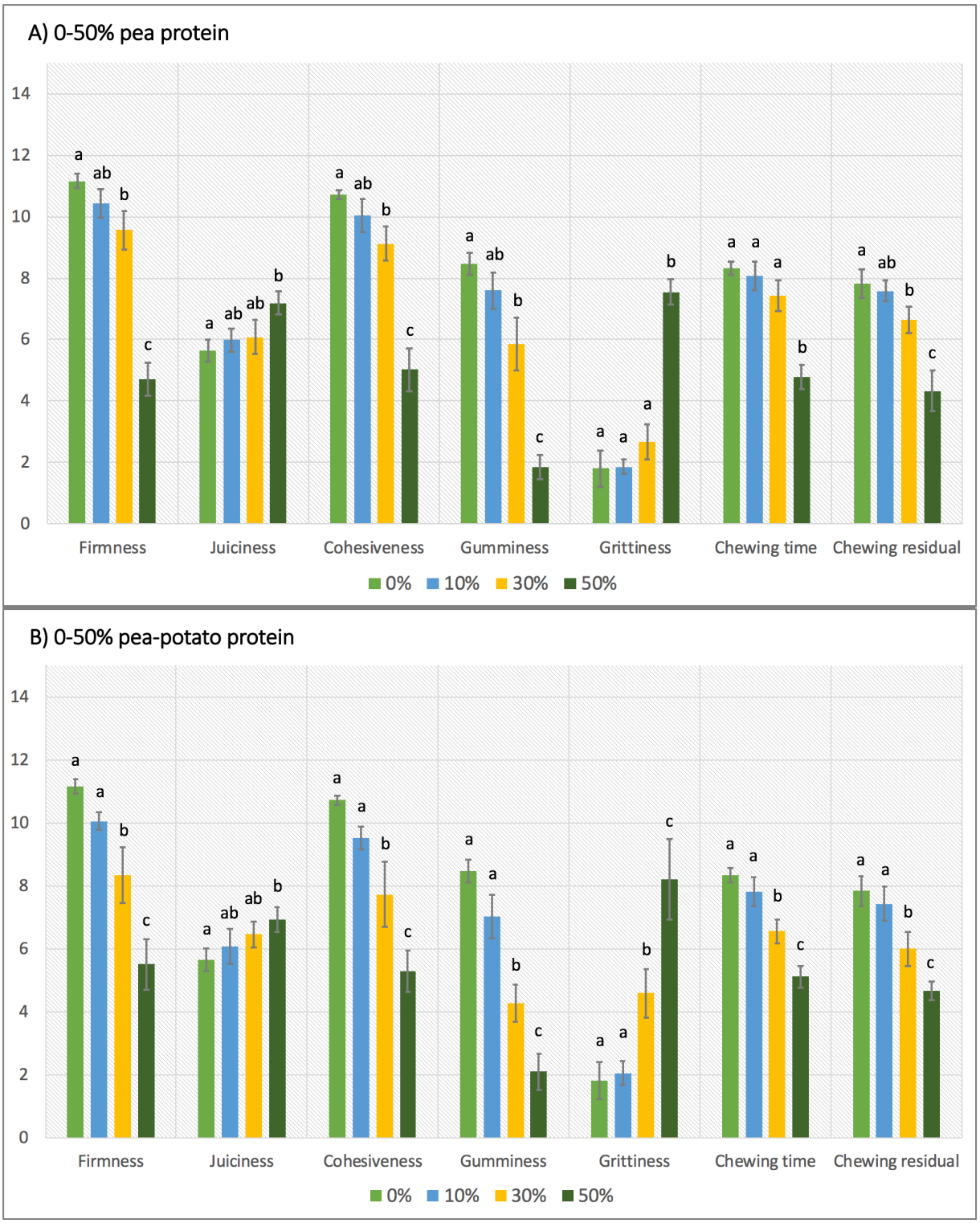

Figure 4.11: Effects of A) partially replacing meat proteins by texturised pea proteins and B) partially replacing meat proteins by texturised pea-potato proteins on sensory texture properties (firmness, juiciness, cohesiveness, gumminess, grittiness, chewing time, and chewing residual) of pork sausages. The reference sausages contained 0% texturised vegetable proteins. All measurements were expressed as the mean with error bars showing ± SD (n = 6 (mean of each evaluated sample during sensory analysis)). Different superscripts (a-c) above bars indicate significant differences ($P < 0.05$) between means of the same attribute.

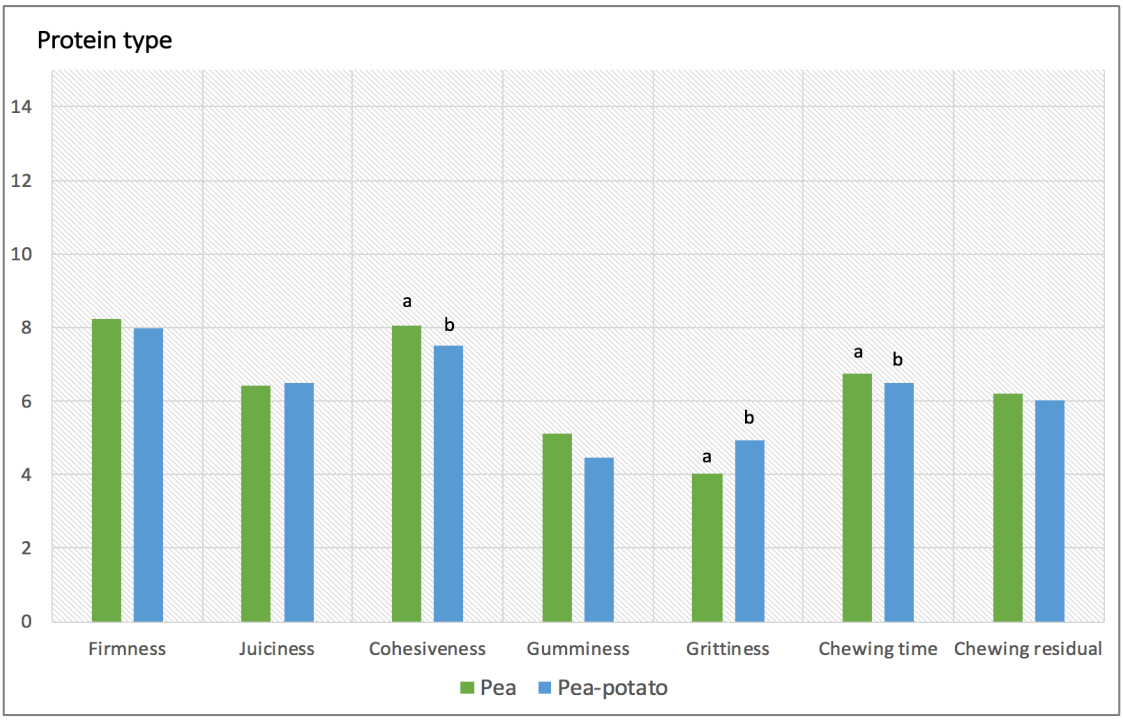

Figure 4.12: Effect of protein type on sensory texture properties (firmness, juiciness, cohesiveness, gumminess, grittiness, chewing time, and chewing residual) of pork sausages. All measurements were expressed as the mean (n = 18 (mean of each evaluated sample during sensory analysis)). Different superscripts (a-b) above bars indicate significant differences ($P < 0.05$) between means of the same attribute.

4.2.5 Instrumental texture

Firmness of each pork sausage type was instrumentally measured as the resistance force during a compression analysis. The results are presented in Figure 4.13.

The data indicate no significant difference in firmness of pork sausages with 0-10% meat proteins replaced by texturised pea proteins (Figure 4.13A). However, when 10% meat proteins were substituted by texturised pea-potato proteins, the sausages was significantly ($P < 0.001$) less firm than the sausages without texturised proteins (Figure 4.13B). Pork sausages with 0-10% or 10% meat proteins replaced by texturised pea or pea-potato proteins, respectively, had significantly higher ($P < 0.001$) firmness than sausages with 30-50% substituted meat proteins by the same texturised protein material. Furthermore, pork sausages with 30% meat proteins replaced by texturised vegetable proteins had significantly higher ($P < 0.001$) firmness than sausages with 50% substituted meat proteins. Hence, pork sausages with the highest concentration of texturised vegetable proteins were significantly ($P < 0.001$) less firm than the other pork sausages. Figure 4.13C reveals that the firmness was significant higher ($P < 0.05$) in sausages containing pea proteins than sausages containing pea-potato proteins. Moreover, a significant interaction ($P < 0.001$) between protein concentration and type was found.

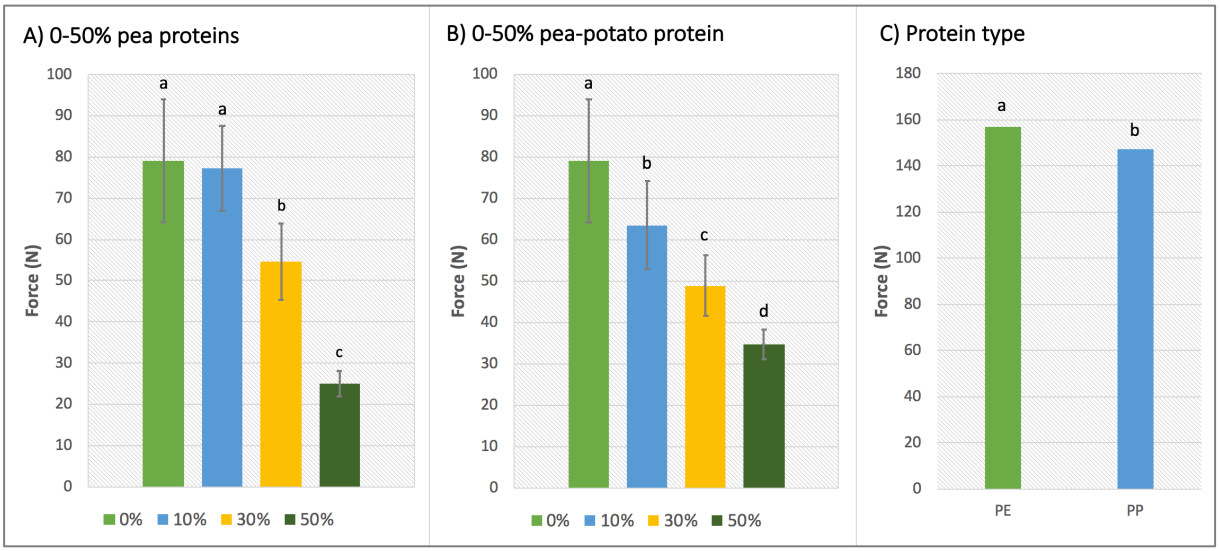

Figure 4.13: Effects of A) partially replacing meat proteins by texturised pea proteins (PE), B) partially replacing meat proteins by texturised pea-potato proteins (PP), and C) protein type on firmness of pork sausages measured by a texture analyser. The reference sausages contained 0% texturised vegetable proteins. All measurements were expressed as the mean with error bars showing ± SD (n = 24, (C: n = 72)). Different superscripts (a-d) above bars indicate significant differences ($P < 0.05$).

4.3 Comparative analyses

4.3.1 Moisture loss, juiciness, and LF-NMR relaxometry

Application of PCA to the mean dataset of total moisture loss, juiciness, and the relative abundance of the three relaxation components T_{21}, T_{22}, and T_{23} was done to assess the correlations between the water binding ability of the sausages during processing and storage, the juiciness of the final sausages, and the water mobility and distribution in sausage protein matrix (Figure 4.14). The PCA biplots present the distribution of the pork sausages samples and the variables with the first three principal components accounting for 99.3% of the total variance.

Figure 4.14A reveals that PC1 and PC2 explain 60.3% and 31.8% of the total variance, respectively. From the PC1 vs. PC2 biplot, three clusters consisting of observations and attributes can be observed (highlighted in circles). These clusters are primarily separated along PC1. The first principal component models the variabilities of pork sausages with meat proteins replaced by texturised pea or pea-potato proteins at different levels (0-50%). Furthermore, PC1 appears to separate the relative abundance of the three relaxation components. The reference sausages are located in the negative PC1 domain and are positively correlated with the relative abundance of T_{22} and high moisture loss. With lower negative PC1 values than the reference sausages, pork sausages with 10-30% meat proteins replaced by texturised vegetable proteins are clustered with the relative abundance of T_{23}. However, this cluster is located close to zero and is therefore least explained by PC1. Finally, pork sausages with 50% substituted meat proteins are located in the positive PC1 domain and are positively correlated with the relative abundance of T_{21} and the texture attribute juiciness. Hence, these results indicate that pork sausages with high moisture loss are negatively correlated with juiciness. However, this correlation was visualised due to autoscaling of the data that showed only small differences between the sausages. Furthermore, pork sausages with high moisture loss are positively correlated with the relative abundance of T_{22}, which is the major relaxation component associated with water trapped within the protein-dense network of sausages. However, assessment of the second principal component reveals less clear correlations as juiciness is not explained (located close to zero) and pork sausages with high moisture loss are positively correlated with the relative abundance of both T_{21} and T_{22}.

In Figure 4.14B, the third principal component explaining 7.2% of the total variance reveals a clear separation of pork sausages with 10% meat proteins replaced by texturised vegetable proteins in the negative domain and pork sausages with 30% meat proteins replaced by pea-potato proteins in the positive domain. In contrast with the results from PC1 and PC2, PC3 indicates that pork sausages with high moisture loss are most correlated with the relative abundance of both T_{23}. Hence, no clear relationship can be established between moisture loss and the relative abundance of the three relaxation components T_{21}, T_{22}, and T_{23}.

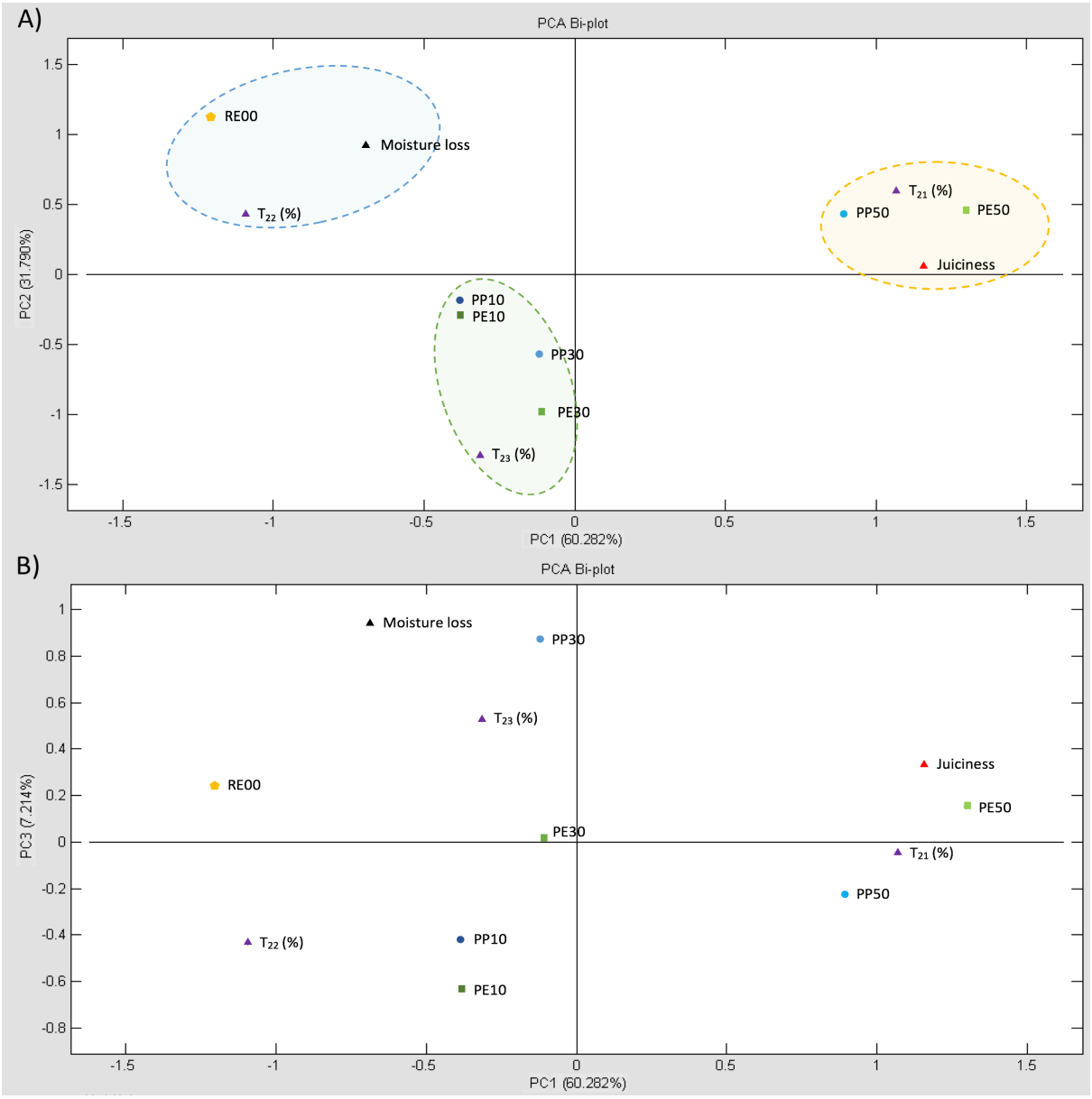

Figure 4.14: PCA of the water related data showing the three first principal components (99.3% of the total variance). A) Biplot of PC1 vs. PC2 and B) Biplot of PC1 vs. PC3. The samples (RE00, PE10, PE30, PE50, PP10, PP30, and PP50) are observations and the moisture loss, juiciness, and relative abundances of the relaxation components (T_{21}, T_{22}, and T_{23}) are variables. Clusters are highlighted in circles.

4.3.2 Sensory and instrumental texture

PCA was performed on the mean sensory and instrumental texture data to examine possible correlations among observations (pork sausages) and variables (texture attributes) (Figure 4.15). Application of PCA to the dataset revealed that 99.2% of the total variance could be extracted by the first two principal components.

Figure 4.15 reveals that PC1 accounts for 98.1% of the total variance indicating high consistency between the data. PC1 seems to model the variabilities between pork sausages containing different levels (0-50%) of texturised pea or pea-potato proteins. Hence, along PC1, a separation between pork sausages with the lowest (0-10%) and the highest (50%) content of texturised vegetable proteins is observed. Pork sausages with 30% substituted meat proteins are least explained by PC1 (located closest to zero). The first principal component reveals that pork sausages with 0-10% meat proteins replaced by texturised vegetable proteins are positively correlated with the following texture properties: gumminess, chewing residual, cohesiveness, chewing time, and sensory and instrumental firmness. Pork sausages with a low concentration (10%) of texturised pea proteins seem to be most similar to the reference sausages in texture attributes as they are located closest to the reference along PC1. The first principal component also reveals that pork sausages with the highest concentration (50%) of texturised pea or pea-potato proteins are positively correlated with grittiness and juiciness.

The second principal component only explains 1.1% of the total variance and reveals a separation between pork sausages with high content of texturised pea and pea-potato proteins (Figure 4.15). Pork sausages with pea-potato proteins seem to correlate more with grittiness, whereas sausages with pea proteins are more associated with juiciness. Finally, PC2 separates the instrumental firmness from sensory firmness, gumminess, chewing residual, cohesiveness, and chewing time.

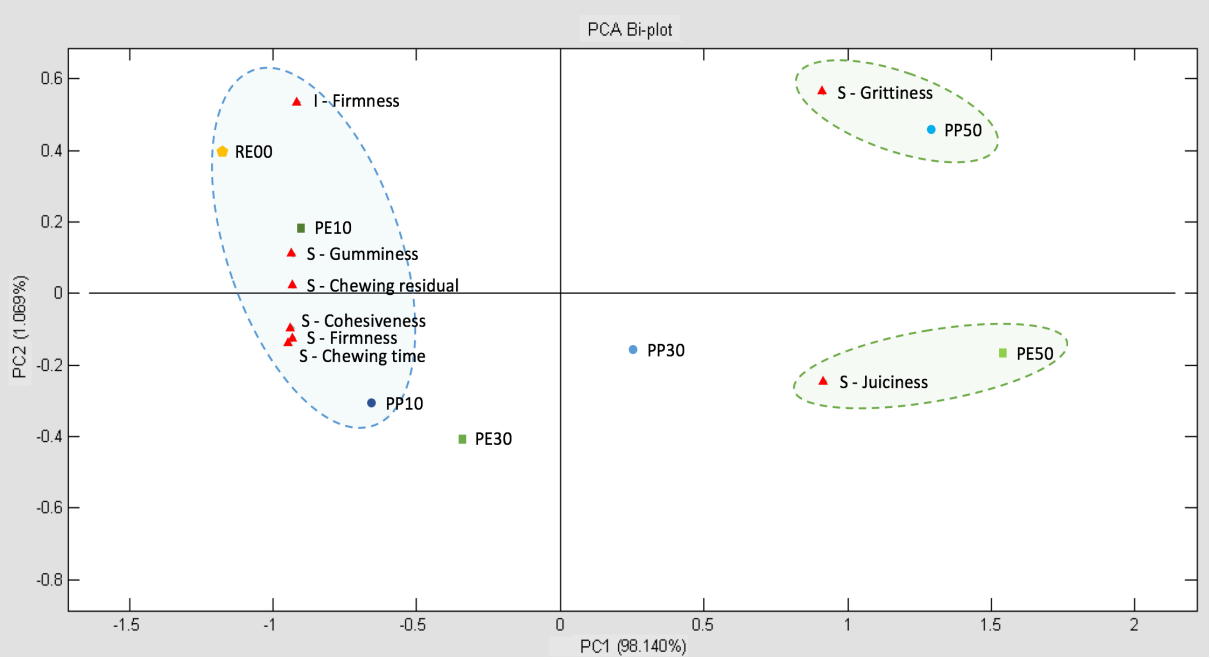

Figure 4.15: PCA biplot showing the two first principal components (99.2% of the total variance) of the texture data with the pork sausage samples (RE00, PE10, PE30, PE50, PP10, PP30, and PP50) as observations and the sensory attributes (S-Firmness, S-Juiciness, S-Cohesiveness, S-Gumminess, S-Grittiness, S-Chewing time, and S-Chewing residual) and instrumental firmness (I-Firmness) as variables. Clusters are highlighted in circles.

5. Discussion

The overall aim of the thesis was to investigate changes in functional and textural properties of pork sausages as a result of partially replacing meat proteins with texturised pea and potato proteins. Pea and potato protein concentrates were texturised during low moisture extrusion cooking. The resulting products, a texturised material of pea protein concentrate and a texturised material of 3:1 pea-potato protein concentrate mix, were replacing 10%, 30%, and 50% of the meat proteins in emulsion-type pork sausages with low content of fat and salt to comply with the Nordic Keyhole nutrition label regulation.

In this section, the results and methods presented in this thesis will be discussed, followed by suggestions for future experiments to further investigate the potential of developing meat applications with partially replaced meat proteins by texturised pea and potato proteins.

5.1 Texturisation of pea and potato protein concentrates

Low moisture extrusion cooking was in this study used to texturised pea and potato proteins. The pea protein concentrate responded well to the extreme extrusion conditions of high temperature, high pressure, and high shear as the material was easily converted into a semisolid, plasticised, and homogenous mass. Thus, texturisation of pea proteins was achieved. In contrast, potato protein concentrate was impossible to texturise in preliminary extrusion trials. The extruded potato protein mass became inhomogeneous, hard, crumbly, and very dry. This is most likely due to a poor solubility of potato proteins in water. In order to be texturised, the raw protein material must be capable of dissolving in the water added during extrusion, as it will otherwise remain inert [94]. Stirring the potato protein concentrate in warm water indicated that the concentrate had very poor water solubility as it quickly sank to the bottom of the water. This was confirmed in liquid-state ^{1}H NMR as the spectra revealed that the potato protein concentrate contained fewer soluble fractions compared to pea protein concentrate. Consistent with our data, Miedzianka et al. (2012) and Waglay et al. (2014) reported very low water solubility index (<15%) of potato protein isolates obtained by thermal/acidic precipitation like the potato protein concentrate used in this study [37, 38]. This insolubility of potato proteins in water was primarily caused by the heat- and acid-induced irreversible structural changes of the main storage protein patatin. Using other extraction techniques to recover potato proteins with better functional properties have shown

to be very costly [33]. Instead, we developed a mixture of 3:1 pea and potato protein concentrates (corresponding to about 66%/34% pea/potato proteins) to obtain a texturised product with incorporated potato proteins. A visual analysis of the texturised pea-potato protein material showed that it was still more crumbly and had a darker colour compared to the texturised pea protein material.

The protein producers reported that the pea protein concentrate on dry basis contained 55% protein, 3% fat, 2% fiber, 8% starch, and 34% other carbohydrates, whereas the potato protein concentrates on dry basis contained 85% protein, 2% fat, 6% fiber, and 7% other carbohydrates. These data were further investigated by solid-state ^{13}C and liquid-state ^{1}H NMR spectroscopy, which can give qualitative information of the ingredients. Consistent with the composition data from the producers, the NMR data revealed that the pea protein concentrate was considerably more dominated by carbohydrates, including starch, compared to the potato protein concentrate. The difference in carbohydrate content would be expected as producers of potato protein concentrate have an interest in separating as much starch from the protein fraction as possible, because potato starch is the main value-added ingredient from potatoes [36].

In extruded mixtures of proteins and starch, feed moisture and extrusion conditions can cause proteins to degrade and starch to gelatinise and degrade. At the die section, proteins will realign and cross-link with the starch molecules forming a stable new complexes. These interactions between proteins and starch, can result in lower viscosities, lower frictions, expansion of the product, and negatively affect texturisation of proteins [49, 95–99]. In this study, only minor interactions between proteins and starch are believed to have occurred due to low starch contents (<8%) in the concentrates and low feed moisture.

The pork muscle contains biologically a high amount of water. It is desirable to retain as much water in meat applications as possible during post mortem aging, processing, and storage as it is associated with juiciness and high texture stability [17]. An important functional property of vegetable proteins in meat applications is therefore high WHC. The higher WHC, the more water can be added and entrapped in the food microstructure achieving a high quality product and making it more profitable for the producers due to higher prices per kg product. Studies by Wang et al. (1999) and Alonso et al. (2000) observed significantly increased WHC of pea ingredients as a result of texturisation [21, 25]. In this study, WHC measurements of the protein materials also showed significant increase in WHC from 1.22 to 3.18 g water per g of pea protein material as a result of texturisation. The new texturised product is therefore a more suitable ingredient in meat applications, such as pork

sausages, compared to the raw concentrate. Surprisingly, the raw potato protein concentrate could initially retain 3.08 g water per g dry sample indicating that the potato proteins are able to interact and bind water in their network before even being texturised. Miedzianka et al. (2012) has reported similar results with a WHC of 3.78 g water per g sample of potato protein isolate [38]. Even though potato proteins had a higher initial WHC, our WHC measurements revealed no significant differences in the WHC of texturised pea and pea-potato protein materials. Thus, their ability to bind and retain water in their protein network are similar despite clear visual and textural differences. Furthermore, when used as an ingredient in emulsion-type pork sausages, the texturised protein materials will have retained similar amounts of water per g product before processing. However, during processing, the differences in protein composition between the sausages may affect how the water is distributed in the sausage matrix.

It can potentially be environmental beneficial to substitute meat proteins with vegetable proteins in food applications as meat has a relatively low delivery efficiencies in terms of energy used or greenhouse gas emitted [9]. However, the transformation of already processed pea and potato protein concentrates into value-added texturised products with water addition, high temperatures, and high pressures during extrusion cooking can be a major source of energy consumption. Thus, it can be discussed how sustainable the use of texturised protein products in meat applications are. A useful tool to quantify the environmental effects of the products from cradle to grave is a Life Cycle Assessment (LCA). More research is needed to do an in-depth description of the food production with LCA and to improve the processing method to become more sustainable [100].

5.2 Nutritional values of texturised pea and potato proteins

The essential amino acid composition is important for the nutritional value of proteins. The essential amino acid composition of the pea and potato protein concentrates has been reported by the producers [22, 92]. These data revealed that none of the concentrates met WHO/FAO/UNU adult essential amino acid requirements [7]. Nevertheless, it was observed that pea and potato proteins' essential amino acid compositions complemented each other well as potato proteins are insufficient in phenylalanine and tyrosine, whereas pea proteins are insufficient in all other essential amino acids than phenylalanine and tyrosine. Hence, it is nutritional favourable to add potato proteins to pea protein mixtures before texturisation.

In this study, the exact ratio of pea and potato protein could not be determined in the texturised pea-potato material, making it difficult to calculate its essential amino acid composition. With solid-state ^{13}C NMR spectroscopy, the pea and potato protein composition was estimated to about 70% pea proteins and 30% potato proteins, which corresponded to the theoretical composition of 66% pea and 34% potato proteins in the 3:1 pea-potato concentrate mix. In contrast, the protein analysis revealed a protein content of only 55.0 g protein per 100 g texturised pea-potato product, which corresponded to a relative pea and potato protein composition of 86% pea proteins and 14% potato proteins. This calculation is provided that no reduction in protein content occur during texturisation as reported in other studies [52, 53, 56, 101]. The texturised pea-potato product was very crumbly and inhomogeneous, which could have resulted in an unevenly distribution of pea and potato proteins. NMR spectroscopy and protein analysis used very small amounts of the texturised sample, which might not have been representative for the total texturised product. Hence, the observed difference in pea/potato proteins (66%/34% vs. 86%/14%) can be caused by a high variability in the protein distribution of the texturised pea-potato material.

In our study, only estimates of the essential amino acid composition of the texturised vegetable protein materials and the final pork sausages could be calculated due to uncertainties of protein composition and processing effects on amino acids. The processing effects of extrusion cooking on the essential amino acid composition of pea flour has been investigated by Alonso et al. (2000). The study revealed that the levels of histidine, tryptophan, and the sulphur-containing methionine and cystine were significantly reduced by the treatment [101]. Generally, pea proteins are deficient in the two sulphur-containing essential amino acids. Thus, it is a concern that these amino acids are further reduced by extrusion cooking. In addition to the reduction in essential amino acids, some amino acids can become unavailable after thermal treatment due to cross-linking and Maillard reactions with reducing sugars. Generally, lysine is an important indicator of these reactions, but Alonso et al. (2000) observed no loss in the lysine content of extruded pea flour [101].

The essential amino acid estimations in our study revealed that only pork sausages with 50% meat proteins replaced by texturised pea proteins could not meet the adult essential amino acid requirements. Hence, increasing potato or meat protein content in these sausages are recommended, as the composition of essential amino acids are better in potato and meat proteins than pea proteins [93]. Importantly, it should be recognised that essential amino acid

values do not describe how digestible the texturised proteins are in the human body. Heat-induced alterations of the proteins and anti-nutrients can highly affect the digestibility [101].

5.3 Emulsion-type pork sausage production

This study succeeded in producing six types of low-fat and low-salt emulsion-type pork sausage batters in which meat proteins were partially (10%, 30%, and 50%) replaced by texturised pea or pea-protein materials. A reference sausage type without vegetable proteins was also produced. The rheology and LF-NMR relaxometry measurements of the pork sausage batters were unsuccessful in demonstrating how the three-dimensional network of swollen and dissolved meat proteins in the batters were affected by the addition of texturised vegetable proteins. High standard deviations between replicates of the batter samples made it difficult to obtain any conclusive results. Other studies have used rheology and LF-NMR to examine meat batters and analysed 2-5 replicates without obtaining high standard deviations [63–65, 102–106]. The high standard deviations measured in our study may be caused by the relatively inhomogeneous batters with big pieces of pork fat.

Upon heating, each pork sausage batters successfully formed a stable gel network that immobilised fat, water, and other constituents in the sausage matrix. The ultimate functionality and texture properties of the pork sausages can be affected by the carbohydrate, fat, protein, and water content [58, 60–62, 107]. The compositional information of the raw and texturised protein materials obtained by NMR spectroscopy revealed that the reference sample greatly differed from the texturised protein materials as it primarily consisted of proteins and lipids with small amounts of polyunsaturated fatty acids. Thus, replacing meat with texturised protein materials in the pork sausages increased the content of carbohydrates and polyunsaturated fatty acids.

In this study, we did not measure the carbohydrate content and the relative composition of polysaccharides, such as starch and fibre, of the pork sausages. The exact effect of the carbohydrates on the functionality and texture of the sausages is therefore difficult to anticipate. However, generally, an increase in starch content would have made the meat more firm [108, 109], whereas the type of fiber affect the texture of sausages differently [110–112].

Several pork sausage studies have investigated the effects of replacing pork fat containing relatively high levels of saturated fat with vegetable oils rich in healthier monounsaturated and polyunsaturated fatty acids [61, 62, 113–116]. Generally, the studies observed a reduction

in cooking loss and improved fatty acid composition, but significantly higher hardness, cohesiveness, gumminess, and chewiness of sausages [61, 62, 113, 116]. Unfortunately, high content of polyunsaturated fatty acids in meat applications are associated with early rancidity due to lipid oxidation resulting in a shorter shelf life [115]. The texturised vegetable protein materials in our study only contained about 5% lipids. Furthermore, the produced pork sausages had a low content of fat varying between 7.6 and 9.4 g per 100 g of sample. The ultimate content of polyunsaturated fatty acids in the sausages is therefore very small and thought to have had minimal impact on sausage functionality and texture.

The protein content measurements of the pork sausages revealed a difference in protein content of 2.7% between the highest (reference sausages) and lowest content (pork sausages with 50% meat proteins replaced by texturised pea proteins). A previous study by Youssef & Badut (2009) revealed that increased protein level of only 1% can significantly increase hardness, chewiness, and gumminess in sausages [107]. The higher firmness, gumminess, chewing time, and chewing residual of the reference sausages observed during instrumental and sensory texture analyses in this study can therefore be a result of the higher protein content in these sausages.

The difference in water content was about 4% between the reference sausages and the sausages with 50% meat proteins replaced by vegetable proteins. Interestingly, the reference sausages contained most water while having the highest total moisture loss during processing and storage. Generally, reduction of water content is related to reduction in juiciness of meat applications [60]. In present study, variation in water content did not cause any effect on juiciness as the pork sausages with 50% substituted meat proteins by texturised vegetable proteins contained the least amount of water while being significantly juicier than the reference sausages.

5.4 Water properties and structure of pork sausages

The ability to bind water during cooking, cooling, and heating, and the resulting water distribution and mobility, and juiciness of emulsion-type pork sausages were investigated using LF-NMR and sensory analysis.

The pork sausages with 10-50% meat proteins replaced by texturised vegetable proteins had significantly better water binding and retaining ability during processing and storage than reference sausages. However, only pork sausages with 50% substituted meat proteins were

significantly juicier than reference sausages. These results suggest that addition of texturised vegetable proteins improve the quality of low-fat pork sausages as low moisture loss and high juiciness are desirable properties in meat applications.

Water properties of pork meat have previously been analysed by LF-NMR relaxometry revealing clear positive correlation between the slowest T_{23} population reflecting extra-myofibrillar water and low water binding ability [117, 118]. LF-NMR relaxometry data can also contain information of moisture loss and sensory properties of pork meat after heat treatment [119]. The present study observed clear effects in water mobility and distribution of pork sausages when substituting up to 30% meat proteins with texturised vegetable proteins. The meat protein replacement caused the T_{22} population to decrease, while the T_{23} population increased. The data suggest that texturised vegetable proteins in the sausage gel network cause more water molecules to become loosely bound in the protein structure [78]. However, pork sausages with 10-30% substituted meat proteins had significant lower moisture loss during processing and storage than the reference sample. Thus, moisture loss could not be explained by increased T_{23} population. In contrast, the relaxation times of the components, T_{22} and T_{23}, revealed that the water became stronger bound in the intra- and extra-myofibrillar structures when the meat protein replacement increased from 0-30%. This suggests that high T_{23} relaxation times are positively correlated with moisture loss.

Interestingly, the water distribution changes from two to three water populations between 30% and 50% meat protein replacement in the pork sausages. Instead of having the highest relative abundance of T_{23}, the pork sausages with 50% meat proteins replaced by texturised vegetable proteins had a relative abundance between 11.5-14.4% of the slowest relaxation component T_{21} reflecting water tightly bound to macromolecules in the sausage network. These results indicate that the pork sausage matrix undergo physicochemical and functional changes as a result of replacing above 30% meat proteins by texturised vegetable proteins. The observed enhanced water entrapment could be caused by the higher content of polysaccharides, because they may cause a disruption of the sausage protein matrix making the water molecules more exposed to strong interactions with macromolecules [120]. This theory is supported by a significant higher T_{21} population in pork sausages with pea proteins containing more carbohydrates than sausages with a combination of pea and potato proteins. However, more in-depth molecular studies are required to elucidate the observed results.

The low moisture loss of pork sausages with 50% meat proteins replaced by texturised vegetable proteins could unlike the other sausages not be explained by T_{23} relaxation times as they were similar to the reference sausages. In addition, juiciness of the pork sausages was

difficult to explain with LF-NMR data due to small differences between the sausages. This has been achieved in a study by Bertram et al. (2005). The authors observed that the myofibrillar structure of pork changes as a result of protein denaturation during cooking causing an increase in expelled water, which correlated positively with the slowest relaxation component and correlated negatively with juiciness [119].

5.5 Texture effects of meat protein replacement

To assess if the partially replacement of meat proteins by texturised pea or pea-potato proteins had an effect on the sensory and instrumental texture properties in emulsion-type pork sausages, we used a trained sensory panel of 10 assessors and a texture analyser, respectively. Overall, the data from the sensory and texture analysis showed the same differences in firmness between the pork sausages, which agrees with previous data suggesting there is a correlation between sensory and instrumental firmness [121]. Our data showed that a 30-50% meat protein replacement resulted in a significantly reduced firmness of the sausages. Thus, the functionality of pea and potato proteins seemed to have an effect on the formation of gel network during processing. It is proposed, that the lesser firmness is caused by vegetable proteins' challenges of forming an organised protein network together with meat proteins due to their highly irreversible crosslinked and denatured state that occurred during texturisation [39]. Furthermore, the higher content of polysaccharides may cause disruption of the sausage network [120].

Our data indicated that the instrumental texture analyser was more sensitive to differences in firmness than the sensory panel, as the texture analysis revealed that pork sausages with texturised pea proteins were a little, but significantly, firmer than pork sausages with texturised pea-potato proteins. Thus, the functionality of potato proteins caused the firmness of the sausage gel network to decrease more than pea proteins. The WHCs of the two texturised vegetable protein materials were not significantly different, and WHC can therefore not explain the observed differences in firmness. The reduced firmness could more likely be caused by more aggregated proteins unable to interact with meat proteins in the texturised pea-potato protein material. It should be stressed that in this study low-fat pork sausages were produced, which previously have been rejected by consumers due to a more firm, rubbery, and less juicy texture [64, 67]. Hence, less firm pork sausages containing texturised protein materials may not be negatively received by the consumers.

PCA revealed a very high consistency between the texture data with clear correlations. Pork sausages with 0-10% meat proteins replaced by texturised vegetable proteins were associated with firmness, cohesiveness, gumminess, chewing time, and chewing residual, whereas pork sausages with 50% substituted meat proteins positively correlated with juiciness and grittiness. Interestingly, the sensory panel perceived the pork sausages with most gritty texture as the most juicy sausages, which is in contrast to other studies that have observed an inverse relationship between grittiness and juiciness of meat applications [122, 123]. Grittiness is an unusual texture attribute for pork sausages and may not be acceptable to the consumers. A reduction of this attribute will be most challenging in pork sausages containing potato proteins as they are significantly more gritty than sausages with meat proteins only replaced by pea proteins.

5.6 Pork sausage development with focus on functionality and texture

The development of a new food product is an iterative process that constitutes several stages for producing the optimal product ready to be sold to the consumers. Figure 5.1 illustrates the iterative product development process of emulsion-type pork sausages with partially replaced meat proteins by texturised vegetable proteins. The product development circle displays the methods used in this study to investigate functional and textural properties of the raw and texturised materials, the pork sausage batters, and the finished pork sausages. Hence, only one round in the development circle was achieved during this study. However, the results from present study can be used to iterate the product development process. During several steps of the process, such as the raw materials, the extrusion cooking, the sausage recipe, or the processing of the final sausages, adjustments can be made in order to obtain pork sausages with optimal functionality and texture. Moreover, the methods used for assessing the functional and textural properties of the pork sausages can be removed or replaced by other methods.

The measurements of WHC, water content, and protein content of the texturised vegetable materials were essential in order to calculate the pork sausage recipes. The results from the solid-state ^{13}C and liquid-state ^{1}H NMR spectroscopy gave valuable compositional information of the raw and texturised protein materials. In this study, the assessment of functionality and texture of the finished pork sausages by sensory and instrumental texture, moisture loss, chemical composition, and LF-NMR relaxometry measurements showed to be

more important than assessment of the pork sausage batters. Thus, rheology and LF-NMR relaxometry of sausage batters may be excluded from the product development process. However, a more comprehensive examination of the sausage batters may reveal important information of the three-dimensional sausage batter network.

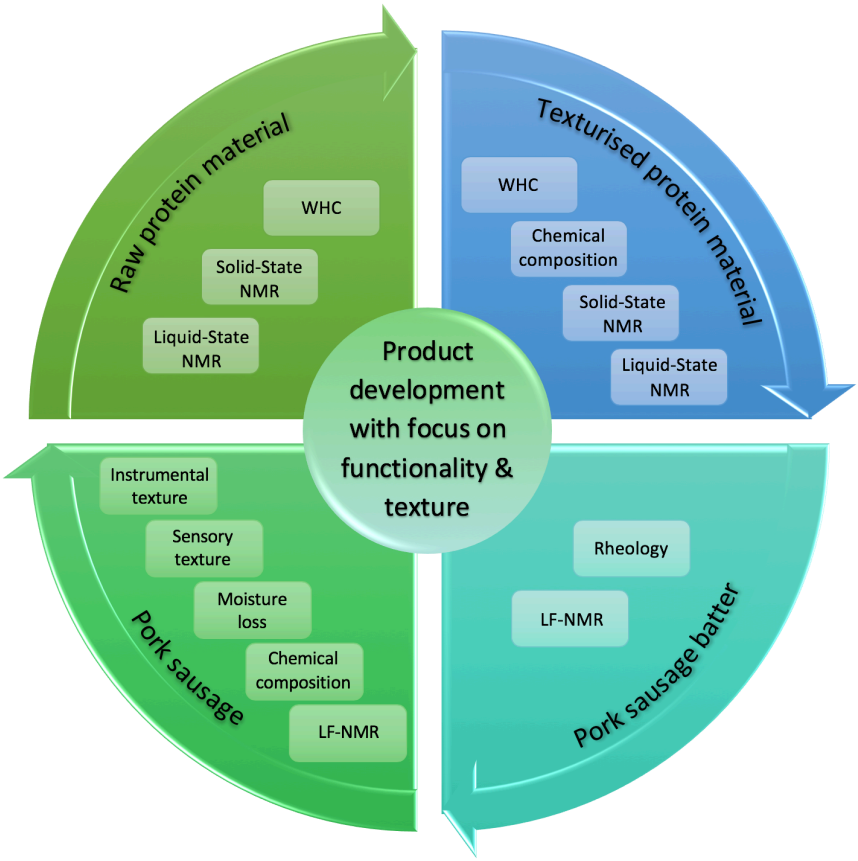

Figure 5.1: Illustration of the iterative process of pork sausage development with methods used for the assessment of functionality and texture.

5.7 Limitations of the study

This study contained some limitations. Firstly, the lack of published work on meat protein replacement by texturised vegetable proteins made it difficult to form the basis of this study. This topic is possibly being investigated in food companies where the research results are being kept a trade secret. For this reason, the materials, recipes, and methods used in this study were based on previous experience or studies involving other research, such as development of pork sausages with dietary fibre [72].

Another limitation of this study was the lack of experience with texturising potato protein concentrate. During preliminary trials, it was observed that the potato protein concentrate did

not respond to extrusion conditions in the same positive way as the pea protein concentrate. As a result, a texturised 3:1 pea-potato protein concentrate mix became our product. This led to some other limitations, as the texturised pea-potato product gave some conflicting results between the experimentally measured protein content and the solid-state NMR data, which made it difficult to determine the composition of pea and potato proteins. Further studies are needed to examine the protein composition and to investigate how pea and potato proteins interact with each other during extrusion cooking.

The time period of this thesis was only six months. Research often require more than six months to obtain careful and detailed results that are thoroughly analysed. As a result of time constraints, no adjustments during each step of the development process were possible.

Finally, this study was challenged by the limited durability of emulsion-type pork batters and sausages. All sausages were produced the same day, but the analyses of the sausages were performed in a time period between a week and a couple of months after production. Preservation of the batters and sausages by cooling or freezing were carefully chosen to avoid affecting the functionality and texture of the products. However, it is recognised that despite these considerations the effects of microbial and enzymatic reactions during cooling and crystallisation of water during freezing can be of great importance for the functional and textural properties of pork batters and sausages.

5.8 Future studies and conclusion

The work presented in this study could inspire a number of exciting new studies. For example, we observed significant differences in functionality and texture of emulsion-type pork sausages with 0-50% meat proteins replaced by texturised vegetable proteins, but further consumer acceptance studies should be performed to investigate the consumer preferences of the sausages.

Further experiments are also needed to investigate how pea and potato proteins interact during extrusion cooking. In addition, assessment of protein and amino acid digestibility are important to establish the PDCAAS of the food.

Overall, more research needs to be done in order to develop high quality products of low-fat and low-salt emulsion-type pork sausages with partially replaced meat proteins by texturised vegetable proteins. Ingredients and recipes of the sausages should be adjusted to determine how much of the meat proteins can be substituted without compromising sausage quality. Our

results indicated that the water distribution and mobility changed markedly between 30% and 50% of meat protein replacement in the pork sausages. Thus, it would be highly interesting to examine the water distribution and mobility in sausages with 40% of substituted meat proteins. In addition, future studies should visually investigate the physicochemical changes of the sausage matrix by computed tomography (CT) or other methods that can visualise microstructures. Although, the pork sausages consist of a complex mixture of meat and vegetable proteins, carbohydrates, and lipids that potentially could make the visual assessment difficult.

The suggested future experiments would provide additional information about the effects of partially replacing meat proteins by texturised vegetable proteins in pork sausages. From the present study it can be concluded that WHC of pea protein concentrate increases as a result of texturisation, suggesting that texturisation makes vegetable protein materials more suitable as an ingredient in meat applications. Furthermore, the functional and textural properties change when meat proteins are partially replaced by texturised pea or pea-potato protein materials in low-fat and low-salt emulsion-type pork sausages, indicating that vegetable proteins and polysaccharides disrupt the organised sausage gel network while increasing its water binding ability during processing and storage. Pork sausages with a low fat content have previously been rejected by the consumers. Hence, the changes in texture attributes caused by meat protein replacement have the potential of improving the consumer acceptability of low-fat pork sausages. The results of this study highlight the feasibility and prospect of making pork sausages that can contribute to a reduction in meat, fat, and salt consumption.

6. References

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7. Appendix

7.1 Texturisation parameters

Table 7.1: Screw profile used for texturisation of protein materials.

Barrel zone	Barrel section	Element type	No. of elements
1	Feed	16/16[a]	1
1-2	Feed	44/22	7
2	Feed	33/33	1
2-3	Compression	KB 45°/5/11[b]	2
3	Compression	33/33	3
4	Compression	KB 45°/5/11	1
4	Compression	KB 90°/5/11	1
4-5	Compression	33/33	3
5	Compression	KB 45°/5/33	1
5	Compression	KB 90°/5/11	1
5	Compression	KB 45°/5/11 LH[c]	1
5	Compression	33/16.5	1
6	Compression	33/33	1
6	Compression	KB 45°/5/22	1
6	Compression	22/11 LH	1

Barrel zone (cont.)	Barrel section	Element type	No. of elements
6	Compression	22/11	1
6	Compression	22/11 LH	1
7	Compression	33/33	1
7	Compression	33/16.5	1
7	Compression	KB 45°/5/33	1
7	Compression	KB 90°/5/11	1
7	Compression	KB 45°/5/11 LH	1
8	Compression	33/33	1
8	Compression	33/16.5	1
8	Compression	KB 45°/5/22	1
8	Compression	22/11 LH	1
8	Compression	22/11	1
8	Compression	22/11 LH	1
9-10	Metering	22/22	10

[a] Screw elements: pitch (mm)/length (mm)

[b] Kneading blocks (KB): stagger °/number of disks/length (mm)

[c] Left-handed (LH)

Table 7.2: Texturisation process parameters.

Process parameters	Pea protein concentrate mix	3:1 pea-potato protein concentrate mix
Screw speed	1000 rpm	1200 rpm
Torque	36%	29%
Mass flow	28 kg/h	20 kg/h
SME	215.7 Wh/kg	291.9 Wh/kg
Melt temperature	151°C	148°C
Temperature in barrel zones:		
1	-	-
2	50°C	50°C
3	95°C	80°C
4	130°C	110°C
5	140°C	140°C
6	140°C	140°C
7	140°C	140°C
8	140°C	140°C
9	130°C	130°C
10	120°C	120°C
Die pressure	20 bar	11 bar

7.2 Semi-finished product assessment

7.2.1 Rheological analysis of pork sausage batters

Table 7.3: Effect of partially replacing meat proteins by texturised pea (PE) or pea-potato (PP) proteins on rheological properties (critical shear strain (γ), critical shear stress (τ), power law index (n), and phase angle (δ)) of pork sausage batters during amplitude sweep test. The reference sausages (RE) contained 0% texturised vegetable proteins. All measurements were expressed as the mean ± SD (n = 3 each).

Product	Oscillation amplitude sweep test			
	Critical shear strain, γ [%]	Critical shear stress, τ [Pa]	Power law index, n	Phase angle δ [°]
RE00	0.96 ± 1.62	47.70 ± 79.09	0.16 ± 0.02	13.47-20.24
PE10	1.00 ± 0.88	28.63 ± 22.37	0.24 ± 0.02	19.80-27.59
PE30	1.26 ± 0.61	40.99 ± 18.77	0.25 ± 0.01	20.93-25.76
PE50	0.30 ± 0.03	15.70 ± 3.12	0.20 ± 0.00	17.94-21.51
PP10	0.82 ± 1.16	22.08 ± 25.77	0.23 ± 0.03	20.00-27.87
PP30	1.06 ± 0.27	17.53 ± 3.13	0.25 ± 0.01	23.00-26.37
PP50	0.16 ± 0.15	6.69 ± 5.73	0.20 ± 0.01	18.17-20.72

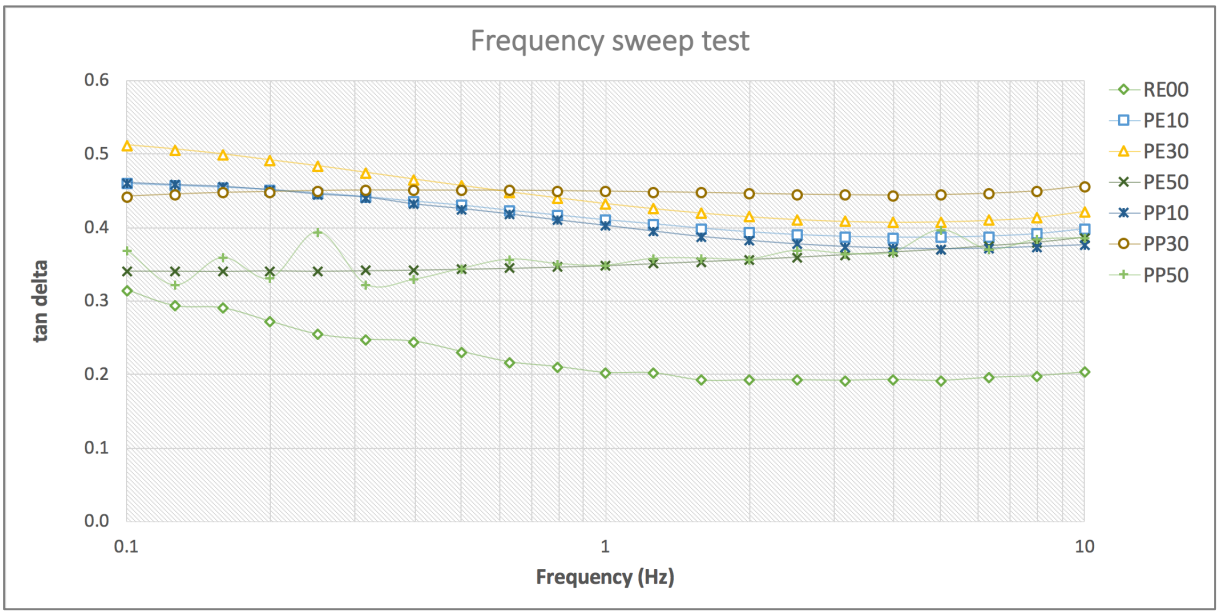

Figure 7.1: Effect of partially replacing meat proteins by texturised pea (PE) or pea-potato (PP) proteins on tan delta (tan δ) values of pork sausage batters during frequency sweep test. The reference sausages (RE) contained 0% texturised vegetable proteins. All measurements were expressed as the mean (n = 3 each).

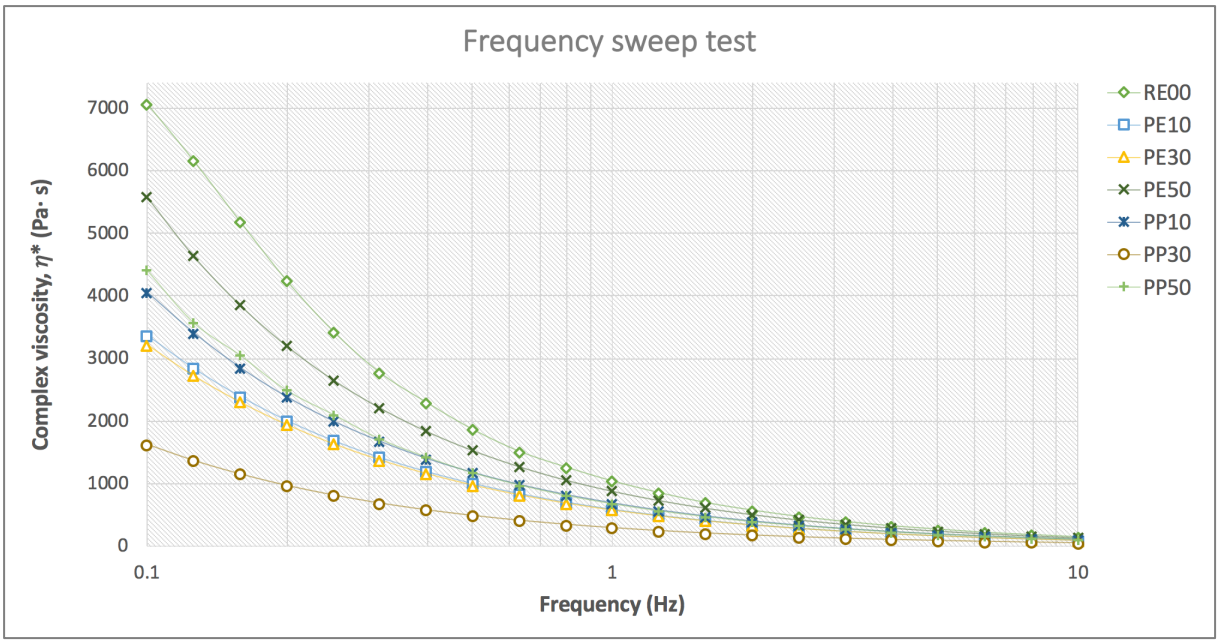

Figure 7.2: Effect of partially replacing meat proteins by texturised pea (PE) or pea-potato (PP) proteins on complex viscosity (η^*) of pork sausage batters during frequency sweep test. The reference sausages (RE) contained 0% texturised vegetable proteins. All measurements were expressed as the mean (n = 3 each).

7.2.2 LF-NMR relaxometry of pork sausage batters

Table 7.4: Effect of partially replacing meat proteins by texturised pea (PE) or pea-potato (PP) proteins on T_2 relaxation times and relative abundance in pork sausage batters. The reference sausages (RE) contained 0% texturised vegetable proteins. All measurements were expressed as the mean ± SD (n = 3 each).

Product	Relaxation time			Relative abundance		
	T_{2b} [ms]	T_{21} [ms]	T_{22} [ms]	T_{2b} [%]	T_{21} [%]	T_{22} [%]
RE00	0.0 ± 0.0	75.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
PE10	0.0 ± 0.0	55.7 ± 10.4	67.6 ± 58.5	0.0 ± 0.0	75.0 ± 21.7	25.0 ± 21.7
PE30	0.0 ± 0.0	31.3 ± 0.7	80.6 ± 0.4	0.0 ± 0.0	31.8 ± 0.9	68.2 ± 0.9
PE50	0.0 ± 0.0	25.5 ± 0.9	82.7 ± 0.7	0.0 ± 0.0	33.2 ± 3.0	66.8 ± 3.0
PP10	0.0 ± 0.0	70.8 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
PP30	0.0 ± 0.0	67.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	100.0 ± 0.0	0.0 ± 0.0
PP50	2.2 ± 3.9	49.6 ± 17.6	77.9 ± 75.7	3.3 ± 5.7	70.4 ± 32.7	26.3 ± 34.1